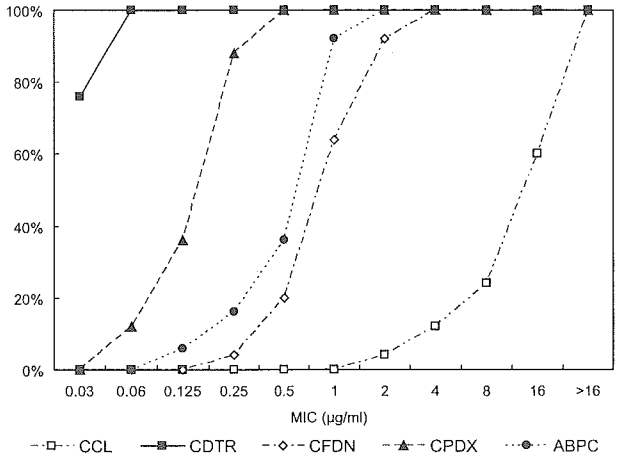

Figure 2. MICs of other β-lactams against *H. influenzae* strains with mutations in the *ftsI* gene ($n=35$).

Age distribution of the strains with mutations in the ftsI gene (BLNAR)

The strains with mutations in the *ftsI* gene (BLNAR) were broadly identified among young children (Figure 3).

Discussion

Nasopharyngeal colonization with causative pathogens is the first step in the development of AOM. Pathogens that colonize the nasopharynx infect the middle ear cavity via the Eustachian tube. *H. influenzae* is one of the major causative pathogens of AOM and is frequently isolated in the nasopharynx. Although the positive predictive value of causative middle ear pathogens remains controversial, a nasopharyngeal swab is less invasive than tympanocentesis and can easily be applied frequently in young children to assess middle ear pathogens. Thus, it is important to characterize pathogens that colonize the nasopharynx of children with AOM [9].

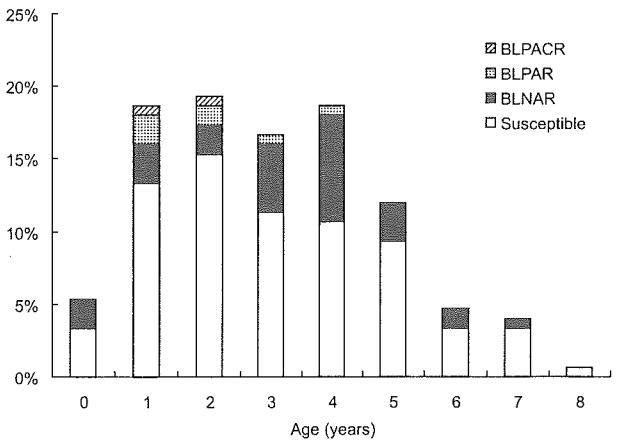

Figure 3. Age distribution of resistant strains of *H. influenzae* ($n=150$).

The incidence of resistant *H. influenzae* strains has gradually increased since the early 1970s [1]. The incidence of BLPAR in the USA is >30% [10], compared to 15–20% in Japan [2,3]. A recent epidemiological study [11] showed a decrease in the incidence of BLPAR strains and a corresponding increase in the incidence of BLNAR strains. BLNAR strains were identified in only <1–2% of isolates until the 1990s. By 1994–95, the prevalence had increased to 2.5–10.1% in the USA. In Japan, BLNAR strains were not identified until 1984. They were identified in 2.1% of cases in 1988 and in 5.0% in 1991. The difference in prevalence of these antimicrobial-resistant strains will depend on the consumption of cephalosporin. The definition of the BLNAR strain differs between the USA and Japan. In Japan, strains with MICs against ABPC of 1–2 μg/ml are interpreted as BLNAR [8]. In contrast, in the USA and Europe, strains with MICs against ABPC of 2–4 μg/ml are defined as BLNAR according to a recommendation from the National Committee for Clinical Laboratory Standards. Although the disk diffusion method is a convenient and inexpensive procedure for estimating antimicrobial susceptibility, it is not appropriate for identifying BLNAR strains. As the MICs of BLNAR strains are close to those of susceptible strains, it is necessary to have a unified definition for clinical isolates based on the mechanism of resistance.

The mechanism of resistance of BLNAR strains is due to mutations in PBP genes which decrease the affinity of antibiotics for PBP [8]. Recently, common substitutions of deduced amino acid residues were identified in the transpeptidase region on the *ftsI* gene encoding PBP-3 in BLNAR strains. The MICs of β-lactams against *H. influenzae* transformans in which the *ftsI* gene from BLNAR strains was introduced were similar to those against BLNAR strains. These findings suggest that mutations in the *ftsI* gene are of greatest important to the development of resistance to β-lactams in BLNAR strains. In this study, 24.6% of *H. influenzae* strains had mutations in the *ftsI* gene. The MICs of ABPC against the strains with mutations in the *ftsI* gene (BLNAR) were 0.5–2 μg/ml. The molecular biological method is useful for identifying BLNAR strains.

The BLNAR strains were broadly identified among children aged <6 years. Our previous study [7] of nasopharyngeal colonization with penicillin-resistant *Streptococcus pneumoniae* (PRSP) showed that PRSP were predominantly isolated from children aged <2 years. Younger children tend to harbor more resistant strains because they are exposed to these pathogens more often through contacts with siblings and attendance at day-care centers [12]. In

addition, they are frequently treated with antibiotics. The high prevalence of BLNAR strains of *H. influenzae* should be taken into account when treating AOM in young children.

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Increase of Macrolide-Resistant *Streptococcus pneumoniae*-Expressing *mefE* or *ermB* Gene in the Nasopharynx among Children with Otitis Media

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Objective: **To evaluate prevalence of macrolide resistant strains and the genotypes of the resistance among *Streptococcus pneumoniae* isolated from the nasopharynx of children with otitis media.** ***Study Design:*** **Retrospective review.** ***Methods:*** **A total of 858 *S. pneumoniae* isolates were collected from the nasopharynx of pediatric patients with acute otitis media at the clinics of Otolaryngology–Head and Neck Surgery, Wakayama Medical University Hospital and six affiliated hospitals in Wakayama prefecture between January 1998 and December 2002. The antibiotic susceptibility patterns were analyzed for penicillin, erythromycin, and clindamycin according to the National Committee for Clinical Laboratory Standards. Macrolide resistance genes of *mefE* and *ermB* were determined by polymerase chain reaction of all *S. pneumoniae*.** ***Results:*** **Of 858 clinical isolates, 259 (30.1%) were strains without *ermB* or *mefE* gene, 279 (32.5%) carrying *mefE*, 292 (34.0%) carrying *ermB*, and 28 (3.4%) carrying both genes. There was a strong correlation between phenotypes and the presence of macrolide resistance genes. The macrolide resistance genes were especially frequently identified among penicillin-resistant *S. pneumoniae*. Strains carrying *ermB* gene gradually increased from 25% in 1998 to 45% in 2002, with a concurrent decrease in strains carrying *mefE* from 36% in 1998 to 1999 to 19% in 2002. Strains having *mefE* were frequently identified among children younger than 2 years old. The current finding suggested that high-level *ermB*-mediated macrolide resistance in *S. pneumoniae* is increasing at an alarming rate in pediatric patients with otitis media, especially among young children. Physicians should pay close attention to such macrolide-resistant bacterial pathogens in the antimicrobial treatment of pediatric patients with otitis media.** ***Key Words: Streptococcus pneumoniae, mefE, ermB,*** **macrolide resistance, PCR.**
Laryngoscope, **115:317–320, 2005**

INTRODUCTION

Streptococcus pneumoniae (*S. pneumoniae*) heavily colonizes in the nasopharynx of healthy children and continues to be a significant cause of otitis media.[1] For many years, pneumococci were universally susceptible to a number of antibiotics until a penicillin-resistant strain was first reported in 1967. The 1990s witnessed an explosive emergence of antimicrobial resistance in pneumococci. Macrolides are alternatives for penicillin and are frequently prescribed among children. However, resistance to macrolides as well as to penicillin and cephalosporin in *S. pneumoniae* has been rapidly increasing worldwide. Increasing antimicrobial resistance in pneumococci further complicates the treatment of otitis media among children. The resistant pathogens are frequently isolated from middle ear fluid from children with otitis media.[2] Recent studies in molecular identification of both middle-ear and nasopharyngeal isolates revealed that the nasopharynx has become the reservoir for antimicrobial-resistant middle-ear pathogens. To characterize prevalent phenotypes or genotypes in antimicrobial resistant *S. pneumoniae* isolated from the nasopharynx among children with otitis media will bring us beneficial information for the treatment of intractable otitis media. Two predominant mechanisms of macrolide resistance in *S. pneumoniae* have been reported. One is a target-site modification by methylation encoded by the *ermB* gene. The other is an efflux pump system encoded by the *mef*A gene.[1]

In the present study, we investigated the predominant mechanism of macrolide resistance by evaluating *ermB* and *mefE* genes by polymerase chain reaction (PCR) in *S. pneumoniae* isolated from the nasopharynx of Japanese children with otitis media.

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MATERIALS AND METHODS

S. pneumoniae Isolates

S. pneumoniae isolates were collected from the nasopharynx of pediatric patients with acute otitis media (AOM) at the clinics of Otolaryngology–Head and Neck Surgery, Wakayama Medical University Hospital and six affiliated hospitals in Wakayama prefecture between January 1998 and December 2002. Nasopharyngeal cultures were obtained with a small rayon-tipped flexible swab at the onset or convalescent period of the diseases. *S. pneumoniae* was identified by α-hemolysis and colony morphology on 5% sheep blood agar, Gram stain, Optochin disk sensitivity, and bile solubility. The autolysin gene (*lytA*) was also amplified by PCR to confirm the isolates were *S. pneumoniae*. Bacteria were routinely cultured on the 5% sheep blood agar plates (Nippon Becton Dickinson Company Ltd., Tokyo, Japan) in a humidified atmosphere supplemented with 5% CO_2. The isolates were stocked in Todd-Hewitt broths (Difco Laboratories, Detroit, MI) supplemented with 0.5% yeast extract (Difco Laboratories) containing 10% glycerol at −80°C before the study. Duplicated isolates were excluded from the study by removing repeat isolates obtained from the same patients within 3 months of the first isolates.

Antimicrobial Susceptibility Test

Minimal inhibitory concentrations (MICs) for penicillin G (PCG), erythromycin (EM), and clindamycin (CLDM) were determined by microbroth dilution methods. The definition of susceptibility was based on the criteria established by the National Committee for Clinical Laboratory Standards.[3] Isolates with PCG MICs 2 μg/mL or greater were interpreted as penicillin-resistant *S. pneumoniae* (PRSP), isolates with PCG MICs of 0.1 to 1 μg/mL as penicillin intermediately resistant *S. pneumoniae* (PISP), and isolates with MICs 0.06 g/mL or less as penicillin-susceptible *S. pneumoniae* (PSSP). Isolates with EM MICs 1 μg/mL or greater were interpreted as EM-resistant *S. pneumoniae*, isolates with EM MIC at 0.5 μg/mL as EM intermediately resistant *S. pneumoniae*, and isolates with EM MICs 0.25 μg/mL of less as EM-susceptible *S. pneumoniae*.

Identification of mefE and ermB Genes by PCR

The oligonucleotide primers to amplify *mefE* and *ermB* genes were used in this study (Wakunaga Co. Ltd., Osaka, Japan). A single colony of *S. pneumoniae* on a 5% sheep blood agar plate was lysed in 30 μL of lysis solution (1 mol/L Tris pH 8.9, 4.5 v/v nonident P-40, 4.5 v/v Tween 20, 10 mg/mL proteinase K) for 10 minutes at 60°C and for 5 minutes at 94°C in a programmable thermal cycler (Gene Amp PCR System 9700, Perkin-Elmer, Norwalk, CT). The reaction mixtures for PCR consisted of 4 μL of bacterial lysate, 8 μL of 25 5mol/L of dNTP mixture, 2.5 U of Tth DNA polymerase (Takara Biomedicals, Kyoto, Japan), 10 μL of 10 × PCR buffer (pH 8.3), and 60 ng of the appropriate sets of primers in a total volume of 100 μL solution. The mixture was subjected to 30 cycles of amplification in a programmable thermal cycler consisting of 20 seconds at 94°C, 20 seconds at 57°C, and 15 seconds at 72°C. PCR fragments were separated using 3% agarose gel electrophoresis.

Statistical Analysis

Comparisons between two groups were assessed by chi-square analysis with Fisher's exact test or analysis of variance test. A P value of less than .05 was considered statistically significant. Calculations were performed using the statistical software package StatView version 5.0 (SAS Institute, Inc, Cary, NC).

RESULTS

Detection of Macrolide Resistance Genes by PCR

Two hundred fifty-eight isolates (30.1%) did not carry the *ermB* or *mefE*, and 600 strains (69.9%) carried either *mefE* or *ermB*. Of these 600 isolates, 277 (32.5%) carried *mefE*, 292 (34.0%) carried *ermB*, and 28 (3.4%) carried both *mefE* and *ermB*.

Relationship between the Presence of Macrolide Resistance Genes and Susceptibilities to EM or CLDM

We randomly selected 165 isolates to determine MICs to EM and CLDM. Among these 165 isolates, 61 isolates (37.0%) carried *mefE*, 50 isolates (30.3%) carried *ermB*, and 6 isolates (3.6%) carried both genes. The remaining 48 isolates (29.1%) were the isolates without macrolide resistance genes. There was a strong correlation between EM MICs and the presence of macrolide resistance genes. Although all the isolates with neither *ermB* nor *mefE* were susceptible to EM (MIC ≤0.125 μg/mL), isolates carrying *mefE* were intermediately resistant or resistant to EM (MIC 0.5–8 μg/mL). All isolates carrying *ermB* were highly resistant to EM (MIC ≥16 μg/mL) (Fig. 1A). There was strong correlation also found between susceptibilities to CLDM and the presence of macrolide-resistant genes. All strains carrying *ermB* showed high-level resistance to CLDM (MIC ≥ 16 μg/mL), and all isolates without macrolide resistance genes were susceptible to CLDM (MIC ≤ 0.125 μg/mL). Approximately 96.7% isolated carrying *mefE* were susceptible to CLDM (only 2 strains were resistant to CLDM) (Fig. 1B).

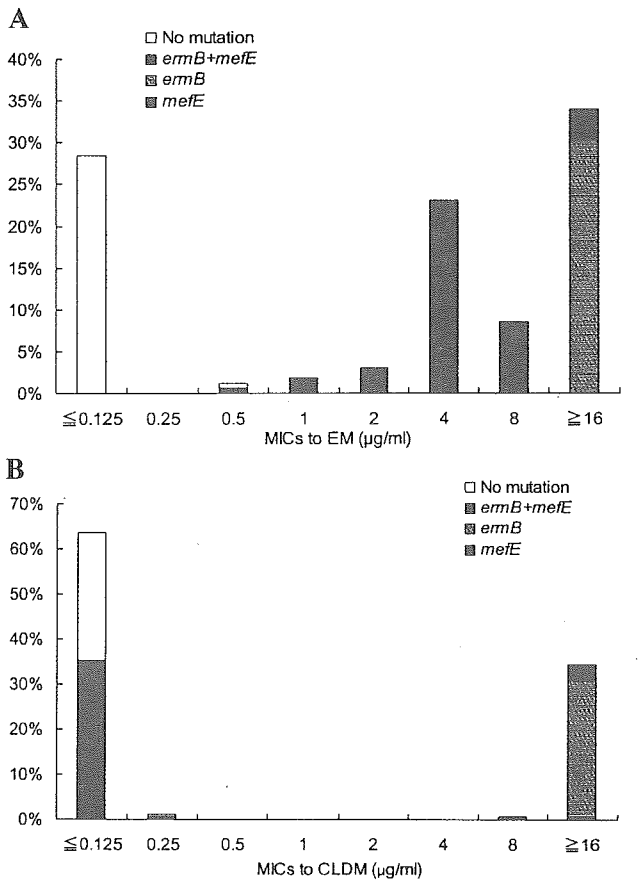

Fig. 1. (A) Macrolide resistance genes and susceptibility of erythromycin (EM). (B) Macrolide resistance genes and susceptibility of clindamycin (CLDM). MICs = minimal inhibitory concentrations.

Relationship between Presence of Macrolide Resistance Genes and Susceptibility to PCG

Susceptibilities to PCG were evaluated in 706 strains. Among these 706 isolates, 280 (39.7%) were PSSP, 220 (31.2%) were PISP, and 206 (29.2%) were PRSP.[3] Among 280 PSSP isolates, 147 (52.5%) carried macrolide-resistant genes, 83 (29.6%) carried *mefE*, 47 (16.8%) carried *ermB*, and 3 (1.1%) carried both genes. Among 220 PISP isolates, 40 (18.2%) carried neither macrolide resistant gene, 103 (46.8%) carried *mefE*, 60 (27.3%) carried *ermB*, and 17 (7.7%) carried both genes. Among 206 PRSP isolates, 22 (10.7%) carried neither macrolide resistance gene, 43 (20.9%) carried *mefE*, 136 (66.0%) carried *ermB*, and 5 (2.4%) carried both genes (Fig. 2). Thus, macrolide resistance genes were highly identified among penicillin nonsusceptible isolates (PISP + PRSP) ($P < .01$).

Distribution of Macrolide-Resistant Isolates according to Year

Overall macrolide resistance rate increased from 66.9% to 76.2% during 1997 to 2002; however, the proportion of *mefE* and *ermB* changed during these years. Isolates carrying *mefE* decreased from 36% in 1998 to 1999 to 19% in 2002. On the other hand, strains carrying *ermB* increased from 25% in 1998 to 45% in 2002 (28.6% from 1998–1999 vs. 38.3% from 2000–2002, $P < .05$) (Fig. 3). Also, the proportion of isolates carrying both *mefE* and *ermB* increased from 4% in 1998 to 8% in 2002 (Fig. 3); thus, high-level macrolide-resistant isolates increased from 32% in 1998 to 53% in 2002.

Age Distribution of Macrolide Resistance Genes

Strains carrying *ermB* were identified in all age groups. However, strains carrying *mefE* were frequently identified among children younger than 2 years old, whereas susceptible strains carrying no macrolide resistance genes were more frequently identified among children older than 6 years old ($P < .05$) (Fig. 4).

DISCUSSION

The emergent increase of multidrug-resistant *S. pneumoniae* has complicated treatment decisions and may lead to treatment failures of otitis media. Surveillance studies in Japan among upper respiratory tract infections revealed 49.6% were PSSP, 28.5% PISP, and 21.9% PRSP.[4] Macrolides are one of the alternatives for penicillin. However, macrolide resistance in *S. pneumoniae* has increased in recent years, and macrolide resistance occurs more frequently in isolates with reduced susceptibility to penicillin (PISP + PRSP). The Asian Network for Surveillance of Resistance Pathogens (ANSORP) study in 1997 showed that 67.9% of *S. pneumoniae* were highly resistant to EM in Asian countries.[5] In the United States and Europe, 60% to 80% of the multidrug-resistant pneumococci exhibited EM resistance.[6–8] Recent reports from all over Japan showed that 21.9% of *S. pneumoniae* isolates were highly resistant to penicillin, and 75.6% of isolates were resistant to EM.[3,4] The MICs to EM of *S. pneumoniae* were also high (MIC_{50} = 4 μg/mL and MIC_{90} = 128 μg/mL) in Japan. In the present study, the overall rate of macrolide resistant *S. pneumoniae* was high, and 70.3% of isolates carried either *ermB* or *mefE*.

Two mechanisms of resistance to macrolides in *S. pneumoniae* were investigated. One is a target-site methylase that prevents the binding of macrolides to 23S rRNA encoded by *ermB*, and the other is an efflux pump encoded by the *mefE*. Methylation of the ribosomal target encoded by *ermB* leads to either inducible or constitutive cross-resistance to 14-, 15-, and 16-membered ring macrolides, lincosamides, and streptogramin B (MLS_B phenotype) and is a high-level resistance mechanism. The efflux pump encoded by *mefA* confers resistance to only 14- and 15-

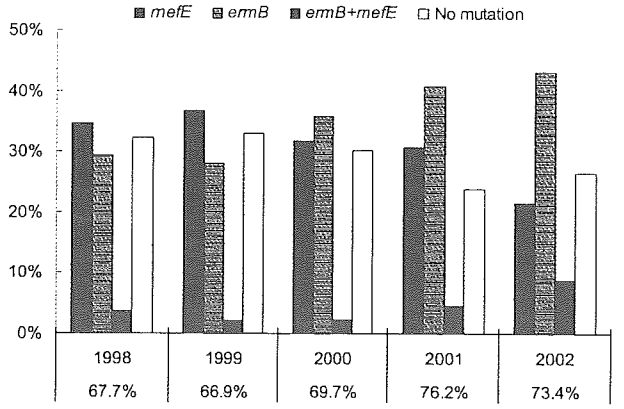

Fig. 3. Annual changes of macrolide resistance genes.

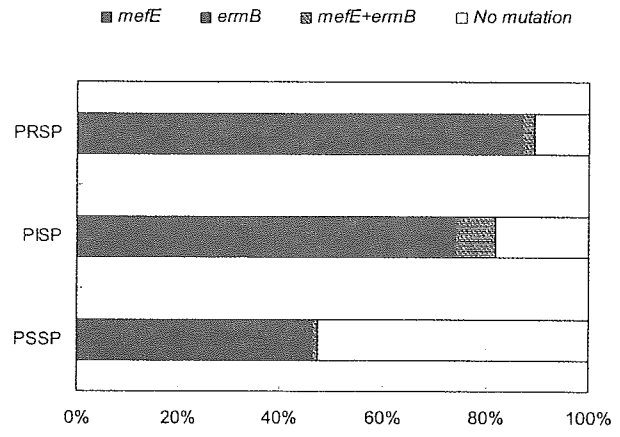

Fig. 2. Macrolide resistance genes and susceptibility to penicillin G (PCG).

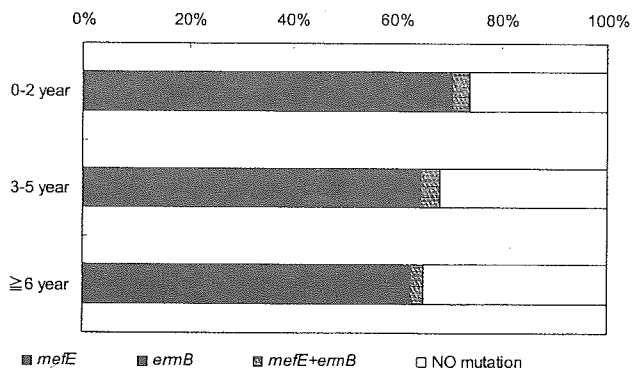

Fig. 4. Age distribution of macrolide resistance genes.

membered ring macrolides and is a low-level resistance mechanism. Macrolide-resistant *S. pneumoniae* carrying this gene remains susceptible to lincosamides and streptogramin B.[1] Ubukata et al.[9] reported that all isolates with resistance to EM possessed *ermB* or *mefE* genes in the countrywide surveillance of *S. pneumoniae* in Japan. The surveillance study consisted of 215 isolates from otitis media related strains and also revealed that *mefE* and *ermB* were equally distributed (*mefE* 37.7%, *ermB* 30.2%). A similar prevalence of these genes among macrolide-resistant *S. pneumoniae* was found in the first years of present study. Comparing the presence of these macrolide resistance genes and susceptibilities to EM and CLDM, we saw that *mefE*-positive isolates showed moderate resistance to EM but remained susceptible to CLDM, whereas the *ermB*-positive isolates were highly resistant to both EM and CLDM. These findings suggested that the macrolide resistance mechanism (either MLS_B type or M phenotype) can be identified by PCR detection of these two types of macrolide resistance genes (i.e., the MLS_B and M phenotypes were associated with the presence of *ermB* or *mefE*). Isolates carrying both genes demonstrated the *ermB*-like MLS_B phenotype. The annual prevalence of macrolide-resistant *S. pneumoniae* by phenotype was also evaluated in the present study. The proportion of isolates carrying *ermB* increased from 1998 thought 2002, whereas *mefE*-positive strains decreased during this period. In the United States, during 1996 through 1997, 7% of isolates carried *mefE*, and 33% of isolates carried *ermB*.[10] In contrast, the PROTEKT study from Korea, Japan, U.S., and Canada during 1999 through 2000 revealed that 6.8% of isolates carried *ermB* and 66% carried *mefE*.[2] Waites et al.[11] also showed that 39.1% of clinical isolates in Korea carried both *ermB* and *mefE*. The ANSORP study also revealed a large increase in resistance from 8.7% in 1998 and 36.6% in 1992 to 65.3% in 1996 to 1997.[4] Thus, the prevalence of macrolide resistance genes among macrolide-resistant *S. pnuemoniae* differs geographically. Variations in the prescription of macrolides in country to country influence the prevalence of the resistant stains.

The high prevalence of macrolide-resistant *S. pneumoniae* coincided with penicillin resistance. Okamoto et al.[12] reported that *S. penumoniae* with reduced susceptibility to amoxicillin isolates were considered to be resistant to EM. In this study, PRSP frequently carried macrolide resistance genes, whereas most PSSP carried neither *ermB* nor *mefE*. These findings suggested that most antimicrobial-resistant *S. pneumoniae* were cross-resistant to macrolide and β-lactams.[13] The strains with macrolide-resistant genes were highly identified in children younger than 2 years old. Our previous study also showed that PRSP were highly identified among children younger than 2 years. Immature immune defense systems among younger children cannot eliminate pathogens effectively.[14] There is a close relationship between siblings or in day-care centers that become the risk factor for frequent transmissions of resistant pathogens.[15] Further studies in molecular epidemiology and predominant clones should be condiucted.[16] Physicians should take the high prevalence of multidrug-resistant *S. pneumoniae* into account for the antimicrobial treatment of pediatric patients with otitis media.

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Respirology (2005) **10**, 208–214

ORIGINAL ARTICLE

Usefulness of the Japanese Respiratory Society guidelines for community pneumonia: a retrospective analysis of community-acquired pneumonia between 2000 and 2002 in a general hospital

KAZUSHI MOTOMURA,[1] HIRONORI MASAKI,[1] MAYUMI TERADA,[1] TOMOKO ONIZUKA,[1] AKITSUGU FURUMOTO,[2] NORICHIKA ASOH,[2] KAZUNORI OISHI[2] AND TSUYOSHI NAGATAKE[2]

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Usefulness of the Japanese Respiratory Society guidelines for community pneumonia: a retrospective analysis of community-acquired pneumonia between 2000 and 2002 in a general hospital

MOTOMURA K, MASAKI H, TERADA M, ONIZUKA T, FURUMOTO A, ASOH N, OISHI K, NAGATAKE T. *Respirology* 2005; **10**: 208–214

Objective: The aim of this study was to investigate the causative organisms of community-acquired pneumonia (CAP) diagnosed between 2000 and 2002 and to evaluate the Japanese Respiratory Society (JRS) guidelines.

Methodology: A total of 124 cases of CAP diagnosed during the study period were analyzed, and the results were compared with those of a previous study by the authors' research group. Determination of the causative organisms of CAP was based on Gram stain, morphology of colonies, quantitative culture of sputum, identification of bacterial isolates, and serological tests.

Results: During the study period, the causative organisms were identified in 42 cases (33.8%). *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* were the major causative organisms. Patients were classified into three groups based on the severity of CAP according to the JRS guidelines. The survival rates of patients with moderate and severe CAP were significantly lower than those of the mild group as evaluated by the Kaplan–Meier method (moderate vs mild, 70% vs 100%; severe vs mild, 40% vs 100%; $P < 0.001$ for both). Seven patients died during the study, and the risk factors were old age, bedridden status with cerebral infarction, and microaspiration, which was associated with recurrent pneumonia within 17 days.

Conclusion: This study indicates that the JRS guidelines for CAP are useful for treating patients with CAP in Japan.

Key words: community-acquired pneumonia, Japanese Respiratory Society guidelines, prognosis factors.

INTRODUCTION

Acute respiratory infection (ARI) is one of the most common causes of death. In particular, community-acquired pneumonia (CAP) is associated with a high mortality rate in non-industrialized countries as well as in industrialized countries.[1,2] In Western countries, guidelines for the diagnosis of CAP were established in the 1990s and included classification of CAP patients by severity and treatment regimens for CAP, which take into account the underlying disease, laboratory tests, and the causative organisms.[3,4] In Japan, the Japanese Respiratory Society (JRS) guidelines were published in 2000.[5]

In the present study, we analyzed 124 patients with CAP, and determined the causative organisms and compared this data with that from our previous study.[6] We previously reported that strains of methicillin-resistant *Staphylococcus aureus* (MRSA) were the major causative organisms of CAP.[6] In this

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study, we analyzed patients with CAP diagnosed at Tagami Hospital, Nagasaki, Japan, on the basis of the JRS guidelines.

SUBJECTS AND METHODS

Setting

Tagami Hospital is a 180-bed private and advanced emergency hospital. However, it is a non-teaching hospital and is located in an urban area. The hospital is of average size when compared with other hospitals in the area. Most of the in-patients are elderly. The hospital is affiliated with Nagasaki University, Japan, and CAP patients admitted from 1 January 2000 to 30 September 2002 were studied. The hospital's ethics committee approved the study.

Subjects

A total of 124 cases of CAP (76 men, aged 69.6 ± 12.7 years (mean ± SD) and 48 women, aged 69.8 ± 6.6 years) were enrolled. Of these, 122 (98.4%) were admitted to the wards and received antimicrobial therapy. The remaining two cases were treated in the outpatient department. Case analyses were based on the medical records of enrolled patients.

Diagnostic criteria for CAP

The diagnosis of CAP was based on the presence of the following two criteria:[7] (i) onset of pneumonia occurred outside the hospital—a new infiltrate shadow was detected on the chest X-ray in the outpatient department or within 24 h of hospitalization; and (ii) the presence of at least one symptom among fever, cough and sputum, or two symptoms among chest pain, dyspnoea and laboratory findings indicative of acute inflammation such as peripheral leukocytosis or elevated C reactive protein (CRP), or both.

Patients with the following diseases were excluded from the study: (i) nosocomial pneumonia; (ii) pneumonia in institutions such as nursing homes for the elderly; (iii) recurrent pneumonia within 7 days of hospital discharge; and (iv) pneumonia due to non-infectious causes such as pulmonary infarction, pulmonary oedema or lung cancer.

Detection of causative organisms

A rabbit blood-agar culture medium (Difco, Detroit, MI, USA) was used for the quantitative culture of sputum, and aerobic and anaerobic blood culture bottles (SEPTI-CHEK; Becton Dickinson, San Jose, CA, USA) were used for the blood cultures. API 20E, API 20NE, API Strept, API Staph, API Coryne, and API Ne (BioMerieux Inc., Mary-I'Etoile, France) were used for bacterial identification. Specimens were obtained by expectoration or using sterilized suction tubes for sputum if respiratory infection was suspected. When it was difficult to obtain a sputum sample, or when recurrent pneumonia occurred, we aspirated bronchial secretion samples from the lower respiratory tract using a bronchoscope, before instituting antimicrobial chemotherapy.

The collected specimen was cultured using Tryptic Soy Agar II (Becton Dickinson) supplemented with 5% rabbit blood agar for 18 h at 35°C. The causative organisms were identified by Gram stain of the purulent portion of the sputum, colony morphology, and the detection of $>10^7$ CFU/mL by quantitative sputum culture.[8] When the presence of *Mycoplasma pneumoniae* was suspected, we measured cold agglutinins and *M. pneumonia* IgG antibodies (complement fixation test) on paired serum samples with at least a 4-week interval. When the presence of *Chlamydia pneumoniae* was suspected, *C. pneumonia* IgG and IgA antibodies were measured in paired serum samples by enzyme immunoassay (EIA). The antibody titres were evaluated according to the diagnostic criteria of Kishimoto *et al.*[9] The test was regarded as positive if the increase in IgG absorbance of the convalescent serum was more than 0.3 compared with the negative baseline in the acute phase, or if the increase in IgG absorbance of the convalescent serum was more than 0.2 compared with the positive baseline in the acute phase. When the presence of *Legionella pneumoniae* was suspected, an enzyme-linked immunosorbent assay (ELISA) for urinary Legionella antigen was performed. If viral pneumonia was suspected, the antibody titre for a presumptive virus was measured in paired serum samples.

The minimum inhibitory concentration (MIC) was determined by the microdilution technique using the antibiotic susceptibility test, according to the revised guidelines of the National Committee for Clinical and Laboratory Standards, USA.[10] Penicillin-intermediate *Streptococcus pneumoniae* (PISP) and penicillin-resistant *S. pneumoniae* (PRSP) were defined by MIC of 0.125–1.0 μg/mL and more than 2.0 μg/mL of penicillin for *S. pneumoniae*, respectively. In our analysis, PRSP included both PISP and PRSP.

Clinical analysis

In this study, we classified CAP cases into three groups: mild, moderate, and severe, according to the JRS guidelines. The patient's background, underlying disease, causative organisms, antimicrobial chemotherapy, and clinical outcomes were compared with our previous results.[6] The antimicrobial therapy was considered effective if the clinical symptoms and acute inflammatory responses improved (decreased cough, reduced sputum volume, and decrease in sputum purulence) or if the causative organisms decreased in number or disappeared from the sputum. For the management of CAP, the severity of pneumonia was classified using the JRS guidelines,[5] based on the physical examination, CXR, WCC, serum CRP value and the arterial PaO_2. Patients were classi-

fied into three groups: mild, moderate, and severe. These groups were compared in terms of duration of admission, duration of treatment, and mortality rate using the Kruskal–Wallis test for multiple group comparisons. *P*-values <0.05 were considered statistically significant. The Kaplan–Meier survival curves were analyzed using the Mini-stat computer software program (ATMS, Tokyo, Japan).

RESULTS

Patient background

The age distribution at onset of CAP was 16–94 years (16–19 years, six patients (4.8%); 20–64 years, 27 patients (21.8%); >65 years, 91 patients (73.4%)). Table 1 shows the analysis of CAP cases using the JRS severity of pneumonia criteria. The mean age was 66.6 ± 20.8 years for the mild group, 75.3 ± 17.5 years for the moderate group, and 79.9 ± 9.2 years for the severe group. Multiple group comparisons showed significant differences (mild vs moderate, $P < 0.001$; mild vs severe, $P < 0.001$). The duration of admission was significantly shorter in the mild group (mild, 30.8 days vs moderate, 53.9 days, $P < 0.001$; mild, 30.8 days vs severe, 58.9 days, $P < 0.001$). The duration of treatment was 18 days in the severe group, 7 days in the mild group and 6 days in the moderate group (mild vs severe, $P < 0.001$; moderate vs severe, $P < 0.001$). The number of deaths during the hospitalization period was zero, three and four in the mild, moderate and severe groups, respectively (mild vs severe, $P < 0.001$; mild vs moderate, $P < 0.001$; moderate vs severe, $P = 0.281$). The subsequent observation period was up to 534 days. Figure 1 shows the Kaplan–Meier survival curves calculated for the three severity groups. The cumulative survival rate decreased to 70% at 70 days in the moderate group, and 40% at 105 days in the severe group (mild vs moderate, $P < 0.01$; mild vs severe, $P < 0.001$).

Laboratory data were similar to those reported in our previous study.[6] The mean level of CRP in the mild group was 5.97 ± 4.36 mg/dL; in the moderate group, 7.25 ± 4.78; and in the severe group, 10.26 ± 6.78. The severity of CAP was significantly associated with the CRP levels in the three severity groups (mild vs moderate and mild vs severe, $P < 0.01$). The mean level of serum albumin in the mild group was 4.43 ± 2.12 g/dL; in the moderate group, 4.17 ± 1.98; and in the severe group, 3.76 ± 1.85. The mean level of serum albumin was significantly lower in the severe group than in the mild or moderate groups ($P < 0.01$ for both).

Underlying diseases

The most common underlying diseases were respiratory, which were present in 53 (43%) of the 124 cases. The breakdown of these included bronchial asthma in 19 (36.4%), emphysema in 14 (26.4%), and old tuberculosis in 12 (22.6%). The second common underlying disease group was cerebrovascular accident, including cerebral infarction, which accounted for 20 patients (16%). Of the 124 patients studied, 11 (9%) had diabetes mellitus.

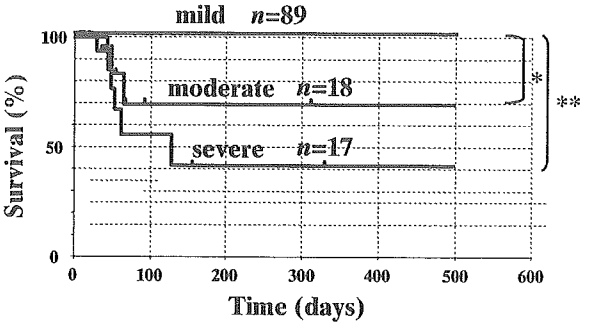

Figure 1 Kaplan–Meier analysis of 124 cases of community-acquired pneumonia in each of the three severity groups. Kaplan–Meier survival plots were computed using Mini-stat statistical software. The *P*-values for the prediction for each severity group were computed using a log-rank test. *$P < 0.01$, **$P < 0.001$.

Table 1 Analysis of the study population in each severity group according to the JRS Guideline for CAP

	Severity by JRS		
	mild	moderate	severe
No. cases	89 (71.8%)	18 (14.5%)	17 (13.7%)
Age (years, mean ± SD)	66.6 ± 20.8	75.3 ± 17.5	79.9 ± 9.2
Duration of admission (days)	30.8	53.9*	58.9*
Duration of treatment (days)	9	9.3	11.9**
Deaths	0	3	4
Serum C-reactive protein (mg/dL, mean ± SD)	5.97 ± 4.36	7.25 ± 4.78*	10.26 ± 6.78*
Serum albumin (g/dL, mean ± SD)	4.43 ± 2.12	4.17 ± 1.98*	3.76 ± 1.85*

*$P < 0.01$ compared to mild group; **$P < 0.01$ compared to moderate group.

Causative organisms of CAP

Causative organisms of CAP were identified in 42 (33.8%) patients. Among these, the diagnosis was based on sputum culture in 38, while in the other four patients it was based on serological tests. Among the 42 cases, 35 (83.3%) were monomicrobial infection. Polymicrobial infection with *C. pneumoniae*, *S. pneumoniae* and *H. influenzae* was detected in one patient (2.4%).

The strains of *S. pneumoniae* were determined in 15 cases (12.1%), and seven (46.7%) of 15 isolates were PRSP. *S. pneumoniae* was the most frequently isolated organism. Figure 2 shows the causative organisms of CAP in this study and in our previous study.[6] The rate of isolated PRSP was not determined in our previous study, as the oxacillin sensitivity test was not performed.[6]

H. influenzae strains were determined in 10 patients (8.1%) and all were non-β-lactamase-producing strains. Only one strain of *H. influenzae* did not disappear after antimicrobial therapy. This strain was shown to be a low β-lactamase negative ampicillin resistant *Haemophilus influenzae* (BLNAR) strain, based on the pattern of penicillin-binding proteins determined by polymerase chain reaction.[11] The infection rates for *H. influenzae* as a causative organism in our previous and present studies were 27.2% and 23.8%, respectively. *Moraxella catarrhalis* was isolated in four patients (Fig. 2). *Pseudomonas aeruginosa* was isolated in three patients, in whom the underlying disease was bronchiectasis. Other organisms isolated included *Klebsiella pneumoniae*, *Corynebacterium pseudodiphtheriticum* and *Corynebacterium propinquum* (Fig. 2).

In this study, serological tests were performed for *M. pneumoniae* and *C. pneumoniae*, using paired serum samples obtained from 68 (54.8%) patients. *Mycoplasma pneumoniae* serology was positive in three (2.4%) patients. Their age range was 18–50 years. Only one patient was positive for *C. pneumoniae* as per the diagnostic criteria of Kishimoto *et al.*[9]

Causative organisms were not determined in 82 (66.1%) patients. There were a number of reasons for this. A total of 26 patients (21%) had received antibiotics before attending the hospital. They had received antibiotics for a mean period of 3.9 days, but prior to any CXR being taken. Cerebrovascular disease was identified as the underlying disease in 10 (8.1%) patients. In such patients, microaspiration-induced pneumonia caused by anaerobic bacteria was suspected. No factor that may have influenced the detection of the causative organisms could be identified in the remaining 46 cases. Unfortunately, blood cultures were performed in only seven (5.6%) of the 124 cases of CAP.

Antimicrobial therapy of CAP

Antimicrobial therapy for CAP was terminated following improvement of clinical symptoms and normalization of the CRP level, even if the pulmonary shadows on the CXR were still present. However, if there was a worsening of clinical symptoms and worsening pneumonia on CXR on the third day after antibiotics were commenced, the antibiotics were immediately changed. Of the 124 patients, 123 were treated by i.v. infusion, and only one patient with suspected atypical pneumonia was treated orally with a quinolone. The first choice antimicrobial agents for CAP were cephalosporins and cephamycin which were used in 36 (29.0%) patients (second generation cephalosporin, five cases; third generation cephalosporin, 31 cases). Penicillins were used in 32 (25.8%) patients, carbapenems in 23 (18.5%), tetracyclines in four (3.2%), macrolides in two (1.6%), and a new quinolone in one (0.8%). Antimicrobial agents were changed in 26 (20.9%) patients with carbapenem being used in 10 patients, third generation cephalosporins in seven, tetracycline in seven, and penicillin, clarithromycin clindamycin and levofloxacin each in one patient. All patients were switched to a single antibiotic. The reasons for changing antibiotics were adverse effects in seven cases, atypical pneumo-

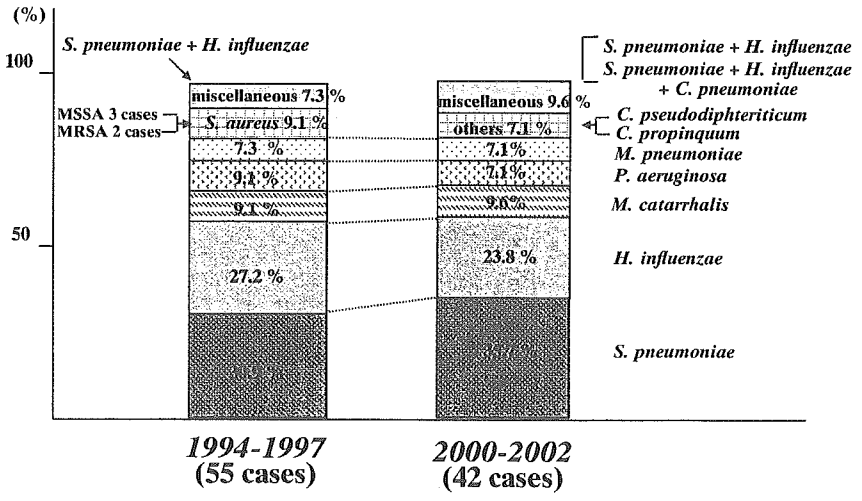

Figure 2 Causative organisms of community-acquired pneumonia during each study period. The 1994–1997 study has been published previously.[6]

nia in three, aspiration pneumonia in four, and others (insufficient therapeutic effect) in 12. A second antimicrobial agent was added in one patient with PRSP-induced pneumonia and in four patients with *H. influenzae* pneumonia.

Analysis of patients who died during treatment of community-acquired pneumonia

Table 2 shows the clinical details of those patients who died during the treatment of CAP. Seven (5.6%) died during the treatment and all were males. The mean age was 81.1 ± 8.6 years. Of these, *H. influenzae* was isolated in one patient but no causative organisms were isolated in the remaining six (85.7%). Pleural effusion was found in five cases (71.4%), of whom three had congestive heart failure. Four had respiratory failure, which was treated either with ventilatory support or non-invasive positive pressure ventilation in addition to corticosteroid therapy. The risk factors for death included male gender, performance status of 4,[12] cerebral infarction, and aspiration pneumonia relapsing within 17 days.

DISCUSSION

The present study was designed to identify the causative organisms of CAP and analyze cases with CAP based on severity as classified by the JRS guidelines. The major causative organisms of CAP were *S. pneumoniae* and *H. influenzae*,[13–15] which accounted for 69% of the cases in our study. PRSP accounted for 46.7%, which is similar to the results from another survey of adults.[16] No relationship was observed between PRSP pneumonia and the severity of CAP in our study. Watanabe *et al.*[17] reported that PISP pneumonia did not differ significantly from PRSP pneumonia in clinical symptoms and severity.

Interestingly, *C. propinquum* in the Corynebacterium absolute non-fermenter (ANF) group has been identified as a new causative organism of CAP.[18] Corynebacterium species are usually known as organisms that constitute the normal bacterial flora. Therefore, it is important to examine the Gram-stained sputum specimen for such microorganisms. The rate of identification of causative organisms in our previous study[6] was 45.8%, however, the rate decreased to 33.8% in the present study. The reasons for this decrease are likely to be the increase in the number of patients receiving antibiotics before presentation and the increase in the number of patients with cerebral infarction and possible anaerobic infection in such patients.[19] A low frequency of *M. pneumoniae* infection was observed in the present study, which was similar to our previous study.[6] The reason for this might be related to the age distribution of our patients, which was biased towards the older age group (the proportion of patients aged >65 years was 73.4%).

Table 2 Analysis of dead cases during the treatment course of community-acquired pneumonia

No.	Age	Gender	PS	Underlying diseases	Consciousness disturbance	Pleural effusion*	Aetiology	Infiltration on chest X-ray	Severity	Days before recurrence
1	78	Male	4	Cerebral infarction	No	No	ND	Three lobes	Moderate	6
2	92	Male	4	Cerebral infarction	Yes	Yes	ND	Two lobes	Severe	14
3	87	Male	4	Cerebral infarction	Yes	Yes	ND	Two lobes	Severe	8
4	79	Male	4	Cerebral infarction	Yes	Yes	ND	One lobe	Moderate	9
5	85	Male	4	Cerebral infarction	Yes	Yes	ND	Two lobes	Severe	17
6	84	Male	4	Cerebral infarction	No	No	ND	One lobe	Moderate	10
7	63	Male	4	Cerebral infarction	Yes	Yes	*H. influenzae*	All	Severe	3

Pleural effusion yes means unilateral or both; ND, not determined; PS, performance status.
*Yes indicates one or both lungs.

Bed-ridden elderly patients with multiple cerebral infarctions and recurrent aspiration pneumonia had a poorer prognosis. Arancibia *et al.*[20] reported that infections caused by Gram-negative bacteria such as *P. aeruginosa*, microaspiration and the presence of underlying diseases indicated a poor prognosis in patients with pneumonia.[20]

In our previous report,[6] we classified the patients' severity according to the Japanese Society of Chemotherapy guidelines.[21] We reported that the severe cases had high CRP and low serum albumin levels.[6] Similar results were obtained in the present study in which the JRS guidelines were applied. Moreover, the duration of admission, duration of treatment, and the cumulative survival rates were significantly different among the three severity groups. The duration of admission was long even in the mild group. The main reason for the long hospitalizations was weakness of lower limb muscles and a further general weakness after CAP treatment, which was seen in most of the elderly patients, and rehabilitation was provided until they were discharged. In some patients, a pulmonary rehabilitation program was added, as COPD was incidentally diagnosed during their admission.

We classified and analyzed those who died from CAP into five stages, based on the classification of Fine *et al.*[22] Our results were different from their prediction (data not shown) as most of their cases were labelled severe, if their underlying disease was malignancy,[22] while cases with multiple cerebral infarctions were not graded as being severe. Thus, their classification was not suitable for our patients, as multiple cerebral infarction was a major underlying disease.

Our results suggest that the JRS guidelines for CAP are a suitable and useful tool for analysis of the treatment of patients with CAP in Japan.

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Comparative Molecular Analysis of *Haemophilus influenzae* Isolates from Young Children with Acute Lower Respiratory Tract Infections and Meningitis in Hanoi, Vietnam

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Thirty-seven *Haemophilus influenzae* strains from nasopharyngeal swabs (NP) and 44 *H. influenzae* strains from cerebrospinal fluid (CSF) were investigated. Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas all of the 44 isolates from CSF were type b. The MICs of 16 antibiotics for the *H. influenzae* isolates from NP and CSF were similar, and no β-lactamase-negative ampicillin-resistant strain was found. Molecular typing by pulsed-field gel electrophoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns, with none predominating, and the 44 *H. influenzae* strains from CSF had 9 PFGE patterns, with patterns α (22 isolates) and β (12 isolates) predominating. Our results indicate that two predominant types of *H. influenzae* type b strains have the potential to spread among children with meningitis in Hanoi, Vietnam.

Nontypeable *Haemophilus influenzae* (NTHi) can cause a variety of infections, including otitis media, bronchitis, and pneumonia (7), whereas *H. influenzae* type b (Hib) is a common cause of meningitis in children (11). Hib infection rates have been dramatically reduced in countries that have implemented Hib conjugate vaccine programs as part of routine infant immunizations (10). It has also recently been reported that β-lactamase-negative ampicillin (AMP)-resistant (BLNAR) strains have increased in some countries (6, 12), although their global prevalence remains low (4, 5). The aim of our study was to investigate the characteristics of *H. influenzae* among children less than 5 years of age in Vietnam.

Thirty-seven *H. influenzae* strains were isolated from the nasopharyngeal swabs (NP) of 37 children aged 2 to 60 months (mean age, 11 months) who were diagnosed with acute lower respiratory tract infections between 2001 and 2002, and 44 *H. influenzae* strains were isolated from the cerebrospinal fluid (CSF) of 44 children aged 1 to 24 months (mean age, 9 months) who were diagnosed with meningitis between 2002 and 2003, in Hanoi, Vietnam. No patient with an acute lower respiratory tract infection overlapped a patient with meningitis. *H. influenzae* isolates were serotyped by slide agglutination with antisera purchased from Difco Laboratories (Detroit, Mich.), and β-lactamase production was detected by a disk impregnated with nitrocefin (Becton Dickinson, Sparks, Md.). PCR was carried out for *H. influenzae* isolates by using mixed primers (Wakunaga Pharmaceutical Co., Hiroshima, Japan), as described previously (3). MICs were determined by the agar dilution method according to the NCCLS guidelines (8). The susceptibilities of 81 *H. influenzae* isolates to the following 16 antibiotics were tested: penicillin G (Meiji Seika Kaisha, Tokyo, Japan), AMP (Meiji Seika Kaisha), amoxicillin-clavulanic acid (AMC) (GlaxoSmithKline K.K., Tokyo, Japan), cefatrizine (Taiyo Yakuhin Co., Nagoya, Japan), cefuroxime (Sankyo Co., Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical Co., Tokyo, Japan), cefotaxime (Aventis Pharma, Tokyo, Japan), imipenem (Banyu Pharmaceutical Co., Tokyo, Japan), minocycline [Lederle (Japan), Tokyo, Japan], chloramphenicol (Sankyo Co.), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Osaka, Japan), gentamicin (Schering-Plough K.K., Osaka, Japan), levofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan), norfloxacin (Kyorin Pharmaceutical Co., Tokyo, Japan), and sulfamethoxazole-trimethoprim (Shionogi & Co., Osaka, Japan). After digestion with SmaI (Takara Shuzo Co., Shiga, Japan), pulsed-field gel electrophoresis (PFGE) was performed on the 37 *H. influenzae* isolates from the NP and the 44 *H. influenzae* isolates from the CSF, as described previously (16), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (13).

Of the 37 *H. influenzae* isolates from NP, the serotypes of 30 isolates were nontypeable, 4 were type b, 2 were type c, and 1 was type a, whereas the 44 isolates from CSF were all type b. Twenty-six strains (70.3%) from NP and 23 strains (52.3%) from CSF were β-lactamase producing, and the remaining strains were β-lactamase negative by the nitrocefin disk assay. PCR analysis to identify the resistance genes indicated that 25 strains from NP and 21 strains from CSF were β-lactamase-producing AMP-resistant isolates which had the TEM-1-type β-lactamase gene; 11 strains from NP and 22 strains from CSF were β-lactamase-negative AMP-susceptible isolates, all of which lacked all resistance genes; and 1 strain each from NP and CSF were β-lactamase-producing AMC-resistant isolates

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TABLE 1. Distribution of MICs against 16 antibiotics for *H. influenzae* strains isolated from nasopharyngeal swabs and cerebrospinal fluid from children in Vietnam

Antibiotic	MIC (μg/ml) for isolates from:					
	NP (n = 37)			CSF (n = 44)		
	Range	50%	90%	Range	50%	90%
Penicillin G	0.5–128	16	32	≤0.004–128	2	32
Ampicillin	0.25–64	8	32	0.125–32	1	8
Amoxicillin-clavulanic acid	0.25–2	0.5	0.5	0.25–1	0.25	0.25
Cefatrizine	2–32	4	8	2–16	4	16
Cefuroxime	0.5–4	1	4	0.016–4	1	2
Ceftriaxone	≤0.004–0.032	0.008	0.016	≤0.004–0.032	0.008	0.008
Cefotaxime	0.008–0.125	0.032	0.032	≤0.004–0.125	0.032	0.063
Imipenem	0.25–4	2	2	0.25–1	0.25	1
Minocycline	0.5–2	1	2	0.5–2	1	1
Chloramphenicol	0.5–16	4	8	0.5–16	8	16
Clarithromycin	0.25–16	8	16	4–16	8	8
Erythromycin	0.25–4	4	4	0.016–8	2	4
Gentamicin	1–2	1	2	0.016–2	0.5	2
Levofloxacin	0.016–0.063	0.032	0.032	≤0.004–0.032	0.032	0.032
Norfloxacin	0.063–0.125	0.125	0.125	0.063–0.125	0.063	0.125
Sulfamethoxazole-trimethoprim	1–≥128	≥128	≥128	0.032–≥128	128	≥128

which had the TEM-1-type β-lactamase gene and the *ftsI* gene with the same substitution as the low-BLNAR strains. Although all isolates from NP which had the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay, one isolate from CSF which had the TEM-1-type β-lactamase gene was β-lactamase negative and two isolates from CSF which did not have the TEM-1-type β-lactamase gene were β-lactamase producing by the nitrocefin disk assay. No BLNAR strain was found. Table 1 shows the MIC range, the MICs at which 50% of isolates were inhibited (MIC_{50}), and the MIC_{90} of 16 antibiotics for 37 *H. influenzae* isolates from NP and 44 *H. influenzae* isolates from CSF. Although the MICs of the *H. influenzae* isolates from NP against penicillin G and AMP appear to be higher than those from CSF, the antimicrobial susceptibilities of the *H. influenzae* isolates from NP and CSF were similar. Molecular typing by pulsed-field gel electrphoresis (PFGE) showed that the 37 *H. influenzae* strains from NP had 22 PFGE patterns (A to V), without any predominant pattern (Fig. 1). The PFGE patterns of *H. influenzae* types a, b, and c were different from those of NTHi. Four isolates of type b had two PFGE patterns (I and K), and two isolates of type c had two PFGE patterns (H and Q). Forty-four *H. influenzae* strains from CSF had nine PFGE patterns (α to ι), with patterns α (22 isolates) and β (12 isolates) predominating. The PFGE patterns of 4 *H. influenzae* type b strains from NP were quite different from those of the 44 *H. influenzae* type b strains from CSF (Fig. 2).

Infants and young children tend to acquire *H. influenzae* in the upper respiratory tract because of their low immunity (16), and subsequent colonization can become a risk factor for invasive diseases caused by *H. influenzae* (2, 11). Since it has recently been reported that BLNAR NTHi and Hib have increased in some countries (3, 6, 12), the primary objective of this study was to investigate such resistant strains among children in Vietnam. In fact, no BLNAR strains were found in either NP or CSF, although more than half the isolates were β-lactamase producing and had the TEM-1-type β-lactamase gene. Hib remains the major cause of meningitis after the introduction of Hib vaccine in many advanced nations, because that vaccine is not usually available in Vietnam (14). Therefore, a secondary objective of this study was to examine the transmission route of *H. influenzae*. It has recently been reported that children can acquire *H. influenzae* at day care centers (9, 16) or from their parents at home (15). Our PFGE studies showed that NTHi did not have dominant genetic patterns but that Hib had two dominant genetic patterns. The results provide evidence to show that at least two types of Hib strains are spreading horizontally among children with meningitis in Vietnam. The Hib conjugate vaccine appears to be effective, not only for the prevention of invasive diseases, but also for the reduction of nasopharyngeal carriage in young children (1, 10).

In conclusion, our results demonstrate that BLNAR strains are not prevalent and that two predominant types of Hib

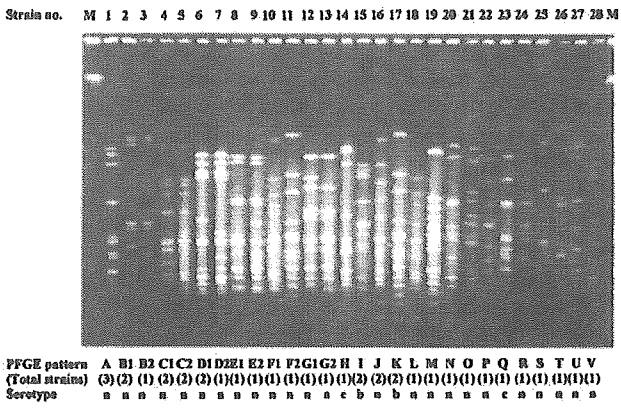

FIG. 1. PFGE patterns of SmaI-digested DNA from 37 *H. influenzae* isolates from NP of 37 children with acute lower respiratory tract infections. Molecular typing by PFGE demonstrated that 37 *H. influenzae* strains from the NP had 22 PFGE patterns (A to V), without any predominant pattern. The PFGE patterns of *H. influenzae* types a, b, and c were different from those of the nontypeable strains.

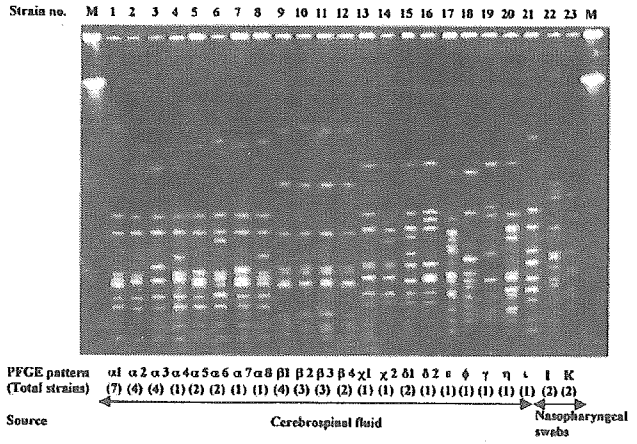

FIG. 2. PFGE patterns of SmaI-digested DNA from 48 Hib isolates from the CSF of 44 children with meningitis and the NP of 4 children with acute lower respiratory tract infections. Molecular typing by PFGE demonstrated that the 44 Hib strains from the CSF had nine PFGE patterns (α to ι), with patterns α (22 isolates) and β (12 isolates) predominating. PFGE patterns of 4 Hib strains from the NP were quite different from those of 44 Hib strains from CSF.

strains have the potential for spreading among children with meningitis in Hanoi, Vietnam. Therefore, the introduction of the Hib conjugate vaccine for young children should be considered in order to prevent invasive diseases caused by Hib.

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SHORT COMMUNICATION

Molecular analysis of intrafamiliar transmission of *Moraxella catarrhalis*

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Abstract

The possible intrafamiliar transmission of *Moraxella catarrhalis* was evaluated in 3 pairs between children and their parents, and 8 pairs between siblings from 11 families. Of the 22 isolates, all were found producing β-lactamase. Molecular typing by pulsed-field gel electrophoresis (PFGE) with Not I and Spe I showed that the PFGE patterns in 2 of 3 pairs between children and their parents, and 4 of 8 pairs between siblings were indistinguishable and those of the remaining pairs were different. These data indicate a possible high rate of intrafamiliar transmission of *M. catarrhalis*.

Keywords: *Moraxella catarrhalis*; PFGE; Intrafamiliar transmission

Introduction

Moraxella catarrhalis is an aerobic Gram-negative diplococcus that colonizes the human nasopharynx and can cause a variety of infections, including otitis media, sinusitis, bronchitis, pneumonia, and meningitis (Zhanel et al., 2003; Daoud et al., 1996). Children are frequent carriers of *M. catarrhalis* and the rate of carriage is high in infancy. Colonization may subsequently lead to the development of infectious diseases (Faden et al., 1997; Garcia-Rodriguez and Fresnadillo Martinez, 2002). It has previously been reported that children can acquire *M. catarrhalis* at day care centers (Yano et al., 2000), and nosocomial transmission of *M. catarrhalis* can occur (Masaki et al., 2003). However, the issue of whether *M. catarrhalis*, when colonizing the upper respiratory tract, can be transmitted between children and their parents or siblings at home and cause invasive diseases is not clear. To address this issue, we conducted the prospective study described below.

Materials and methods

New patients with infections (e.g., pharyngitis, sinusitis, otitis media) caused by *M. catarrhalis* who visited the Sugita Otorhinolaryngologic Clinic from March 2001 to June 2003 were asked to bring their family as soon as possible for a clinical examination and collection of biological specimens (e.g., nasopharynx, middle nutus for diagnosis of acute otitis media, acute pharyngitis or acute bacterial sinusitis). During this study, *M. catarrhalis* could be detected as often as *Streptococcus pneumoniae* and *Haemophilus influenzae*.

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To diagnose infectious diseases caused by *M. catarrhalis*, Gram-stained smears and cultures of good-quality specimens, according to the criteria described by Bartlett (1974), obtained as recently as possible, were performed by standard methods. Twenty-two *M. catarrhalis* strains that were collected from 3 pairs between children and their parents, and 8 pairs between siblings in 11 families were evaluated. The mean age of the children was 2.4 years (35.3 months) and that of the adults was 31 years. β-Lactamase production was detected by means of a disc impregnated with nitrocefin (Becton Dickinson, Sparks, MD, USA). Minimal inhibitory concentrations (MICs) were determined by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1998). The MICs of 22 *M. catarrhalis* isolates to the following 4 antibiotics was tested: ampicillin (AMP, Meiji Seika Kaisha, Tokyo, Japan), amoxicillin–clavulanic acid (AMC, GlaxoSmithKline, Tokyo), cefaclor (CEC, Shionogi Co., Osaka, Japan) and cefditoren (CDN, Meiji Seika Kaisha). Pulsed-field gel electrophoresis (PFGE) after digestion with Not I and Spe I (Takara Bio Inc., Shiga, Japan) was performed for the 22 *M. catarrhalis* isolates as described previously (Yano et al., 2000), and the interpretation of PFGE patterns was based on the criteria described by Tenover et al. (1995).

Results

Twenty-two *M. catarrhalis* strains were isolated from the middle nutus ($n = 16$) and nasopharynx ($n = 6$), and were all found producing β-lactamase. Twenty strains were detected in patients with infections and 2 strains colonized the nasopharynx without causing any symptoms. The respective range of MICs (μg/ml) against *M. catarrhalis* was 0.125–8.0 for AMP, 0.032–0.25 for AMC, 0.5–8.0 for CEC, and 0.032–0.5 for CDN (Table 1). Molecular typing by PFGE showed that the 22 *M. catarrhalis* strains had 12 patterns (A–L) by

Table 1. Epidemiological characteristics of *Moraxella catarrhalis* from children and their parents or siblings

Family	Strain no.	Age[a]	Specimen	Infection	Date	MIC (μg/ml)				PFGE pattern	
						AMP[e]	AMC[f]	CEC[g]	CDN[h]	Not I	Spe I
a	1	0(11)	Nasopharynx	AOM[b]	3/10/2001	1.0	0.125	8.0	0.5	A	M
	2	33	Nasopharynx	Colonization	3/17/2001	1.0	0.125	0.5	0.25	B	N1
b	3	1(12)	Middle nutus	ABS[c]	5/8/2003	4.0	0.25	2.0	0.5	C	N2
	4	30	Nasopharynx	AP[d]	5/8/2003	4.0	0.25	2.0	0.5	C	N2
c	5	1(21)	Middle nutus	ABS	5/20/2003	4.0	0.125	2.0	0.125	D1	O
	6	30	Middle nutus	ABS	5/24/2003	2.0	0.125	2.0	0.125	D1	O
d	7	0(3)	Middle nutus	ABS	6/6/2003	4.0	0.125	1.0	0.25	E	P
	8	3(45)	Middle nutus	ABS	6/6/2003	2.0	0.125	4.0	0.125	E	P
e	9	4(57)	Middle nutus	ABS	5/27/2003	2.0	0.125	0.5	0.5	F	Q
	10	4(57)	Middle nutus	ABS	5/27/2003	2.0	0.25	1.0	0.25	F	Q
f	11	4(58)	Middle nutus	ABS	5/20/2003	0.25	0.032	0.5	0.032	D2	N3
	12	8(101)	Middle nutus	ABS	6/4/2003	2.0	0.25	1.0	0.5	G	R
g	13	1(18)	Nasopharynx	AOM	7/21/2001	1.0	0.125	8.0	0.125	H	S
	14	5(63)	Nasopharynx	Colonization	7/21/2001	1.0	0.125	8.0	0.063	D3	T1
h	15	0(4)	Middle nutus	AOM, ABS	7/31/2001	2.0	0.25	1.0	0.5	I	U
	16	3(36)	Middle nutus	ABS	7/28/2001	2.0	0.25	1.0	0.25	I	U
i	17	1(21)	Nasopharynx	ABS	6/12/2001	8.0	0.125	1.0	0.25	J	V
	18	3(41)	Middle nutus	ABS	5/7/2001	2.0	0.125	1.0	0.25	D4	T2
j	19	0(11)	Middle nutus	ABS	6/16/2001	2.0	0.25	4.0	0.5	K	W
	20	1(22)	Middle nutus	ABS	5/12/2001	2.0	0.125	1.0	0.5	K	W
k	21	2(25)	Middle nutus	ABS	3/17/2001	0.5	0.032	1.0	0.032	J	V
	22	5(64)	Middle nutus	ABS	3/17/2001	0.125	0.032	0.5	0.125	L	X

All strains were β-lactamase positive.

[a]Ages are given in years for adults and in years (months) for children.

[b]AOM, acute otitis media.

[c]ABS, acute bacterial sinusitis.

[d]AP, acute pharyngitis.

[e]AMP, ampicillin.

[f]AMC, amoxicillin–clavulanic acid.

[g]CEC, cefaclor.

[h]CDN, cefditoren.

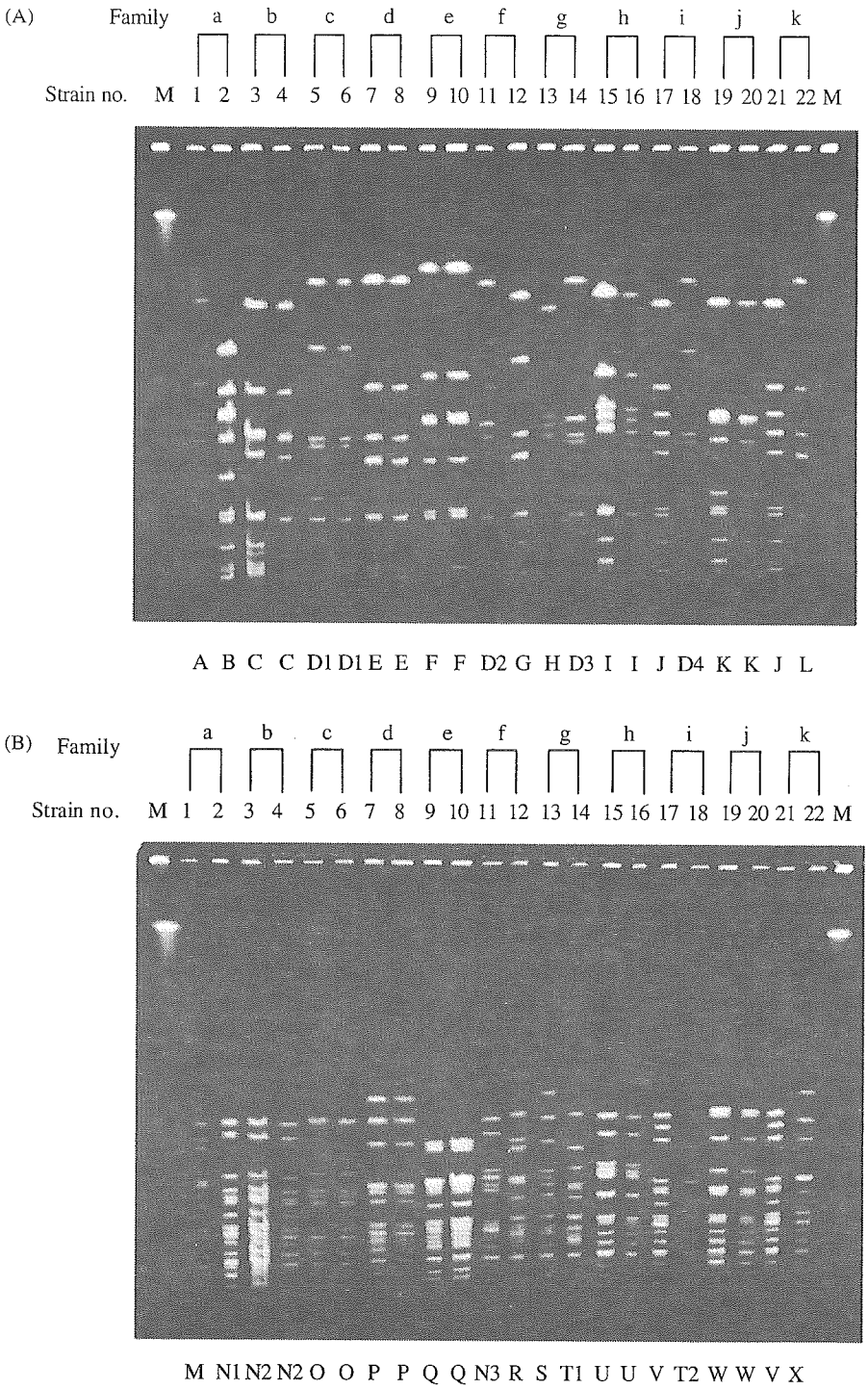

Fig. 1. PFGE patterns of Not I- (A) and Spe I- (B) digested DNA of *M. catarrhalis* isolates from children and their parents or siblings in 11 families (a–k). The PFGE patterns of *M. catarrhalis* isolates in children and their parents or siblings are indistinguishable in b–e, h, and j, and different in a, f, g, i, and k. Lanes M contain a molecular size marker.

Not I and (M–X) by Spe I. And the PFGE patterns in 2 of 3 pairs between children and their parents, and 4 of 8 pairs between siblings were indistinguishable. Those of the remaining pairs were different (Table 1, Fig. 1).

Discussion

M. catarrhalis is one of the most common causes of otitis media in children (Faden et al., 1997; Pichichero and Casey, 2002) and lower respiratory tract infections

in adults with chronic obstructive pulmonary disease (Klingman et al., 1995). In particular, the nasopharyngeal carriage rate of *M. catarrhalis* in infants and young children is higher compared to adults because of their low immunity, and colonization can become a risk factor for infectious diseases caused by *M. catarrhalis* (Faden et al., 1997; Garcia-Rodriguez and Fresnadillo Martinez, 2002). Otitis media rarely results in death, but leads to hearing loss as a complication at a critical stage in the development of speech, language and cognitive abilities in children (Cripps and Kyd, 2003). In our study, a possible high rate of correlation in *M. catarrhalis* colonization between children and their parents or siblings at home was confirmed by PFGE with Not I and Spe I. In addition, it has been reported that some patients were colonized by multiple strains with different genetic patterns (Klingman et al., 1995), raising the possibility that the mismatching strain is tested instead of the matching one, resulting in apparently discordant pairs. However, multiple strains of *M. catarrhalis* from the same individual could not be found in our study. It has recently been reported that *S. pneumoniae* and *H. influenzae* can be potentially transmitted between children and their parents or siblings at home (Shimada et al., 2002; Watanabe et al., 2004). Our data confirms this and reveals that infants and young children can acquire *M. catarrhalis* not only at day care centers or hospitals (Yano et al., 2000; Masaki et al., 2003) but at home, as well. Pneumococcal and *H. influenzae* type b conjugate vaccine appears to be effective not only for the prevention of invasive disease but also for the reduction of nasopharyngeal carriage in young children (Barbour et al., 1995; Kyaw et al., 2001). On the other hand, a vaccine to prevent infections caused by *M. catarrhalis* is not available at present, although several laboratories have active programs with the goal of developing such a vaccine (McMichael, 2000).

In conclusion, our results demonstrate a possible high rate of intrafamiliar transmission of *M. catarrhalis*. Therefore, it is clear that young children can potentially acquire *M. catarrhalis* in various places – day care centers, hospitals and home, for example.

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