

## RESEARCH NOTE

### A review of the serological results obtained in a routine diagnostic laboratory for *Mycoplasma pneumoniae* infections

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#### Abstract

The results of a *Mycoplasma pneumoniae* serology test performed routinely at the Bacteriology Division, Institute for Medical Research were reviewed. A total of 1402 patients were screened over a period of 4 years (January, 1990 - December, 1993), of which 327 (23.3%) were seropositive. The seropositivity rates among Malays, Chinese and Indians were 25.2, 25.4 and 17.8% respectively. The male to female ratio was 1:1. The age specific rate was highest amongst patients of the 6-12 years (35.1%) followed by the 13-20 years age groups (35.0%). In general, infection with *M. pneumoniae* appears to be relatively common in this country.

*Key words:* *Mycoplasma pneumoniae*, serology test.

#### INTRODUCTION

*Mycoplasma pneumoniae* is an important human lower respiratory pathogen and is prevalent all year round in most countries, with a relatively high incidence during winter.<sup>1,2</sup> The infection has been associated with extrapulmonary complications such as arthritis, meningoencephalitis, haemolytic anaemia and rash.<sup>3,4</sup> In Singapore, *M. pneumoniae* was the causative agent in 33% of children hospitalized for severe lower respiratory tract infection and was twice as frequent for children above 5 years of age.<sup>5</sup> In Malaysia, in a study conducted by the Ministry of Health in 1986 for children below 5 years of age,<sup>6</sup> *M. pneumoniae* was identified as the second most common cause of acute respiratory infection, after respiratory syncytial virus, with an isolation rate of 2.9%.

*M. pneumoniae* is a fastidious organism and the culture is time consuming and labour intensive. Although the detection of bacterial genome by molecular biology techniques such as PCR and DNA probe are promising, these are still in the developmental stage. Hence, serological techniques are the primary means of diagnosis for *M. pneumoniae* infections in routine laboratories. A wide range of serological methods are available including complement fixation test, indirect immunofluorescent assay, indirect haemagglutination, enzyme-linked immunoabsorbent assay and microtiter particle agglutination test.

This research note reviews the results of a serological test for *M. pneumoniae* infection

over a 4 year period (January, 1990 - December, 1993) with regard to age, sex and ethnicity of the patients and the monthly distribution.

#### MATERIALS AND METHODS

##### Sera

Sera and clinical reports of patients suspected of *M. pneumoniae* infections were received by the Bacteriology Division, IMR from several government hospitals in Malaysia, mainly Hospital Kuala Lumpur, Klang, Pulau Pinang, Ipoh and Alor Setar over the four-year period from January, 1990 to December, 1993.

##### Microtiter particle agglutination test

The presence of *M. pneumoniae* specific antibody in the sera was determined and quantitated using a commercial microtiter particle agglutination test (SERODIA MYCOII; Fujirebio, Inc., Tokyo, Japan) according to the manufacturers' instructions. Briefly, artificial gelatin particles sensitized with cell membrane components of *M. pneumoniae* (Mac strains) were mixed with the same volume of serum in U-shaped microplates. Sera were diluted in twofold steps from 1:40 to 1:640 and the plate was read after 3h at room temperature. Positive control serum supplied by the manufacturer was included in each test. An antibody titre of 40 or more was regarded as positive to *M. pneumoniae* infections. Sera of 100 healthy blood donors were also examined by the test to determine the prevalence of antibody among the healthy population.

The age specific rate was based on the total

number of seropositive cases from a particular age-group over the total number of the cases from the same age-group.

## RESULTS AND DISCUSSION

The SERODIA-Myco II microtiter particle agglutination test is a commercially available serological test which is based on the principle that the sensitized particles cause indirect agglutination in the presence of anti-mycoplasmal antibody in the serum. Although claimed by the manufacturer to "exclusively detect IgM antibody" the kit has been found to detect other immunoglobulin classes in addition to IgM<sup>7</sup> and was therefore recommended as a screening assay for *M. pneumoniae* infections as an adjunct to the complement fixation test. The test has similar sensitivity to the p-capture ELISA and indirect immunofluorescent assay but was less specific for detecting *M. pneumoniae* IgM than the other two tests.<sup>7</sup> However, in another study,<sup>8</sup> the test was of high sensitivity and specificity.

The cut-off titre recommended by the manufacturer (2 40) could not be used in our local population. Sillis<sup>9</sup> reported that the use of the designated cut-off titre would produce false positive results particularly in sera from patients with extrapulmonary conditions. In this study, we found antibody titres of 2 40 in 45% of blood donors. This high seropositivity rate could be due to the high sensitivity but low specificity of the test or a high endemicity of the infection in the local population. A titre of 160 or more was arbitrarily chosen as presumptive of a recent infection. At this titre, 92% of the donors had antibody levels below the cut-off value and a total of 8% of the healthy blood donor would be considered seropositive.

Analyses were based on laboratory records from January 1990 to December 1993. A total of

1416 blood specimens from 1402 patients were tested. Paired sera were only obtained from 14 cases, of which two demonstrated a fourfold rise in antibody levels. The interpretation of this laboratory test was therefore mainly based on a single specimen. Mycoplasmal antibody was detected at a titer of 2 160 in 327 (23.3%) of the cases (Table 1). Although *M. pneumoniae* epidemics occurred seasonally especially in winter and during rainy seasons in some countries, no seasonal variation could be inferred from the current data due to limited information. However, the number of positive cases was highest in October 1990, September 1991, October 1992 and March 1993 (data not shown).

The male to female ratio of *M. pneumoniae* seropositive cases was 1:1. The percentages of seropositive cases among the three major races were 25.2% in Malays, 25.4% in Chinese, 17.8% in Indians and 10.8% in others. The age-specific rate was highest in children aged 6-12 years and young adults in the 13-20 years age-group and was lowest among infants of less than 1 year old and in persons above 30 years of age (Table 2).

Of the 1402 patients, 265 was diagnosed as having pneumonia at the time of admission, of which 74 (27.9%) were serologically positive for *M. pneumoniae* infections. Other clinical conditions recorded were asthma (8 cases), Guillain-Barré Syndrome (5 cases) and Steven-Johnson Syndrome (1 case). Fever and cough were the most common clinical manifestations.

In general, *M. pneumoniae* infection appears to be relatively common in this country. An average of 23.3% cases suspected of *M. pneumoniae* infection were seropositive by the microparticle agglutination test. The true incidence of *M. pneumoniae* infection in our population however cannot be inferred from these findings.

**TABLE 1: Results of a *M. pneumoniae* serological test over a 4 year period (January 1990 - December 1993)**

Year	No. cases	No. (%) positive
1990	150	37 (24.7)
1991	231	67 (29.0)
1992	330	95 (28.8)
1993	691	128 (18.5)
Total	1402	327 (23.3)

Note: Titres of  $\geq 160$  were regarded as positive

**TABLE 2: Age specific seropositive rates of *M. pneumoniae* infections over a 4 year period (January, 1990 - December, 1993)**

Age-groups	No. cases	No. positive	Age specific seropositive rates (%)
≤1	82	12	14.6
>1-5	150	42	28.0
6-12	225	79	35.1
13-20	137	48	35.0
21-30	209	55	26.3
31-40	191	27	14.1
41-50	147	27	18.4
51-60	112	14	12.5
≥61	108	8	7.4

Note: 41 sera tested were without age records

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