Original Article

Seroprevalence of *Mycoplasma pneumoniae* in Healthy Adolescents in Taiwan

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**SUMMARY:** This study was designed to understand the seroprevalence of *Mycoplasma pneumoniae* infection in healthy Taiwanese adolescents. The study included 2,233 college freshmen (female: male = 1.29:1; mean age, 19.7 years). The percentages of subjects residing in northern, central, southern, and eastern Taiwan were 66.91, 15.89, 9.0, and 8.2%, respectively. All enrolled subjects underwent a serologic agglutination test to detect serum concentrations of antibodies to *M. pneumoniae*. The test results showed that 19.84% of the subjects were infected and, of those, 6.0% were estimated to have subsequently acquired a current or acute infection during this study period. Moreover, the percentage of seropositive females (22.77%) was significantly higher than that of seropositive males (16.07%) (odds ratio, 1.54; 95% confidence interval, 1.241 - 1.911). Subjects residing in eastern counties were more likely to contract *M. pneumoniae* than those residing in other areas of Taiwan.

**INTRODUCTION**

*Mycoplasma pneumoniae*, a common causative agent of community-acquired pneumonia (CAP), which is transmitted mainly through aerosols or by direct contact, causes both upper and lower respiratory tract infection (or primary atypical pneumonia), especially in children and young adults. *M. pneumoniae* is reported to account for 3.3-40% of CAP cases worldwide (1-10) and 20-30% in Taiwan (11,12).

*M. pneumoniae* infection occurs both endemically and epidemically throughout the world. Although cases may present sporadically throughout the year, the incidence of this infection reportedly increases by up to 70% in the summer and early fall (13-16). Although most cases are asymptomatic, clinical indications may include influenza-like symptoms including malaise, cough, headache, fever, dyspnea, purulent sputum, sore throat, and bronchitis. More serious indications are bronchiolitis, pneumonia, or extrapulmonary manifestations with pleural effusion, hepatitis, erythema multiforme, meningocerebralitis, encephalitis, polyneuropathy, or Stevens-Johnson syndrome (17-19).

To diagnose *M. pneumoniae* infection, most laboratories use high-sensitivity and -specificity serologic tests rather than bacterial culture, which is less sensitive and more laborious (2,20,21). Available serologic methods include complement fixation test (CF), enzyme-linked immunoassay (ELISA), particle agglutination assay (PA), and immunofluorescence assay (IFA) (2,22,23). However, for rapid or large-scale screening, PA and ELISA are usually the methods of choice. A significant rise or fall in specific antibody titers (IgG and IgM) between sera from acute and convalescent cases may confirm the diagnosis.

Although studies have verified the prevalence of *M. pneumoniae* in many countries throughout the world, few surveillance reports have documented the prevalence of *M. pneumoniae* infection in teens who are neither hospitalized nor outpatients. Hence, this study investigated the seroprevalence of *M. pneumoniae* in healthy Taiwanese adolescents.

**MATERIALS AND METHODS**

This study included 2,233 volunteer freshmen enrolled at a university in northern Taiwan (1,256 females, 977 males, 1.29:1; mean age, 19.7 years). The percentages of volunteers from northern, central, southern, and eastern Taiwan were 66.91, 15.89, 9.0, and 8.2%, respectively (Table 1).

Specific *M. pneumoniae* antibodies were measured by passive particle agglutination kits (SERODIA-myco II; Fujirebio, Inc., Tokyo, Japan). This method is more effective than the CF test and ELISA, and is suitable for testing large numbers of sera samples (24-27). Briefly, the sera were serially diluted to 1:20, 1:40, 1:80, and 1:160 in diluents, co-incubated with quantities of sensitized gelatin particles (test group) and unsensitized gelatin particles (control group) in U-shaped 96-well microtiter plates (FASTEC microtiter plate U; Fujirebio), then mixed fully and sated for 4 h at room temperature (22-25°C). Antibody titers equal to or greater than 1:40 or 1:80 are considered to indicate a past *M. pneumoniae* infection, and titers under 1:40 are regarded as a negative reaction. Moreover, antibody titers equal to or exceeding 1:160 are considered to indicate recent acute infection by *M. pneumoniae* (28).

The descriptive statistics and significance analyzed using ANOVA, the chi-square test, and independent *t* testing were included in this investigation. All data were processed by SPSS software package (SPSS13; SPSS, Inc., Chicago, Ill., USA). A *P* value less than 0.05 was considered statistically significant.

**RESULTS**

The seroprevalence of *M. pneumoniae*: The test results were negative in 80.16% of the 2,233 volunteers; the remaining 19.84% were positive for a past infection, as indicated by antibody titers above 1:40. Among the positive cases, 6.0% were in the acute phase of *M. pneumoniae* infection during the study period, as indicated by antibody titers above 1:160 (Table 1).

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Gender variations in *M. pneumoniae* infection: Gender differences were revealed in this investigation. The mean percentage of patients with antibody titers equal to or above 1:40 was higher in females (22.77%) than in males (16.07%) (Odds ratio 1.54, 95% confidence interval, 1.241-1.911) (Table 1).

<table>
<thead>
<tr>
<th>Area</th>
<th>Antibody titer of <em>M. pneumoniae</em></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Male n = 977 (%) (within males)</td>
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<tr>
<td></td>
<td>&lt;1:40</td>
</tr>
<tr>
<td>Northern</td>
<td>571 (84.84)</td>
</tr>
<tr>
<td>Central</td>
<td>134 (80.72)</td>
</tr>
<tr>
<td>Southern</td>
<td>62 (86.11)</td>
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<tr>
<td>Eastern</td>
<td>53 (80.30)</td>
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<tr>
<td>Total</td>
<td>820 (83.00)</td>
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</tbody>
</table>

DISCUSSION

Previous studies (29,30) have demonstrated that *M. pneumoniae* is the most common pathogen in both pediatric and adult patients in Taiwan, although the contagious agents of CAP may also include *Streptococcus pneumoniae*, *Legionella* spp. and *Chlamydia pneumoniae*, (9,31). However, the focus of this study was the prevalence of *M. pneumoniae* in the healthy population, particularly adolescents, throughout the country.

So far, serologic tests are the most common methods of clinically diagnosing *M. pneumoniae* infection. However, this investigation revealed that 19.84% of healthy subjects, particularly females, were infected with this organism recently. In serologic methods, it would be very difficult to correctly determine CAP etiology if only a single serum sample is used, though not if paired samples are used. However, for sera samples collected 4 weeks apart, paired sera tests are not routinely available in clinical laboratories or the emergency rooms in Taiwan. Additionally, although serological tests using single sample have limited value for clinical diagnosis, the tests are extremely valuable for the epidemiological analysis of *M. pneumoniae* infection at approximately 1 year post-infection (2.9). The current gold standard is PCR testing using nasopharyngeal swabs, which offers more sensitive and accurate diagnosis of acute *M. pneumoniae* infection (32-34); however, due to inhibitor interference, technical problems, and the limited number of large-scale studies, the clinical application of PCR is limited.

The experimental results indicated that 19.84% of the seropositive population were recently infected by *M. pneumoniae* or had been in the recovery stage for 1 year or less (35,36). Interestingly, this result was consistent with clinical data reported in CAP studies conducted elsewhere (2,4-12). The findings of this study indicate that approximately one-fifth of the healthy population carried *M. pneumoniae*, and 6% of those persons with antibody titers equal to or exceeding 1:160 had been in the acute phase recently. These results can help healthcare authorities construct a new epidemiologic model for preventing outbreaks or limiting the spread of *M. pneumoniae* in areas of high population density.

For the comparison of geography-related differences, it is noted that Taiwan is one of the most densely populated countries in the world, and the population is concentrated in the coastal regions surrounding the metropolitan areas. Cites in northern Taiwan are municipalities directly under the jurisdiction of the central government, and their budgetary resources are more plentiful than other areas, which are under the authority of local governments. Most subjects enrolled in this study had recently left their residence of origin to attend university in the north. These demographic data may offer more information regarding the incidence of *M. pneumoniae*. Although residents of northern Taiwan have advantages in public health systems and hygiene issues, its population density is the highest (followed by southern, central, and eastern Taiwan), and its climate is the most humid. The present study found no statistically significant differences in the rate of *M. pneumoniae* infection in different geographic locations of Taiwan. *M. pneumoniae* infection can easily spread from person to person simply by close contact. Moreover, the incidence of *M. pneumoniae* infection is higher in females than in males in all areas except central Taiwan (Table 1). Further
epidemiological investigations based on gender variation offer clear clues about the exact mechanism that is responsible for M. pneumoniae infection.

Clearly, according to this study, M. pneumoniae is an infectious microorganism. Previous reports have verified that M. pneumoniae infection can trigger or exacerbate other diseases (37-39). The term “walking pneumonia” has been used widely by physicians to describe a persistent M. pneumoniae infection that may develop gradually, with only mild infection or asymptomatic conditions. The present study revealed that approximately one-fifth of the seropositive population might have active or mixed infection with microorganisms that may exacerbate M. pneumoniae infections and transmit easily before favorable outbreak conditions arise.

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REFERENCES