

Macrolide-Resistant *Mycoplasma pneumoniae* Infection, Japan, 2008–2015

Takaaki Tanaka, Tomohiro Oishi, Ippei Miyata, Shoko Wakabayashi, Mina Kono, Sahoko Ono, Atsushi Kato, Yoko Fukuda, Aki Saito, Eisuke Kondo, Hideto Teranishi, Yuhei Tanaka, Tokio Wakabayashi, Hiroto Akaike, Satoko Ogita, Naoki Ohno, Takashi Nakano, Kihei Terada, Kazunobu Ouchi

We evaluated isolates obtained from children with *Mycoplasma pneumoniae* infection throughout Japan during 2008–2015. The highest prevalence of macrolide-resistant *M. pneumoniae* was 81.6% in 2012, followed by 59.3% in 2014 and 43.6% in 2015. The prevalence of macrolide-resistant *M. pneumoniae* among children in Japan has decreased.

Since the early 2000s, macrolide-resistant *Mycoplasma pneumoniae* isolates have been identified in Japan. We previously reported the results of our national surveillance study that investigated the prevalence of macrolide-resistant *M. pneumoniae* infection among children in Japan during 2008–2012 (1). An *M. pneumoniae* pandemic occurred in Japan during 2010–2012, especially among children. Similar pandemics also occurred in other countries (2). However, the prevalence of macrolide-resistant *M. pneumoniae* infection also gradually increased at the same time in Asia, including Japan (1,2). We investigated the prevalence of macrolide-resistant *M. pneumoniae* infection after the pandemic.

The Study

Children with respiratory tract infections who visited 68 medical institutions in Japan were classified according to district: 1) Kyushu (population 14 million); 2) Chugoku-Shikoku (11 million); 3) Kinki (20 million); 4) Kanto-Chubu (62 million); and 5) Tohoku-Hokkaido (14 million) (Figure). Most patients had been enrolled in the Atypical Pathogen Study Group before the 2010–2012 pandemic; however, some did not participate in our previous study reported in 2013 (1). Here, we report the data for January 2008–December 2015.

As previously reported (1), pediatricians collected nasopharyngeal swab samples and sputum samples, when available, from children with respiratory tract infections. Participants' parents provided informed consent before

sample collection. The Ethics Committee of the Kawasaki Medical School (Kurashiki, Japan) approved the study protocol on December 8, 2014 (no. 286–3).

Nasopharyngeal swab specimens were collected with a sterile swab (Nippon Menbo, Saitama, Japan). After collection, each swab was placed into 3 mL of Universal Vial Transport Medium (Becton, Dickinson and Company, Sparks, MD, USA) and transported at room temperature within 2 days to Kawasaki Medical School by a parcel delivery system. Crude DNA extracts were obtained with the following procedure: 300 μ L of resuspended transport medium was transferred into a 1.5-mL microtube centrifuged at 4°C, 20,000 \times g for 30 min, after which 285 μ L of supernatant was discarded; the remainder was transferred into a thin-wall 200- μ L PCR tube after resuspension with 85 μ L lysis buffer by gentle pipetting. This suspension was incubated at 55°C for 60 min, followed by 100°C for 10 min before cooling to 4°C. The composition of the lysis buffer was Tris-HCl (pH 8.3) 2 mmol/L, KCl 10 mM, MgCl₂ 0.045 mM, Triton X-100 0.45%, Tween 20 0.45%, and RNA-grade Proteinase K (Thermo Fisher Scientific Inc., Waltham, MA, USA) 0.4 μ g/ μ L. *M. pneumoniae* DNA was detected by real-time PCR targeting a conserved part of the gene coding for the P1 adhesin (3).

We searched for mutations at sites 2063, 2064, and 2617 in domain V of 23S rRNA of *M. pneumoniae* using a direct sequencing method with isolates or samples with a positive PCR result, as reported previously (3). For this study, we investigated 1,448 samples obtained from patients in Japan who had respiratory tract infections; we detected *M. pneumoniae* DNA by real-time PCR and searched for mutations using a direct sequencing method.

The overall prevalence rate of macrolide-resistant *M. pneumoniae* in Japan was 70.2% (1,016/1,448) and ranged from 43.7% in Kyushu to 89.3% in Kanto-Chubu (Table 1). When divided into 3 time periods (prepandemic, pandemic, and postpandemic), the overall rate of macrolide-resistant *M. pneumoniae* was substantially lower in the post epidemic period than in the pandemic period; macrolide-resistant *M. pneumoniae* isolates decreased in 4 of the 5 locations.

The peak rate of macrolide-resistant *M. pneumoniae* infection was 81.6% in 2012 (493/604) (Table 2). Rates of macrolide-resistant *M. pneumoniae* infection gradually decreased as follows: 65.8% (25/38) in 2013, 59.3% (16/27) in 2014, and 43.6% (65/149) in 2015. The most frequent mutation was A2063G mutation (95.8%), followed

Author affiliation: Kawasaki Medical School, Okayama, Japan

DOI: <https://doi.org/10.3201/eid2310.170106>



Figure. Districts of origin for patients with *Mycoplasma pneumoniae* infection, Japan, 2008–2015: 1, Kyushu; 2, Chugoku-Shikoku; 3, Kinki; 4, Kanto-Chubu; 5, Tohoku-Hokkaido.

by A2063T (3.1%), A2064G (0.6%), A2063C (0.3%), C2617G (0.2%), and C2617T (0.1%).

Conclusions

The prevalence of macrolide-resistant *M. pneumoniae* infection was high during 2008–2012 but gradually decreased throughout Japan during 2013–2015. One reason for this decrease might be the 2011 publication of guidelines for treating *M. pneumoniae* pneumonia (4). Because of the higher prevalence of macrolide-resistant *M. pneumoniae* infection in children than in adults (5), respiratory fluoroquinolone/tosufloxacin was recommended for use in patients in whom *M. pneumoniae* pneumonia responded poorly to macrolide treatment in these guidelines. After the pandemic, the guideline committee addressed the concerns of further accumulation of macrolide-resistant *M. pneumoniae* in children resulting from constant macrolide use and teeth damage from tetracycline use in children <8 years of age (4). Tosufloxacin was approved for use in children

in 2010 in Japan and has been used to treat *M. pneumoniae* infection. Because the guidelines recommend tosufloxacin as a second-line drug, pediatricians in Japan may be using the appropriate antimicrobial drugs for *M. pneumoniae* infection in accordance with these guidelines, which might have led to the decrease in incidence. Unfortunately, to our knowledge, no reports have been published to support these hypotheses. However, prescriptions for oral antimicrobial drugs in Japan comprise most of the prescriptions for antimicrobial drugs (6); therefore, we believe that the rate of macrolide-resistant *M. pneumoniae* might be affected by changes in the use of oral macrolide agents.

In addition, the prevalence of macrolide-resistant *M. pneumoniae* infection varies among countries: for example, 13.2% in the United States (7), 8.3% in France (8), and 3.1% in Germany (9). These variations might be attributed to differences in the number of prescription macrolide agents among countries. Although accurately assuming the number of prescription macrolide agents in each country is

Table 1. *Mycoplasma pneumoniae* infections diagnosed by real-time PCR and prevalence of macrolide-resistant *M. pneumoniae*, by district, Japan

District	Average age, y (range)	No. patients (M:F)	Macrolide resistance, % (no. positive/total no. patients)*			
			2008–2010	2011–2012	2013–2015	Total
Kyushu	6.4 (0–14)	239 (1.2:1)	–	64.1 (82/128)	27.9 (31/111)	47.3 (113/239)
Chugoku-Shikoku	7.3 (0–15)	623 (1.4:1)	68.6 (59/86)	70.6 (339/480)	80.7 (46/57)	71.3 (444/623)
Kinki	7.1 (1–15)	227 (1:1)	33.3 (7/21)	86.1 (162/188)	66.7 (12/18)	79.7 (181/227)
Kanto-Chubu	7.5 (0–13)	268 (1.1:1)	–	80.1 (197/246)	72.7 (16/22)	79.5 (213/268)
Hokkaido-Tohoku	7.7 (0–13)	91 (1.2:1)	84.2 (32/38)	68.1 (32/47)	16.7 (1/6)	71.4 (65/91)
Total	7.3 (0–15)	1,448 (1.3:1)	67.6 (98/145)	74.6 (812/1,089)	49.5 (106/214)	70.2 (1,016/1,448)

*2008–2010, pre-pandemic; 2011–2012, pandemic; 2013–2015, post-pandemic.

Table 2. Rates of macrolide-resistant *Mycoplasma pneumoniae* and point mutations in domain V of 23S rRNA, Japan

Variable	2008	2009	2010	2011	2012	2013	2014	2015	Total
Macrolide resistance, % (no. positive/total no. patients)	55.6 (10/18)	72.7 (8/11)	69.0 (80/116)	65.8 (319/485)	81.6 (493/604)	65.8 (25/38)	59.3 (16/27)	43.6 (65/149)	70.2 (1,016/1,448)
Point mutations, no. (%)									
A2063G	10 (100)	8 (100)	80 (100)	301 (94.4)	471 (95.5)	25 (100)	16 (100)	62 (95.2)	97.3 (95.8)
A2063C	0	0	0	3 (0.9)	0	0	0	0	3 (0.3)
A2063T	0	0	0	15 (4.7)	14 (2.3)	0	0	2 (3.2)	31 (3.1)
A2064G	0	0	0	0	6 (1.0)	0	0	0	6 (0.6)
C2617G	0	0	0	0	2 (0.3)	0	0	0	2 (0.2)
C2617T	0	0	0	0	0	0	0	1 (1.6)	1 (0.1)

difficult, we can estimate the macrolide resistance rate of *Streptococcus pneumoniae* among those countries. A recent report supported the hypothesis that antimicrobial selection pressure results in clonal expansion of existing resistant strains (10). In Japan and China, which have a high prevalence of macrolide-resistant *M. pneumoniae* (3,5), the rates of macrolide-resistant *S. pneumoniae* also are very high (11,12). In countries with low rates of macrolide-resistant *M. pneumoniae*, such as the United States, France, and Germany (7,8,9), the prevalence rates of macrolide-resistant *S. pneumoniae* are low (13,14). However, the mechanisms of macrolide resistance are difficult to compare between *Streptococcus* and *Mycoplasma* (15). Therefore, we also interviewed the pediatricians who collected the samples for this study, and analysis of their responses is ongoing. Upon completion of these interviews, we will be able to report patients' background characteristics, such as previous use of macrolides and medical examination histories; this information may provide further insight into the decreased prevalence of macrolide-resistant *M. pneumoniae* infection.

In summary, the prevalence of macrolide-resistant *M. pneumoniae* infection in children in Japan was high and increased between 2008 and 2012 but declined thereafter. Careful continuous monitoring of macrolide-resistant *M. pneumoniae* infection rates in Japan and other countries is needed.

Acknowledgments

We thank Reiji Kimura and Mika Suwa for their technical assistance and all the clinicians who participated by taking samples in the Atypical Pathogen Study Group.

Dr. Tanaka is a senior lecturer in the Department of Pediatrics at Kawasaki Medical School. His primary research interest is vaccines for travelers.

References

- Kawai Y, Miyashita N, Kubo M, Akaike H, Kato A, Nishizawa Y, et al. Nationwide surveillance of macrolide-resistant *Mycoplasma pneumoniae* infection in pediatric patients. *Antimicrob Agents Chemother.* 2013;57:4046–9. <http://dx.doi.org/10.1128/AAC.00663-13>
- Zhao F, Liu G, Wu J, Cao B, Tao X, He L, et al. Surveillance of macrolide-resistant *Mycoplasma pneumoniae* in Beijing, China, from 2008 to 2012. *Antimicrob Agents Chemother.* 2013; 57:1521–3. <http://dx.doi.org/10.1128/AAC.02060-12>
- Miyashita N, Kawai Y, Yamaguchi T, Ouchi K, Oka M; Atypical Pathogen Study Group. Clinical potential of diagnostic methods for the rapid diagnosis of *Mycoplasma pneumoniae* pneumonia in adults. *Eur J Clin Microbiol Infect Dis.* 2011;30:439–46. <http://dx.doi.org/10.1007/s10096-010-1107-8>
- Joint Committee of Japanese Society of Pediatric Infectious Disease and Japanese Society of Pediatric Pulmonology. Pneumonia. In: Ouchi K, Kurosaki T, Okada K, editors. Guidelines for management of respiratory infectious diseases in children in Japan 2011 [in Japanese]. Tokyo: Kyowa Kikaku Ltd.; 2011. p. 44
- Miyashita N, Kawai Y, Akaike H, Ouchi K, Hayashi T, Kurihara T, et al.; Atypical Pathogen Study Group. Macrolide-resistant *Mycoplasma pneumoniae* in adolescents with community-acquired pneumonia. *BMC Infect Dis.* 2012;12:126. <http://dx.doi.org/10.1186/1471-2334-12-126>
- Muraki Y, Yagi T, Tsuji Y, Nishimura N, Tanabe M, Niwa T, et al. Japanese antimicrobial consumption surveillance: first report on oral and parenteral antimicrobial consumption in Japan (2009–2013). *J Glob Antimicrob Resist.* 2016;7:19–23. <http://dx.doi.org/10.1016/j.jgar.2016.07.002>
- Zheng X, Lee S, Selvarangan R, Qin X, Tang YW, Stiles J, et al. Macrolide-resistant *Mycoplasma pneumoniae*, United States. *Emerg Infect Dis.* 2015;21:1470–2. <http://dx.doi.org/10.3201/eid2108.150273>
- Pereyre S, Touati A, Petitjean-Lecherbonnier J, Charron A, Vabret A, Bébéar C. The increased incidence of *Mycoplasma pneumoniae* in France in 2011 was polyclonal, mainly involving *M. pneumoniae* type 1 strains. *Clin Microbiol Infect.* 2013;19:E212–7. <http://dx.doi.org/10.1111/1469-0691.12107>
- Dumke R, Schnee C, Pletz MW, Rupp J, Jacobs E, Sachse K, et al.; Capnetz Study Group. *Mycoplasma pneumoniae* and *Chlamydia* spp. infection in community-acquired pneumonia, Germany, 2011–2012. *Emerg Infect Dis.* 2015;21:426–34. <http://dx.doi.org/10.3201/eid2103.140927>
- Keenan JD, Klugman KP, McGee L, Vidal JE, Chochua S, Hawkins P, et al. Evidence for clonal expansion after antibiotic selection pressure: pneumococcal multilocus sequence types before and after mass azithromycin treatments. *J Infect Dis.* 2015;211:988–94. <http://dx.doi.org/10.1093/infdis/jiu552>
- Okade H, Funatsu T, Eto M, Furuya Y, Mizunaga S, Nomura N, et al. Impact of the pneumococcal conjugate vaccine on serotype distribution and susceptibility trends of pediatric non-invasive *Streptococcus pneumoniae* isolates in Tokai, Japan over a 5-year period. *J Infect Chemother.* 2014;20:423–8. <http://dx.doi.org/10.1016/j.jiac.2014.03.010>
- Liu WK, Liu Q, Chen DH, Liang HX, Chen XK, Chen MX, et al. Epidemiology of acute respiratory infections in children in Guangzhou: a three-year study. *PLoS One.* 2014;9:e96674. <http://dx.doi.org/10.1371/journal.pone.0096674>

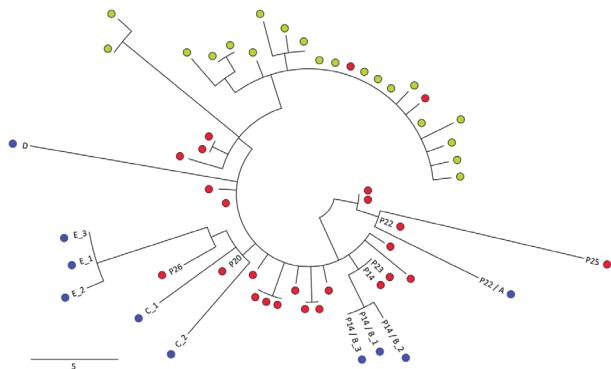
13. Jenkins SG, Farrell DJ. Increase in pneumococcus macrolide resistance, United States. *Emerg Infect Dis*. 2009;15:1260–4. <http://dx.doi.org/10.3201/eid1508.081187>
14. Farrell DJ, Couturier C, Hryniewicz W. Distribution and antibacterial susceptibility of macrolide resistance genotypes in *Streptococcus pneumoniae*: PROTEKT year 5 (2003–2004). *Int J Antimicrob Agents*. 2008;31:245–9. <http://dx.doi.org/10.1016/j.ijantimicag.2007.10.022>
15. Bley C, van der Linden M, Reinert RR. *mef(A)* is the predominant macrolide resistance determinant in *Streptococcus*

pneumoniae and *Streptococcus pyogenes* in Germany. *Int J Antimicrob Agents*. 2011;37:425–31. <http://dx.doi.org/10.1016/j.ijantimicag.2011.01.019>

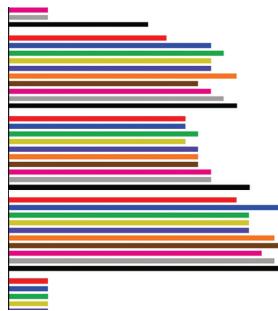
Address for correspondence: Tomohiro Oishi, Kawasaki Medical School—Pediatrics, 577, Matsushima, Kurashiki Kurashiki Okayama 701-0192, Japan; email: oo0612@med.kawasaki-m.ac.jp

September 2016: Antimicrobial Resistance

- Co-Infections in Visceral Pentastomiasis, Democratic Republic of the Congo
- Multistate US Outbreak of Rapidly Growing Mycobacterial Infections Associated with Medical Tourism to the Dominican Republic, 2013–2014
- Virulence and Evolution of West Nile Virus, Australia, 1960–2012
- Phylogeographic Evidence for 2 Genetically Distinct Zoonotic *Plasmodium knowlesi* Parasites, Malaysia
- Hemolysis after Oral Artemisinin Combination Therapy for Uncomplicated *Plasmodium falciparum* Malaria



- Enterovirus D68 Infection in Children with Acute Flaccid Myelitis, Colorado, USA, 2014
- Middle East Respiratory Syndrome Coronavirus Transmission in Extended Family, Saudi Arabia, 2014



- Exposure-Specific and Age-Specific Attack Rates for Ebola Virus Disease in Ebola-Affected Households, Sierra Leone
- Outbreak of *Achromobacter xylosoxidans* and *Ochrobactrum anthropi* Infections after Prostate Biopsies, France, 2014

- Human Babesiosis, Bolivia, 2013
- Assessment of Community Event-Based Surveillance for Ebola Virus Disease, Sierra Leone, 2015
- Probable Rabies Virus Transmission through Organ Transplantation, China, 2015
- Cutaneous Melioidosis Cluster Caused by Contaminated Wound Irrigation Fluid
- Possible Role of Fish and Frogs as Paratenic Hosts of *Dracunculus medinensis*, Chad
- Time Lags between Exanthematous Illness Attributed to Zika Virus, Guillain-Barré Syndrome, and Microcephaly, Salvador, Brazil
- Use of Unamplified RNA/cDNA-Hybrid Nanopore Sequencing for Rapid Detection and Characterization of RNA Viruses
- Importation of Hybrid Human-Associated *Trypanosoma cruzi* Strains of Southern South American Origin, Colombia
- Lyssavirus in Indian Flying Foxes, Sri Lanka
- Survival and Growth of *Orientia tsutsugamushi* in Conventional Hemocultures
- Chagas Disease Screening in Maternal Donors of Publicly Banked Umbilical Cord Blood, United States
- Multilocus Sequence Typing Tool for *Cyclospora cayatanensis*

