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CASE REPORT

Transmission of macrolide-resistant *Mycoplasma pneumoniae* within a family

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Abstract Outbreaks of *Mycoplasma pneumoniae* have occurred in closed surroundings, including among families, university students, in military camps, and in schools, but available data on outbreaks of macrolide-resistant (MR) *M. pneumoniae* are limited. We encountered a family outbreak of MR *M. pneumoniae* pneumonia in four sisters (16, 14, 10, and 8 years of age). *M. pneumoniae* was isolated from all four patients, and an A-to-G transition at position 2063 in domain V of the 23S rRNA gene was identified. Although three of four patients received azithromycin, which is the first-choice antimycoplasmal agent, this agent was not effective. All isolates had an identical antibiotic susceptibility pattern. The MIC values for 14- and 15-membered macrolides, such as erythromycin, clarithromycin, and azithromycin, were >128, >128, and 64 μg/ml, respectively. On admission, all four patients were diagnosed with suspected *M. pneumoniae* pneumonia using the Japanese Respiratory Society (JRS) guidelines scoring system. We carried out culture and polymerase chain reaction tests for the detection of *M. pneumoniae* in their parents (mother, 49 years old, and father, 56 years old) four times, but no *M. pneumoniae* organism was detected using either test. In conclusion, MR *M. pneumoniae* strains can occur in outbreaks in closed surroundings, such as within families, as well as macrolide-sensitive strains. To prevent outbreaks of *M. pneumoniae* infection, especially MR *M. pneumoniae*, in closed populations, physicians should pay careful attention to the potential occurrence of infections involving MR *M. pneumoniae*.

Keywords *Mycoplasma pneumoniae* · Macrolide resistant · Family outbreak · Community-acquired pneumonia · Japanese Respiratory Society guidelines

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Introduction

Mycoplasma pneumoniae is a common causative pathogen of respiratory infections in school-aged children and young adults, accounting for as many as 10–30 % of all cases of community-acquired pneumonia (CAP) [1]. *M. pneumoniae* pneumonia is specified for weekly reporting by specially designated sentinel clinics in accordance with the Japanese Infectious Diseases Control Law. In 2011 and 2012, an epidemic of *M. pneumoniae* infection occurred throughout Japan, and the incidence was the highest observed during the past decade [2]. Although *M. pneumoniae* pneumonia is relatively mild, the increase in macrolide-resistant (MR) *M. pneumoniae* has become a problem in Japan. MR *M. pneumoniae* has become widespread in Japan and China [3–9] and is now spreading throughout Europe and North America, especially in children [10–15]. In cases with genetic resistance, macrolides are less effective compared with macrolide-sensitive (MS) *M. pneumoniae* [16–18].

Outbreaks of *M. pneumoniae* have occurred in closed surroundings including among families, university students, in military camps, and in schools. Such outbreaks among younger population groups have been observed repeatedly. However, the available data in outbreaks of MR *M. pneumoniae* are limited in Japan [19]. Recently, we encountered a family outbreak of MR *M. pneumoniae*

pneumonia in four sisters. Here, we report the clinical and microbiological findings of this outbreak of MR *M. pneumoniae* pneumonia in a family. We also investigated the transmission of MR *M. pneumoniae* in the asymptomatic parents using culture, real-time polymerase chain reaction (PCR), and serology for the detection of *M. pneumoniae*.

Case report

Outbreak in a family

A 16-year-old, previously healthy girl visited a clinic on December 28, 2010 complaining of sore throat and cough for 4 days. She received cefditoren pivoxil (300 mg/day), but her clinical condition did not improve. At the visit to our hospital on December 31, her temperature was 39.1 °C, pulse rate 102 beats/min, blood pressure 108/66 mmHg, respiratory rate 17/min, and SpO_2 96 % (room air). Her breath sounds were normal, and no abnormal physical findings were observed. The laboratory data revealed a normal white blood cell (WBC) count and mild elevation of C-reactive protein. Her chest radiograph revealed a homogeneous infiltrate in the left upper to lower lung fields (Fig. 1a). All six parameters with the Japanese Respiratory Society (JRS) guidelines scoring system to differentiate between atypical and bacterial pneumonia were matched in this case [1, 20–22].

Microbiological tests, such as Gram stain, cultures, and real-time polymerase chain reaction (PCR), using the sputum sample and nasopharyngeal swab specimen and serological tests, were performed as described previously [21, 22]. Cultures for *M. pneumoniae* and *Legionella* species were performed on pleuropneumonia-like organism broth (Difco, Detroit, MI, USA) and buffered charcoal-yeast extract alpha agar, respectively. Cultures for *Chlamydophila pneumoniae* and *C. psittaci* were performed using cycloheximide-treated HEp-2 cells grown in a 24-well cell culture plate. All specimens were passed twice. Culture confirmation was done using fluorescent-antibody staining with *C. pneumoniae* and *C. psittaci* species-specific and genus-specific monoclonal antibodies. These specimens were also used for real-time PCR of *M. pneumoniae*, *Chlamydophila* species, and *Legionella* species. The target sequences were in the region of the 53-kDa protein gene for *C. pneumoniae*, the major outer membrane protein gene for *Chlamydophila*, the P1 cytadhesin gene for *M. pneumoniae*, and the nucleotide sequence of the 5S-ribosomal DNA for *Legionella*. DNA was extracted from respiratory samples using a QIAamp DNA Mini Kit (Qiagen, Tokyo, Japan) in accordance with the manufacturer's instructions. The assays were performed as described previously [21, 22].

Antibodies to *M. pneumoniae* were measured using a particle agglutination (PA) test (Serodia-Myco II kit; Fujirebio, Tokyo, Japan) and ImmunoCard *Mycoplasma* kit for the detection of *M. pneumoniae*-specific IgM (Meridian Bioscience, Cincinnati, OH, USA), and *Legionella* species using a microagglutination test (detection of *L. pneumophila* serogroups 1–6, *L. bozemanii*, *L. dumoffii*, *L. gormanii*, and *L. micdadei*). A microimmunofluorescence test was used for the titration of IgG and IgM antibodies against chlamydial species, using formalinized elementary bodies of *C. pneumoniae* KKpn-15, *C. trachomatis* L2/434/Bu, and *C. psittaci* Budgerigar-1 strains as antigens. Rheumatoid factors were absorbed using GullSORB (Meridian Bioscience) before IgM titrations. In addition to serology, culturing, and/or PCR, urinary antigen tests (Binax NOW; Binax, Portland, ME, USA) for *Streptococcus pneumoniae* and *L. pneumophila* were performed.

ImmunoCard *Mycoplasma* antibody was positive and the JRS scoring system indicated atypical pneumonia. Thus, the patient was diagnosed as *M. pneumoniae* pneumonia and was started on intravenous minocycline 200 mg/day after admission. After 7 days antibiotic therapy, her condition improved and no relapse was observed. In history taking, some other students in the same high school class had complained of cough and fever from the end of November.

Her three younger sisters visited the clinic on January 13, 16, and 17, 2011, respectively, complaining of sore throat, cough and fever. They received azithromycin (10 mg/kg/day), but their clinical condition did not improve. At the visit to our hospital, their chest radiographs revealed a homogeneous infiltrate in the right lower lung field (14-year-old girl on January 19; Fig. 1b), a homogeneous infiltrate in the right middle to lower lung field (10-year-old girl on January 20; Fig. 1c), and a homogeneous infiltrate in the left lower lung field (8-year-old girl on January 20; Fig. 1d). ImmunoCard *Mycoplasma* antibody was positive in all patients, and minocycline was started after admission. After 7 days antibiotic therapy, the condition of the patients improved and no relapse was observed.

M. pneumoniae was detected by culture and PCR, and no other microorganisms were detected in all four patients. Clinical and laboratory findings of the four patients are summarized in Table 1. All four patients were suspected as having *M. pneumoniae* pneumonia using the JRS scoring system even though they were pediatric and adolescent patients. There was a correlation of six parameters in patients 1 and 4 and five parameters in patients 2 and 3. In two patients (patients 1 and 2), seroconversion of *M. pneumoniae* antibody measured by a PA test was observed in paired serum samples. In the other two patients (patients 3 and 4), high antibody titer of *M. pneumoniae* was observed in a single serum sample.