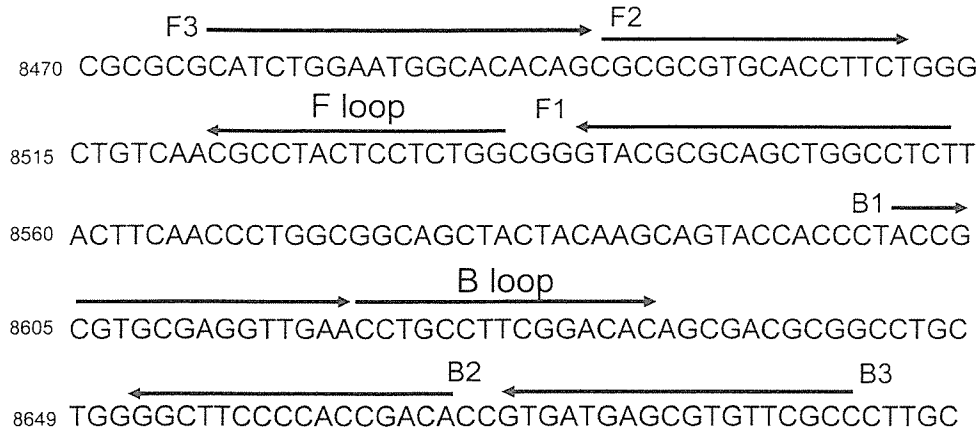


F3: GCATCTGGAATGGCACACAG
FIP: AGAGGCCAGCTGCGCGTACCGCGCGTGCACCTTCT
F loop: CCAGAGGAGTAGGCG
B3: CCGCTTGTGCGAGTAGTG
BIP: ACCGCGTGCGAGGTTGAATGTCGGTGGGGAAGCC
B loop: CCTGCCTTCGGACAC

FIG. 1. Diagram of RT-LAMP primers for the detection of the rubella virus genome. The eight primer sites are shown in the upper panel. Sequence alignments of six LAMP primers are shown in the lower panel. Positive-sense F3 and complementary B3 were used as outer primers. FIP contains the sequence complementary to F1 linked with the F2 sequence. BIP contains the B1 sequence linked with the sequence complementary to B2. Two additional loop primers (F and B) are synthesized between F1 and F2 and between B1 and B2, respectively. The arrows show the direction of DNA synthesis.

numbers AB238919, AB238920, and AB238921, respectively. They were classified into genotype 1D. Rubella virus genome was detected in six patients (66.7%) by RT-PCR and seven (77.8%) by RT-LAMP. The rubella virus genome was detected by RT-PCR and RT-LAMP in all three patients positive for virus isolation. Among six patients negative for virus isolation, the virus genome was detected in three by RT-PCR and in four by RT-LAMP. The backgrounds of the

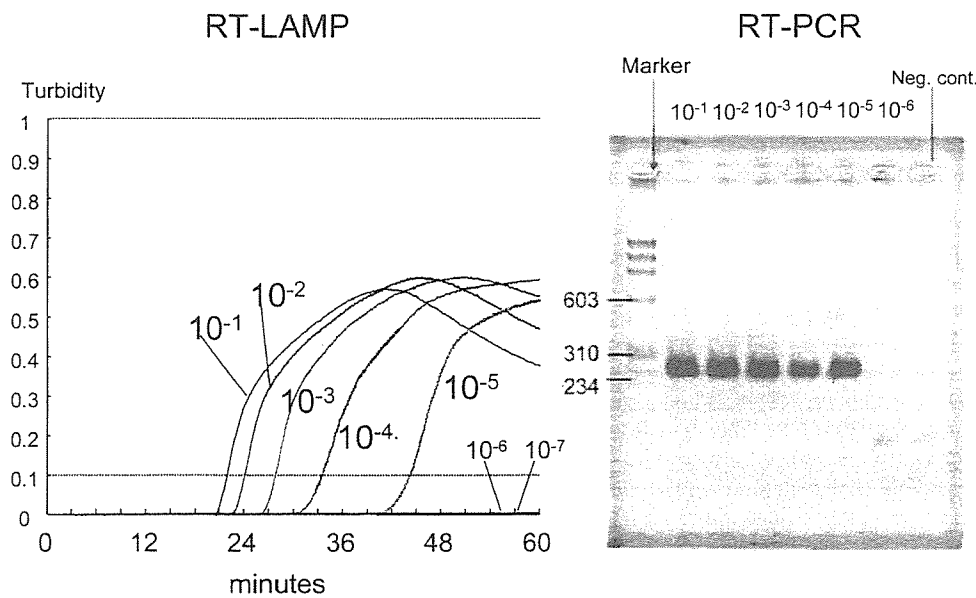


FIG. 2. Detection limit of RT-LAMP and RT-PCR. The Takahashi vaccine strain containing $10^{5.5}$ PFU/0.1 ml was used. RNA was serially diluted by 1:10, and each dilution was subjected to RT-LAMP and RT-PCR. A result with a turbidity of ≥0.1 was considered to be LAMP positive. Numbers at left of the right panel are sizes in base pairs of the DNA marker.

TABLE 1. Results of virological examinations: virus isolation, RT-PCR, and RT-LAMP, using nine clinical samples obtained from nine patients suspected of rubella virus infection

| Method and type of result (*n*) | No. of results by method and type (*n*) | | | |
|---|---|---|---|---|
| | RT-PCR | | RT-LAMP | |
| | Positive (6) | Negative (3) | Positive (7) | Negative (2) |
| Virus isolation | | | | |
| Positive (3) | 3 | 0 | 3 | 0 |
| Negative (6) | 3 | 3 | 4 | 2 |
| RT-PCR | | | | |
| Positive (6) | | | 6 | 0 |
| Negative (3) | | | 1 | 2 |

TABLE 2. Immunization history and the results of serology, virus isolation, RT-PCR, and RT-LAMP for nine patients

| Patient | Immunization history | Result by method[a] | | | |
|---|---|---|---|---|---|
| | | Rubella IgM EIA | Virus isolation | RT-PCR | RT-LAMP |
| Case 1 | Unknown | ND | − | + | + |
| Case 2 | No | ND | − | + | + |
| Case 3 | No | ND | − | − | − |
| Case 4 | No | ND | − | + | + |
| Case 5 | No | + | − | − | − |
| Case 6 | No | + | − | − | + |
| Case 7 | No | + | + | + | + |
| Case 8 | No | ND | + | + | + |
| Case 9 | No | + | + | + | + |

[a] +, positive; −, negative; ND, not done.

patients are shown in Table 2, together with the results of virological examinations. Eight patients did not have a history of immunization against rubella, and the rubella immunization history of case 1 was unknown. The rubella IgM EIA was performed in four (cases 5, 6, 7, and 9) out of nine patients and was found to be positive in all. Of two patients negative for all three tests, one patient (case 5) was positive for IgM EIA antibodies and another patient (case 3) had family contact with a laboratory-confirmed patient (case 6). Among four patients (cases 5, 6, 7, and 9) who were serologically confirmed to have rubella virus infection by IgM EIA, the rubella virus genome was detected in three by RT-LAMP and RT-PCR. Among five patients who were clinically diagnosed without serological confirmation, RT-PCR or RT-LAMP detected rubella virus genome in four patients.

RT-LAMP detected the genome of different genotypes, 1E (RVi/Tokyo.JPN/87CRS-w, RVi/Tokyo.JPN/87CRS-o, and RVi/Tokyo.JPN/95CRS-n) and 1a (RVi/Kanagawa.JPN/04-s). The difference between the Takahashi vaccine strain and the wild strains in this study was 2.8 to 5.8% of nucleotides and 1.0 to 2.4% of amino acids in the E1 region. There was no significant difference in the sensitivity of rubella RT-LAMP among the circulating wild strains and vaccine strain in Japan (data not shown).

## DISCUSSION

The rubella virus genomes from the Takahashi vaccine strain and the current wild strains were successfully amplified by RT-LAMP. The detection limit was calculated as 30 PFU/ml in culture fluid for both RT-LAMP and RT-PCR and estimated as 1 PFU of infectious particles in 5 μl of RNA materials in a single test. Actually, RT-LAMP detected the rubella virus genome in all six samples positive for RT-PCR and also in one of three samples negative for RT-PCR. The detection limit of RT-LAMP for rubella (30 PFU/ml in culture fluids) showed the slightly lower sensitivity of this method compared with that for other viruses: 0.15 to 0.4 50% tissue culture infective doses ($TCID_{50}$)/ml of measles virus (10), 3 PFU/ml of mumps virus (20), and 0.5 to 1.5 $TCID_{50}$/ml of RSV (27). RT-LAMP for measles virus, mumps virus, and RSV has been used for routine clinical examinations in our laboratory.

The RT-LAMP primers were designed based on the Takahashi vaccine strain, since the wild strains of rubella (clades 1 and 2) were found to differ by 8 to 10% at the nucleotide level (5). The genetic differences in the E1 region between the wild strains and Takahashi vaccine strain were 2.8 to 5.8% in our study. Although RT-LAMP was not performed for clade 2, we suppose that RT-LAMP would detect other genotypes because the RT-LAMP primers were designed in the conserved region. RT-LAMP amplifies 200 to 250 bp of nucleotides with high sensitivity and specificity. The products of RT-LAMP are not appropriate for sequence analysis because most of the region amplified by RT-LAMP is occupied by the RT-LAMP primers as shown in Fig. 1. WHO recommended the E1 coding region for molecular epidemiology or a window of 739 nucleotides, 8731 to 9469, for routine molecular epidemiological analysis (30, 32, 33). As for genotyping, E1 gene should be amplified by conventional RT-PCR.

RT-LAMP for rubella would be expected to be a reliable and rapid diagnostic method in the clinical setting, because RT-LAMP showed an equivalent or higher detection rate compared with RT-PCR and virus isolation for nine samples obtained from clinically diagnosed patients. The RT-LAMP procedure has clinical advantages of simplicity and rapidity, in comparison with virus isolation and RT-PCR. Virus isolation requires complex procedures for cell culture, is not always successful, and is not appropriate for a clinical laboratory diagnostic tool. The genome amplification method always has the possibility of false positives due to cross-contamination. Since RT-LAMP is performed as a simple procedure in a single tube with sensitivity similar to that of nested PCR, cross-reaction would less likely occur in RT-LAMP than in RT-PCR.

The detection limit of RT-PCR primers used in our study (30 PFU/ml) was similar to that in previous studies, and the RT-PCR primers were designed to have the same target as the RT-LAMP primers in order to compare the sensitivities of RT-LAMP and RT-PCR. In previous reports, RT-PCR for rubella targeting the E1 region detected up to 8 infectious units of WHO international standard/ml in amniotic fluid (8, 15) and a $10^{-6}$ dilution of culture fluid containing $10^{6.8}$ $TCID_{50}$/ml of rubella virus, reported by Bosma et al. (3). Cooray et al. (6) also reported that the rubella genome RNA was detected in oral fluid containing 100 PFU/ml.

In several laboratories, real-time PCR has been developed for the detection of measles virus (21, 23), mumps virus (14),

and RSV (9, 12), but real-time PCR for rubella virus has not been reported. This method is based on PCR temperature shifts and shows higher sensitivity than RT-PCR with a reduction of the risk of cross-contamination. As it takes 2 to 3 h to obtain the results by real-time PCR, RT-LAMP has the distinct benefit of a faster reaction, within 60 min. Also, Tzeng et al. (26) improved the methodology of virus isolation developed using a replicon-based reporter gene assay for the detection of rubella virus. LAMP is a simpler, more sensitive, and more rapid method than virus isolation.

Misclassification of the clinical diagnosis of the nine patients from whom we collected samples in our study might have occurred, since we did not require a serological confirmation for the definition of the cases. Among the nine patients in this study, IgM EIA was performed in four patients. The remaining five did not receive an IgM test, but they were strongly suspected to have rubella virus infection. These five samples from patients with suspected rubella were appropriate for use in our study for the following two reasons. The outbreak of rubella in this area in April 2004 was confirmed by isolating rubella virus from patients. Also, the positive predictive value of case definition is considered to be high during outbreaks (11).

This study had some limitations, including the different criteria for the clinical diagnosis of rubella, the differences in timing and procedure for sample collection, and the limited number of clinical samples. However, at least 100 samples should be tested for the assessment of the procedure according to the review on the validation of nucleic acid-based tests published by Dimech et al. (7). The purpose of this study was to establish a rapid, sensitive, and simple method of RT-LAMP for the detection of rubella virus. The detection of the rubella virus genome by RT-LAMP can be confirmed using clinical samples from patients with rubella or CRS. We should further evaluate RT-LAMP for rubella diagnosis in the field.

During the progress of rubella control toward CRS elimination, the clinical application of RT-LAMP would be helpful for rapid diagnosis when managing patients or determining the response to an outbreak. A rapid response should be made to cope with outbreaks of rubella or CRS. RT-LAMP would contribute to the rapid diagnosis of index cases for the control of outbreaks. Methods for laboratory-confirmed diagnosis such as RT-LAMP will improve the surveillance of rubella and CRS by enabling the early detection of outbreaks and decreasing the risk of misclassification of cases that may occur with clinical diagnoses.

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## REFERENCES

1. **Atkison, W., J. Hamborsky, and S. Wolfe.** 2004. Rubella, p. 145–158. *In* W. Atkinson, J. Hamborsky, and S. Wolfe (ed.), Centers for Disease Control and Prevention. Epidemiology and prevention of vaccine-preventable diseases, 8th ed. Public Health Foundation, Washington, D.C.
2. **Banatvala, J. E., and D. W. G. Brown.** 2004. Rubella. Lancet **363:**1127–1137.
3. **Bosma, T. J., K. M. Corbett, S. O'Shea, J. E. Banatvala, and J. M. Best.** 1995. PCR for detection of rubella virus RNA in clinical samples. J. Clin. Microbiol. **33:**1075–1079.
4. **Centers for Disease Control and Prevention.** 2001. Control and prevention of rubella: evaluation and management of suspected outbreaks, rubella in pregnant women, and surveillance for congenital rubella syndrome. MMWR Recomm. Rep. **50**(RR-12):1–23.
5. **Chantler, J., J. S. Wolinsky, and A. Tingle.** 2001. Rubella virus, p. 963–990. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, 4th ed. Lippincott Willians & Wilkins, Philadelphia, Pa.
6. **Cooray, S., L. Warrener, and L. Jin.** 2006. Improved RT-PCR for diagnosis and epidemiological surveillance of rubella. J. Clin. Virol. **35:**73–80.
7. **Dimech, W., D. S. Bowden, B. Brestovac, K. Byron, G. James, D. Jardine, T. Sloots, and E. M. Dax.** 2004. Validation of assembled nucleic acid-based tests in diagnostic microbiology laboratories. Pathology **36:**45–50.
8. **Eggerding, F. A., J. Peters, R. K. Lee, and C. B. Inderlied.** 1991. Detection of rubella virus gene sequences by enzymatic amplification and direct sequencing of amplified DNA. J. Clin. Microbiol. **29:**945–952.
9. **Falsey, A. R., M. A. Formica, J. J. Treanor, and E. E. Walsh.** 2003. Comparison of quantitative reverse transcription-PCR to viral culture for assessment of respiratory syncytial virus shedding. J. Clin. Microbiol. **41:**4160–4165.
10. **Fujino, M., N. Yoshida, S. Yamaguchi, N. Hosaka, Y. Ota, T. Notomi, and T. Nakayama.** 2005. A simple method for the detection of measles virus genome by loop-mediated isothermal amplification (LAMP). J. Med. Virol. **76:**406–413.
11. **Gordis, L.** 2004. Assessing the validity and reliability of diagnostic and screening tests, p. 71–940. *In* L. Gordis (ed.), Epidemiology, 3rd ed. Elsevier Saunders, Philadelphia, Pa.
12. **Gueudin, M., A. Vabret, J. Petitjean, S. Gouarin, J. Brouard, and F. Freymuth.** 2003. Quantitation of respiratory syncytial virus RNA in nasal aspirates of children by real-time RT-PCR assay. J. Virol. Methods **109:** 39–45.
13. **Ho-Terry, L., G. M. Terry, and P. Londesborough.** 1990. Diagnosis of foetal rubella virus infection by polymerase chain reaction. J. Gen. Virol. **71:**1607–1611.
14. **Kubar, A., M. Yapar, B. Besirbellioglu, I. Y. Avci, and C. Guney.** 2004. Rapid and quantitative detection of mumps virus RNA by one-step real-time RT-PCR. Diagn. Microbiol. Infect. Dis. **49:**83–88.
15. **Macé, M., D. Cointe, C. Six, D. Levy-Bruhl, I. P. du Châtelet, D. Ingrand, and L. Grangeot-Keros.** 2004. Diagnostic value of reverse transcription-PCR of amniotic fluid for prenatal diagnosis of congenital rubella infection in pregnant women with confirmed primary rubella infection. J. Clin. Microbiol. **42:**4818–4820.
16. **Mori, Y., M. Kitao, N. Tomita, and T. Notomi.** 2004. Real-time turbidimetry of LAMP reaction for quantifying template DNA. J. Biochem. Biophys. Methods **59:**145–157.
17. **Nagamine, K., K. Watanabe, K. Ohtsuka, T. Hase, and T. Notomi.** 2001. Loop-mediated isothermal amplification reaction using a nondenatured template. Clin. Chem. **47:**1742–1743.
18. **Nagamine, K., T. Hase, and T. Notomi.** 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol. Cell. Probes **16:**223–229.
19. **Notomi, T., H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, and T. Hase.** 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. **28:**e63.
20. **Okafuji, T., N. Yoshida, Y. Motegi, T. Ihara, Y. Ota, and T. Notomi, and T. Nakayama.** 2005. Rapid diagnostic method for detection of mumps virus genome by loop-mediated isothermal amplification. J. Clin. Microbiol. **43:** 1625–1631.
21. **Ozoenmena, L. C., P. D. Minor, and M. A. Afzal.** 2004. Comparative evaluation of measles virus specific TaqMan PCR and conventional PCR using synthetic and natural RNA templates. J. Med. Virol. **73:**79–84.
22. **Plotkin, S. A., and S. Reef.** 2004. Rubella vaccine, p. 707–743. *In* S. A. Plotkin and W. A. Orenstein (ed.), Vaccine, 4th ed. W. B. Saunders, Philadelphia, Pa.
23. **Schalk, J. A. C., C. van den Elzen, H. Ovelgönne, C. Baas, and P. M. J. Jongen.** 2004. Estimation of the number of infectious measles viruses in live virus vaccines using quantitative real-time PCR. J. Virol. Methods **117:**179–187.
24. **Thomas, H. I. J., E. Barrett, L. M. Hesketh, A. Wynne, and P. Morgan-Capner.** 1999. Simultaneous IgM reactivity by EIA against more than one virus in measles, parvovirus B19 and rubella infection. J. Clin. Virol. **14:**107–118.
25. **Tipple, G. A., R. Hamkar, T. Mohktari-Azad, M. Gray, J., Ball, C. Head, and S. Ratnam.** 2004. Evaluation of rubella IgM enzyme immunoassays. J. Clin. Virol. **30:**233–238.
26. **Tzeng, W.-P., Y. Zhou, J. Icenogle, and T. K. Frey.** 2005. Novel replicon-based reporter gene assay for detection of rubella virus in clinical specimens. J. Clin. Microbiol. **43:**879–885.
27. **Ushio, M., I. Yui, N. Yoshida, M. Fujino, T. Yonekawa, Y. Ota, T. Notomi, and T. Nakayama.** 2005. Detection of respiratory syncytial virus genome by subgroups-A, B specific reverse transcription loop-mediated isothermal amplification (RT-LAMP). J. Med. Virol. **77:**121–127.
28. **Watson, J. C., S. C. Hadler, C. A. Dykewicz, S. Reef, and L. Phillips.** 1998. Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps:

recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 47(RR-8):1–57.
29. **World Health Organization.** 1999. Guidelines for surveillance of congenital rubella syndrome and rubella. WHO/V&B/99.22. [Online.] http://www.who.int/gpv-documents/.
30. **World Health Organization.** 2005. Standardization of the nomenclature for genetic characteristics of wild-type rubella viruses. Wkly. Epidemiol. Rec. **80:**125–132.
31. **World Health Organization.** 2000. Rubella vaccines. WHO position paper. Wkly. Epidemiol. Rec. **75:**161–172.
32. **World Health Organization.** 2005. Global measles and rubella laboratory network—update. Wkly. Epidemiol. Rec. **80:**384–388.
33. **Zheng, D., T. K. Frey, J. Icenogle, S. Katow, S. Abernathy, K. Song, W. Xu, V. Yarullin, R. G. Desjats, Y. Aboudy, G. Enders, and M. Croxson.** 2003. Global distribution of rubella virus genotypes. Emerg. Infect. Dis. **9:**1523–1530.

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# Antimicrobial susceptibility and serotype distribution of *Streptococcus pneumoniae* isolated from patients with community-acquired pneumonia and molecular analysis of multidrug-resistant serotype 19F and 23F strains in Japan

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## SUMMARY

A nationwide study was undertaken to determine the susceptibility to penicillin and serotypes of *Streptococcus pneumoniae* in Japan. *S. pneumoniae* was isolated from 114 adult patients with community-acquired pneumonia over 22 months at 20 hospitals and medical centres in different regions in Japan. All but five isolates were from sputum. Forty-eight isolates (42·1%) were susceptible, 40 (35·1%) showed intermediate resistance (MIC, 0·12–1·0 μg/ml) and 26 (22·8%) were resistant (MIC, ⩾2·0 μg/ml) to penicillin G. All isolates were susceptible to ceftriaxone (breakpoint 1 μg/ml), imipenem (4 μg/ml) and vancomycin (4 μg/ml). Most were resistant to erythromycin, clarithromycin and azithromycin; only two were resistant to levofloxacin. Differences were found in the distribution of serotypes among isolates showing susceptibility to penicillin (predominant types 3, 6B, and 19F), intermediate resistance (6B, 14, 19F, and 23F) and full resistance (19F and 23F). PFGE typing showed that 14 of the 25 strains of serotype 19F had a single DNA profile, pattern A, a pattern closely similar to that of the Taiwan multidrug-resistant 19F clone. Twelve pattern A strains were not susceptible to penicillin but carried the macrolide resistance gene *mef*(A). The DNA profiles of the 15 strains of 23F were also heterogeneous but six were highly similar (pattern b) yet distinct from the Spanish multidrug-resistant 23F clone although possibly related to the Taiwan multidrug-resistant 23F clone. The pattern b strains were not susceptible to penicillin and also harboured either *mef*(A) or *erm*(B). Our results indicate that multidrug-resistant pneumococci are spreading rapidly in Japan. Efforts to prevent the spread of the pandemic multidrug-resistant serotypes should be intensified.

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## INTRODUCTION

*Streptococcus pneumoniae* is the major cause of community-acquired pneumonia (CAP) as well as otitis media, sinusitis, septicaemia, and meningitis [1]. Although susceptible to penicillin in the past, penicillin-resistant and multidrug-resistant pneumococci are now widespread in the world [1, 2]. In Japan, *S. pneumoniae* is the most common cause of CAP [3], and the proportion of multidrug-resistant strains is increasing at a dramatic rate [4, 5]. Nevertheless, there are few reports on the distribution of various serotypes and the genetic relatedness of pneumococci in this country [6]. In this study, we describe the results of a nationwide survey of the antimicrobial susceptibilities and the distribution of pneumococcal serotypes isolated from patients with CAP. We also examined the genetic relatedness of the predominant multidrug-resistant serotype 19F and 23F strains by pulsed-field gel electrophoresis (PFGE) and compared their DNA profiles with selected pandemic reference strains.

## METHODS

### Bacterial strains

A total of 114 isolates of pneumococci were recovered from 114 adult patients (mean age 67·4, range 20–99 years) presenting with CAP between November 2001 and August 2003 at 20 hospitals and medical centres located in different regions of Japan. Pneumonia was diagnosed if there was an appearance of a new abnormal shadow and likely infiltration on a chest roentgenogram and if at least two of the following clinical and laboratory findings were present: fever (temperature $>37{\cdot}8$ °C), cough, production of purulent sputum, dyspnoea, and leukocytosis (WBC count $>10\,000/\mu$l). The sources of the isolates were sputum ($n=109$), blood ($n=3$), pleural effusion ($n=1$) and bronchoalveolar lavage ($n=1$). Culture plates were incubated overnight in a 5% $CO_2$ atmosphere, and optochin sensitivity and bile solubility tests were performed to confirm *S. pneumoniae*.

### Antimicrobial susceptibility test

MICs were determined by an agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards [7]. All isolates were tested for susceptibility to the following nine antibiotics: penicillin G (Meiji Seika Kaisha, Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Tokyo, Japan), cefditoren (Meiji Seika Kaisha), imipenem (Banyu Pharmaceutical Co., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Osaka, Japan), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), azithromycin (Pfizer Japan Inc., Tokyo, Japan), levofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan), and vancomycin (Shionogi Co., Osaka, Japan).

### Serotyping

Isolates were serotyped by the capsular swelling (quellung reaction) observed microscopically after suspension in pneumococcal typing antisera (Statens Serum Institut, Copenhagen, Denmark).

### PCR

PCR was performed to detect alterations in penicillin-binding protein genes *pbp1a*, *pbp2x*, and *pbp2b* and macrolide resistance genes *mef*(A) and *erm*(B) by using a commercially available test kit (Wakunaga Pharmaceutical Co., Hiroshima, Japan) with primers modified as reported previously [8]. Briefly, a single colony from the blood agar medium was suspended in a microtube containing 30 $\mu$l of a lysis solution. The tube was placed in a thermal cycler, and bacterial cells were lysed at 60 °C for 10 min followed by 94 °C for 5 min. Next, 2 $\mu$l lysate was placed in a PCR tube containing 25 $\mu$l reaction mixture (1 ml reaction mixture contained 60 ng of a primer for each of the target genes, 80 $\mu$l of 10 mM dinucleoside triphosphate, 40 U *Tth* DNA polymerase, and 100 $\mu$l of 10× PCR buffer). The PCR conditions were 94 °C for 20 s, 52 °C for 20 s, and 72 °C for 15 s for 30 cycles total.

### PFGE

PFGE was performed on strains of serotype 19F, 23F. The Spanish multidrug-resistant serotype 23F clone (ATCC 700669), the Taiwan multidrug-resistant serotype 19F clone (ATCC 700905) and the Taiwan multidrug-resistant serotype 23F clone (ATCC 700906) were used as reference standards [9]. Strains were grown overnight in brain heart infusion broth at 35 °C, and PFGE of *Sma*I chromosomal digests was performed as described previously [10]. DNA banding patterns were interpreted according to the criteria of Tenover *et al.* [11] with a greater than three bands

Table 1. *Distribution of MICs for various antibiotics for 114 isolates of pneumococci in Japan*

| | No. of isolates for which MIC ($\mu$g/ml) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antibiotic | ⩽0·004 | 0·008 | 0·016 | 0·032 | 0·063 | 0·125 | 0·25 | 0·5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | ⩾128 |
| Penicillin G | | 1 | 3 | 26 | 18 | 11 | 9 | 5 | 15 ⇓ | 25 | 1 | | | | | |
| Ceftriaxone | | | 4 | 6 | 1 | 22 | 25 | 23 | 33 | | | | | | | |
| Cefditoren | | 1 | 3 | 9 | 4 | 26 | 18 | 40 | 10 | 3 | ⇓ | | | | | |
| Imipenem | 9 | 36 | 15 | 6 | 5 | 14 | 28 | ⇓ | 1 | | | | | | | |
| Erythromycin | | | | 3 | 10 | 7 | 2 | 6 ⇓ | 13 | 10 | 3 | 3 | 1 | | | 56 |
| Clarithromycin | | | 1 | 6 | 11 | 7 | | 8 | 12 ⇓ | 9 | 2 | | 2 | | | 56 |
| Azithromycin | | | | 2 | | 14 | 6 | 8 | 14 | 3 ⇓ | 5 | | 2 | | 1 | 59 |
| Levofloxacin | | | | | | | | 3 | 50 | 59 | 1 ⇓ | | | 1 | | |
| Vancomycin | | | | | | 1 | 93 | 20 | | | | | | | | |

⇓ Breakpoints for each antibiotic.

difference in profile needed to distinguish between PFGE types.

## RESULTS

### Antimicrobial susceptibility test

The MICs of the nine antibiotics tested for the 114 *S. pneumoniae* isolates are shown in Table 1. Using NCCLS breakpoints all isolates were found to be susceptible to ceftriaxone, imipenem and vancomycin. The majority were resistant to erythromycin, clarithromycin and azithromycin; only two isolates were not susceptible to levofloxacin. Forty-eight isolates (42·1%) were susceptible to penicillin G, 40 (35·1%) showed intermediate resistance and 26 (22·8%) were fully resistant to ⩾2·0 $\mu$g/ml of this antibiotic.

### Serotyping

All isolates but one, were grouped into 21 different serotypes (Table 2). The serotypes of penicillin-susceptible isolates varied widely but with serotypes 3 (22·9%), 6B (12·5%) and 19F (12·5%) predominating among 18 other serotypes. In contrast, the 26 fully penicillin-resistant isolates fell into only six serotypes, the most frequent being 19F (50·0%), and 23F (23·1%); intermediate susceptible isolates were represented by 11 serotypes with 23F (20·0%), 14 (17·5%), 6B (15·0%) and 19F (15·0%) being the most common.

### Molecular characterization of serotypes 19F and 23F

PFGE typing revealed 10 different DNA profiles (A–J) among the 25 strains of serotype 19F (Fig. 1). One pattern, type A, accounted for 14 strains and this was indistinguishable from the profile of the Taiwan multidrug-resistant serotype 19F clone. Three strains of serotype 19F were classified as pattern B and the other eight strains gave unique patterns. Twelve of the pattern A strains were not susceptible to penicillin and all 14 harboured the *mef*(A) gene. Overall 21 (84%) of the serotype 19F strains were *mef*(A) and/or *erm*(B) positive. Eighteen of the serotype 19F strains had alterations in *pbp1a*, *−2x*, and *−2b*; four in *pbp2x* and *−2b*; two in *pbp2x*; and one had no alteration. These strains originated from different geographical regions of Japan (Table 3).

The 15 strains of serotype 23F were characterized by nine different DNA profiles (b–j) with pattern b predominating and represented by six strains (Fig. 2). Two strains were typed as pattern g and the remainder were unique. None of the DNA profiles of serotype 23F matched that of the Spanish multidrug-resistant 23F clone but pattern b was similar to the profile of the Taiwan multidrug-resistant serotype

Table 2. *Serotypes of susceptible, intermediate, and fully penicillin-resistant pneumococci*

| Serotype | Susceptible ($n=48$) | Intermediate ($n=40$) | Resistant ($n=26$) |
|---|---|---|---|
| 3 | 11 | 2 | 0 |
| 6A | 3 | 1 | 3 |
| 6B | 6 | 6 | 2 |
| 7F | 1 | 0 | 0 |
| 9A | 1 | 0 | 0 |
| 9V | 4 | 0 | 1 |
| 11A | 2 | 2 | 1 |
| 14 | 2 | 7 | 0 |
| 15A | 1 | 0 | 0 |
| 15C | 2 | 0 | 0 |
| 16F | 0 | 3 | 0 |
| 18A | 1 | 0 | 0 |
| 18B | 1 | 0 | 0 |
| 18C | 1 | 0 | 0 |
| 19A | 3 | 2 | 0 |
| 19F | 6 | 6 | 13 |
| 22F | 1 | 0 | 0 |
| 23A | 0 | 1 | 0 |
| 23F | 1 | 8 | 6 |
| 29 | 0 | 1 | 0 |
| 34 | 1 | 0 | 0 |
| Non-typable | 0 | 1 | 0 |

23F clone although there were at least four band differences between them (Fig. 2). Strains of pattern b were not susceptible to penicillin and all positive by PCR for either the *mef*(A) or *erm*(B) gene as were strains of the other DNA types. These strains were also widespread in the country (Table 3). Thirteen of the serotype 23F strains were altered in *pbp1a*, *−2x*, and *−2b*; and one each in *pbp2x* and *−2b*, and *pbp2x* respectively.

Two strains each of serotype 19F (patterns A2 and B) and 23F (patterns e and i) had both *mef*(A) and *erm*(B) genes, and these four strains were all penicillin non-susceptible (Table 3).

## DISCUSSION

Penicillin-resistant *S. pneumoniae* are already distributed worldwide and resistance appears to be expanding to include multiple antimicrobial agents [1, 4]. In Asia, the proportion of *S. pneumoniae* reported to be non-susceptible to penicillin over the last decade ranges from 68·8% in Thailand [12], to 3·8% in India, 9·0% in Malaysia, 9·8% in China, 21·0% in Indonesia, 23·1% in Singapore, 38·7% in Taiwan, 41·2% in Sri Lanka, 60·8% in Vietnam, 79·7% in

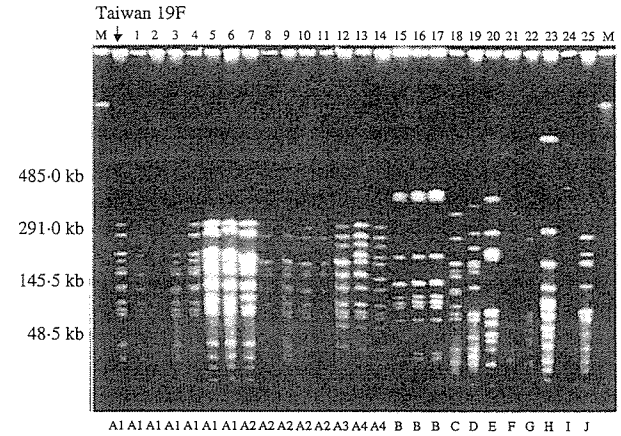


**Fig. 1.** Pulsed-field gel electrophoresis (PFGE) patterns of *Sma*I-digested DNA from 25 strains of serotype 19F *S. pneumoniae* and a representative of the Taiwan multi-resistant serotype 19F clone. M, Molecular size marker.

Korea and 65·3% in Japan in 1996 to 1997 [13]. Indeed, a survey of a single prefecture in Japan from 2001 to 2003 reported a frequency of 60·9% [6]. In this study, 57·9% of the *S. pneumoniae* isolates from patients with CAP were penicillin non-susceptible and some of these were also resistant to various other antibiotics. Thus, it appears that the frequency of multidrug-resistant *S. pneumoniae* in Japan is among the highest in Asia. A previous study demonstrated that the aetiology of CAP in Japan did not differ substantially from Western countries and that *S. pneumoniae* was the most common pathogen in this disease [3]. Some earlier surveys indicate that the clinical outcome between penicillin-susceptible and non-susceptible isolates does not differ under current resistance levels [5, 14]. However, invasive pneumococcal isolates with very high-level penicillin resistance (MIC $\geqslant 8{\cdot}0\ \mu g/ml$) have been reported to be prevalent in the United States [15]. Caution should be exercised concerning such strains, although we did not detect them in Japan.

The 23-valent pneumococcal polysaccharide vaccine (PS23) has been promoted worldwide, although it fails to protect children $<2$ years old [16, 17]. It is well known that children aged $<5$ years and adults of $\geqslant 65$ years are the most susceptible to invasive pneumococcal infection and have a high mortality rate [16, 18]. Vaccination, therefore, seems to be the only available method for preventing pneumococcal disease [16, 19]. In this study, all the isolates were classified into 21 serotypes, 82·5% of which are accounted for in PS23. Furthermore, 56 (59·6%) of the isolates were penicillin non-susceptible isolates.

Table 3. *Correlation of PFGE patterns, penicillin resistance, macrolide resistance genes and mutation in penicillin binding protein genes in strains of* S. pneumoniae *serotypes 19F and 23F*

| PFGE line | PFGE pattern | Penicillin resistant | autolysin | *pbp1a* | *pbp2x* | *pbp2b* | *mef*(A) | *erm*(B) | Region |
|---|---|---|---|---|---|---|---|---|---|
| 19F | | | | | | | | | |
| 1 | A1 | R | + | + | + | + | + | − | Tohoku |
| 2 | A1 | S | + | − | + | − | + | − | Kyushu |
| 3 | A1 | S | + | − | + | − | + | − | Kyushu |
| 4 | A1 | R | + | + | + | + | + | − | Kinki |
| 5 | A1 | R | + | + | + | + | + | − | Kanto |
| 6 | A1 | R | + | + | + | + | + | − | Kinki |
| 7 | A2 | R | + | + | + | + | + | + | Kyushu |
| 8 | A2 | I | + | + | + | + | + | − | Chubu |
| 9 | A2 | R | + | + | + | + | + | − | Kanto |
| 10 | A2 | I | + | + | + | + | + | − | Kanto |
| 11 | A2 | R | + | + | + | + | + | − | Kanto |
| 12 | A3 | R | + | + | + | + | + | − | Chubu |
| 13 | A4 | R | + | + | + | + | + | − | Kinki |
| 14 | A4 | I | + | + | + | + | + | − | Kanto |
| 15 | B | I | + | + | + | + | + | − | Kanto |
| 16 | B | R | + | + | + | + | + | + | Kanto |
| 17 | B | S | + | − | + | + | − | + | Kyushu |
| 18 | C | I | + | + | + | + | + | − | Kanto |
| 19 | D | R | + | + | + | + | + | − | Kinki |
| 20 | E | I | + | − | + | + | − | + | Kanto |
| 21 | F | R | + | + | + | + | − | + | Kanto |
| 22 | G | S | + | − | + | + | − | − | Kanto |
| 23 | H | S | + | − | + | + | − | − | Chugoku |
| 24 | I | S | + | − | − | − | − | − | Kanto |
| 25 | J | R | + | + | + | + | − | − | Kanto |
| 23F | | | | | | | | | |
| 1 | b2 | R | + | + | + | + | − | + | Kinki |
| 2 | b3 | I | + | + | + | + | + | − | Kanto |
| 3 | b2 | I | + | + | + | + | + | − | Kanto |
| 4 | b2 | I | + | + | + | + | − | + | Kanto |
| 5 | b3 | I | + | + | + | + | − | + | Chuguku |
| 6 | b3 | I | + | + | + | + | + | − | Kanto |
| 7 | c | I | + | − | + | + | − | − | Kyushu |
| 8 | d | R | + | + | + | + | − | + | Kanto |
| 9 | e | R | + | + | + | + | + | + | Kanto |
| 10 | f | I | + | + | + | + | − | + | Kanto |
| 11 | g | S | + | − | + | − | − | + | Kanto |
| 12 | g | R | + | + | + | + | − | + | Kyushu |
| 13 | h | R | + | + | + | + | + | − | Kanto |
| 14 | i | I | + | + | + | + | + | + | Kinki |
| 15 | j | R | + | + | + | + | − | + | Kanto |

S, penicillin-susceptible. I, penicillin intermediate resistant. R, penicillin-resistant.
Penicillin binding protein genes *pbp1a*, *pbp2x*, and *pbp2a*: +, altered; −, not altered.
Macrolide resistant genes *mef*(A) and *erm*(B): +, positive; −, negative.

Although the efficacy of PS23 against pneumonia without bacteraemia is controversial [20], a combination of influenza and pneumococcal vaccination appears to be beneficial for the reduction of mortality from all causes in individuals aged ⩾65 years [21].

In the group of serotype 23F pneumococci, six out of 15 strains showed the predominant PFGE pattern b which was different from the pattern of the Spanish multidrug-resistant 23F clone but similar to the Taiwan multidrug-resistant serotype 23F clone.

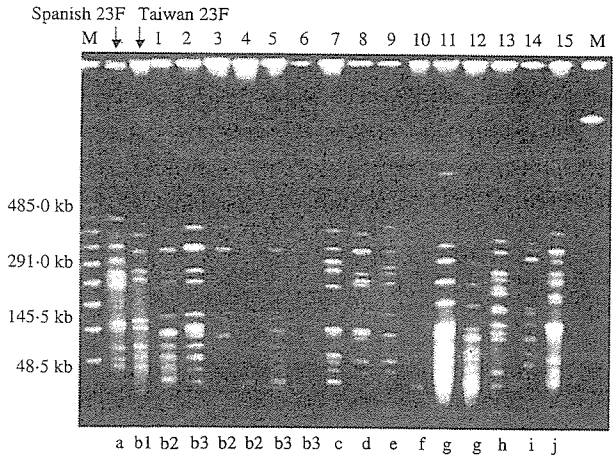


**Fig. 2.** Pulsed-field gel electrophoresis (PFGE) patterns of *Sma*I-digested DNA from 15 strains of serotype 23F *S. pneumoniae* and representatives of the Spanish and Taiwan multiresistant serotype 23F clone. M, Molecular size marker.

Although a previous study indicated that the Spanish multidrug-resistant 23F clone was spreading in Asia [13], it was not detected in this survey. On the other hand, 14 out of 25 strains of serotype 19F showed the predominant pattern A which was closely related to the pattern for the Taiwan multidrug-resistant 19F clone. Since these predominant serotypes were collected from different regions in Japan and most were penicillin non-susceptible with multiple alterations in *pbp* and all isolates had macrolide resistant genes, these results suggest that derivatives of the Taiwan multidrug-resistant 19F and 23F clones have the potential to spread further in Japan. Recently, it was reported by Kasahara *et al.* [6] that these clones had already spread in Japan. However, theirs was a pilot study and they recognized the need for an urgent nationwide surveillance for these strains. Our nationwide study focusing on CAP-associated pneumococci has yielded results consistent with their earlier findings.

Moreover, it has been suggested in a recent report that pneumococcal isolates containing both the *mef*(A) and *erm*(B) genes may have originated from the Taiwan multidrug-resistant serotype 19F clone containing the *mef*(A) gene after introduction of the *erm*(B) gene [22]. However, one serotype 19F strain different from the Taiwan serotype 19F clone and two serotype 23F strains containing both the *mef*(A) and *erm*(B) genes were identified in our study suggesting that other types of antibiotic-resistant pneumococci might appear in Japan.

In conclusion, our results indicate that multidrug-resistant pneumococci are spreading rapidly in Japan and that efforts to prevent spread of pandemic multidrug-resistant serotype 19F and 23F clones should be intensified.

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## DECLARATION OF INTEREST

None.

## REFERENCES


1. **Appelbaum PC.** Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. *Clinical Infectious Diseases* 1992; **15**: 77–83.
2. **Linares J, *et al.*** Trends in antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitge Hospital, Barcelona, Spain (1979–1990). *Clinical Infectious Diseases* 1992; **15**: 99–105.
3. **Ishida T, *et al.*** Etiology of community-acquired pneumonia in hospitalized patients: a 3-year prospective study in Japan. *Chest* 1998; **114**: 1588–1593.
4. **Rikitomi N, *et al.*** Rapid increase of pneumococcal resistance to β-lactam and other antibiotics in isolates from the respiratory tract (Nagasaki, Japan: 1975–1994). *Microbiology and Immunology* 1996; **40**: 899–905.
5. **Watanabe H, *et al.*** A comparative clinical study of pneumonia by penicillin-resistant and -sensitive *Streptococcus pneumoniae* in a community hospital. *Respirology* 2000; **5**: 59–64.
6. **Kasahara K, *et al.*** Clonal dissemination of macrolide-resistant and penicillin-susceptible serotype 3 and penicillin-resistant Taiwan 19F-14 and 23F-15 *Streptococcus pneumoniae* isolates in Japan: a pilot surveillance study. *Journal of Clinical Microbiology* 2005; **43**: 1640–1645.
7. **NCCLS.** Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7–A4. National Committee for Clinical Laboratory Standards, Wayne, PA, 1998.
8. **Ubukata K, Iwata S, Sunakawa K.** *In vitro* activities of new ketolide, telithromycin, and eight other macrolide antibiotics against *Streptococcus pneumoniae* having mefA and ermB genes that mediate macrolide resistance. *Journal of Infection and Chemotherapy* 2003; **9**: 221–226.
9. **McGee L, *et al.*** Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the Pneumococcal Molecular Epidemiology Network. *Journal of Clinical Microbiology* 2001; **39**: 2565–2571.
10. **Yano H, *et al.*** Pulsed-field gel electrophoresis analysis of nasopharyngeal flora in children attending a day care center. *Journal of Clinical Microbiology* 2000; **38**: 625–629.
11. **Tenover FC, *et al.*** Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology* 1995; **33**: 2233–2239.
12. **Watanabe H, *et al.*** Antimicrobial susceptibility and serotype distribution of *Streptococcus pneumoniae* and molecular characterization of multidrug-resistant serotype 19F, 6B, and 23F pneumococci in North Thailand. *Journal of Clinical Microbiology* 2003; **41**: 4178–4183.
13. **Song JH, *et al.*** Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Clinical Infectious Diseases* 1999; **28**: 1206–1211.
14. **Aspa J, *et al.*** Drug-resistant pneumococcal pneumonia: clinical relevance and related factors. *Clinical Infectious Diseases* 2004; **38**: 787–798.
15. **Schrag SJ, *et al.*** Emergence of *Streptococcus pneumoniae* with very-high-level resistance to penicillin. *Antimicrobial Agents and Chemotherapy* 2004; **48**: 3016–3023.
16. **Bogaert D, *et al.*** Pneumococcal vaccines: an update on current strategies. *Vaccine* 2004; **22**: 2209–2220.
17. **U.S. Centers for Disease Control.** Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *Morbidity and Mortality Weekly Report* 1997; **46 (RR-8)**: 1–24.
18. **Mantese OC, *et al.*** Prevalence of serotypes and antimicrobial resistance of invasive strains of *Streptococcus pneumoniae*. *Journal of Pediatrics* 2003; **79**: 537–542.
19. **Martens P, *et al.*** Serotype-specific mortality from invasive *Streptococcus pneumoniae* disease revisited. *BMC Infectious Diseases* 2004; **4**: 21.
20. **Mangtani P, Cutts F, Hall AJ.** Efficacy of polysaccharide pneumococcal vaccine in adults in more developed countries: the state of the evidence. *Lancet Infectious Diseases* 2003; **3**: 71–78.
21. **Christenson B, *et al.*** Effects of a large-scale intervention with influenza and 23-valent pneumococcal vaccines in adults aged 65 years or older: a prospective study. *Lancet* 2001; **357**: 1008–1011.
22. **Ko KS, Song JH.** Evolution of erythromycin-resistant *Streptococcus pneumoniae* from Asian countries that contains *erm*(B) and *mef*(A) genes. *Journal of Infectious Diseases* 2004; **190**: 739–747.

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ORIGINAL ARTICLE

# Drug-resistant genes and serotypes of pneumococcal strains of community-acquired pneumonia among adults in Japan

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**Drug-resistant genes and serotypes of pneumococcal strains of community-acquired pneumonia among adults in Japan**
OISHI K, YOSHIMINE H, WATANABE H, WATANABE K, TANIMURA S, KAWAKAMI K, IWAGAKI A, NAGAI H, GOTO H, KUDOH S, KURIYAMA T, FUKUCHI Y, MATSUSHIMA T, SHIMADA K, MATSUMOTO K, NAGATAKE T. *Respirology* 2006; **11**: 429–436

**Background:** A high frequency of drug-resistant pneumococci has been reported in Asian countries. Few data on the drug-resistance or serotype of pneumococcal strains responsible for community-acquired pneumonia (CAP), however, are available for the past two decades in Japan.

**Methodology:** Susceptibility to antibiotics and the genotype of antibiotic-resistant genes and serotypes of *Streptococcus pneumoniae* isolates from 114 adult patients with CAP were examined in a nationwide study in Japan between 2001 and 2003.

**Results:** Most of the cases were non-bacteraemic pneumonia and the case fatality rate was 4.4%. The most frequent genotype of the *pbp* gene was *pbp1a+2x+2b* (gPRSP; 36.8%) followed by *pbp 2x* (28.1%) and of the macrolide-resistant gene, it was *ermB* (50.0%). The most common serotype was 19F (29.1%), followed by serotype 23F (13.2%), 6B (12.3%) and 3 (11.4%). The coverage of serotypes of isolates by a 23-valent pneumococcal polysaccharide vaccine (PPV) would be 82.5% in these patients with CAP. Most of strains with serotypes 19F and 23F were gPRSP. A cluster of serotype 3 strains associated with the *pbp 2x* and *ermB* gene was also noted.

**Conclusion:** A high frequency of altered *pbp* gene mutations or of macrolide-related genes and a high serotype coverage by the 23-valent PPV found in our study of pneumococcal pneumonia facilitates attempts to increase the coverage rate of the 23-valent PPV in adults older than 65 years in Japan.

**Key words:** community-acquired pneumonia, drug-resistance, pneumococcal polysaccharide vaccine, serotype, *Streptococcus pneumoniae*.

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## INTRODUCTION

Pneumonia is currently the fourth leading cause of death in Japan and 94 900 persons (75 per 100 000) died of this disease in 2003.[1] *Streptococcus pneumoniae* (*S. pneumoniae*) is the most commonly identified cause of community-acquired pneumonia (CAP),

accounting for 16.5–38.9% of the CAP cases among adult patients in Japan.[2–5] An increasing prevalence of pneumococcal strains that are resistant to β-lactam antibiotics has been observed in both developing and developed countries during the last decade.[6] Recent studies have also shown high frequencies of penicillin- and macrolide-resistant pneumococci as a respiratory pathogen in several Asian countries, including Japan.[7–10] The resistance of *S. pneumoniae* to penicillin and other β-lactams has been shown to be associated with mosaic mutations in the *pbp1a, pbp2b* and *pbp2x* genes.[11,12] On the other hand, macrolide resistance in pneumococci is generally mediated by one of two possible mechanisms: (i) methylation of the 23S rRNA methylase encoded by the *ermB* gene; and (ii) macrolide efflux via the *mef A* gene.[13,14]

The increased rate of appearance of drug-resistant pneumococci in recent years emphasizes the need for preventing pneumococcal infections by vaccination with pneumococcal polysaccharide vaccine (PPV).[15] Although the effects of 23-valent PPV have been established for invasive pneumococcal diseases, such as bacteremia and meningitis, its effect on reducing the risk of pneumonia in adults remains a controversial issue.[16–19] Starting in 2001, the vaccination law in Japan permits the subsidization of the influenza vaccination of persons older than 65 years. Because of this, an additional preventive effect of the influenza vaccine and 23-valent PPV on reducing hospital mortality for pneumonia would be expected for persons older than 65 years.[20] The rate of vaccine coverage appears to be much lower in Japan (about 2%) than in the USA, where the proportion of subjects reporting ever having had the 23-valent PPV is 64.2% in adults older than 65 years.[21]

Few data on the drug-resistance or serotype distribution of pneumococcal strains responsible for CAP, however, are currently available in Japan.[4,7,8] This study was undertaken to examine the distribution of *pbp* genes or macrolide-resistant genes and serotypes that are responsible for CAP among adults in Japan.

## METHODS

### Patients

One hundred and fourteen patients with CAP and older than 16 years were enrolled at 20 hospitals nationwide in Japan between 2001 and 2003. Patients were enrolled only if all of the following findings were present: clinical symptoms and signs of pneumonia, the appearance of a new pulmonary infiltrative shadow on a CXR and isolation of *S. pneumoniae* from blood and/or lower respiratory tract specimens with compatible Gram stain findings.[22] After enrolment, the demographic data including underlying diseases, the severity of disease, the length of hospital stay and the outcome were evaluated. The severity of the disease was classified into the following three grades: mild, moderate and severe according to the Japanese Respiratory Society guidelines, based on a physical examination, CXR, white blood cell count, serum CRP value and arterial $PaO_2$.[5,23] Of the 114 patients, 89 (78.1%) were hospitalized. The other 25 patients (21.9%) were followed at outpatient clinics. All studies described here were approved by the Institutional Review Boards of our institutions and signed consent form was obtained from each subject.

### Bacterial strains and polymerase chain reaction (PCR) of drug-resistant genes

Each strain of *S. pneumoniae* was isolated from 114 patients with CAP. These strains were obtained from sputum ($n = 109$), BAL fluid ($n = 1$), blood ($n = 3$) and pleural effusion ($n = 1$). *S. pneumoniae* was identified by optochin sensitivity and bile solubility tests and confirmed by PCR for the autolysin (*lyt A*) genes.[11] Of these 114 strains, three penicillin-binding protein (PBP) genes (*pbp1a, pbp2x, pbp2b*) only in susceptible strains[11] or macrolide-resistant genes (*mefA* and *ermB*)[24] were amplified by means of PCR (Wakunaga Pharmaceutical Co., Hiroshima, Japan) according to the manufacturer's instructions. Amplification of DNA fragments were carried out with the oligonucleotide primer pairs: 5′-AAACAAGGTCGGACTCAACC-3′ and 5′-AGGTGCTACAAATTGAGAGG for *pbp1a*, 5′-CCAGGTTCCACTATGAAAGTG-3′ and 5′-CATCCGT CAAACCGAAACGG-3′ for *pbp2x* and 5′-CAATCTA GAGTCTGCTATGGA-3′ and 5′-GGTCAATTCCTGTCG CAGTA-3′ for *pbp2b* and 5′-CGTACCTTGGATATTC ACC and 5′-GTAAACAGTTGACGATATTG *ermB* for 5′-CTGTATGGAGCTACCTGTGG-3′ and CCCAGCTTAG GTATACGTAC-3′ for *mefA* as previously described.[11,24] The amplified regions of each PBP gene were positioned in highly divergent sequences of penicillin-resistant *S. pneumoniae* (PRSP).[11]

### Antimicrobial susceptibility test

Minimum inhibitory concentrations (MICs) were determined by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards.[25] The susceptibilities of the 114 *S. pneumoniae* isolates to the following three β-lactam antibiotics, penicillin G (Meiji Seika Kaisha, Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Tokyo, Japan) and imipenem (Banyu Pharmaceutical Co., Tokyo, Japan) and clarithromycin (Taisho Pharmaceutical, Tokyo, Japan) and azithromycin (Pfizer Pharmaceutical) were tested. Standard quality control strains (gifts from Dr K Ubukata, Kitasato Institute for Life Science and Graduate School of Infection Control Sciences, Kitasato University) including the R6 strain (penicillin- and macrolide-sensitive), KM-99 and H69 strains (genotype *pbp 2x* and *ermB*), KK-133 strain (genotype *pbp1a + 2x + 2b* and macrolide-sensitive), KM-90 strain (genotype *pbp1a + 2x + 2b* and *mefA*) and H-29 and SHA-3 strains (genotype *pbp1a + 2x + 2b* and *ermB*) and T-197 strain (genotype *pbp1a + 2x + 2b*, mefA and *ermB*) were also examined in this study.

### Serotyping

Pneumococci were serotyped on the basis of the quellung reaction observed microscopically after suspension in pneumococcal diagnostic antisera (Statens Serum Institut, Copenhagen, Denmark).

### Statistical analysis

Statistical analyses were performed by the Krusukal-Wallis test or Mann–Whitney's *U*-test. Data were considered to be statistically significant, if the *P*-value was less than 0.05. All data are expressed as the mean ± SD.

## RESULTS

The mean age (±SD) of the total 114 patients was 67.4 ± 15.8 years (range, 20–99 years) (Fig. 1). One small peak at 30 years and another large peak at 70 years were found in the age distribution. There were more male patients (59.6%) than female patients, except for the 80- and 90-year-old patients. A decreased number of patients between 80 and 90 is probably due to a decreased population in this age group in Japan.[1] Of these 114 patients, 82 (71.9%) had underlying conditions, such a chronic lung disease (39.5%), diabetes mellitus (12.3%), cerebrovascular accident (8.8%), hypertensive heart disease (7.9%), chronic liver disease (4.5%) or malignancy (4.5%). The severity of the disease for these patients was classified into three groups; mild (33.3%), moderate (42.1%) and severe (24.6%). The mean period (±SD) of hospital stay was 15.9 ± 11.5 days for patients with mild disease ($n = 20$), 24.1 ± 22.3 days for those with moderate disease ($n = 38$) and 34.3 ± 31.8 days for those with severe disease ($n = 24$). No significant difference in the length of hospital stay was found among these three groups. Of the 114 patients, 109 (95.6%) showed clinical improvement and five (4.4%) died within 30 days after the diagnosis of pneumonia. All of the fatal cases were associated with severe disease.

The MIC distributions for penicillin G, ceftriaxone and imipenem against the 114 *S. pneumoniae* isolates (one strain per patient) based on the PCR results are shown in Figure 2. The *in vitro* activity of penicillin G against these strains indicated that 26 patients (22.8%) were infected with penicillin-resistant strains (the MIC value was 2 μg/mL in 25 strains and 4 μg/mL in one strain of penicillin G) (Fig. 2A). Penicillin-resistant strains (MIC > 0.12 μg/mL for penicillin G) were isolated from 66 patients (57.9%). The pneumococcal isolates were classified into six groups according to the genotype of the *pbp* gene (Table 1). Only 13 strains (11.4%) were associated with no *pbp* gene mutation, which closely corresponded to penicillin-sensitive *S. pneumoniae* on the basis of the MIC distribution for penicillin G. The frequency of strains with genotype *pbp 1a+2x+2b*, closely corresponding to PRSP was the highest (36.8%). This genotype was named gPRSP in the present study.[26] Most strains (51.8%) possessed one or two *pbp* gene mutations with the highest frequency for the *pbp 2x* gene (28.1%). An alteration in the *pbp 2x* gene had no effect on the MIC value for penicillin G and imipenem (Fig. 2A,C). In contrast, the MICs for ceftriaxone were affected by alteration of the *pbp 2x* gene, consistent with a previous report (Fig. 2B).[26] As a result, a small cluster of antibiotic-susceptible strains, comprised of strains without a *pbp* gene mutation, was found for ceftriaxone.

Erythromycin-resistant strains (MIC ≥ 1 μg/mL) were isolated from 86 patients (75.4%). The pneumococcal strains were also classified into four groups according to the genotype of the macrolide-resistant genes (Table 1). The genotype of the *ermB* strains were highly resistant to erythromycin and the frequency (50%) was much higher than that for *mefA* (22.6%) in patients with CAP. Macrolide resistance in pneumococcal strains for CAP was mainly due to the *ermB* gene in Japan. Only seven strains (6.1%) possessed both gene mutations. While most strains with genotype *ermB* showed a high resistance to erythro-

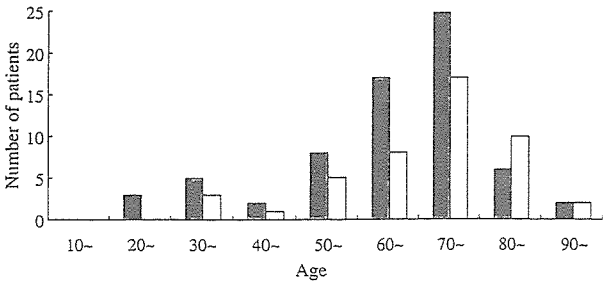


**Figure 1** Distribution of gender and age of the 114 adult patients enrolled in this study with community-acquired pneumococcal pneumonia in Japan. Closed column is male and open column is female, respectively.

**Table 1** Genotype of drug-resistant genes and MICs distribution for penicillin G or erythromycin in 114 clinical isolates of *Streptococcus pneumoniae*

| | | MIC of penicillin G (μg/mL) | | |
|---|---|---|---|---|
| Genotype | *n* (%) | Range | 50 | 90 |
| No mutation | 13 (11.4) | 0.01–0.13 | 0.03 | 0.13 |
| *pbp 2x* | 32 (28.1) | 0.03–0.13 | 0.03 | 0.06 |
| *pbp 2b* | 2 (1.8) | 0.06–0.13 | 0.06 | 0.13 |
| *pbp 2x+2b* | 15 (13.2) | 0.03–1.0 | 0.25 | 1 |
| *pbp 1a+2x* | 10 (8.8) | 0.06–0.25 | 0.13 | 0.25 |
| *pbp 1a+2x+2b* | 42 (36.8) | 0.25–4.0 | 2 | 2 |
| | | MIC of erythromycin (μg/mL) | | |
| Genotype | *n* (%) | Range | 50 | 90 |
| No mutation | 24 (21.1) | 0.03–0.13 | 0.06 | 0.13 |
| *mefA* | 26 (22.8) | 0.25–4.0 | 1 | 2 |
| *ermB* | 57 (50.0) | 0.5–128 | 128 | 128 |
| *mefA+ermB* | 7 (6.1) | 8.0–128 | 128 | 128 |

MIC, minimum inhibitory concentration.

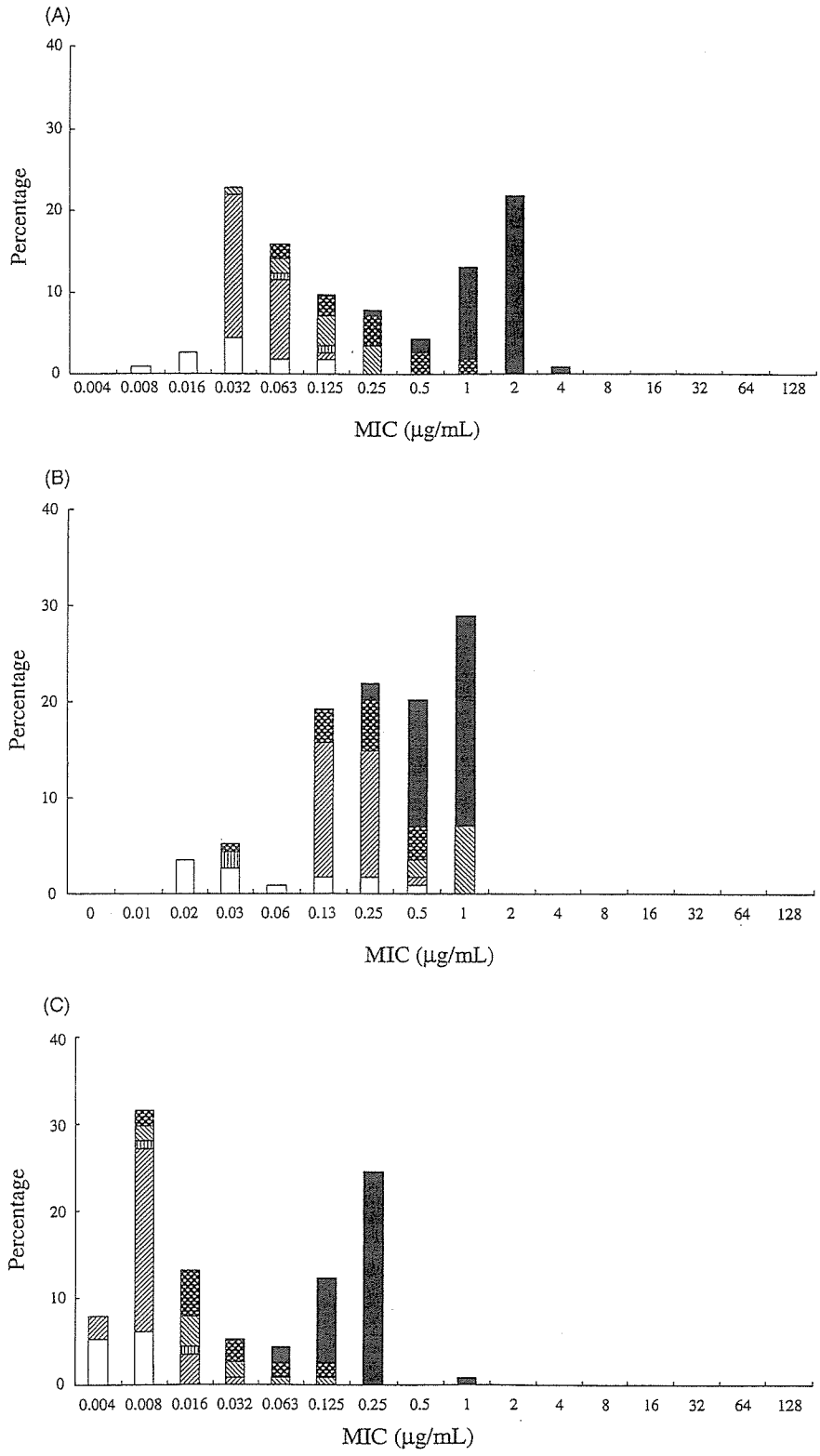


**Figure 2** Relationship between MICs values for three β-lactam antibiotics: (A) penicillin G, (B) ceftriaxone and (C) imipenem; and the genotype of the *pbp* genes in 114 clinical isolates of *S. pneumoniae* from adults patients with community-acquired pneumonia. (□) No mutation; (▨) *pbp 2x*; (▥) *pbp 2b*; (▧) *pbp 1a+2x*; (▦) *pbp 2x+2b*; (■) *pbp 1a+2x+2b*.

mycin, in the case of the genotype *mefA* the resistance was low. The MIC range (μg/mL) and the $MIC_{50}$ and $MIC_{90}$ (μg/mL) against 114 isolates of *S. pneumoniae* were 0.02–128 and 16 and 128 for clarithromycin and 0.032–128 and 128 and 128 for azithromycin, respectively.

Relationships between the genotype patterns of the *pbp* gene (Table 2) or the macrolide-resistant gene (Table 3) among the 114 isolates are shown. The most frequent serotype was 19F (29.1%), followed by serotypes 23F (13.2%), 6B (12.3%) and 3 (11.4%) (Tables 2,3). Of the 114 strains, 94 (82.5%) possessed

**Table 2** Genotype of the pbp genes and serotype distribution in 114 clinical isolates of *Streptococcus pneumoniae*

| | No. of isolates with serotype (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | 19F | 23F | 6B | 3 | 14 | 6A | 11A | 19A | 9V | 16F | 15C | Other | Total |
| No mutation | 1 | | 1 | 1 | | | | 1 | 1 | 1 | | 7 | 13 |
| *pbp 2x* | 2 | 1 | 4 | 11 | 2 | 3 | 2 | 2 | 3 | | 1 | 1 | 32 |
| *pbp 2b* | | | 1 | | | | | | | | | 1 | 2 |
| *pbp 2x+2b* | 4 | 1 | 3 | 1 | 3 | | | 1 | | | | 2 | 15 |
| *pbp 1a+2x* | | | 1 | | 4 | | 2 | 1 | | 1 | 1 | | 10 |
| *pbp 1a+2x+2b* | 18 | 13 | 4 | | | 4 | 1 | | 1 | 1 | | | 42 |
| Total | 25 (29.1) | 15 (13.2) | 14 (12.3) | 13 (11.4) | 9 (7.9) | 7 (6.1) | 5 (4.4) | 5 (4.4) | 5 (4.4) | 3 (2.6) | 2 (1.8) | 11 (9.6) | 114 |

**Table 3** Genotype of the macrolide-resistant genes and serotype distribution in 114 clinical isolates of *Streptococcus pneumoniae*

| | No. of isolates with serotype (%) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | 19F | 23F | 6B | 3 | 14 | 6A | 11A | 19A | 9V | 16F | 15C | Other | Total |
| No mutation | 4 | 1 | 2 | 1 | | 1 | 4 | 1 | 3 | 1 | | 6 | 24 |
| *mefA* | 16 | 4 | 3 | | | | | | 1 | 2 | | | 26 |
| *ermB* | 3 | 8 | 9 | 12 | 9 | 5 | 1 | 2 | 1 | | 2 | 5 | 57 |
| *mefA+ermB* | 2 | 2 | | | | 1 | | 2 | | | | | 7 |
| Total | 25 (29.1) | 15 (13.2) | 14 (12.3) | 13 (11.4) | 9 (7.9) | 7 (6.1) | 5 (4.4) | 5 (4.4) | 5 (4.4) | 3 (2.6) | 2 (1.8) | 11 (9.6) | 114 |

serotypes that are involved in the 23-valent PPV. Non-covered serotypes in these strains were 6A (seven strains), 16F (three strains), 15C (two strains) and others (each one strain). The involvement of subtypes of the vaccine serotype as related serotypes led to a high vaccine coverage (94.7%). Most of strains with serotypes 19F and 23F were gPRSP (72% for serotype19F and 86.7% for serotype 23F). Interestingly, most of the type 3 strains were associated with the *pbp* 2x genotype (11/13) and the *ermB* genotype (12/13), respectively. Furthermore, all of nine serotype 14 strains contained the *ermB* mutation.

## DISCUSSION

This is the first report demonstrating the clinical features and the frequency of an altered *pbp* gene or macrolide-resistant genes and serotype of pneumococcal strains responsible for CAP among adults in Japan. Although the number of patients examined was limited, the patients were enrolled at 20 hospitals which were widely distributed in Japan. The clinical and microbiological data shown in this study therefore might represent a trend for this disease in Japan. The rate of bacteraemic pneumonia in this study was lower than that reported in previous studies in Japan (4.7–9.3%) or in a study in the Asian Network (30.9%).[2,27,28] The case fatality in our study was similar to that reported in previous studies of pneumococcal pneumonia in Japan (0–3.4%),[2–4,8] but much lower than those reported in Asia and Europe (13.3–14.3%).[28,29] A low rate of mortality might be attributable to the low rate of bacteraemic pneumococcal pneumonia in this study. These rates are comparable to those reported for other Asian countries in recent years.[9,10] The grade of resistance was milder for third-generation cephems and imipenem than for penicillin G. A new breakpoint for ceftriaxone also defined all of the 114 pneumococcal strains, including gPRSP strains, as being susceptible.[30] Because the reduced affinity of penicillin to PBPs is much prominent by virtue of several mutations of *pbp* gene than that of third-generation cephem or imipenem, the resistance is more pronounced for penicillin than for third-generation cephem or imipenem.[12] On the other hand, the frequent use of macrolide antibiotics has resulted in an increasing incidence of macrolide resistance among pneumococcal isolates. The prevalence *mefA* gene and *ermB* gene mediated-mechanisms of macrolide resistance varies in different geographical areas. While strains with *ermB* genes are predominant in Europe and the Far East, a strain with the *mefE* gene is predominant in North America.[10,31–33] In this study, more than half of the pneumococcal strains possessed the *ermB* gene, which induced a high level of macrolide resistance. This rate was higher than that (39.1%) reported in an earlier study conducted between 1995 and 1997 in Japan.[24]

A nationwide study of the distribution of serotype of isolates from 590 patients with pneumococcal diseases was carried out between 1980 and 1984 and the rank order of isolates was reported to be serotype 3 (12.7%), 19F (9.3%), 23F (6.8%) and 6B (5.9%).[34] The coverage of these isolates by 23-valent PPV was 72.9% in this study. In the present study, the relative frequency of serotypes was higher by three times for serotype 19 and two times higher for serotype 23F and 6B and the vaccine coverage by 23-valent capsular polysaccharide was also increased, compared with a previous study in Japan.[34] The distribution of serotype in the present study was similar to the recent studies in Asian countries.[7–9] An association of serotype 3 strains with genotype *pbp2x* and genotype *ermB* was also found in the present study. A recent study by Japanese investigators suggested a clonal spread of serotype 3 strains which are susceptible to penicillin and positive for the *ermB* gene in a pilot study performed in a single institution in Japan.[35] Because an alteration in the *pbp 2x* gene did not affect the MIC for penicillin, strains with the *pbp 2x* gene were penicillin susceptible. Although the authors did not examine the *pbp gene* mutation in pneumococcal strains, their data are consistent with our results of a cluster of serotype 3 strains with genotype *pbp 2x* and genotype *ermB*. Collectively, our data are consistent with a clonal expansion of serotype 3 strains associated with altered *pbp 2x* gene and with *ermB* gene among adults across Japan.

A nationwide, prospective study in Japan demonstrated that the rates of β-lactam or macrolide-resistant pneumococcal strains responsible for CAP remain high. The coverage of serotypes of isolates by a 23-valent PPV would be 82.5% in these patients with CAP. The data reported on pneumococcal pneumonia should facilitate attempts to increase coverage by the 23-valent PPV in Japanese adults older than 65 years.

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patients); Toshima H, Minoh Municipal Hospital (six patients); Matsushima T, Department of Respiratory Medicine, Kawasaki Medical School (three patients); Satoh S, Kyorin Hospital (seven patients), Masaki H, Tagami Hospital (two patients); Kawakami K, Nagasaki Medical Center of Neurology (nine patients); and Takasugi M, Takasugi Clinic (two patients).

## REFERENCES


1 Health and Welfare Statistics Association. Annual report of national health and hygiene in Japan. *J. Health. Welfare. Stat.* 2003; **51**: 1–488.
2 Ishida T, Hashimoto T, Arita M *et al.* Etiology of community-acquired pneumonia in hospitalized patients. *Chest* 1998; **114**: 1588–93.
3 Ishida T, Hashimoto T, Arita M *et al.* A 3-year prospective study of a urinary antigen-detection kit for *Streptococcus pneumoniae* in the community-acquired pneumonia: utility and clinical impact on the reported etiology. *J. Infect. Chemother.* 2004; **10**: 359–63.
4 Nishiyama O, Taniguchi H, Kondoh Y *et al.* The utility of the pneumonia PORT prediction rule for evaluating the severity of community-acquired pneumonia in Japan. The relationship between disease severity and drug-resistant *Streptococcus pneumoniae. Jpn. J. Infect. Dis.* 2002; **76**: 1003–9.
5 Motomura K, Masaki H, Terada M *et al.* Usefulness of the Japanese Respiratory Society guidelines for community-acquired pneumonia: a retrospective analysis of community-acquired pneumonia. *Respirology* 2005; **10**: 208–14.
6 Baquero F. Pneumococcal resistance to β-lactam antibiotics: a global geographic overview. *Microb. Drug Resist.* 1995; **1**: 115–20.
7 Rikitomi N, Papa SS, Watanabe K *et al.* Rapid increase of pneumococcal resistance to β-lactam and other antibiotics in isolates from the respiratory tract (Nagasaki, Japan: 1975–1994). *Microbiol. Immunol.* 1996; **40**: 899–905.
8 Watanabe H, Sato S, Kawakami K *et al.* A comparative clinical study of pneumonia by penicillin-resistant and -sensitive *Streptococcus pneumoniae* in a community hospital. *Respirology* 2000; **5**: 59–64.
9 Song JH, Lee NY, Ichiyama S *et al.* Spread of drug-resistant *Streptococcus pneumoniae* in Asian countries: Asian Network for Surveillance of Resistant Pathogens (ANSORP) study. *Clin. Infect. Dis.* 1999; **28**: 1206–11.
10 Inoue M, Lee NY, Hong SW *et al.* PROTEKT 1999–2000: a multicenter study of the antibiotic susceptibility of respiratory tract pathogens in Hong Kong, Japan and South Korea. *Int. J. Antimicrob. Agents* 2004; **23**: 44–51.
11 Ubukata K, Muraki T, Igarashi A *et al.* Identification of penicillin and other beta-lactam resistance in *Streptococcus pneumoniae* by polymerase chain reaction. *J. Infect. Chemother.* 1997; **3**: 190–7.
12 Hakenbeck R, Grebe T, Zahner D *et al.* β-Lactam resistance in *Streptococcus pneumoniae*: penicillin-binding proteins and non-penicillin-binding proteins. *Mol. Microbiol.* 1999; **33**: 673–8.
13 Tait-Kamradt A, Clancy J, Cronan M *et al.* mefE *is necessary for the erythromycin-resistant M phenotype in* Streptococcus pneumoniae. *Antimicrob. Agents. Chemother.* 1997; **41**: 2251–5.
14 Roberts. MC, Sutcliffe J, Courvalin P *et al.* Nomenclature for macrolide and macrolide-lincosamide-streptogamin B resistance determinants. *Antimicrob. Agents. Chemother.* 1999; **43**: 2823–30.
15 CDC. Prevention of pneumococcal disease. Recommendation of the advisory committee on immunization practice (ACIP). *Morbid. Mortal. Wkly. Rep.* 1997; **46**: 1–24.
16 Butler JC, Shapiro ED, Carlone GM. Pneumococcal vaccines: history, current status and future directions. *Am. J. Med.* 1999; **107**: 69S–76S.
17 Ortqvist A, Hedlund J, Burman LA *et al.* Randomised trail of 23-valent pneumococcal capsular polysaccharide vaccine in prevention of pneumonia in middle-aged and elderly people. *Lancet* 1998; **351**: 399–403.
18 Shapiro ED, Berg AT, Austrian R *et al.* The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *N. Engl. J. Med.* 1991; **325**: 1453–60.
19 Jackson LA, Neuzil KM, Yu O *et al.* Effectiveness of pneumococcal polysaccharide vaccine in older adults. *N. Engl. J. Med.* 2003; **348**: 1747–55.
20 Christenson B, Hedlund J, Lundberg P, Ortqvist A. Additive preventive effect of influenza and pneumococcal vaccines in elderly persons. *Eur. Respir. J.* 2004; **23**: 363–8.
21 CDC. Influenza and pneumococcal vaccination coverage among persons aged >65 years and persons aged 18–64 years with diabetes or asthma-United States. *M.M.W.R.* 2003; **53**: 1007–12.
22 Yoshimine H, Oishi K, Mubiru FH *et al.* Community-acquired pneumonia in Ugandan adults: short-term parenteral ampicillin therapy for bacterial pneumonia. *Am. J. Trop. Med. Hyg.* 2001; **64**: 172–7.
23 The Japanese Respiratory Society. *The Japanese Respiratory Society Guidelines for the Management of Community-Acquired Pneumonia in Adults.* The Japanese Respiratory Society, Tokyo, 2000; 20–1.
24 Ubukata K, Iwata S, Sunakawa K. *In vitro* activities of new ketolide, telithromycin and eight other macrolide antibiotics against *Streptococcus pneumoniae* having *mefA* and *ermB* genes that mediate macrolide resistance. *J. Infect. Chemother.* 2003; **9**: 221–6.
25 National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing. Eleventh Information Supplement.* National Committee for Clinical Laboratory Standards, Wayne, PA, 2001.
26 Ubukata K, Chiba N, Hasegawa K *et al.* Antibiotic susceptibility in relation to penicillin-binding protein genes and serotype distribution of *Streptococcus pneumoniae* strains responsible for meningitis in Japan, 1999–2002. *Antimicrob. Agents. Chemother.* 2004; **48**: 1488–94.
27 Watari M, Ohe M, Kunimoto E *et al.* Community-acquired pneumococcal pneumonia: a comparative study of bacteraemic and nonbacteraemic patients. *J. Jpn. Respir. Soc.* 2000; **38**: 253–8.
28 Song J-H, Jung S-I, Ki HK *et al.* Clinical outcomes of pneumococcal pneumonia caused by antibiotic-resistant strains in Asian countries: a study by the Asian Network for Surveillance of resistant pathogens. *Clin. Infect. Dis.* 2004; **38**: 1570–8.

29 Aspa J, Rajas O, Rodriguez de Castro F *et al.* Drug-resistant pneumococcal pneumonia: clinical relevance and related factors. *Clin. Infect. Dis.* 2004; **38**: 787–98.
30 Mandell LA, Bartlett JG, Dowell SF *et al.* Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. *Clin. Infect. Dis.* 2003; **37**: 1405–33.
31 Perez-Trallero E, Garcia-de-la-Fuente C, Garcia RC *et al.* Geographical and ecological analysis of resistance, coresistance and coupled resistance to antimicrobials in respiratory pathogenic bacteria in Spain. *Antimicrob. Agents. Chemother.* 2005; **49**: 1965–72.
32 Kresken M, Henrichfreise B, Bagel S *et al.* High prevalence of the *ermB* gene among erythromycin-resistant *Streptococcus pneumoniae* isolates in Germany during the winter of 2000–2001 and *in vitro* activity of telithromycin. *Antimicrob. Agents Chemother.* 2004; **48**: 3193–5.
33 Shortridge VD, Doern GV, Brueggemann AB *et al.* Prevalence of macrolide resistance mechanisms in *Streptococcus pneumoniae* isolates from multicenter antibiotic resistance surveillance study conducted in the United States in 1994–1995. *Clin. Infect. Dis.* 1999; **29**: 1186–8.
34 Fukumi H, Kaneko Y, Agata T *et al.* Studies on clinical application of pneumococcal vaccine: distribution of *Streptococcus pneumoniae* in Japan. *Jpn. J. Infect. Dis.* 1984; **58**: 39–52.
35 Kasahara K, Maeda K, Mikasa K *et al.* Clonal dissemination of macrolide-resistant and penicillin-susceptible serotype 3 and penicillin-resistant Taiwan 19F-14 and 23F-15 *Streptococcus pneumoniae* isolates in Japan: a pilot surveillance study. *J. Clin. Microbiol.* 2005; **43**: 1640–5.

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# Significant Reduction of Nosocomial Pneumonia after Introduction of Disinfection of Upper Airways Using Povidone-Iodine in Geriatric Wards

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**Key Words**
Nosocomial pneumonia · Methicillin-resistant *Staphylococcus aureus* · *Pseudomonas aeruginosa* · Upper airways · Povidone-iodine · Geriatric long-term care ward · Pulsed-field gel electrophoresis · Respiratory infection

**Abstract**
We investigated the efficacy of disinfection of the upper airway using povidone-iodine against nosocomial pneumonia in geriatric wards. Cases of nosocomial pneumonia were retrospectively analyzed between January 1991 and March 1995 in geriatric wards (190 beds). Moreover, the relationship concerning methicillin-resistant *Staphylococcus aureus* (MRSA) isolates between patient and environment was investigated using pulsed-field gel electrophoresis (PFGE) with the *Sma*I restriction enzyme. The incidence of nosocomial pneumonia decreased significantly ($p < 0.05$). Major causative organisms of nosocomial pneumonia were MRSA and *Pseudomonas aeruginosa*, which significantly decreased. PFGE studies showed that the patterns of MRSA isolates show a strong association between patient and environment. Our study indicates that disinfection of the upper airways by povidone-iodine is very important in the prevention of nosocomial pneumonia in geriatric wards.



## Introduction

*Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), is an important nosocomial pathogen. In particular, MRSA is of great concern in both hospitals and nursing homes [1–3]. Previous studies have shown that the phage groups of *S. aureus* in the hospital environment of a nursing home only infrequently matched the phage group that colonized the patients [1]. However, little information, including molecular analysis, is available regarding the relationship between colonization and environmental contamination of *S. aureus* in geriatric long-term-care institutions. In this study, we investigated the possible relationship between *S. aureus* types colonizing the respiratory tract and rectum, and *S. aureus* types isolated from the hospital environment.

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