ORIGINAL PAPERS

Adv Clin Exp Med 2009, **18**, 4, 337–344
ISSN 1230-025X
© Copyright by Wroclaw Medical University

Beata Mączyńska[1], Marzenna Bartoszewicz[1], Urszula Kasprzykowska[1], Barbara Sozańska[2], Adam Junka[1]

Comparison of the Detectability of *Mycoplasma pneumoniae* Infection in Children Using PCR and Serological Methods: Indirect Immunofluorescence and Enzyme Immunnoassays

Porównanie wykrywalności zakażeń *Mycoplasma pneumoniae* u dzieci z użyciem metody PCR oraz metod serologicznych: immunofluorescencji pośredniej i odczynów immunoenzymatycznych

[1] Department of Microbiology, Wrocław Medical University, Poland
[2] Department of Pediatrics, Allergology and Cardiology, Wrocław Medical University, Poland

Abstract

Background. *Mycoplasma pneumoniae* is an important etiological agent of upper and lower respiratory tract infections, mainly atypical pneumonia, in children and young adults. A specific diagnosis is important because the treatment of *M. pneumoniae* infections with β-lactam antibiotics is ineffective, whereas macrolides or tetracyclines may markedly reduce the duration of the illness.
Objectives. Determination of the specificity and sensitivity of routine diagnostic procedures of *M. pneumoniae* using PCR and serological methods.
Material and Methods. Throat swabs and sera from children in the acute phase of atypical pneumonia were analyzed. The PCR assay was performed with the Venor Mp Diagnostic Kit (Minerva, Germany). The levels of IgM and IgG antibodies were evaluated in serum samples using ELISA from DiaSorin, USA, ELISA from Euroimmun, Germany, and an indirect immunofluorescence test with the BIOCHIP technique from Euroimmun.
Results. Fifty-two percent of the throat swabs were positive by PCR. Depending on the serological test used, different percentages of positive results (IgM or both IgM and IgG antibodies) were detected in the serum samples. In 15% of the cases, positive results with PCR were negative in all the serological methods.
Conclusions. The indirect fluorescence test was considered the most reliable and sensitive in comparison with PCR assay (**Adv Clin Exp Med 2009, 18, 4, 337–344**).

Key words: *Mycoplasma pneumoniae,* PCR, serological diagnosis.

Streszczenie

Wprowadzenie. *Mycoplasma pneumoniae* jest ważnym etiologicznym czynnikiem zakażeń górnych i dolnych dróg oddechowych, głównie atypowego zapalenia płuc, u dzieci oraz młodych dorosłych. Postawienie trafnej diagnozy jest niezwykle istotne, ponieważ leczenie zakażeń *Mycoplasma pneumoniae* antybiotykami β-laktamowymi nie przynosi rezultatów, kuracja makrolidami lub tetracyklinami natomiast znacząco skraca czas trwania choroby.
Cel pracy. Oszacowanie swoistości i czułości procedur diagnostycznych stosowanych w diagnostyce zakażeń *M. pneumoniae* – metody PCR oraz metod serologicznych.
Materiał i metody. Pobierano wymazy gardła oraz surowicę od dzieci, w ostrej fazie infekcji zdiagnozowanej jako atypowe zapalenie płuc. Technikę PCR wykonano z użyciem Diagnostic Kit Venor Mp (Minerva, Germany). Stężenia przeciwciał IgG oraz IgM z próbek surowicy oznaczono z użyciem testu ELISA DiaSorin, USA**,** ELISA Euroimmun, Germany, test immunofluorescencji pośredniej przeprowadzono używając zestawu Biochip technique (Euroimmun, Germany).

Wyniki. Dla 52% przypadków wymazów z gardła zbadanych techniką PCR uzyskano wynik pozytywny. W zależności od rodzaju użytego testu serologicznego, uzyskano różny odsetek wyników dodatnich (dla przeciwciał IgM lub przeciwciał IgM oraz IgG) w zbadanych próbkach osocza. Dla 15% przypadków uzyskano pozytywne wyniki testu PCR i negatywne wyniki z użyciem wszystkich metod serologicznych.
Wnioski. Test immunofluorescencji pośredniej okazał się najbardziej wiarygodny i czuły w porównaniu z techniką PCR (**Adv Clin Exp Med 2009, 18, 4, 337–344**).

Słowa kluczowe: *Mycoplasma pneumoniae,* PCR, diagnostyka serologiczna.

Mycoplasma pneumoniae is an important etiological agent of community-acquired infections of the upper and lower respiratory tracts, mainly atypical pneumonia, in children and young adults [1–3]. *M. pneumoniae* has been reported as the cause of 10–20% of pneumonia cases in hospitalized and 20–40% in ambulatory pediatric patients [4–6]. This pathogen has also been associated with non-respiratory diseases such as meningitis, encephalitis, pancreatitis, and arthritis [7–10].

A specific diagnosis is important because the treatment of *M. pneumoniae* infections with β-lactam antibiotics is ineffective, whereas macrolides and tetracyclines may markedly reduce the duration of the illness [11–13]. The standard laboratory methods for the diagnosis of *M. pneumoniae* infection have been culture and serology. Using culture, the diagnosis of this microorganism is difficult because culture is specific but slow (it may require three weeks for the results) and insensitive. Therefore the diagnostics is usually based on serological methods. Serological diagnosis has conventionally been made by the complement fixation (CF) test, which measures immunoglobulins M and G, but it does not determine the classes of antibodies. The CF tests are time-consuming, have frequently demonstrated a lack of sensitivity, and also have a tendency to give false-positive reactions with some cross-reactive antibodies [14, 15]. Alternative serological procedures, such as commercial enzyme immunoassays (EIA) and indirect immunofluorescence, require the demonstration of a rise in antibody titer or the detection of a different class of immunoglobulin, mainly IgG and IgM antibodies. These serological methods also give false-positive or false-negative results and the diagnosis is often retrospective [16]. Sometimes the results are difficult to interpret and do not allow the rapid application of an effective treatment. IgM antibodies in adult serum does not always indicate a current infection because in some cases IgM has been shown to persist for up to one year after infection. In addition, the IgM response is either minimal or undetectable in some cases of re-infection with *M. pneumoniae* [15–18].

It is generally difficult to establish criteria for a "gold standard" to detect acute or remote *M. pneumoniae* infection. Molecular techniques, especially polymerase chain reaction (PCR), have recently become optional methods for the rapid detection of *M. pneumoniae* in clinical specimens. PCR methods allow detecting *Mycoplasma* DNA in direct materials from patients with high sensitivity and specificity and the results always suggest current infection [19–22]. Thus the aim of this study was to compare serological procedures used in routine diagnostics (enzymatic and fluorescence assays) and PCR, which is currently the most reliable and sensitive method applied in *M. pneumoniae* diagnostics.

Material and Methods

Material from 100 children 5–14 years of age hospitalized at the Department of Pediatrics, Allergology, and Cardiology of the Wroclaw Medical University due to atypical pneumonia were analyzed. There was also a specific ambulatory control group consisting of 30 children diagnosed with throat infection at the "ALAB" Laboratory of the Microbiology Department. Serum samples for antibody testing and throat swabs for PCR were obtained from the children with acute infection. Due to difficulties with the choice of a control group of healthy children, the selection of hospitalized children to study was very precise. Atypical pneumonia without auscultatory symptoms, mild fever, and characteristic RTG were diagnosed in all the patients. Typical pathogens (*S. pneumoniae*, *H. influenzae*, *K. pneumoniae*) were also excluded. The levels of IgM and IgG antibodies were evaluated in the serum samples using two different enzyme immunoassays (EIAs) and an indirect immunofluorescence test with the BIOCHIP technique.

PCR: VenorMp Diagnostic Kit, Minerva Biolabs, Germany

The PCR test, validated by the European in Vitro Diagnostic Directive, ensures a qualitative determination of infection level. The test is almost 100% specific, determined through the clinical evaluation of primers which exclusively amplify *Mycoplasma pneumoniae* subtypes I and II encod-

ing the P1 adherent protein. Because of the very highly specificity, electrophoretic analysis is the only necessary post-PCR step needed to evaluate the results. It is reliable with as little as 1–5 *Mycoplasma pneumoniae* particles per sample. The reliability of the test is guaranteed by the internal control (PCR quality check), i.e. a 263-bp sequence of the HTLV-I-tax gene. There is a positive control included, i.e. a DNA fragment of *M. pneumoniae* prepared by PCR, to verify results. Templates for PCR analysis were prepared by DNA extraction from the material obtained by washing out throat swabs using a manual method with guanidine isothocianiate. Similar effects were obtained using a very simple thermal method in which the material obtained from the swabs was heated to 94°C for 10 minutes [20] and Qiagen DNA kits. Preparation of the PCR samples, dNTPs, buffer, polymerase, and internal and positive control concentrations are specified by the producer. The temperature profile is also given. Amplified products are separated and visualized by standard agarose electrophoresis in 2% gel stained with ethidium bromide (TAE buffer, 80 V). In samples containing *Mycoplasma pneumoniae* DNA (as well as the positive control), a 207-bp band is detected. A successfully performed reaction is indicated by a 263-bp internal control product, although this product might not be seen in positive samples with a high concentration of the template competition between templates in the PCR.

Indirect Immunofluorescence Test: Euroimmun, Germany

M. pneumoniae infected and non-infected cells covering the reaction areas of a BIOCHIP slide are incubated with diluted serum samples. The important step before detecting specific IgM antibodies is removing IgG immunoglobulins from the patient's serum by immunoabsorption (EUROSORB reagent). This is to prevent a false IgM-positive result caused by possible IgM rheuma factors reacting with specifically bound IgG. False IgM-negative results brought about by specific IgG competing with IgM for antigenic binding sites are also avoided. Furthermore, the immunoadsorption of IgG leads to the elimination of rheuma factors from the sample. If the sample is positive, specific IgM and IgG antibodies attach to the bacterial antigens. In the second step the attached antibodies are stained with fluorescein-labeled anti-human antibodies and made visible with a fluorescence microscope. Antibodies against *M. pneumoniae* cause a granular to coarse drop-like fluorescence in infected cells, mainly in the area of the cytoplasm. If the serum sample contains anti-*M. pneumoniae*, the same pattern is essentially observed as for the positive control serum. For comparison, the second BIOCHIP of each test field is coated with non-infected cells. Important is that in this assay the antibody titer may be detected. The serum samples showed the presence of IgM (titer ≥ 10) or both IgM and IgG antibodies (Table 2) and samples with a high titer (> 200) of IgG antibodies (Table 3) are considered positive (current, acute infection).

Table 1. Percentages of positive and negative samples with all the investigated methods in the children with atypical pneumonia

Tabela 1. Odsetek próbek dodatnich oznaczonych różnymi metodami u dzieci z atypowym zapaleniem płuc

N = 100 Method (Użyta metoda)	Percentage of samples detected as: (Odsetek próbek określonych jako:)	
	positive (dodatnie)	negative (ujemne)
PCR VenorMp – Minerva Biolabs	52	48
Indirect Immuno-fluorescence – Euroimmun	36	64
ELISA ETI-MP – DiaSorin	32	68
ELISA – Euroimmun	14	86

Enzyme immunoassay: ETI-MP IgG, IgM ELISA (DiaSorin, USA)

The antigen used in the ETI-MP test, i.e. a purified fraction of the *M. pneumoniae* membrane protein P1, is coated on microtiter plates. Cover plates with serum samples diluted 1/105, a negative control, and three calibrators (P10, P50, P100) are incubated for 1 h at 37°C. Then anti-human antibodies conjugated with horseradish peroxidase (HRP) are added and the plates were incubated for 1 h at 37°C. The next step is the incubation with the substrate. After stopping the reaction, the optical densities (ODs) of the samples are transformed to arbitrary units determined from a calibration curve (10, 50, and 100 BU/ml). Antibody titers between 10 and 50 BU/ml are considered significant and a value > 100 BU/ml is considered a high titer. Serum samples showing the presence of IgM or both IgM and IgG antibody in a significant titer are regarded as positive.

Table 2. Detection of *M. pneumoniae* IgM and IgG antibodies by the two ELISA tests and the immunofluorescence test in the children with atypical pneumonia

Tabela 2. Oznaczenie przeciwciał IgM i IgG anty-*M. pneumoniae* w różnych testach ELISA oraz w teście immunofluorescencji u dzieci z atypowym zapaleniem płuc

N = 100	Percentage of serum samples detected: (Odsetek próbek surowicy oznaczonych testem:)		
	ELISA DiaSorin	ELISA Euroimmun	Immunofluorescence Euroimmun
Absence of IgM and IgG antibodies (Brak przeciwciał obu klas)	57	73	51
Presence of IgM and IgG antibodies (Obecność przeciwciał IgM oraz IgG)	14	14	23
Presence of IgM antibodies only (Obecność jedynie przeciwciał IgM)	18	0	4
Presence of IgG antibodies only (Obecność jedynie przeciwciał IgG)	11	13	22

Table 3. IgG antibody levels detected by the immunofluorescence test in the children with atypical pneumonia

Tabela 3. Analiza stężenia przeciwciał IgG oznaczanych testem immunofluorescencji u dzieci z atypowym zapaleniem płuc

N = 22 (100%)	Number (%) of serum samples with IgG antibodies only detected by immunofluorescence (Liczba (%) próbek surowicy w których z użyciem testu immunofluorescencji wykryto jedynie przeciwciała klasy IgG)
IgG antibodies with titer > 200 (Przeciwciała IgG w mianie > 200)	9 (40%)
IgG antibodies with titer > 100 (Przeciwciała IgG w mianie > 100)	5 (24%)
IgG antibodies with titer > 10 (Przeciwciała IgG w mianie > 10)	8 (36%)

Enzyme Immunoassay: IgG, IgM ELISA, Euroimmun, Germany

Patient samples diluted 1/101 are incubated in the wells of plates coated with whole cell extract of *M. pneumoniae* strain FN for 30 minutes at room temperature. Afterwards, an enzyme conjugate is added. After further incubation the chromogen/substrate is used. The reaction is stopped by means of a stop solution. IgG antibodies are removed by immunoabsorption before a patient's serum is tested for specific IgM antibodies. The extinction values are interpreted by calculating the ratio of the extinction of controls to the extinction of the calibrator (cut-off). Ratios > 1.0 are considered positive and ratios < 1.0 negative.

Results

There were 100 serum and throat swab samples from the children hospitalized with atypical pneumonia with the characteristic clinical view. In the ambulatory control group with throat infections, 30 samples of serum and throat swabs were tested for *M. pneumoniae.*

IgM and IgG antibodies were determined in the serum samples. Samples with both IgM and IgG or only IgM antibody were considered positive and signifying a current *M. pneumoniae* infection. Samples in which only IgG was detected were classified as considerable and signifying the trace of past infection. A more exact analysis of IgG presence was made with the use of the immunofluorescence method only when a determination of antibody titer was possible (samples with a titer above 100 were considered positive).

In the throat swabs the presence of *Mycoplasma pneumoniae* DNA was determined with the use of PCR. In positive samples both the 207-bp and 263-bp products were observed. The 263-bp product represents an internal control of a correct amplification process. The numbers of positive samples number determined by PCR and serological methods are presented in Table 1.

Over 50% of the samples from the children with atypical pneumonia were considered positive using PCR. Using serological methods, the results diverged. Only 15 samples (15%) were analyzed identically using all three methods. For 36 samples

Table 4. Detection of *M. pneumoniae* DNA and IgM and IgG antibodies in the children of the control group

Tabela 4. Oznaczanie DNA *M. pneumoniae* oraz przeciwciał IgM i IgG anty-*M. pneumoniae* u dzieci z grupy kontrolnej

Control group (Grupa kontrolna)	PCR	ELISA DiaSorin		ELISA Euroimmun		Immunofluorescence Euroimmun	
N = 30	IgM	IgG	IgM	IgG	IgM	IgG	
Number of positives samples (Liczba dodatnich próbek)	0	0	4	0	3	0	5

the results were distinct (the presence of antibodies was shown using two or one method only). Of the serological test, the majority of positive results (32 serum samples) was observed using the DiaSorin ELISA test, 36 using the immunofluorescence method, and 14 using the ELISA method from Euroimmun (Table 1). As many as 14 of the 32 samples marked as positive by DiaSorin ELISA on the basis of IgM antibody presence were considered negative using the two other serological tests. This was probably due to false-positive results. Simultaneously, as many as 13 samples showing a lack of antibodies or the presence of IgG class only by the Euroimmun ELISA test were positive in the other two tests. These results may be considered false negative (Table 2).

The indirect immunofluorescence method showed the highest sensitivity and specificity of the serological tests, especially for detecting IgG antibodies. The advantage of this method is the possibility of determining the exact titer of antibody. Due to this it is possible to specify a "current infection" in cases when only IgG is observed. Fourteen of the 22 samples classified as doubtful (signifying a trace of past infection) showed a titer of > 100 (including 9 samples with titer above 200). Undoubtedly, despite the lack of IgM antibodies, such results prove a current *M. pneumoniae* infection (Table 3). Eight samples with IgG titers of about 10, which indicates past infection, were classified as negative.

The sensitivity and specificity of all the serological methods with reference to the PCR method are juxtaposed in Table 5.

In the control group there was no case of *M. pneumoniae* DNA detected in the throat swabs. There were also no anti-*M. pneumoniae* IgM antibodies in this group found with any of the tested serological methods, but the presence of IgG antibodies in three samples of serum was found with those methods. In two samples, IgG antibodies were found only by one or two serological methods (Table 4) and only in one case was the titer of antibodies in the immunofluorescence method higher than 200.

The amplified PCR products were visualized by standard gel electrophoresis. A sample containing *Mycoplasma pneumoniae* DNA produces a distinct 207-bp band, as seen with the positive control reaction. This band is absent in a reaction performed with negative patient samples and in the negative control. The internal control DNA should be present in every reaction and produces a 263-bp band. The DNA samples in a high quantity of 207-bp product were also considered positive because *M. pneumoniae* DNA competes for primers with the DNA internal control. The results of the detection of different *M. pneumoniae* DNA samples are presented in Fig. 1.

Table 5. Sensitivity and specificity of the serological methods using PCR as a reference test

Tabela 5. Czułość i swoistość metod serologicznych w stosunku do metody PCR jako testu referencyjnego

	ELISA DiaSorin	ELISA Euroimmun	Immuno-fluorescence Euroimmun
Sensitivity (Czułość)	77%	59%	87%
Specificity (Swoistość)	81%	76%	82%

Discussion

The comparison of the detectability *Mycoplasma pneumoniae* using serological methods (determining IgM and IgG antibodies in enzyme immunoassay and indirect fluorescence methods) and PCR revealed significant discrepancies in the results depending on the test used. Many reports consider differences in the detectability of *M. pneumoniae* antibodies using different serological tests based on the enzyme immunoassay method [23, 16]. The credibility of these tests varies considerably and depends mostly on the applied antigen as well as the number of controls and calibrators [18, 24]. The DiaSorin ELISA test used in the studies, in the present authors' previous experience, shows high sensitivity and specificity [24]. There are four controls applied in the test: three positive and a membrane protein, P1, as antigen.

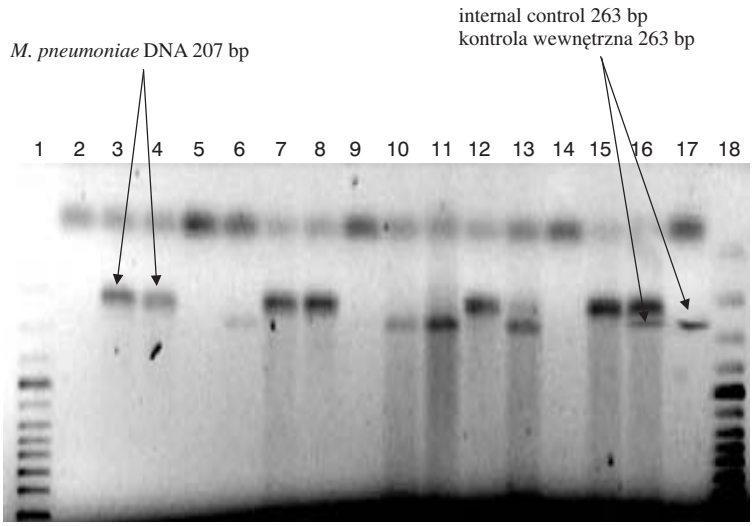

Fig. 1. Electrophoretic discrimination of amplified *M. pneumoniae* DNA isolated from throat swabs of the children with atypical pneumonia

Ryc. 1. Rozdział elektroforetyczny amplifikowanego DNA *M. pneumoniae* izolowanego z wymazów krtaniowych dzieci z atypowym zapaleniem płuc

Lines 1, 18 – molecular weight marker
Lines 3, 4, 7, 8, 12, 13, 15 – positive samples
Lines 2, 6, 10, 11 – negative samples
Lines 2, 5, 9, 14 – uncertain samples (amplification should be repeated)
Line 16 – positive control with internal control
Line 17 – negative control with internal control

Ścieżki 1,18 – molekularny wzorzec masowy
Ścieżki 3, 4, 7, 8, 12, 13, 15 – próbki dodatnie
Ścieżki 6, 10, 11 – próbki ujemne
Ścieżki 5, 9, 14 – próbki niepewne (amplifikacja powinna zostać powtórzona)
Ścieżka 16 – kontrola pozytywna z kontrolą wewnętrzną
Ścieżka 17 – kontrola negatywna z kontrolą wewnętrzną

However, there is no immunoadsorption stage to eliminate rheumatoid factor and IgG antibodies which may influence the determination of IgM antibodies. The very high level of positive reactions obtained in this test (32%, including 18% only on the basis of IgM presence) suggests the possibility of false-positive results. In comparison, the Euroimmun ELISA test reveals relatively low sensitivity according to IgM class detectability. Taking into consideration the results using the two other methods as well as the quite strict criteria of patient selection (all cases with clinically demonstrated atypical pneumonia), such a result seems to be highly improbable. Therefore it is possible that the immunoadsorption stage which should eliminate false-positive results is improperly constructed and might be responsible for removing some specific IgM antibodies directed against *M. pneumoniae*. The presence of IgM antibodies, an important marker of current *Mycoplasm*a-related infection in children, has been proven and underlined in many scientific publications [5, 17, 18, 23, 25]. The situation is different in adults; chronic infection or re-infection might manifest as a high level of class G immunoglobulins [15, 17, 26].

It has been demonstrated that the indirect immunofluorescence test is the most reliable of the applied methods. The proportion of positive results corresponds with published data (24%) [1, 2, 4]. At the same time, the test kits show considerably higher IgG sensitivity than enzyme immunoassay. The ability to determine the titer enables the detection of current infection even if there is a lack of IgM antibodies (Table 3). The indirect immunofluorescence method, used quite rarely in routine diagnosis so far, also seems specific. The Euroimmun test is based on BIOCHIP technology. Slides are coated with fibroblasts infected with *M. pneumoniae* cells. Negative controls with only non-infected fibroblasts enable eliminating false-positive nonspecific results. Additional control sera, both positive and negative, are also included. However, this method requires some practice and experience of the operator as well as a high-quality fluorescence microscope to interpret the result properly. The recommended method, showing very high sensitivity and specificity, in diagnosing *Mycoplasma* infections is determining *Mycoplasma* DNA using PCR [19–22]. The present investigations performed with the throat swab method revealed the highest percentage of positive results (52%) (Table 1). Considering the patient group selected (children with atypical lungs changes and with excluded *S. pneumoniae* infection), such

a result seems highly probable. The negative results of *Mycoplasma pneumoniae* DNA in the control group and lack of antibodies (especially IgM) also seem to confirm this (Table 4). Moreover, in this group the most common result of throat swab diagnosed by routine inoculation was *Streptococcus pyogenes.*

At the moment there are many commercial tests available detecting *M. pneumoniae* DNA that are based on PCR or real-time PCR. These tests are, similarly to the Venor Mp, equipped with suitable internal controls preventing false-positive and false-negative reactions. The commercial tests often contain more than one pair of primers similar to sequences characteristic of *M. pneumoniae* (such as P1 protein, the 16sRNA and 23sRNA region, elongation factor Tu) [27, 22], which improves the specificity of the test. What is more important, with the use of a DNA-based method it is possible to diagnose infection when there are as few as 100 cells in the diagnostic material [28]. The available commercial PCR tests seem to have one disadvantage, namely a lack of reagents which could be recommended for DNA isolation. Moreover, it has been demonstrated that the efficiency of PCR depends on the DNA isolation methods. Because the PCR results may differ depending on the isolated amount of DNA, using methods with diverse efficiency of DNA isolation makes the subsequent PCR test less reliable.

In conclusion, the present investigation concerning the diagnosis of *M. pneumoniae* infections showed that not all commercially available serological tests are equally adequate. Moreover, the combination of genetic and serological methods could serve as a "gold standard", increasing the possibility of detecting the etiological quickly in atypical pneumonia and thus correct therapy.

References

[1] Mac Farlane J: An overview of community-acquired pneumonia with lessons learned from the British Thoracic Society Study. Semin Respir Infect 1994, 9, 153–165.

[2] Grendrel D, Raymond J, Moulin F, Inigues JL, Ravilly S, Habb F, Lebon P, Kalifa G: Etiology and response to antibiotic therapy of community-acquired pneumonia in French children. Eur J Clin Microbiol Infect Dis 1997, 16, 388–391.

[3] Principi N, Esposito S, Blasi F, Allegra L: Role of *Mycoplasma pneumonia* in children with community-acquired lower respiratory tract infections. Clin Infect Dis 2001, 32, 1281–1289.

[4] Kałużewski S, Jagielski M, Rastawicki W, Kochman M: The estimation of appearance of *Mycoplasma pneumoniae* infections in Poland 1970–1993 years, on the basis of serological study (in Polish). Przeg Epid 1994, 48, 166–175.

[5] Waris ME, Toikka P, Nikkari S, Meurman O, Vainionpaa R, Mertsol J, Ruuskanen O: Diagnosis of *Mycoplasma pneumoniae* pneumonia in children. J Clin Microbiol 1998, 36, 3155–3159.

[6] Mączyńska B, Stankiewicz M: Detection of *Mycoplasma pneumoniae* antibodies in patients with respiratory tract infections. Clin Microbiol Infect 2000, 6, (Suppl. 1), abstr TuP81: 55.

[7] Foy HM: Infections caused by *Mycoplasma pneumoniae* and possible carrier state in different populations of patients. Clin Infect Dis 1993, 17 (1), 37–47.

[8] Kenny RT, Li JS, Clyde WA, Wall TC, O'Connor CM, Campbell PT, Van Trigt P, Corey GR: Mycoplasmal pericarditis: evidence of invasive disease. Clin Infect Dis 1993, 17, 558–562.

[9] Rastawicki W, Jagielski M: *Mycoplasma pneumoniae*; Characteristic of the microbe, mechanisms of pathogenesis and immunological response of man to *M pneumoniae* infection (in Polish). Post Mikrobiol 1998, 37, 261–271.

[10] Bencina D, Dove P, Muelle-Premru M, Avsic-Zupanc T, Socan M, Beovic B, Arnez M, Narat M: Intrathecal synthesis of specific antibodies in patients with invasion of the central nervous system by *Mycoplasma pneumoniae.* Eur J Clin Microbiol Infect Dis 2000, 19, 521–530.

[11] Cassell GH, Waites KB, Pate MS, Canupp KC, Duffy LB: Comparative susceptibility of *Mycoplasma pneumoniae* to erythromycin, ciprofloxacin and lomefloxacin. Diagn Microbiol Infect Dis 1989, 12, 433–435.

[12] Waites KB, Duffy LB, Schmid T, Crabb D, Pate MS, Cassell GH: *In vitro* susceptibilities of *Mycoplasma pneumoniae, Mycoplasma hominis* and *Ureaplasma urealyticum* to sparfloxacin and PD 127391. Antimicrob Agents Chemother 1991, 6, 1181–1187.

[13] Rennie KA, Prasad ES, Wenman WN: *In vitro* activity of Dirithromycin, a new macrolide antibiotic, against *Mycoplasma* species. Diagn Microbiol Infect Dis 1994, 20, 57–61.

[14] Lind K, Madsen H, Wiik A: Autoantibodies to the mitotic spindle apparatus in *Mycoplasma pneumoniae* disease. Infect Immun 1998, 56, 714–715.

[15] Jacobs E: Serological diagnosis of *Mycoplasma pneumoniae* infections: a critical review of current procedures. Clin Infect Dis 1993, 17 (Suppl.1), abstr S79–S82.

[16] Tuuminen T, Suni J, Kleemola M, Jacobs E: Improved sensitivity and specificity of enzyme immunoassays with P1-adhesin enriched antigen to detect acute *Mycoplasma pneumoniae* infection. J Microbiol Methods 2001, 1, 44, 27–37.

[17] Sillis M: The limitations of IgM assays in the serological diagnosis of *Mycoplasma pneumoniae* infections. J Med Microbiol 1990, 33, 253–258.
[18] Thacker WL, Talkington DF: Analysis of complement fixation and commercial enzyme immunoassays for detection of antibodies to *Mycoplasma pneumoniae* in human serum. Clin Diagn Labor Immunol 2000, 7, 778–787.
[19] Lüneberg E, Jensen JS, Frosch M: Detection of *Mycoplasma pneumoniae* by polymerase chain reaction and nonradioactive hybridization in microtiter plates. J Clin Microbiol 1993, 31, 1088–1094.
[20] Blackmore TK, Reznikov M, Gordon DL: Clinical utility of the polymerase chain reaction to diagnose *Mycoplasma pneumoniae* infection. Pathology 1995, 27, 177–181.
[21] Grattard F, Bourlet T, Galambrun C, Berger C, Stephan JL, Lauras B, Pozzetto B: Interest of gene amplification by PCR for the diagnosis of *Mycoplasma pneumoniae* infections in the child. Pathol Biol 1998, 46, 464–469.
[22] Rastawicki W, Jagielski M, Gierczyński R: Application of PCR for detection of the selected *Mycoplasma pneumoniae* genes (in Polish). Med Dośw Mikrobiol 2000, 52, 67–74.
[23] Matas L, Dominquez J, De Ory F, Garcia N, Cardona PJ, Hernandez A, Rodrigo C, Ausina V: Evaluation of Meridian ImmunoCard Mycoplasma test for the detection of *Mycoplasma pneumoniae*-specific IgM in pediatric patients. Scand J Infect Dis 1998, 30, 289–293.
[24] Mączyńska B, Chiciak J, Stankiewicz M, Przondo-Mordarska A: Detectability of *Mycoplasma pneumoniae* antibodies using different serological methods in children with atypical pneumonia (in Polish). Adv Clin Exp Med 2002, 11, 451–456.
[25] Cimolai N, Cheong ACH: An assessment of new diagnosis indirect enzyme immunoassay for the detection of anti-*Mycoplasma pneumoniae* IgM. Am J Clin Pathol 1996, 105, 205–209.
[26] Rastawicki W, Jagielski M: *Mycoplasma pneumoniae.* II. Clinical features, epidemiology and diagnosis of *M. pneumoniae* infections (in Polish). Post Mikrobiol 1998, 37, 273–288.
[27] Kai M, Kamiya S, Yale H, Tkatura I, Shiozawa K, Ozawa A: Rapid detection of *Mycoplasma pneumoniae* in clinical samples by the polymerase chain reaction. J Med Microbiol 1993, 38, 166–170.
[28] Haima P, Oerdijk M, Loens K, Ieven M, Van Roosmalen I, Sillekens P: Molecular diagnosis of *Mycoplasma pneumoniae* infections by using NASBA with "Real time" detection. Clin Microbol Infect 2001, 8 (Suppl. 1), abstr. P731: 148.

Address for correspondence:

Beata Mączyńska
ul. Sielska 19a
54-144 Wrocław
Poland
Tel.: +48 71 784 13 01
E-mail: beciam@mbio.am.wroc.pl

Conflict of interest: None declared

Received: 13.01.2009
Revised: 5.08.2009
Accepted: 10.08.2009