

RESEARCH NOTE

DETECTION OF *MYCOPLASMA PNEUMONIAE* FROM CEREBROSPINAL FLUID SAMPLES OF PEDIATRIC PATIENTS

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Abstract. Central nervous system manifestations are probably the most frequent extrapulmonary complications of infections due to *Mycoplasma pneumoniae*, occur mostly in children. In this study, we attempted to isolate *M. pneumoniae* and to detect the organism by polymerase chain reaction (PCR) from cerebrospinal fluid samples (CSF) of pediatric patients. Of the 244 CSF samples, no *M. pneumoniae* was isolated. Six (2.5%) of the CSF samples were positive by PCR amplification. More effort are necessary to isolate the organism from CSF samples in order to ascertain the role of *M. pneumoniae* in causing neurological complications.

INTRODUCTION

Mycoplasma pneumoniae is a common respiratory tract pathogen that generally produces a mild self-limited disease characterized by cough, malaise, myalgia and headache. Tracheobronchitis and pneumonia are the most common reasons for the patients to seek medical care (Lind, 1983). An increasing number of unusual and severe clinical pictures of *M. pneumoniae* disease have been described, involving multiorgan systems such as skin, mucous membranes, central nervous system (CNS) and heart (Lind, 1983; Koskiniemi, 1993). The neurologic manifestations are reported to be the most common nonpulmonary manifestations (Smith and Eviatar, 2000).

M. pneumoniae was not usually included in the diagnosis of childhood meningitis due to the unavailability and shortcomings of diagnostic tests. Clinical diagnosis of *M. pneumoniae* pneumonia is difficult because many viral and other pneumonias present clinically similar pictures. *M. pneumoniae* has been isolated from cerebrospinal fluid (CSF) samples with CNS manifestations (Fleischhauer *et al*, 1972; Suzuki *et al*, 1983; Kasahara *et al*, 1985; Abramovitz *et al*, 1987; Nagayama *et al*, 1987). Culture, ideally regarded as the gold standard, requires skill and preparation of complex media, is time consuming and is not available in routine clinical laboratories. Serological procedures are the most widely used and require the demonstration of a rise in antibody titer. However, it takes too long for results of isolation and serological methods to be obtained to allow for the rapid application of an effective treatment. Recent

advances in molecular biology has allowed specific, sensitive and rapid detection of *M. pneumoniae* by PCR (Abele-Horn *et al*, 1998; Dorigo-Zetsma *et al*, 1999). Narita *et al* (1992) detected successfully in CSF samples from four of six patients and in serum samples from three of four patients with clinically and serologically confirmed mycoplasma central nervous system infection.

In this study, an attempt to isolate *M. pneumoniae* and PCR detection was performed on 244 CSF samples submitted to the Bacteriology Division of the Institute for Medical Research from 1996-1997. The samples were obtained from pediatric patients admitted to the Kuala Lumpur Hospital with CNS manifestations.

Isolation of *M. pneumoniae* was performed as described by Velleca *et al* (1980). Three hundred microliters of the sample was inoculated into glucose diphasic media and 0.1 ml was inoculated onto glucose agar medium. These media were incubated aerobically at 37°C. The diphasic medium was examined daily for a shift to acid pH as demonstrated by a color change from blue or red to yellow. When a color change was apparent, subculture to another fresh glucose agar medium was performed. For presumptive identification of *M. pneumoniae*, guinea pig hemadsorption test was performed.

For detection of *M. pneumoniae* by PCR, CSF sample (approximately 1 ml) was first centrifuged at 13,000 rpm for 10 minutes following which the pellet was resuspended in lysis buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100 and 200 mg/ml proteinase K]. The

mixture was incubated at 55°C for 2 hours before subjected to heating at 95°C for 30 minutes. A volume of 10 µl of the treated sample was used as DNA sample. Primer set MP-P11 (5'-TGCCATCAACCCGCGCTTAAC-3') and MP-P12 (5'-CCTTTGCAACTGCTCATAGTA-3') as described by de Barbeyrac *et al* (1993) were used. A total volume of 10 µl DNA sample was incubated in a 50 µl reaction volume containing 0.1 µM each primer, 200 µl each deoxynucleoside triphosphate, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ and 1 U of *Taq* polymerase (Boehringer Mannheim). Amplification was carried out for 35 cycles (1 minute at 95°C, 1 minute at 55°C and 1 minute at 72°C). Positive control (DNA extracted from the *M. pneumoniae* ATCC 10119) and negative control (H₂O) were run simultaneously. A positive signal was defined by a 466-bp fragment visualized in ethidium bromide-stained agarose gel.

Antimycoplasmal antibody in serum and CSF samples was measured by microparticle agglutination test according to the instruction of the manufacturer (SERODIA MYCO II, Fujirebio Inc, Japan) whenever samples were available.

Of the 244 CSF samples, no *M. pneumoniae* was isolated. Six (2.5%) of the CSF samples were positive by PCR amplification. Table 1 shows the demographic and clinical findings of these patients. Only 70 CSF samples were tested for antimycoplasmal antibody, all were negative at the titer of <1:40 except one at the titer of 1:80.

The isolation of *M. pneumoniae* in this study was similar to those reported by Narita *et al* (1992), in which no isolation was obtained despite positive detection of *M. pneumoniae* by PCR in the CSF samples. The recovery of *M. pneumoniae* from CSF samples is associated with the survival capability of *M. pneumoniae*. Kasahara *et al* (1985) reported that the success of isolation of *M. pneumoniae* in their study was due to the collection of a large amount of specimen (1.5 ml) at an early stage of the disease and its immediate inoculation into the culture medium. The failure to isolate *M. pneumoniae* in this study therefore might be due to the small size (300 µl) of samples used for culturing, the low number of the organisms present in the CSF samples and some delay in the culturing of the samples.

With the use of PCR, more direct confirmation of *M. pneumoniae* in the CSF samples is possible (Narita *et al*, 1992; Fink *et al*, 1995). PCR allowed the detection of *M. pneumoniae* DNA

regardless of the viability of the organism. In this study, oligonucleotide primers were chosen from the published nucleotide sequences of the P1 adhesion gene from *M. pneumoniae* (Inamine *et al*, 1988). The adhesins are presumably essential for the pathogenicity of these organisms and specific for the organism to be detected. The sensitivity for detection of DNA using the primers was found to be to a level of one copy per sample in clinical material (CSF, serum or sputum), and is 4-5 logs more sensitive than culture or antigen detection (Fink *et al*, 1995).

CNS complications of *M. pneumoniae* infection include encephalitis, meningitis, myelitis, polyradiculitis, or acute psychosis. These neurologic complications are usually preceded by respiratory symptoms although some patients gave no history of the symptoms (Thomas *et al*, 1993). In this study, three patients had evident of respiratory tract infections preceding the development of neurological signs (Table 1). In two cases, patients were having pneumonia before developing neurological illness. In another case, a 3 year-old female patient presented with cough and fever for 5 days and was clinically diagnosed as having atypical pneumonia before developed acute ascending paralysis and Guillain Barre syndrome. *M. pneumoniae* DNA was detected by PCR from the CSF sample on day 12th. The serum antimycoplasmal antibody was significantly positive at a titer of 1:160. However, the antimycoplasmal antibody in the CSF sample was negative (1:40).

Only one (1.4%) of 70 CSF samples tested was positive for antimycoplasmal antibody. The positive sample was obtained from a 4 year-old male patient with diagnosis of acute lymphoblastic leukemia. Isolation and PCR detection of *M. pneumoniae* from the CSF sample was however negative. Although it has been reported that low titers of antimycoplasmal antibodies in CSF samples would allow isolation of *M. pneumoniae* (Klimek *et al*, 1976; Kasahara *et al*, 1987), no *M. pneumoniae* was isolated in this study. It has been reported that when measured by a particle agglutination method, the CSF antibody titers are not necessarily able to predict the presence of this organism in the CNS. *M. pneumoniae* DNA were detected by PCR in three of the four cases in which the antibody titers were below its detection limit (Narita *et al*, 1992).

CNS symptoms are present in up to 7% of patients treated at hospitals for *M. pneumoniae*

Table 1

Demographic and other clinical findings of patients with PCR detection of *M. pneumoniae* DNA in cerebrospinal fluid samples.

Patient	Age (years)	Race	Sex	Other clinical diagnosis	Respiratory symptoms or signs
1	10	Other	F	-	Bronchopneumonia
2	3	Malay	F	-	Bronchopneumonia
3	3	Malay	F	Acute placcid paralysis, Guillain Barre syndrome	Bronchopneumonia
4	9	Malay	F	Acute lymphoblastic leukemia	NA
5	NA	Malay	F	-	NA
6	10/12	Indian	F	-	NA

NA = not available

infection (Levine and Lerner, 1978; Ponka, 1980). In turn, among patients with neurological syndromes, *M. pneumoniae* is associated with 5-10% of cases (Lind *et al*, 1979; Urquhart, 1979). Based on the PCR findings in this study, *M. pneumoniae* was probably associated with 2.5% of CNS manifestations among pediatric patients. *M. pneumoniae* has also been described as a cause of the Guillain-Barre syndrome (Hodges and Perkins, 1969; Meseguer *et al*, 1998; Hughes *et al*, 1999) and acute lymphoblastic leukemia (Alexander, 1997). In this study, *M. pneumoniae* DNA was detected from the CSF sample of a patient who had Guillain Barre syndrome. Of 2 patients with acute lymphoblastic leukemia, one had *M. pneumoniae* DNA detected by PCR and another with a low titer of anti-mycoplasmal antibody presence in the CSF sample. The pathophysiology of *M. pneumoniae* infections in these two diseases needs further investigation.

There are 3 suggested possibilities for *M. pneumoniae* associated with neurological illness (Thomas *et al*, 1993): autoimmune mechanisms, direct neurotoxicity and direct invasion of the CSF and brain. The detection of *M. pneumoniae* in CSF samples by isolation and PCR detection in this and other studies (Fleischhauer *et al*, 1972; Suzuki *et al*, 1983; Kasahara *et al*, 1985; Abramovitz *et al*, 1987; Nagayama *et al*, 1987) suggested that neurological complication in *M. pneumoniae* infection is directly related to invasion of the CNS by *M. pneumoniae*.

Complications of *M. pneumoniae* infection, particularly neurological illness in childhood, should alert clinicians for early diagnosis and treatment of respiratory infections. In view of the nonspecific clinical syndromes of *M. pneumoniae* infections, particularly in neurological cases and the

presence of *M. pneumoniae* in CSF samples, it is suggested that the diagnosis of neurological complications of *M. pneumoniae* infections should be considered in all cases of acute encephalopathy, even if there is no antecedent respiratory illness (Thomas *et al*, 1993). A diagnostic technique such as PCR for detection of *M. pneumoniae* for extrapulmonary cases, in particular CNS infection is needed. Attempts to isolate *M. pneumoniae* from CSF samples should be continued in order to clarify the mechanism of neurological complication in infections with *M. pneumoniae*.

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