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BRIEF REPORT

Laboratory diagnosis of *Mycoplasma pneumoniae* respiratory tract infections in children

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Mycoplasma pneumoniae is a common respiratory tract pathogen that causes up to 40% of cases of community-acquired pneumonia in children [1]. A specific diagnosis is essential because treatment of *M. pneumoniae* infection with β-lactam antibiotics is ineffective. In routine laboratories, serology remains an important diagnostic tool [2, 3]. However, it can only provide a retrospective diagnosis and paired samples are required. Recently developed PCR techniques show high levels of specificity and sensitivity for the rapid detection of *M. pneumoniae* in clinical specimens [4, 5]; however, PCR alone is not always sufficient for a diagnosis [3]. In lieu of a gold-standard diagnostic method, we aimed to evaluate the laboratory methods currently used to diagnose *M. pneumoniae* infection in order to find the one most suitable for rapidly diagnosing the illness, especially in the early phase of disease.

During a 15-month period, a throat swab (viscose swab) and first serum specimen were taken on admission from a total of 75 children (42 males, 33 females; mean age 6.2 years; range 4 months–14 years) who were hospitalized for treatment of a respiratory tract infection (RTI). According to the guidelines of the British Thoracic Society, 53 of these children had pneumonia, 16 pharyngitis and 6 tracheobronchitis. A second serum specimen was obtained 9–24 days later. The throat specimens were examined for the presence of *M. pneumoniae* using culture in methylene blue–glucose diphasic medium (Oxoid, Basingstoke, UK), a sandwich enzyme-immunoassay for antigen detection (EIA-Ag Virion/Serion, Germany) and a PCR technique for DNA detection (extracted DNA was amplified by the primer pair P1-1 and P1-3 for the P1 adhesin gene [6]). Antibodies against *M. pneumoniae* were measured using the immunofluorescence assay (IFA) for IgG and IgM (Vircell, Granada, Spain), enzyme-linked immunosorbent assay (ELISA) for IgG (Platelia BioRad, Marnes-la-Coquette, France) and IgA (Virotech, Rüsselsheim, Germany), and capture-ELISA for IgM (Platelia BioRad). To confirm the specificity of IgM and IgA antibody detection, the Western blot technique (Virotech) was additionally performed. This method is currently the most specific for detecting anti-*M. pneumoniae* antibodies [3]. For IgM, a positive result requires ≥9 bands including P1 protein; for IgA, >24 bands are required including P1 protein. In the present study a current or recent infection was considered to have occurred definitely if at least two of the following criteria were met: (1) positive PCR and/or culture, (2) positive IgM antibodies (in the first and/or the second sample), (3) seroconversion or significant increase of IgG antibodies (fourfold increase for IFA, twofold increase for ELISA), or high IgG titers >40 AU/ml for ELISA.

M. pneumoniae was detected by PCR in the throat-swab specimens of 11 of 75 patients, by culture in three and by the antigen detection test in only one. All culture-positive samples were also positive by PCR. The complete results obtained using the various methods for the samples of each of the 75 children studied are shown in Table 1.

According to the diagnostic criteria, a definite diagnosis of current or recent *M. pneumoniae* infection was obtained for a total of 12 of the 75 pediatric patients (Table 1; patients 1–12; ten with pneumonia and two with pharyngitis). Four

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Table 1 Results of *M. pneumoniae* detection and serology in 75 children with respiratory tract infections

Patient no.	Culture	AgT	PCR	First serum specimen					Interval (days)[a]	Second serum specimen				
				IgG-IFA[b]	IgG-ELISA[c]	IgM-IFA	IgM-ELISA	IgA-ELISA		IgG-IFA	IgG-ELISA	IgM-IFA	IgM-ELISA	IgA-ELISA
1	+	+	+	0	10	−	−	−	9	64	95	−	+	+
2	−	−	+	64	122	+	+	−	10	64	120	+	+	−
3	−	−	+	64	49	+	+	−	10	128	127	+	+	+
4	−	−	+	0	0	−	−	−	9	64	24	+	+	−
5	−	−	+	0	0	−	−	−	11	64	22	+	+	−
6	−	−	+	0	0	−	−	−	22	128	110	+	+	−
7	+	−	+	0	53	+	+	−	9	256	102	+	+	+
8	−	−	+	0	0	+	+	−	11	0	1	+	+	+
9	+	−	+	64	23	+	+	−	23	128	34	+	+	−
10	−	−	−	128	55	−	+[d]	−	10	128	47	+	+	−
11	−	−	−	64	80	−	+[d]	−	9	64	70	−	+	−
12	−	−	−	64	8	−	+[d]	−	15	128	34	−	+	−
13	−	−	−	64	15	−	+[d]	−	10	64	17	−	+	−
14	−	−	−	128	37	+[e]	−[d]	+[d]	17	128	18	−	−[d]	+[d]
15	−	−	+	0	0	−	−[d]	−	12	0	0	−	−[d]	−
16	−	−	+	0	0	+[e]	−[d]	−	14	0	0	+[e]	−[d]	−
17	−	−	−	0	8	−	−	+	9	128	9	−	−	+[e]
18	−	−	−	0	9	−	−	−	10	0	1	+[e]	−	−
19	−	−	−	0	0	−	−	−	24	0	0	+[e]	−	−
20	−	−	−	0	5	+[e]	−	−	20	0	5	+[e]	−	−
21	−	−	−	0	3	+[e]	−	−	24	0	2	+[e]	−	−
22	−	−	−	0	2	+[e]	−	−	14	0	2	+[e]	−	−
23	−	−	−	0	8	−	−	+	28	0	9	−	−	+[e]
24	−	−	−	0	9	−	−	+	23	0	9	−	−	+[e]
25	−	−	−	128	3	−	−	−	23	64	3	−	−	−
26–33	−	−	−	0	12–56	−	−	−	10–24	0	10–50	−	−	−
34–38	−	−	−	64	10–27	−	−	−	9–21	64	12–24	−	−	−
39–75	−	−	−	0	<10	−	−	−	9–28	0	<10	−	−	−

AgT: antigen detection test, *PCR*: polymerase chain reaction, *IFA*: immunofluorescence assay, *ELISA*: enzyme-linked immunosorbent assay, +: positive, −: negative

[a] Interval between the first and second serum specimens

[b] ≥64=positive

[c] <10 AU/ml=insignificant, 10–20 AU/ml=low antibody rate (LAR), >20–40 AU/ml=moderate antibody rate (MAR), >40 AU/ml=high antibody rate (HAR)

[d] Result confirmed by Western blot

[e] Result not confirmed by Western blot

patients did not meet the criteria and were considered to be probably infected, while another eight patients were considered possibly infected (Table 1; patients 13–16 and 17–24, respectively). Of the remaining patients, 14 had an old mycoplasma infection and 37 had no evidence of mycoplasma infection (Table 1; patients 25–38 and 39–75, respectively).

The sensitivity and specificity of the methods was calculated based on the 12 cases with a definite diagnosis. The sensitivity values were as follows: culture 25%, antigen detection 8.3%, PCR 75%; IgM-IFA 41.7% and 75% (for the first and the first and second serum specimens combined, respectively), IgM-ELISA 66.7% and 100%, IgA 0% and 33.3%, respectively. The specificity values were as follows: culture 100%, antigen detection 100%, PCR 96.8%, IgM-IFA 92.1% and 88.9% (for the first and the first and second serum specimens combined, respectively), IgM-ELISA 98.4% and 98.4%, and IgA 93.7% and 93.7%, respectively.

Different PCR techniques have been compared to serology with varying results depending on the population studied, the types of specimens tested, the serological and molecular methods used, and the diagnostic criteria applied [2–4]. In this study we evaluated almost all the laboratory methods currently used to diagnose *M. pneumoniae* infection and we confirmed the specificity of IgM and

IgA antibody detection by immunoblotting. We also applied strict criteria for defining a recent or current infection.

The diagnostic sensitivity of PCR (75%) we found was higher than that of culture (25%) and antigen detection (8.3%), in accordance with other studies [3, 7], but it remained at a relatively low level; in 3 of the 12 patients with definite infection, the PCR result was actually negative. Using a 16S rDNA-PCR, Nadal et al. [4] found a very similar sensitivity value for throat swabs (79.3%) but a higher value for nasopharyngeal aspirates (NPAs) (90%). In contrast, Waris et al. [2] reported a lower sensitivity (50%) for the NPAs, while Ieven et al. [6] found PCR inhibitors in 26% of undiluted NPAs, and Reznikov et al. [8] found inhibitors in 36% of NPAs and none in throat swabs. We did not use NPAs or sputum specimens because these samples are difficult to obtain from children in routine practice. Moreover, sputum is not the most suitable specimen for diagnosing an upper RTI. Although the specificity of PCR in this study was very high (96.8%), two patients with a positive PCR result had a negative serology result in both the acute and convalescent serum specimens and the diagnosis of *M. pneumoniae* infection was only considered probable. The presence of *M. pneumoniae* in the throat of a healthy individual decreases the clinical significance of PCR (Gnarpe et al. [9] found a prevalence of 13.5%). Consequently, despite advanced in vitro technology, PCR alone is not always sufficient for diagnosing RTI caused by *M. pneumoniae* [2, 3, 5].

Of the serological methods used in this study, IgM-capture ELISA was found to be the most sensitive and specific, which is in agreement with the results obtained by Waris et al. [2]. However, a significant proportion of patients (33.3%) was not diagnosed at the initial visit using this method alone. Its specificity was found to be high, but the specificity of commercial kits, in general, is not always sufficient, and recent papers suggest that these kits need further improvement and that serology in a single serum sample is not always enough [10]. Concerning the detection of specific IgA antibodies, this study shows it is not relevant for the diagnosis in children. To the best of our knowledge, it has only been evaluated in adults to date.

The most interesting finding in our study concerned the combination of PCR and μ-capture ELISA. This combination revealed positive results for all 12 cases with definite infection during the early phase of disease (in five cases both methods were positive, in four cases only PCR was positive, and in three only μ-capture ELISA was positive). Thus, the sensitivity increased to 100%. (Using the same combination, Warris et al. [2] reported an increase of sensitivity to 95%). When the results of both tests are positive, the diagnosis is definite. When one of them is positive, antimicrobial therapy for a mycoplasma infection is strongly indicated; however, a second serum sample is necessary for confirmation. In conclusion, the combination of PCR and IgM-capture ELISA is highly sensitive for rapidly diagnosing *M. pneumonia* infection in children during the acute phase.

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