IMP- and VIM-type MBLs. In this case, the source of bla_{IMP-19} remains unclear, but given the patient’s history, a direct cross-border origin could be excluded. Since the bla_{IMP-19}-carrying plasmid could not be mobilized, its contribution in terms of carriage of additional resistance genes could not be assessed and further investigations will be necessary to understand the potential advantage conferred by acquisition of this plasmid by the VIM-producing strain. Nevertheless, the detection of bla_{IMP-19} in a P. aeruginosa strain of the CC235 epidemic lineage is of concern, since this could promote bla_{IMP-19} dissemination in the clinical setting.

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**Transparency declarations**

None to declare.

**References**


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**Two cases of macrolide resistance in Mycoplasma pneumoniae acquired during the treatment period**

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Sir,

*Mycoplasma pneumoniae* is a common cause of upper and lower respiratory tract infections, especially in children and young adults. *M. pneumoniae* infections can be treated with macrolides, which are generally considered to be the first-choice antibiotics for children. However, the prevalence of macrolide-resistant *M. pneumoniae* has gradually increased worldwide and has reached a rate of 90% in China. In Japan, it has been reported that the prevalence of macrolide-resistant *M. pneumoniae* isolates reached 65.5% in 2010 and 89.5% in 2011.2 *M. pneumoniae* resistance to macrolides is caused by point mutations in domain V of the 23S rRNA gene that interfere with the binding of macrolides to rRNA.3,4 An A-to-G transition at position 2063 (A2063G) is the most frequently detected mutation among resistant strains, closely followed by an A-to-G transition at position 2064 (A2064G).5,6 Although resistant strains of *M. pneumoniae* have been generated in vitro by selection with subinhibitory concentrations of macrolides,3,4 in humans there is only one report of the acquisition of a macrolide resistance mutation during the course of infection.5 We report herein two cases in which macrolide-resistant *M. pneumoniae* isolates are thought to have emerged during treatment with a macrolide.

Patient 1 is an 8-year-old child (Table 1). He visited the Yamanobe Paediatric Clinic with a 1 day history of fever of up to 39.8°C and a mild cough in November 2011. His temperature rapidly decreased 24 h later. Because of the persistent dry cough without fever, he visited the same clinic at 6 days after starting the antibiotic treatment and a second pharyngeal swab sample was taken. The patient’s guardian gave written informed consent for the publication of these data.

Patient 2 is a 5-year-old child (Table 1). She was brought to the Yamanobe Paediatric Clinic in January 2012, with a 4 day history of fever up to 39.8°C and coughing. On initial physical examination, her temperature was 38.7°C and rales were...
M. pneumoniae. In both cases, it is apparent that the macrolide-resistant M. pneumoniae still emerged. These cases suggest that care must be taken when determining the appropriate dose and treatment duration for macrolides and other first-choice antibiotics to inhibit the emergence of resistant strains.

### Table 1. Characteristics of two cases with M. pneumoniae infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td>upper respiratory tract infection</td>
<td>pneumonia</td>
</tr>
<tr>
<td>Days from onset of fever to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>taking antibiotics</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>resolution of fever</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>re-onset of fever</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Antibiotics:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>drug used</td>
<td>azithromycin</td>
<td>clarithromycin</td>
</tr>
<tr>
<td>dosage, medication days</td>
<td>10 mg/kg/day, 3 days</td>
<td>10 mg/kg/day, 3 days</td>
</tr>
<tr>
<td>Days from onset to isolation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>drug-susceptible M. pneumoniae</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>drug-resistant M. pneumoniae</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>23S rRNA mutation</td>
<td>A2063G</td>
<td>A2064G</td>
</tr>
</tbody>
</table>

heard during lung auscultation. Initial laboratory tests showed a leucocyte count of 9300 cells/μL and a C-reactive protein level of 0.8 mg/dL. A chest X-ray showed infiltrates in the inferior lobe of her left lung, a result compatible with pneumonia. After taking a pharyngeal swab sample, she was given clarithromycin (10 mg/kg/day) for 5 days. On day 2 of the oral antibiotic treatment, the patient was brought to the same clinic because she vomited whenever she took the medicine; the clarithromycin was stopped on day 3 because her temperature decreased. On day 6 after starting the antibiotic treatment, her temperature increased again up to 38.0°C. The next day, her leucocyte count was 28300 cells/μL and her C-reactive protein level was 0.4 mg/dL. A second pharyngeal swab sample was taken and she was treated with tosufloxacin (12 mg/kg/day) for 5 days. Her condition resolved within 1 week. The patient’s guardian gave written informed consent for the publication of these data.

In both cases, M. pneumoniae was isolated from the first and second samples using pleuropneumonia-like organism broth (Difco, Detroit, MI, USA). PCR amplification and sequence analysis of the 23S rRNA gene were performed on the DNA extracted from four strains of M. pneumoniae using the procedures reported by Matsuoka et al.6 The MICs of the prescribed antibiotics were determined using a broth microdilution method as described by Okazaki et al.3 No mutation in domain V of the 23S rRNA gene was detected in the M. pneumoniae isolates from the first samples of both patients, which were obtained before the initiation of macrolide treatment. However, an A2063G transition was detected in the M. pneumoniae isolate from the second sample of Patient 1 and an A2064G transition was detected in the M. pneumoniae isolate from the second sample of Patient 2. The MICs of azithromycin for the strains isolated from the first and second samples of Patient 1 were 0.000488 and 16 mg/L, respectively, and the MICs of clarithromycin for the strains isolated from Patient 2 were 0.00195 and 64 mg/L, respectively, indicating the acquisition of resistance to the initially prescribed antibiotics. The MICs of tosufloxacin for the strains isolated from Patient 2 were 0.25 and 1 mg/L, respectively.

In the cases reported by Chironna et al.,5 it could not be determined when the macrolide resistance mutation occurred. In our two cases, it is apparent that the macrolide-resistant M. pneumoniae strains emerged within 6 or 7 days after the initiation of azithromycin or clarithromycin treatment. It is likely that the emergence of macrolide resistance in Patient 2, who was treated with clarithromycin, was the result of treatment with subinhibitory concentrations due to the vomiting that occurred whenever the patient took the medicine. In Patient 1, who was treated with the proper dosage of azithromycin, the fever decreased immediately; however, macrolide-resistant M. pneumoniae still emerged. These cases suggest that care must be taken when determining the appropriate dose and treatment duration for macrolides and other first-choice antibiotics to inhibit the emergence of resistant strains.

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This study was carried out as part of our routine work.

### Transparency declarations
None to declare.

### References