Low incidence of macrolide-resistant *Mycoplasma pneumoniae* between April 2016 and March 2017 in Akita Prefecture, Japan

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Received: April 25, 2018. Accepted: August 14, 2018
Published online: August 31, 2018
DOI:10.7883/yoken.JJID.2018.170

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
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Keywords: *Mycoplasma pneumoniae* pneumonia, macrolide, *p1* gene

Running head: Prevalence of ML-resistant *M. pneumoniae* in Akita
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Laboratory and epidemiology communications

*Mycoplasma pneumoniae* is one of the leading causes of community-acquired pneumonia. It is naturally resistant to β-lactam antibiotics because of the lack of a cell wall; thus, macrolides (MLs) are recommended as the first-line drug against *M. pneumoniae* infection. However, the emergence of ML-resistant *M. pneumoniae* strains has become a major public health concern (1).

In Japan, pneumonia caused by *M. pneumoniae* (*M. pneumoniae* pneumonia) is monitored under the National Epidemiological Surveillance of Infectious Diseases (NESID) program. Since 2000, the number of patients with *M. pneumoniae* pneumonia has increased, with significant elevation noted during 2011–2012 and 2015–2016 (2). Yamazaki et al. (2) estimated that the prevalence of ML-resistant *M. pneumoniae* in Japan was 50%–90%, depending on the area. However, whether ML resistance contributes to the epidemic of *M. pneumoniae* pneumonia remains unknown. In Akita Prefecture, located in the northern part of Japan, the epidemic of *M. pneumoniae* pneumonia was observed in 2016 and 2017 (3). The present study aims to provide the recent prevalence of ML-resistant *M. pneumoniae* and genetic characteristics of *M. pneumoniae* in Akita Prefecture.

Between April 2016 and March 2017, we collected 829 throat swab specimens from pediatric patients with respiratory illness at nine general hospitals in Akita Prefecture in accordance with the NESID program. All specimens were suspended in Eagle’s minimum essential medium (MP Bio, Tokyo, Japan) supplemented with 0.5% lactalbumin, 0.2% bovine serum albumin, 500 µg/mL penicillin, 1 mg/mL streptomycin, and 0.1 mg/mL kanamycin, which was used as a transport medium. In addition, crude DNA was extracted using MagNA Pure LC2.0 (Roche Diagnostics, Tokyo, Japan) as per the manufacturer’s instructions. To detect *M. pneumoniae*, we performed real-time PCR targeting community-acquired respiratory distress syndrome toxin gene (4). In *M. pneumoniae*-positive samples, we sequenced the domain V region of the 23S ribosomal
RNA gene to detect the point mutation conferring ML resistance as well as typed the \( p \)\( 1 \) gene, which classifies into two types based on the sequence polymorphism, using nested PCR-restriction fragment length polymorphism analysis, as per the protocol of the National Institute of Infectious Diseases and previous studies, with slight modifications (5, 6).

Real-time PCR detected \( M. \) pneumoniae DNA in 31 samples; of these, the presence or absence of ML-resistant mutation was determined in 25 samples (Table 1). We found only the A2063G mutation in domain V of the 23S ribosomal RNA gene in seven samples. In this study, the incidence of ML-resistant \( M. \) pneumoniae was 28.0% (7/25), suggesting that the present prevalence of ML-resistant \( M. \) pneumoniae in Akita Prefecture was low unlike the previous estimate of the prevalence of ML-resistant \( M. \) pneumoniae in Japan (2). Tanaka et al. (7) reported that the prevalence of ML-resistant \( M. \) pneumoniae in Japan was at the peak (81.2%) in 2012 but declined after that.

Table 2 presents the \( p \)\( 1 \) gene types examined in this study. Of 31 \( M. \) pneumoniae-positive samples, we determined \( p \)\( 1 \) gene types in 23 samples, including in a sample with unknown ML resistance. Based on several \( p \)\( 1 \) typing studies of recent \( M. \) pneumoniae isolates in Japan (6, 8), the \( p \)\( 1 \) gene type associated with ML resistance is considered to be type 1. In addition, all four samples genotyped as type 1 in this study were also ML-resistant mutation positive. In contrast, the most prevalent \( p \)\( 1 \) type in this study was type 2 (52.2%, 12/23), and all samples genotyped as type 2 were ML-resistant mutation negative. Diaz et al. (9) reported no correlation between the \( p \)\( 1 \) type and ML resistance. However, the prevalence of ML-resistant \( M. \) pneumoniae in Japan increased after 2003 when type 1 \( M. \) pneumoniae became dominant, whereas type 2 was replaced by its variants and became rare (2, 10). Therefore, ML resistance seems not to disseminate in type 2 \( M. \) pneumoniae in Japan. Furthermore, the low ML-resistance rate in this study could be attributed to the dominance of type 2 \( M. \) pneumoniae.
In conclusion, this study suggests low prevalence of ML resistance and dominance of type 2 p1 gene in *M. pneumoniae* in Akita Prefecture. The findings of this study would be useful for future surveillance programs and better understanding for the regional epidemiology of *M. pneumoniae* pneumonia.

**Conflict of interest**
None to declare.
References


10. Morozumi M, Ubukata K. Mechanisms of macrolide-resistant *Mycoplasma*
Table 1. Incidence of ML-resistant *M. pneumoniae* in this study

<table>
<thead>
<tr>
<th>ML-resistant mutation</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7 (28.0)</td>
<td>18 (72.0)</td>
<td>25</td>
</tr>
</tbody>
</table>
Table 2. Distribution of the *p1* gene types.

<table>
<thead>
<tr>
<th>ML-resistant mutation</th>
<th>Type 1</th>
<th>Type 2</th>
<th>p1 typing</th>
<th>Type 2 variants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2a</td>
<td>2b</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>1</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ND*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>4 (17.4)</td>
<td>12 (52.2)</td>
<td>3 (13.0)</td>
<td>0 (0)</td>
<td>4 (17.4)</td>
</tr>
</tbody>
</table>

*ND: not determined.*