Molecular and Serologic Approaches for Diagnosis of *Mycoplasma pneumoniae* Infections in Children and Typing of the Pathogen.

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Introduction

M. pneumoniae is a common cause of upper and lower respiratory tract infections of variable severity (4). Infections of this pathogen occur worldwide throughout the year (7), although they are best documented as a significant cause of respiratory disease in temperate climates (3). The incidence of *M. pneumoniae* is greatest among school-age children and declines after adolescence (1). However, *M. pneumoniae* may occur endemically and occasionally epidemically in older persons, as well as in children under five years of age (2).
The clinical presentation of patients with *M. pneumoniae* infection is not significantly different from that of patients with infections caused by other bacteria or viruses, so diagnosis of *M. pneumoniae* infection relies primarily on special laboratory testing (6). The lack of rapid and accurate diagnostic laboratory tests to detect *M. pneumoniae* directly or the serologic response it elicits has hampered understanding of the epidemiology and contributed to the unawareness of the potential clinical significance of this common pathogen (9). A specific diagnosis is important, because b-lactam antibiotics treatment of an infection due to *M. pneumoniae* is ineffective, whereas the use of other types of antibiotics, such as macrolides, can markedly reduce the duration of the illness (5). The clinical isolates of *M. pneumoniae* have been shown to exhibit some nucleotide sequence variation in the P1 gene. Accordingly, the *M. pneumoniae* strains were divided into two groups, I and II (8).

Materials and Methods

The study included 225 children hospitalized for respiratory tract infections during a13-month period (1 May 2003 to 1 June 2004), in the Department of Pediatrics of AHEPA Hospital-Aristotle University of Thessaloniki. The children were 2 months to 14 years old. From each patient a throat swab and a serum specimen were taken upon admission. A second serum specimen was taken 5-20 days later. Throat swab specimens from these children were examined for the detection of *M. pneumoniae* by: Methylene blue-glucose diphasic medium, EIA-antigen detection and PCR. Both of the first and second serum specimens from these patients were examined for the presence of antibodies against *M. pneumoniae* by complement fixation test (CFT), Indirect Immunofluorescence assay-IFA (IgM and IgG) and Enzyme Linked Immunoassay-ELISA (IgM, IgG, and IgA). A region in the P1 gene (1110 bp) was sequenced for typing of *M. pneumoniae* strains. Using CLUSTAL W program the DNA sequences of the study strains were compared to each other as well as to DNA sequences of P1 gene of reference strains.

Results

A confirmed laboratory result suggestive of current *M. pneumoniae* infection was obtained for a total of 23 children (10.2%) of the patients. In these 23 children, culture, antigen detection test and PCR were positive in 4 cases (sensitivity 17.4%), 1 (4.4%) and 17 (73.9%), respectively. In the acute phase first serum specimens CFT, IgM-IFA, IgM-ELISA and IgA-ELISA were positive in 7 (sensitivity 30.4%), 11 (47.8%), 16 (69.6%) and 2 (8.7%), respectively. The specificities of these methods were found as follows: culture 100%, antigen detection test 100%, PCR 96.5% and for serologic methods: CFT 97.5%, IgM-IFA 91.6% and IgM-ELISA 98%. The combination of PCR and IgM-capture ELISA provides highly sensitive and accurate tool for the rapid diagnosis of *M. pneumoniae* infection in children. In

Greece both types (I and II) of *M. pneumoniae* existed and type I was predominant in 2003. There were a considerable differences in the nucleotide sequence between the 2 types of *M. pneumoniae* most of which were reflected in the amino acid sequence of the P1 protein.

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