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Mycoplasma pneumoniae Isolated from Patients with Respiratory Infection in Kanagawa Prefecture in 1976-2006: Emergence of Macrolide-Resistant Strains

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We have been closely following the trend of *Mycoplasma pneumoniae* infections in Kanagawa Prefecture since 1976. We report here its isolation rates and the frequency of macrolide-resistant strains during the period.

Pharyngeal swabs obtained from patients with respiratory infections were used for isolation of *M. pneumoniae*. The liquid media used were PPLO broth (Difco Laboratories, Detroit, Mich., USA) and BBL™ Mycoplasma broth base (Becton, Dickinson and Co., Baltimore, Md., USA), both supplemented with 20% horse serum (Invitrogen Corp., Carlsbad, Calif., USA) inactivated by heating at 56°C for 30 min, 10% house-made yeast extract, 1% glucose, 1,000 U/ml penicillin G and 0.025% thallium acetate. The agar medium (prepared in 60 mm Petri dishes) was 1.5% agar (Bacto™ agar; Becton, Dickinson) prepared in the above liquid medium. The diphasic medium was prepared in screw-cap tubes (12 mm in diameter and 100 mm in height); the upper layer consisted of 2 ml of the liquid medium and the bottom layer 1 ml of the agar medium, both supplemented with 0.002% phenol red and 0.001% methylene blue. Color change of the medium indicated a positive culture of *M. pneumoniae*. All the cultures were performed aerobically at 37°C.

Typical colonies appearing on the agar plates were selected and inoculated into the liquid medium containing 0.002% phenol red for expansion. *M. pneumoniae* was identified by serological (1) or polymerase chain reaction (2) studies.

Among 2,414 swab specimens obtained from acute respiratory infection cases in 1976-2006, 585 (24.2%) were positive for *M. pneumoniae*. Frequency peaks for isolation of *M. pneumoniae* appeared regularly at 4-year intervals until the first half of the 1990's, but from then the pattern started to degrade (Fig. 1). This trend parallels that of reported *M. pneumoniae* cases across Japan (3).

We tested 381 *M. pneumoniae* isolated in 1985-2006 for sensitivities (MIC; μg/ml) to erythromycin (EM), josamycin (JM), minocycline and tetracycline. The sensitivity test was done by a broth microdilution method previously described (4). The results are shown in Table 1. While none of the isolates before 1999 were resistant to these antibiotics, strains resistant to EM and JM were found among isolates in 2003. Resistant strains were isolated every year from 2003 to 2006. In total, among the 85 isolates in the period 2000-2006, 15 (17.6%) were resistant to EM and JM. The nucleotide sequencing of the V domain of 23S rRNA in the resistant strains revealed replacement of adenine by guanine at a nucleotide position 2063 or 2064, i.e., A2063G or A2064G (5). These

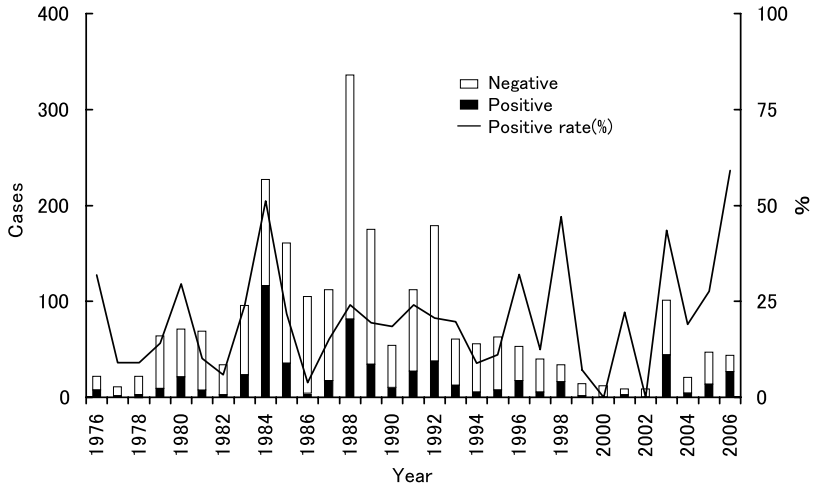

Fig. 1. Isolation of *M. pneumoniae* from throat swabs in patients from 1976 to 2006 in Kanagawa Prefecture.

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Table 1. Detection of macrolide-resistant strains in *M. pneumoniae* isolates from 1985 to 2006 in Kanagawa Prefecture

Year	Strains tested	Resistant strains	Mutation[1)] of the resistant strains
1985 - 1989	171	0	
1990 - 1994	87	0	
1995 - 1999	38	0	
2000 - 2004	48	7	A2063G, A2064G
2005 - 2006	37	8	A2063G, A2064G

[1)]: Point mutation in 23S rRNA gene.

mutant strains were tested for cross-resistance among macrolides such as azithromycin, clarithromycin, leucomycin, midekamycin, oleandomycin and spiramycin. They were resistant to all these macrolides (data not shown).

Okazaki et al. detected macrolide-resistant strains among the isolates in Hokkaido in 2000 (4), and Morozumi et al. reported emergence of such resistant strains across Japan (6). Therefore, at least a part of the mycoplasma infections observed in Japan after 2000 must have been caused by macrolide-resistant strains.

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