Tohoku J. Exp. Med., 2010, **220**, 47-57

Trends in Antimicrobial Susceptibility of *Streptococcus pneumoniae* in the Tohoku District of Japan: A Longitudinal Analysis from 1998 to 2007

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Streptococcus pneumoniae is a common cause of respiratory tract infections (RTIs). The prevalence of *Streptococcus pneumoniae* strains with reduced susceptibility to antimicrobial agents has dramatically increased worldwide. Susceptibility to nine antimicrobial agents and serotypes were determined among 1,644 *Streptococcus pneumoniae* strains isolated from patients with RTIs in the Tohoku district of Japan from October to December every year from 1998 to 2007. The prevalence of penicillin G-nonsusceptible *Streptococcus pneumoniae* (PNSP) strains increased gradually from 48.5% in 1998, reached a statistical peak in 2004 (65.1%) and then decreased to 51.5% in 2007. *Streptococcus pneumoniae* strains with each serotype 3, 6, 19 and 23 were constantly detected, and the distribution of these serotypes in PNSP strains did not significantly change during the study period. A trend of *Streptococcus pneumoniae* strains nonsusceptible to other β-lactams tested was similar to that of PNSP strains, except for cefditoren, to which the resistance rate was < 20% throughout the analysis period. The prevalence of strains nonsusceptible to erythromycin and minocycline were consistently > 60%. Almost all penicillin G-resistant *Streptococcus pneumoniae* (PRSP) strains were resistant to both erythromycin and minocycline throughout the analysis period. The prevalence of strains resistant to fluoroquinolones tested were < 3% over the study period. Our longitudinal surveillance demonstrated for the first time that decreased prevalence of both β-lactam- and multidrug-resistant strains has been occurring since 2004 in a region of Japan. Careful monitoring of antimicrobial susceptibility of *Streptococcus pneumoniae* should be continued.

Keywords: *S. pneumoniae*/susceptibility/penicillin resistance/serotype/multidrug resistance
Tohoku J. Exp. Med., 2010, **220** (1), 47-57.

Received October 23, 2009; revision accepted for publication November 20, 2009. doi:10.1620/tjem.220.47
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Streptococcus pneumoniae (*S. pneumoniae*) is a common cause of community-acquired pneumonia, bronchitis and otitis media, as well as a frequent cause of invasive infections such as bacteremia and meningitis. In the late 1970s, strains of *S. pneumoniae* displaying penicillin resistance were described in South Africa and Spain. Over the past three decades, the prevalence of *S. pneumoniae* strains with reduced susceptibility to penicillin has dramatically increased worldwide. Of greater concern is the emergence of a multidrug-resistant phenotype (i.e., resistant to penicillin and ≥ 2 other non-β-lactam classes, such as macrolides and tetracyclines). Certain β-lactams (ceftriaxone, carbapenem), the respiratory fluoroquinolones and telithromycin are among several antimicrobials that remain effective against drug-resistant *S. pneumoniae* (Hoban et al. 2001; Örtqvist et al. 2005; File 2006). Initial antibacterial therapy should ideally be pathogen-directed, based on culture results and antibacterial sensitivity tests. However, treatment is usually empirical because the necessary results are rarely available at initial diagnosis. Therefore, epidemiological information on changes in local antimicrobial susceptibility patterns is important for choosing antimicrobials for infectious disease treatment, especially those caused by drug-resistant microbes such as *S. pneumoniae*.

In Japan, *S. pneumoniae* strains that were highly resistant to penicillin and other antimicrobial agents appeared during the mid-to-late 1980s. According to a nationwide surveillance study conducted between 1998 and 2000, the prevalence of penicillin-intermediate resistant *S. pneumoniae* (PISP) and penicillin-resistant *S. pneumoniae* (PRSP) were 34.4% and 49.0%, respectively (Ubukata 2003). In parallel, macrolide resistance increased to 70% (Ubukata et al. 2003). Since there was no epidemiological information in the Tohoku district of Japan, the present study was conducted to investigate the ratios of drug-resistant *S. pneumoniae* isolates and their serotypes in this area over a recent 10-year period, from 1998 to 2007.

Materials and methods

S. pneumoniae isolates

A total of 1,644 *S. pneumoniae* isolates were collected at 21 hospitals in the Tohoku district of Japan: 3 hospitals in Aomori, 6 in Akita, 3 in Iwate, 4 in Miyagi, 3 in Yamagata and 2 in Fukushima prefecture, from October to December every year from 1998 to 2007. These hospitals are main institutes for the treatment and prevention of infectious diseases in each prefecture. All of *S. pneumoniae* isolates during the period in the hospitals were subjected to this study. The isolates were obtained from inpatients and outpatients with well-diagnosed respiratory tract infections (acute sinusitis, acute otitis media, acute pharyngitis, acute bronchitis, pneumonia and acute bacterial exacerbations of chronic sinusitis, chronic otitis media and chronic obstructive pulmonary disease). Sources for the isolation were nasal swabs, pharyngeal swabs or aspirates, middle ear fluids, sputum, bronchial lavage and others, including eye swabs and blood. Characteristics of the patients and sources were summarized in Table 1. This study was conducted by the Tohoku Infectious Diseases Study Group.

Table 1. Patient demographics and culture sources of 1644 isolates of *S. pneumoniae* collected.

		1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Isolate No.		132	142	171	163	211	135	189	178	187	136
Gender	male	59	97	109	80	115	79	105	105	99	77
	female	38	45	61	73	82	48	82	73	62	57
	male / female ratio	1.55	2.16	1.79	1.1	1.4	1.65	1.28	1.44	1.6	1.35
	not recorded	35	0	1	10	14	8	2	0	26	2
Age	< 5	35	30	48	49	65	63	88	70	61	44
	5-17	5	6	8	7	13	10	12	16	4	7
	18 - 64	19	25	41	32	47	24	38	28	29	33
	≥ 65	32	73	70	56	66	32	48	62	65	52
	< 5 / 5 ≤ ratio	0.63	0.29	0.4	0.52	0.52	0.95	0.9	0.66	0.62	0.48
	not recorded	41	8	4	19	20	6	3	2	28	0
Patient status	inpatient	54	68	78	69	93	80	115	92	57	70
	outpatient	43	72	93	83	103	47	73	86	96	57
	in / out ratio	1.26	0.94	0.84	0.83	0.9	1.7	1.58	1.07	0.59	1.23
	not recorded	35	1	0	11	15	8	1	0	34	9
Sources	upper airways	36	30	51	24	86	66	105	90	81	60
	lower airways	78	86	99	85	103	41	74	77	83	68
	upper / lower ratio	0.46	0.35	0.52	0.28	0.83	1.61	1.42	1.17	0.98	0.88
	others	18	26	21	54	22	28	10	11	23	8

upper airways ; nasal swabs, pharyngeal swabs and middle ear fluids. lower airways ; sputum and bronchial lavage.
others ; pus, eye swabs, blood and unknown.

Antimicrobial susceptibility testing

Susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) (CLSI 2006) for the microbroth dilution method, using microdilution frozen plates: cation-adjusted Mueller-Hinton broth (CA-MH broth) plus 15 μg/ml nicotinamide, 5 mg/ml yeast extract and 2% lysed horse blood (Eiken Kagaku, Tokyo, Japan). The nine antimicrobial agents that were tested were penicillin G (PCG; Meiji Seika Kaisha, Tokyo, Japan), ampicillin (ABPC; Meiji), cefdinir (CFDN; Astellas Pharma, Tokyo, Japan), cefcapene (CFPN; Shionogi, Tokyo, Japan), cefditoren (CDTR; Meiji), erythromycin (EM; Dainippon Sumitomo Pharma, Tokyo, Japan), minocycline (MINO; Wyeth Pharmaceuticals, Pennsylvania, PA, U.S.A.), levofloxacin (LVFX; Daiichi-Sankyo, Tokyo, Japan) and tosufloxacin (TFLX; Taisyo-Toyama, Tokyo, Japan). *S. pneumoniae* isolates were subcultured twice before performing susceptibility testing. An approximately 0.004-ml aliquot of the organism solution, grown to a turbidity of McFarland number 0.5 and diluted tenfold with saline, was inoculated on the respective CA-MH broth to a final volume of 100 μl (5×10^5 CFU of organism/ml) per well of microplates. In the microplates, a serially diluted test agent was placed in to each well. The microplates were incubated for 20 h at 35°C in ambient air before MICs were visually determined. The accuracy of determination of MICs of antibacterial agents was controlled, using *S. pneumoniae* ATCC49619.

Although CLSI published new breakpoints for penicillin therapy in 2008 (CLSI 2008), the original breakpoints were retained for oral penicillin therapy of nonmeningitis. Accordingly, antimicrobial susceptibility of the isolates in this study was determined by CLSI M100-S17 breakpoints (CLSI 2007). Susceptibility to antibiotics for which breakpoints are not determined by CLSI was determined by Japanese Society of Chemotherapy breakpoints (Saito 1995; Saito et al. 1999). Resistant isolates were defined as those other than susceptible isolates (Table 2).

Serotyping

Serotyping was conducted using the quellung reaction, which was observed microscopically after suspension in pneumococcal diagnostic antisera (Statens Serum Institut, Copenhagen, Denmark) according to the manufacturer's protocol. Serotypes were determined for types 3, 6, 14, 19 (including 19A, 19B, 19C and 19F) and 23 (including 23A, 23B and 23F), since the proportion of these serotypes in Japan has been reported to be comparatively higher than others (Chiba et al. 2005; Oishi et al. 2006; Hotomi et al. 2008).

Statistical Analysis

To evaluate the peak year of the proportion resistant to each medicine, linear spline models that have two or three segments of the time axis were used, where resistant and sensitive were coded as 1 and 0, respectively. We selected the model by AIC (Akaike information criterion). Drug comparisons for the resistant proportion were made using Fisher's exact test. P values < 0.05 were considered to be statistically significant. Statistical analysis was performed using SAS ver. 9 (SAS Institute Inc., NC, U.S.A.).

Results

Susceptibility to penicillin G

The prevalence of PCG-resistant *S. pneumoniae* (PRSP; MIC of PCG ≥ 2 μg/ml) isolates increased gradually, reaching a statistical peak in 2003 (35.6%) in the estimate of the selected model, then decreasing to 12.5% in 2007. The prevalence of *S. pneumoniae* isolates exhibiting intermediate susceptibility to PCG (PISP; 0.125 μg/ml ≤ MIC of PCG ≤ 1 μg/ml) increased from 28.0% in 1998 to 40.9% in 2000 and was statistically stable thereafter (33.1-43.9%). The prevalence of PCG-nonsusceptible *S. pneumoniae* (PNSP = PRSP+PISP; MIC of PCG ≥ 0.125 μg/ml) isolates increased gradually from 48.5% in 1998, reaching a statistical peak in 2004 (65.1%) in the estimate of the selected model, then decreasing to 51.5% in 2007 (Fig. 1). Accordingly, the increasing rate of PNSP isolates until 2003 was attributable to those of PRSP and PISP isolates, and the decreasing rate of PNSP isolates after 2004 was attributable to that of PRSP isolates.

There was no significant difference in the male/female patient ratio between the periods up to and after 2004 (Table

Table 2. Interpretative categories for *S. pneumoniae*.

Antimicrobial agents	Interpretative categories		
	Susceptible	Nonsusceptible	
		Intermediate	Resistant
Penicillin G*	≤ 0.06[+]	0.125 - 1	≥ 2
Ampicillin[#]	≤ 0.125		≥ 0.25
Cefdinir*	≤ 0.5	1	≥ 2
Cefcapene[#]	≤ 0.25		≥ 0.5
Cefditoren[#]	≤ 0.5		≥ 1
Erythromycin*	≤ 0.25	0.5	≥ 1
Minocycline[#]	≤ 1		≥ 2
Levofloxacin*	≤ 2	4	≥ 8
Tosufloxacin[#]	≤ 1		≥ 2

*Susceptible, Intermediate and Resistant based on Clinical and Laboratory Standards Institute (CLSI) definitions. [#]Susceptible and Resistant based on the criteria recommended by Japanese Society of Chemotherapy. [+]μg/ml

1). The prevalence of PNSP isolates from either male or female patients reached a statistical peak in 2003 (70.9% and 66.7%, respectively) in the estimate of the selected model (Fig. 2). The factor of gender did not influence the change in prevalence of PNSP isolates because statistically similar change patterns were estimated for males and females.

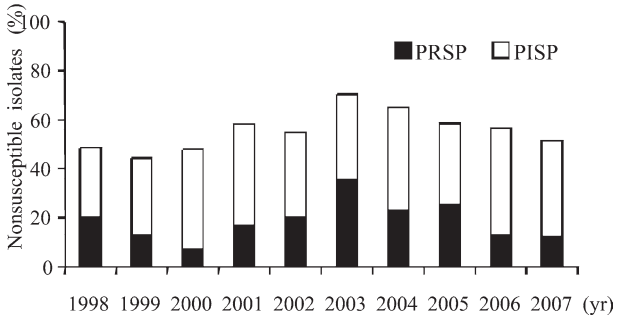

Fig. 1. The prevalence of *S. pneumoniae* isolates nonsusceptible to penicillin G. PRSP: resistant isolates, PISP: intermediate isolates.

Since patients less than 5 years old and over 65 years old accounted for more than 60% of the isolates every year, we investigated the influence of patient age in two groups, patients less than 5 years old and all other patients. There was no significant difference in the ratio of the two patient groups between the periods up to and after 2004 (Table 1). Until 2004, different change patterns were estimated between the two patient groups. The prevalence of PNSP isolates from the patients less than 5 years old was statistically consistent from 1998 (71.5%) to 2004 (70.5%). In contrast, in patients over 5 years old, the prevalence of PNSP isolates increased gradually from 37.5% in 1998 to a statistical peak in 2004 of 61.2% in the estimate of the selected model. The prevalence of PNSP isolates from both patient groups decreased after 2004 (Fig. 3). After 2004, the factor of age did not influence the change in prevalence of PNSP isolates because statistically similar change patterns were estimated for patients less than 5 years old and patients over 5 years old.

There was no significant difference in the inpatient/outpatient ratio between the periods up to and after 2004

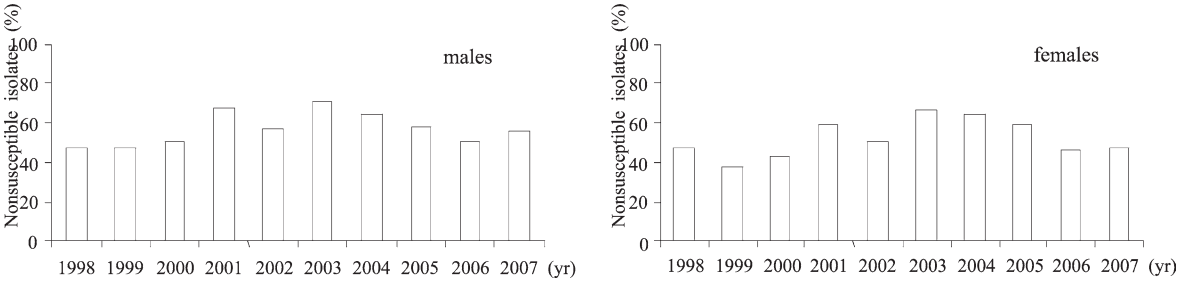

Fig. 2. The prevalence of *S. pneumoniae* isolates nonsusceptible to penicillin G in male and female patients.

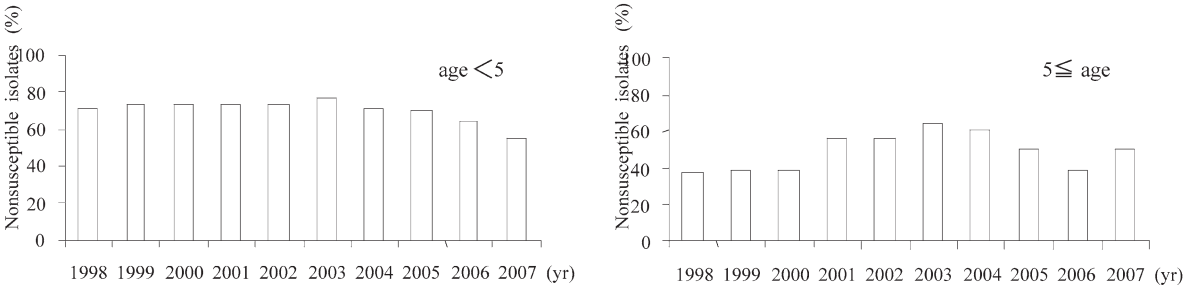

Fig. 3. The prevalence of *S. pneumoniae* isolates nonsusceptible to penicillin G in patients of two age groups.

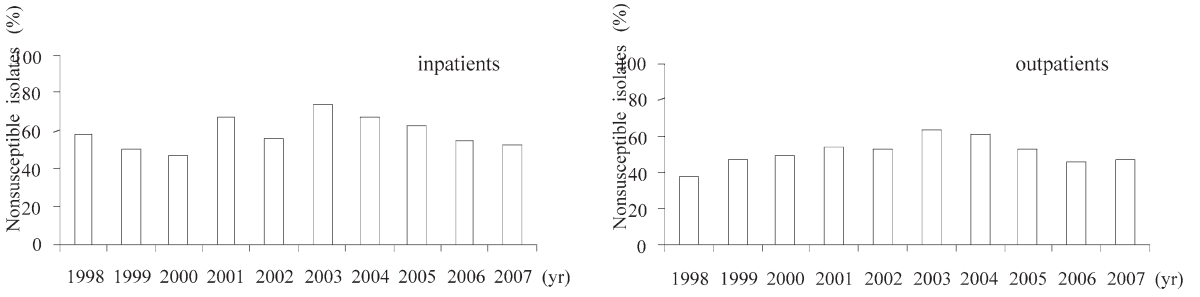

Fig. 4. The prevalence of *S. pneumoniae* isolates nonsusceptible to penicillin G in inpatients and outpatients.

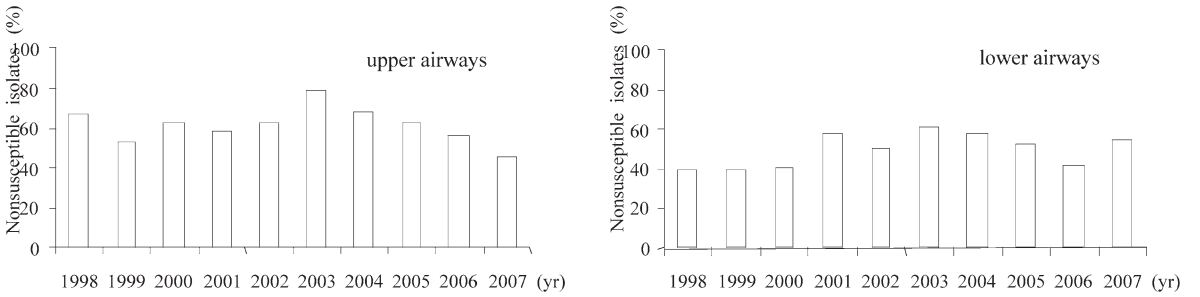

Fig. 5. The prevalence of *S. pneumoniae* isolates nonsusceptible to penicillin G sampled from upper and lower airways.

Table 3. Serotype distribution of *S. pneumoniae* isolates.

	2001	2002	2003	2004	2005	2006	2007
Serotype							
3	0 / 32*	0 / 44	0 / 13	0 / 25	0 / 22	1 / 32	0 / 17
6	30 / 38	26 / 37	26 / 33	24 / 37	26 / 39	37 / 49	14 / 26
14	4 / 6	1 / 1	8 / 8	8 / 13	5 / 10	6 / 12	2 / 5
19	34 / 39	41 / 48	21 / 22	38 / 42	41 / 45	26 / 29	24 / 28
23	8 / 9	29 / 30	21 / 22	36 / 36	21 / 22	22 / 22	19 / 20
Others	19 / 39	19 / 51	19 / 37	17 / 36	11 / 40	14 / 43	11 / 40
Total No.	95 / 163	116 / 211	95 / 135	123 / 189	104 / 178	106 / 187	70 / 136

*No. of penicillin G -nonsusceptible isolates / No. of all isolates

(Table 1). The prevalence of PNSP isolates from either inpatients or outpatients reached a statistical peak in 2003 (73.8% or 63.8%, respectively) in the estimate of the selected model (Fig. 4). The factor of the patient status did not influence the change in prevalence of PNSP isolates because statistically similar change patterns were estimated for inpatients and outpatients.

The ratio of samples from upper and lower airways up to 2004 was significantly lower than the ratio after 2004 ($p < 0.01$) (Table 1). The prevalence of PNSP isolates from either upper or lower airways reached a statistical peak in 2004 (78.8% or 61.0%, respectively) in the estimate of the selected model (Fig. 5). The factor of sample source did not influence the change in prevalence of PNSP isolates because statistically similar change patterns were estimated for upper and lower airways.

The serotypes of 1,199 *S. pneumoniae* isolates during the period from 2001 to 2007 were studied (Table 3). The distribution of 6 serotypes in PNSP isolates was not statistically different between years; when comparing all isolates, the distribution was significantly different (Chi-square test, $p < 0.01$). There were no significant peaks or trends in the prevalence of PNSP isolates in serotypes 3, 6, 19 and 23 in the estimate of the selected model (Fig. 6). The isolate number for serotype 14 was too small to be evaluated statistically for a peak and trend of the prevalence of PNSP isolates.

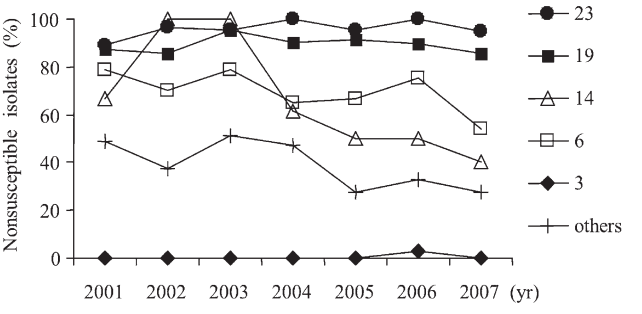

Fig. 6. The prevalence of *S. pneumoniae* isolates nonsusceptible to penicillin G in each serotype.

Susceptibility to other antimicrobial agents

The prevalence of *S. pneumoniae* isolates nonsusceptible to other antimicrobials during the period studied was shown in Fig. 7. The prevalence of *S. pneumoniae* isolates nonsusceptible to other β-lactam antibiotics, similar to PCG, reached a statistical peak in 2003 for ABPC (64.4%) and in 2004 for CFDN (60.8%) and CFPN (65.6%) in the estimate of the selected model. There were no significant differences in the prevalence of nonsusceptible isolates between PCG, ABPC, CFDN and CFPN at any time point. The prevalence of isolates nonsusceptible to CDTR was statistically consistent during the period (4.9-25.8%) and significantly lower than those of any other β-lactam tested at any time point. Although the statistical peak of prevalence was in 2004 (84.1%) for MINO and 2005 (86.5%) for EM in the estimate of the selected model, the prevalence of isolates nonsusceptible to these agents was always greater than

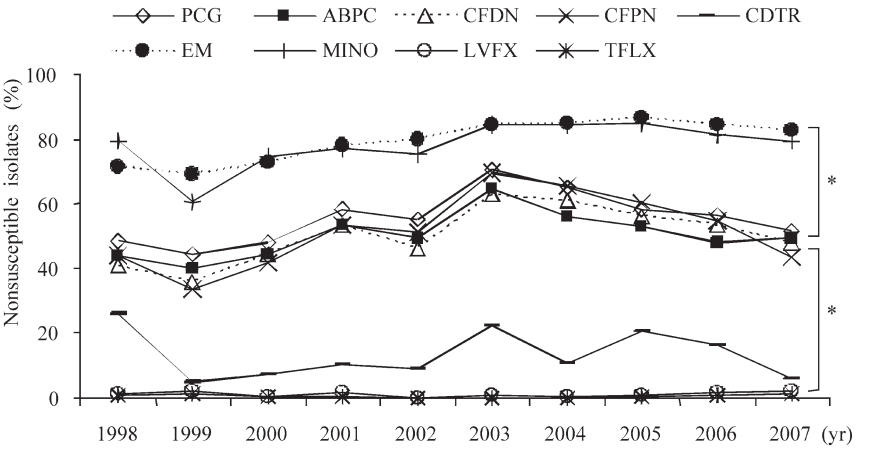

Fig. 7. Antibiotic -nonsusceptible trends for S. *pneumoniae*. *$P < 0.05$

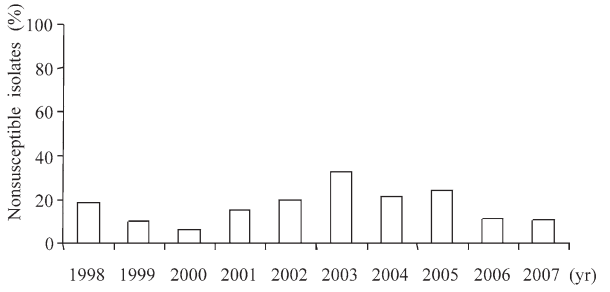

Fig. 8. The prevalence of *S. pneumoniae* isolates resistant to penicillin G, erythromycin and minocycline.

60% during the period studied. There was no significant difference in the prevalence of nonsusceptible isolates between MINO and EM at any time point, and the prevalence of isolates nonsusceptible to these antimicrobials was always higher than for any other antibiotics tested during the study period. As shown in Fig. 8, the prevalence of isolates exhibiting resistance to PCG, EM and MINO decreased from 18.9% in 1998 to 6.4% in 2000, after which time the prevalence increased and reached 32.6% in 2003. Thereafter, it decreased to 10.3% in 2007. The proportion of PRSP strains that were resistant to both EM and MINO ranged from 77.8% to 97.7%. The prevalence of isolates nonsusceptible to LVFX and TFLX was less than 3% throughout the study period and was lower than any other antimicrobials tested throughout the period except for 1999 (2.1% and 1.4%, respectively) and 2007 (2.2% and 1.5%, respectively), when the prevalence was similar to that of CDTR-resistant isolates (4.9% and 5.9%, respectively) (Fig. 7). The MIC_{90}s of the antibacterial agents did not remarkably change during the period (Table 4). Among the β-lactams, PCG, ABPC and CFDN showed high MIC_{90} values (2- > 4 μg/ml), while the MIC_{90}s of CFPN and CDTR were relatively low (0.5-1 μg/ml). On the other hand, EM and MINO were substantially less active with MIC_{90} values of 8- > 16 μg/ml. With regard to the fluoroquinolones, LVFX showed moderate activity (MIC_{90}s of 1-2 μg/ml), while TFLX was very active with MIC_{90} values of 0.25-0.5 μg/ml.

Discussion

We have analyzed a longitudinal data set over the 10-year period from 1998 to 2007 for *S. pneumoniae* isolates sampled from the Tohoku district of Japan. Although the region, the number of strains and the antimicrobial agents examined were limited, the isolates were collected from the same 21 hospitals throughout the district during the entire study period. This led to the generation of valuable information regarding geographic changes in antibacterial resistance patterns; an increasing prevalence of *S. pneumoniae* isolates nonsusceptible to β-lactam antibiotics declined after 2004, and CDTR-susceptibility of the isolates remained higher than the susceptibilities to other β-lactams examined throughout the study period. It was statistically confirmed that the change in prevalence of PNSP isolates was not attributable to the gender or age of the patients, patient status (inpatients or outpatients) or source of bacterial isolation.

The prevalence of *S. pneumoniae* strains resistant to β-lactams has increased around the world since the 1980s (Hoban et al. 2001; Örtqvist et al. 2005; File 2006). In Japan, several studies have shown an increasing prevalence of β-lactam- and macrolide-resistant strains (Rikitomi et al. 1996; Song et al. 1999; Watanabe et al. 2000; Ubukata 2003; Ubukata et al. 2003). A longitudinal analysis from 1994 to 2002 demonstrated that the prevalence rate of PNSP and PRSP strains was 45.4-51.0% and 4.9-13.6%, respectively (Yamaguchi and Ohno 2005). A recent nationwide study by Oishi K., et al., which included 2 hospitals in the Tohoku district, reported that the prevalence of PNSP and PRSP strains isolated from adult patients with community-acquired pneumonia was 57.9% and 22.8%, respectively, between 2001 and 2003 in Japan (Oishi et al. 2006). A longitudinal surveillance study, Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin

Table 4. Trends in MIC_{90} against *S. pneumoniae* isolates.

Antibacterial agents	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Penicillin G	2*	2	1	2	2	2	2	2	2	2
Ampicillin	2	2	1	2	2	4	2	4	2	2
Cefdinir	> 4	4	4	4	4	> 4	4	> 4	4	4
Cefcapene	1	1	1	1	1	1	1	1	2	1
Cefditoren	1	0.5	0.5	1	0.5	1	1	1	1	0.5
Erythromycin	> 16	> 16	> 16	> 16	> 16	> 16	> 16	> 16	> 16	> 16
Minocycline	> 8	> 8	> 8	> 8	8	> 8	> 8	> 8	8	8
Levofloxacin	2	2	1	2	1	2	1	1	1	1
Tosufloxacin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.25

*MIC_{90} μg/ml

(PROTEKT) study, collected pathogens from adult and pediatric patients with respiratory tract infections throughout Japan, including 1 hospital in the Tohoku district, during the period from 1999 to 2004 (Inoue et al. 2008). In the study, PNSP and PRSP isolates were consistently found in 62.8% (54.9%-67.6%) and 37.0% (30.9%-44.5%), respectively, of isolates. These nationwide studies have shown trends of increases in both PRSP and PISP strains during the period from 1998 to 2004 in Japan. The trend of the prevalence rate of PRSP and PISP in the Tohoku district was similar to the national trend during the same period. The prevalence of PNSP/PRSP strains in other local areas in Japan, for example, 44.9%/10.2% in Nagasaki (2001-2002) (Hirakata et al. 2005), 58.0%/8.0% in Sapporo (2001-2004) (Harimaya et al. 2006) and 62.0-70.0%/10.0-26.0% in Tokyo (2002-2004) (Noguchi et al. 2007), was similar to the prevalence in the Tohoku district, although there were differences between regions. Our study pointed out a trend of decreasing resistance to penicillin after 2004 in this district. However, nationwide surveillance following those studies has not been reported. In 2006, a nationwide surveillance of bacterial respiratory pathogens was started by the Japanese Society of Chemotherapy (JSC) to build up a set of controls for future surveillance studies. The prevalence of PNSP and PRSP isolates was 39.0% and 4.0% in 2006 (Niki et al. 2008) and 35.4% and 5.1% in 2007 (Niki et al. 2009). The incidence of PRSP in 2006 (12.8%) and 2007 (12.5%) in our study was higher than that found in the JSC study. The prevalence of PRSP strains isolated from pediatric infections has been reported to be higher (16.9-49.0%) than the prevalence found in adult infections (Karlowsky et al. 2003; Chiba et al. 2005). Therefore, the discrepancy in the incidence of PRSP between the JSC and the current study might be due to the fact that pediatric patients were included in the current study, but were excluded from the JSC study.

The prevalence of resistance to commonly used antimicrobial agents in *S. pneumoniae* isolates in the Tohoku district as well in Japan and Far East countries is higher than in European and North/South American countries (Felmingham et al. 2007). The Tracking Resistance in the United States Today (TRUST) study demonstrated that an increase of prevalence of PNSP strains from 33.1% (1999) to 35.6% (2001) was due to the increased frequency of PRSP strains but not of PISP strains, which was stable at approximately 18% (Karchmer 2004). Another US nationwide surveillance study indicated the same trend in PRSP and PISP strains (Doern et al. 2001). After 2003, penicillin-resistance trends in the US have changed, as indicated in the PROTEKT study and other reports (Doern et al. 2005; Jenkins et al. 2008; Richter et al. 2009). The prevalence of PRSP strains appears to be stable or decreasing, from 21.5% (2000) to 14.6% (2005), while PISP has increased from 12.7% (2000) to 17.9% (2005). In 2000, a pediatric 7-valent pneumococcal conjugate vaccination (PCV-7) was introduced in many countries except in Japan, targeting major PRSP serotypes, which has been thought to have direct and herding effects on the changes in penicillin resistance patterns among children and adults. However, the vaccine does not cover all *S. pneumoniae* serotypes, so the increasing rate of nonvaccine serotypes such as 19A is an ongoing concern (Kyaw et al. 2006); this may also be the case for the increase in PISP strains. In Europe, the European Antimicrobial Resistance Surveillance System (EARSS) has monitored the prevalence of antimicrobial resistance of invasive *S. pneumoniae* infections since 1999 by connecting national surveillance systems. Data for isolates collected over a five-year period (1999-2004) showed that the prevalence of PNSP strains, ranging from < 5% to > 40%, was higher in southern and central than in northern European countries (Bruinsma et al. 2004); the prevalence decreased in Belgium (13% to 9%), Ireland (19% to 10%), UK (7% to 3%) and Spain (33% to 29%) during this time. This decrease is thought to be due to the use of PCV-7 vaccination programs (Vallès et al. 2006).

In Japan, while PCV-7 has been recently licensed, a 23-valent pneumococcal polysaccharide vaccine (23-valent PPV) was introduced in 1988. The 23-valent PPV has been reported to cover 82-83% of serotypes of isolates (Oishi et al. 2006; Hotomi et al. 2008). Although rarely used up to 2001, the use of the vaccine increased dramatically after 2002 (by more than 150 times), resulting in a rate of vac-

cine coverage that reached 2% in adults over 65 in 2006 (Oishi et al. 2006). This increased use of 23-valent PPV might contribute to the decreasing prevalence of PNSP, especially PRSP, isolates observed after 2004 in the current study. Serotypes such as 23A and 6A, found frequently in PISP but not PRSP (Chiba et al. 2005), are not covered by 23-valent PPV. These nonvaccine serotypes of PISP isolates could possibly increase in prevalence, leading to the stable prevalence of PISP strains after 2004 found in our study. In the current study, serotypes 3, 6, 19 and 23, which were found in 15.4%, 21.6%, 21.1% and 13.4%, respectively, of isolates overall, were the most common serotypes, in accordance with previously reported data from nationwide studies in Japan (Chiba et al. 2005; Oishi et al. 2006; Hotomi et al. 2008). The distribution of 6 serotypes investigated in PNSP isolates was not significantly different between years. Furthermore, there was no significant trend in the proportion of PNSP isolates for each serotype. Therefore, the trend in PNSP prevalence observed in the Tohoku district is not a serotype-specific phenomenon. Further examination would be needed to determine subtypes of serotypes for isolates, especially for non-23-valent PPV serotypes.

Inappropriate use of antimicrobial drugs is increasingly being recognized as a major risk factor for the development of antimicrobial resistance (Niederman 2005). In Europe, studies combining data from the European Surveillance of Antimicrobial Consumption and EARSS showed that there was a high degree of consistency between β-lactam use and the rate of resistance in *S. pneumoniae* isolates. Rates of antibiotic resistance that were higher in southern and central Europe than in northern Europe were shown to be related to higher consumption of antibiotics in southern and central Europe (Bronzwaer et al. 2002; Goossens et al. 2005; Sande-Bruinsma et al. 2008). In Japan, no database for antimicrobial prescriptions at the national or regional level is currently available. Therefore, it is of interest and importance to investigate the relationship between the prescribing rate of β-lactams and the prevalence rate of PNSP in the Tohoku district. Increasing usage of respiratory quinolones and 23-valent PPV, as mentioned above, may reduce β-lactam use, resulting in the decline of PNSP after 2004 in the Tohoku district, as suggested by other reports (Jenkins et al. 2008; Cohen 2009). The introduction of guidelines for community-acquired pneumonia by the American Thoracic Society (ATS) (Niederman et al. 2001) and the Infectious Diseases Society of America (IDSA) (Bartlett et al. 2000) has been thought to contribute to the decrease in resistance rates of bacterial pathogens, including *S. pneumoniae*, by promoting appropriate prescribing of antibacterials (Jenkins et al. 2008). In Japan, "the Japanese Respiratory Society guidelines for the management of community-acquired pneumonia in adults" was issued in 2000 (The Japanese Respiratory Society 2000), as well as "the Japanese Respiratory Society guidelines for the management of respiratory tract infection" in 2003 (The Japanese Respiratory Society 2003). Using antibiotics appropriately by following these guidelines may exert downward pressure on resistance rates in the Tohoku district. The prevalence of *S. pneumoniae* isolates nonsusceptible to ABPC, CFDN and CFPN reached a peak in 2003-2004, similar to PCG. Inappropriate and/or increasing use of the antimicrobial agents must be leading to the increase of the prevalence rate of the nonsusceptible isolates from 1998. As discussed above, the decrease of these β-lactams-resistant isolates might be due to the decreased use of these agents resulting from the decrease of patients with *S. pneumoniae* infections in association with the increased use of 23-valent PPV and to appropriate use of these agents following the two guidelines for respiratory tract infections in Japan.

CDTR has sustained strong antibacterial activity against clinical isolates; this activity was statistically higher than for the other β-lactams tested throughout the 10-year period. The superior potency of CDTR compared to other oral β-lactams has been demonstrated by other studies (Biedenbach and Jones 2009). Although the detailed reason is not clear, there is a clear difference in binding affinity toward penicillin-binding proteins (PBPs), which β-lactam antibiotics target. This difference can be attributed to their chemical structures, namely the substitutive effect at the C-3 position. CFDN and CFPN have a small and neutral substitute, a vinyl group and a carbamoyloxymethyl group, respectively, while CDTR possesses a bulky and basic (*Z*)-2-(4-methylthiazol-5-yl)ethenyl group. An analysis of crystal structure of CDTR combined with *S. pneumoniae* PBP 2X, which is frequently associated with cephalosporin resistance in *S. pneumoniae* (Zapun et al. 2008), revealed properties related to the ability of the bulkiness and basicity of the C-3 substitute to strengthen the binding affinity of CDTR toward PBP (Yamada et al. 2007). Our findings suggest the development of β-lactams with structures that are able to maintain and enhance antibacterial activity. Investigation of the alterations of PBPs in the isolates is also needed. Breakpoints for antimicrobial agents based on in vivo pharmacokinetic (PK) and pharmacodynamic (PD) data have been shown to correlate with clinical and bacteriological success. PK/PD breakpoints (μg/ml) determined by serum concentrations present for 40 to 50% of the dosing interval for time-dependent agents such as β-lactams are reported to be 0.50-0.56 for CFDN, 0.36 for CFPN and 0.25-0.31 for CDTR. Based on the percentage of strains sensitive at PK/PD breakpoints, CDTR (58.5-66.7%) was relatively more active than CFDN (49.1-58.9%) and CFPN (41.5%) (Peric et al. 2003; Nakamura and Takahashi 2004; Anzueto et al. 2007). The percentages of our strains sensitive at PK/PD breakpoints for CFDN (0.50 μg/ml), CFPN (0.36 μg/ml) and CDTR (0.25 μg/ml) were 37.0-64.1, 11.9-39.2 and 41.5-74.3%, respectively. These data suggest the advantage of CDTR also in vivo situation among oral cephems.

The prevalence of *S. pneumoniae* isolates resistant to EM and MINO in the Tohoku district, which was 60% or

more throughout the study period, was similar to that seen in nationwide studies (Song et al. 1999; Watanabe et al. 2000; Yamaguchi and Ohno 2005; Oishi et al. 2006). Since almost all of the PRSP strains were resistant to both EM and MINO, the trend of multidrug-resistant strains (Fig. 8) could be affected by that of PRSP strains (Fig. 1). Although the prevalence rate of macrolide resistance in the US (25-30%) (Doern et al. 2001; Karchmer 2004; Doern et al. 2005; Jenkins et al. 2008; Richter et al. 2009) and European countries (3-42%) (Sande-Bruinsma et al. 2008) was much lower than in Japan, it has been increasing, leading to an increase in multidrug resistance. The prevalence rates of the isolates nonsusceptible to LVFX and TFLX in the Tohoku district were less than 3% throughout the study period, similar to those in previous reports in Japan (Inoue et al. 2008; Niki et al. 2008, 2009) and slightly higher than those in the US (roughly 1%) (Jenkins et al. 2008; Richter et al. 2009).

In summary, our longitudinal surveillance of *S. pneumoniae* susceptibility in the Tohoku district over a ten-year period demonstrated, for the first time, that a decreasing prevalence of β-lactam and multidrug-resistant strains has been occurring since 2004 in Japan. This epidemiological information would be useful in selecting antimicrobials for the treatment of infectious diseases caused by *S. pneumoniae* in this region. It is of clinical importance to discover the reasons for the improvement in antibiotic sensitivity of *S. pneumoniae*, especially with regard to the correlation with the prescribing frequency of antibacterial drugs. Careful monitoring of not only the prevalence rates of antibiotic resistance but also the trend of serotypes among *S. pneumoniae* should be continued, because it is possible that increased use of 23-valent PPV and PCV-7 would lead to an increase in disease caused by nonvaccine types (replacement disease), which is a growing concern in Japan.

Acknowledgments

This work was partially supported by grants from the Shimabara Science Promotion Foundation and from Miyagi Organization for Industry Promotion. We thank all of the staff in the participating institutions of this study: Department of Laboratory Medicine, Hirosaki University Graduate School of Medicine; Kuroishi City Hospital; Aomori Prefecture Central Hospital; Akita Kumiai General Hospital; Department of Clinical and Laboratory Medicine, Akita University Hospital; Hiraka General Hospital; Nakadori General Hospital; Yuri Kumiai General Hospital; Ogachi Central Hospital; Department of Laboratory Medicine, Iwate Medical University School of Medicine; Morioka Japanese Red Cross Society Hospital; National Hospital Organization Iwate Hospital; Osaki Citizen Hospital; Tome City Sanuma Hospital; Sendai Kousei Hospital; Department of Infection Control and Laboratory Diagnostics, Internal Medicine, Tohoku University Graduate School of Medicine; Yamagata Prefecture Central Hospital; Yamagata Saisei Hospital; Department of Clinical Laboratories, Yamagata University Hospital; Iwaki Kyouritsu Hospital; Fukushima Prefecture Aizu General Hospital. Members of the Tohoku Infectious Diseases Study Group are MY, JC, AS, YS, CT, HT, SI, MK, AW, TN, KN, KK, and IO.

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