Title:

The prevalence and antimicrobial susceptibility of *Streptococcus pneumoniae* isolated from patients at Jikei University Hospitals after the implementation of the pneumococcal vaccination program in Japan

Authors:

Takashi Ando [a,b], Takahiro Masaki [a,c,*], Midori Kono [a], Yuko Nagano [d], Kazumi Sakamoto [e], Taku Tamura [c], Masaki Abe [b], Masato Matsushima [f], Koji Nakada [a,b], Tomokazu Matsuura [a,c]

Affiliations:

[a] Department of Laboratory Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan

[b] Department of Clinical Laboratory, Jikei University Daisan Hospital, 4-11-1 Izumihoncho, Komae-shi, Tokyo 201-8601, Japan

[c] Department of Central Clinical Laboratory, Jikei University Hospital, 3-19-18 Nishi-shimbashi, Minato-ku, Tokyo 105-8471, Japan

[d] Department of Clinical Laboratory, Jikei University Kashiwa Hospital, 163-1 Kashiwashita, Kashiwa-shi, Chiba 277-8567, Japan

[e] Department of Clinical Laboratory, Jikei University Katsushika Medical Center, 6-41-2 Aoto, Katsushika-ku, Tokyo 125-8506, Japan

[f] Division of Clinical Epidemiology, Research Center for Medical Sciences, The Jikei University School of Medicine, 3-25-8 Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan

Corresponding author:

*Takahiro Masaki, M.D., Ph.D.

Department of Laboratory Medicine, The Jikei University School of Medicine

3-25-8 Nishi-shimbashi, Minato-ku, Tokyo 105-8461, Japan

Tel: +81-3-3433-1111 (ext. 2291)

Fax: +81-3-5401-0467

E-mail: tmasaki@jikei.ac.jp

Authorship statement:

All authors meet the ICMJE authorship criteria.

Conflict of interest:

None

Abbreviations:

invasive pneumococcal disease, IPD; 7-valent pneumococcal conjugate vaccine, PCV7; 13-valent pneumococcal conjugate vaccine, PCV13; 23-valent pneumococcal polysaccharide vaccine, PPSV23; minimum inhibitory concentration, MIC; penicillin G, PCG; cefotaxime, CTX; ceftriaxone, CTRX; cefepime, CFPM; meropenem, MEPM; erythromycin, EM; vancomycin, VCM; levofloxacin, LVFX

Abstract

Studies have shown that pneumococcal vaccination reduces the incidence of *Streptococcus pneumoniae* infections but does not change the prevalence of *S. pneumoniae* nasopharyngeal colonization. To comprehensively and longitudinally assess the epidemiology of *S. pneumoniae* after the introduction of pneumococcal vaccination, we monitored the prevalence and antimicrobial susceptibility of *S. pneumoniae*, irrespective of its serotypes or pathogenicity, by analyzing specimens collected from a large number of patients at Jikei University Hospitals from 2009 to 2017. A total of 5,763 *S. pneumoniae* isolates were identified out of 375,435 specimens from various sources of patients in different age groups. The prevalence of *S. pneumoniae* isolated only from patients <5 years old was significantly reduced with the widespread use of pneumococcal vaccines, although this reduction differed by areas where patients resided. The incidence of pneumococcal infections, including bacteremia and otitis media, clearly decreased among patients <5 years old after the introduction of pneumococcal vaccination, while the prevalence of *S. pneumoniae* isolated from blood specimens of patients 15-64 years old increased, suggesting the involvement of non-vaccine serotypes in the incidence of invasive pneumococcal infections. The antimicrobial susceptibility of *S. pneumoniae* improved after the introduction of pneumococcal vaccination. Our results show that pneumococcal vaccination has a suppressive effect on the prevalence of *S. pneumoniae* and the incidence of pneumococcal infections, at least for children <5 years old, in association with an improvement in the antimicrobial susceptibility of *S. pneumoniae*. However, further measures will be needed to control invasive pneumococcal infections caused by non-vaccine serotypes.

Keywords

Streptococcus pneumoniae; vaccination; PCV; PPSV; IPD; antimicrobial susceptibility

Streptococcus pneumoniae is a facultative anaerobic Gram-positive diplococci that causes pneumonia, otitis media, and occasionally invasive pneumococcal diseases (IPDs), such as meningitis and bacteremia, in children and adults [1-3]. Approximately 14.5 million people worldwide develop pneumococcal disease annually, and 800,000 children <5 years old die [1]. *S. pneumoniae* also asymptomatically colonizes the upper respiratory tract mucosa of healthy individuals. Approximately 40%-60% of infants and 3%-5% of adults are reportedly asymptomatic carriers and play an important role in the transmission of the bacteria in the community [4, 5].

S. pneumoniae has a capsule, and the capsular type is called the serotype. There are currently 97 serotypes [2]. The pneumococcal vaccine is made based on the capsular polysaccharide and used to prevent infection of *S. pneumoniae* with the serotypes contained in the vaccine [3, 6]. In November 2010, the pneumococcal vaccination program started in Japan. A public subsidy program for the vaccination expanded in many local governments, and immunization with a 7-valent pneumococcal conjugate vaccine (PCV7) for children <5 years old was implemented nationwide. In April 2013, PCV7 was adopted as a routine vaccine for all infants before being replaced with a 13-valent pneumococcal conjugate vaccine (PCV13) in November 2013. In addition, routine immunization with a 23-valent pneumococcal polysaccharide vaccine (PPSV23) started for elderly people (≥65 years old) in October 2014. The burden of pneumococcal infections decreased significantly after the introduction of PCV7 to children [6]. However, a relative increase in the incidence of IPD caused by non-vaccine serotype strains has been reported following the implementation of the vaccination program, which might provoke controversy concerning the benefit of vaccination in the control of pneumococcal infections [2, 6].

Jikei University manages four regional core hospitals in and around the Tokyo metropolitan area, making it possible to perform large-scale epidemiological studies using these facilities. To assess the impact of pneumococcal vaccination on the epidemiology of *S. pneumoniae*, we analyzed a large

number of patient specimens at Jikei University Hospitals and comprehensively and longitudinally investigated the prevalence and antimicrobial susceptibility of *S. pneumoniae*, regardless of serotype or pathogenicity, after the introduction of pneumococcal vaccination.

A total of 375,435 specimens were collected from various sources of patients at four Jikei University Hospitals (Main Hospital, Katsushika Medical Center, Daisan Hospital, and Kashiwa Hospital) from 2009 to 2017 (Supplementary Table S1). Among them, more than a third of the specimens were collected at the Main Hospital. The details of the number of specimens from patients in each age group was as follows: 49,032 specimens (13.1%) from patients <5 years old, 12,645 (3.4%) from patients 5-14 years old, 153,714 (41.0%) from patients 15-64 years old, and 160,044 (42.6%) from patients ≥65 years old. A total of 5,763 *S. pneumoniae* isolates were identified from the specimens, and the overall isolation rate was 1.54% (Supplementary Table S1). The largest number of *S. pneumoniae* isolates were collected from patients <5 years old (3,372 isolates [58.5%]), followed by patients ≥65 years old (1,077 [18.7%]), patients 15-64 years old (714 [12.4%]), and patients 5-14 years old (600 [10.4%]). *S. pneumoniae* was isolated more frequently from patients at the Katsushika Medical Center and the Daisan Hospital, especially from patients <5 years old, than from those at other locations. In contrast, markedly fewer *S. pneumoniae* specimens were isolated from patients at the Main Hospital than from other sites, despite the largest number of specimens being collected from the Main Hospital.

Regarding the sources of specimens from which *S. pneumoniae* was isolated, nasopharyngeal mucus was the most frequent specimen (3,854 specimens [66.9%]), followed by sputum (1,394 [24.2%]), and blood (159 [2.8%]) (Supplementary Table S2). The majority of nasopharyngeal mucus specimens was collected from patients <5 years old (3,108 specimens [80.6%]). Isolation of *S. pneumoniae* from sputa, bronchial lavage fluids, and blood was more frequent in patients ≥65 years old (849 [60.9%], 35 [76.1%], and 85 isolates [53.5%], respectively) than in any other patient group, while

the frequency of *S. pneumoniae* isolated from otorrhea or middle ear fluids was the highest in patients <5 years old (69 isolates [46.9%]).

To assess the impact of pneumococcal vaccination on the prevalence of *S. pneumoniae*, we examined the frequency of *S. pneumoniae* isolated from patients in different age groups after the introduction of the routine PCV7 vaccination (Fig. 1). In 2009 to 2010 (prior to the widespread introduction of PCV7 vaccination), the isolation rates of *S. pneumoniae* were 8.98% in patients <5 years old, 4.91% in patients 5-14 years old, 0.52% in patients 15-64 years old, and 0.72% in patients ≥65 years old. In patients <5 years old, the number and frequency of *S. pneumoniae* isolates gradually but significantly decreased after 2011 and reached its nadir (270 isolates and 5.27%) in 2014 (Fig. 1A). In contrast, the number and frequency of *S. pneumoniae* isolated from patients in the other age groups (≥5 years old) temporarily decreased in 2012 and 2013 but increased thereafter (Fig. 1B-D).

Because the suppressive effect of pneumococcal vaccination was clearly observed on the prevalence of *S. pneumoniae* isolated from patients <5 years old, we next focused on this patient group and examined the prevalence of *S. pneumoniae* at each of four Jikei University Hospitals after the introduction of the pneumococcal vaccination (Supplementary Fig. S1). At the Katsushika Medical Center and the Daisan Hospital, both of which are general hospitals and located in the suburbs of Tokyo, the prevalence of *S. pneumoniae* had significantly decreased since 2011 (after the widespread introduction of PCV7 vaccination) (Supplementary Fig. S1B, C). At the Kashiwa Hospital, which is a regional core hospital located in Chiba Prefecture, adjacent to Tokyo, this significant decline was slightly delayed and first observed in 2014 (after the introduction of routine PCV7 vaccination for children and replacement with PCV13) (Supplementary Fig. S1D). At the Main Hospital, which is located in the business district of Tokyo and provides advanced medical treatment to patients with a severe condition, there was no obvious change in the pneumococcal prevalence throughout the

observation period (Supplementary Fig. S1A).

Taken together, these findings demonstrate the suppressive impact of pneumococcal vaccination on the prevalence of *S. pneumoniae* isolated from patients <5 years old but not on that isolated from patients ≥5 years old. However, even in patients <5 years old, this suppressive impact was affected by differences in areas where patients resided or patients' underlying conditions.

We subsequently examined the sources of specimens positive for *S. pneumoniae* collected from patients in different age groups after the introduction of pneumococcal vaccination (Fig. 2). In 2009 and 2010, the majority (over 90%) of positive specimens collected from patients <15 years old was nasopharyngeal mucus (Fig. 2A, B), while in the patients ≥15 years old, *S. pneumoniae* was isolated most frequently from sputum specimens (Fig. 2C, D). No year-by-year changes were observed in the frequencies of the major positive specimens, except for that of nasopharyngeal mucus specimens from patients <5 years old. The frequency of these specimens suddenly decreased in 2017, whereas the frequency of sputum specimens increased (Fig. 2A). The Japanese guidelines for pediatric respiratory infections were revised in November 2016 and stated that the nasopharyngeal mucus was not recommended for the isolation of causative microorganisms. The phenomenon occurring in 2017 was most likely affected by this statement. In patients <5 years old, the frequencies of *S. pneumoniae* isolated from blood specimens and otorrhea or middle ear fluid specimens were 1.3%-1.5% and 3.1%-4.2%, respectively, from 2009 to 2010. Notably, the frequencies of *S. pneumoniae* isolated from these two sources had gradually decreased after 2011, and *S. pneumoniae* was not isolated from blood in 2016 or 2017 (Fig. 2A). In contrast, the frequency of *S. pneumoniae* isolated from blood specimens of patients 15-64 years old had slightly but steadily increased since 2015 (Fig. 2C). In the other patient groups, there were no obvious annual changes in the frequency distribution of *S. pneumoniae* isolated from each source (Fig. 2B, D).

Because *S. pneumoniae* isolated from blood or middle ear fluid is diagnosed as a causative pathogen for pneumococcal disease, these results suggest that pneumococcal vaccination may directly contribute to a reduction in the incidence of pneumococcal infections, including IPD, at least for children <5 years old.

To evaluate changes in antimicrobial susceptibility of *S. pneumoniae* after the introduction of pneumococcal vaccination, we performed susceptibility testing for 4,963 isolates from patients at four Jikei University Hospitals from 2009 to 2017 (Fig. 3 and Supplementary Table S3). Although the susceptibility rates of *S. pneumoniae* isolates tested to penicillin G (PCG), cefotaxime (CTX), and ceftriaxone (CTRX) were already high (>90%) in 2009 and 2010, the rates further increased in association with decreases in the minimum inhibitory concentration (MIC or $MIC_{50/90}$) values after the introduction of PCV7 vaccination (Fig. 3A-C, and the corresponding parts of Supplementary Table S3). The susceptibility to cefepime (CFPM) or meropenem (MEPM) was not too high (susceptibility rates <80%) in 2009 and 2010, but the susceptibility rates had increased since 2011 (Fig. 3D, E, and the corresponding parts of Supplementary Table S3). Similarly, the susceptibility of *S. pneumoniae* isolates to erythromycin (EM) had improved from 2011, although most of the isolates were still non-susceptible to EM in 2017 (Fig. 3F and the corresponding part of Supplementary Table S3). The susceptibility rates to vancomycin (VCM) and levofloxacin (LVFX) were quite high (nearly 100%), and their $MIC_{50/90}$ values did not markedly change throughout the observation period, but the frequency of isolates responding to the lower MICs of each antimicrobial agent appeared to increase with the widespread introduction of pneumococcal vaccination (Fig. 3G, H, and the corresponding parts of Supplementary Table S3).

These results suggest that pneumococcal vaccination is involved not only in a reduction in the prevalence of *S. pneumoniae* but also in the suppression of the emergence of isolates resistant to

antimicrobial agents.

In the present study, we investigated the prevalence and antimicrobial susceptibility of *S. pneumoniae* isolated from a large number of patients at Jikei University Hospitals from 2009 to 2017 and found that pneumococcal vaccination led to (1) a reduction in the prevalence of *S. pneumoniae* isolated from patients <5 years old; (2) a decrease in the frequency of *S. pneumoniae* isolated from the blood, otorrhea, or middle ear fluid of patients <5 years old; and (3) an improvement in the antimicrobial susceptibility of *S. pneumoniae*. These results show that pneumococcal vaccination is beneficial in the prevention and control of pneumococcal infections, at least for patients <5 years old.

The prevalence of *S. pneumoniae* isolated from patients <5 years old has decreased since 2011 (Fig. 1A). Most of the specimens from these patients were nasopharyngeal mucus (Fig. 2A), which was frequently used for the isolation of causative microorganisms for pediatric respiratory infections instead of sputum. Colonizing *S. pneumoniae* may also be isolated from nasopharyngeal mucus specimens, but previous studies have shown that its prevalence did not decrease with the introduction of pneumococcal vaccination due to serotype replacement [7, 8]. The results shown in Figure 1A thus indicate that pneumococcal vaccination had a direct suppressive effect on the prevalence of pathogenic or invasive *S. pneumoniae*. This suppressive effect can also be seen through the reduction in the prevalence of *S. pneumoniae* isolated from the blood and otorrhea/middle ear fluid of patients <5 years old after the introduction of pneumococcal vaccination (as shown in Fig. 2A). In contrast, there were no evident changes in the prevalence of *S. pneumoniae* isolated from patients ≥5 years old during the observation period (Fig. 1B-D). This was presumably due to lower vaccination rates in the generations including those patients. Pneumococcal vaccination is not mandatory, at least for individuals ≥5 years old, in Japan. PPSV23 was adopted as a routine vaccine for elderly people in October 2014, but its vaccination rate was around 40% even in 2016 [9]. In addition, since the PPSV23 vaccine had already been

administered to elderly individuals with underlying diseases before 2014 [3], it may have been difficult to detect any effect of routine PPSV23 vaccination during the observation period in this study. A slight and temporary decline in the prevalence of *S. pneumoniae* isolates was observed among patients ≥5 years old in 2012 and 2013, but conversely, the prevalence increased after 2014 (Fig. 1B-D). These two changes most likely reflect suppression of the transmission of vaccine serotype strains at the community level (herd immunity) [10] and serotype replacement (a relative increase in the prevalence of non-vaccine serotypes) [6, 11] with the widespread use of pneumococcal vaccines.

Annual changes in the prevalence of *S. pneumoniae* isolated from patients <5 years old differed among the four hospitals after the introduction of PCV7 (Supplementary Fig. S1). At the Main Hospital, there was no significant change in the *S. pneumoniae* prevalence throughout the observation period (Supplementary Fig. S1A). Since the Main Hospital mainly accepts patients requiring advanced treatment, the number of patients with typical community-acquired infection, which is frequently caused by *S. pneumoniae*, is relatively small. This presumably accounts for the lack of any evident impact of pneumococcal vaccination on the *S. pneumoniae* prevalence at the Main Hospital. At Katsushika Medical Center and Daisan Hospital, the prevalence of *S. pneumoniae* significantly decreased from 2011 with the widespread use of PCV7 (Supplementary Fig. S1B, C), while at Kashiwa Hospital, this decline was observed from 2014 after the introduction of routine PCV7 vaccination and replacement with PCV13 (Supplementary Fig. S1D). These three hospitals are regional core hospitals; Katsushika Medical Center and Daisan Hospital are located within the Tokyo area, and Kashiwa Hospital is located in Chiba Prefecture, just outside of Tokyo. The community differs between these areas. There was no obvious difference in the vaccination rate before the routine use of PCV7 between the areas according to information from the corresponding local governments, suggesting the possibility that different serotypes were prevalent in the two areas. A previous study demonstrated that the incidence of IPD

among children <5 years old decreased in Chiba Prefecture starting in 2011, before the introduction of routine PCV7 vaccination [12], which differed from our findings in Kashiwa Hospital. However, that study focused on only IPD patients at 58 hospitals in Chiba Prefecture, while in addition to IPD patients, the present study included non-IPD patients or pneumococcal carriers and was conducted at 1 hospital in Kashiwa-shi, Chiba Prefecture. These differences in the research design may have contributed to the discrepancy in the results of the two studies.

The frequency of *S. pneumoniae* isolated from blood or otorrhea/middle ear fluid specimens decreased among patients <5 years old after the use of PCV7, suggesting the suppressive impact of PCV7 vaccination on pneumococcal infections, including bacteremia and otitis media (Fig. 2A) [6, 13]. Previous studies have shown that pneumococcal vaccination reduces the incidence of IPD [6, 10] and that the incidence of pneumococcal meningitis decreases by 87% in children after the introduction of PCV vaccination [10]. There were only a few pediatric patients with pneumococcal meningitis throughout the observation period in this study, and its incidence was not observed after 2014 (Fig. 2A, B). In contrast, an increase in the incidence of IPD caused by non-vaccine serotypes has been reported following the introduction of pneumococcal vaccination [6, 10], believed to be the result of serotype replacement induced by vaccination. In this study, the frequency of *S. pneumoniae* isolated from blood specimens of patients 15-64 years old increased after 2015 (Fig. 2C), suggesting the involvement of non-vaccine serotypes in the incidence of IPD. In patients ≥65 years old, there was no apparent suppressive effect of PPSV23 on the incidence of IPD (Fig. 2D). The low vaccination rate in the same generation may be one of the reasons accounting for this.

The susceptibilities of *S. pneumoniae* to the eight antimicrobial agents tested improved by varying degrees after the introduction of pneumococcal vaccination (Fig. 3 and Supplementary Table S3). These improvements most likely resulted from serotype replacement with the use of pneumococcal vaccines.

Decreases not only in the prevalence of vaccine serotype strains but also in the emergence of resistant strains have been reported after the introduction of PCV7 vaccination [14]. In contrast, a recent study found that the prevalence of pneumococci with reduced susceptibility to penicillin (e.g. serotypes 15A, 19B, and 35B) was increased among non-PCV13 serotypes [11, 15]. Therefore, measures to ensure the rational use of antimicrobial agents and sustained surveillance of antimicrobial resistance are needed in order to ensure effective continued control of pneumococcal disease.

This study was limited by the lack of information on the serotype of pneumococcal isolates, disease severity, patient background, and vaccination history. However, our comprehensive epidemiological study clearly shows that pneumococcal vaccination has a suppressive effect on the prevalence of *S. pneumoniae* and the incidence of pneumococcal infections, at least among individuals <5 years old, in association with an improvement in the antimicrobial susceptibility of *S. pneumoniae*.

Acknowledgements

The authors are grateful to all of the members of the Clinical Laboratory departments at the four Jikei University Hospitals and all staff members of the Department of Laboratory Medicine of the Jikei University School of Medicine for their kind help and support. This study was supported in part by the Research Program on Hepatitis of the Japan Agency for Medical Research and Development, AMED (Grant Numbers JP19fk0210053 and JP19fk0210062).

References

[1] O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by Streptococcus pneumoniae in children younger than 5 years: global estimates. Lancet. 2009;374:893-902.

[2] Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, et al. Pneumococcal Capsules and Their Types: Past, Present, and Future. Clin Microbiol Rev. 2015;28:871-899.

[3] Suzuki M, Dhoubhadel BG, Ishifuji T, Yasunami M, Yaegashi M, Asoh N, et al. Serotype-specific effectiveness of 23-valent pneumococcal polysaccharide vaccine against pneumococcal pneumonia in adults aged 65 years or older: a multicentre, prospective, test-negative design study. Lancet Infect Dis. 2017;17:313-321.

[4] Otsuka T, Chang B, Shirai T, Iwaya A, Wada A, Yamanaka N, et al. Individual risk factors associated with nasopharyngeal colonization with Streptococcus pneumoniae and Haemophilus influenzae: a Japanese birth cohort study. Pediatr Infect Dis J. 2013;32:709-714.

[5] Flamaing J, Peetermans WE, Vandeven J, Verhaegen J. Pneumococcal colonization in older persons in a nonoutbreak setting. J Am Geriatr Soc. 2010;58:396-398.

[6] Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. Lancet. 2011;378:1962-1973.

[7] Oikawa J, Ishiwada N, Takahashi Y, Hishiki H, Nagasawa K, Takahashi S, et al. Changes in nasopharyngeal carriage of Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis among healthy children attending a day-care centre before and after official financial support for the 7-valent pneumococcal conjugate vaccine and H. influenzae type b vaccine in Japan. J Infect Chemother. 2014;20:146-149.

[8] Southern J, Andrews N, Sandu P, Sheppard CL, Waight PA, Fry NK, et al. Pneumococcal carriage in children and their household contacts six years after introduction of the 13-valent pneumococcal conjugate vaccine in England. PLoS One. 2018;13:e0195799.

[9] Murakami Y, Nishiwaki Y, Kanazu SI, Oba M, Watanabe A. A nationwide survey of PPSV23 vaccine coverage rates and their related factors among the elderly in Japan, 2016. Nihon Koshu

Eisei Zasshi. 2018;65:20-24. [Japanese]

[10] Ubukata K, Takata M, Morozumi M, Chiba N, Wajima T, Hanada S, et al. Effects of Pneumococcal Conjugate Vaccine on Genotypic Penicillin Resistance and Serotype Changes, Japan, 2010-2017. Emerg Infect Dis. 2018;24:2010-2020.

[11] Nakano S, Fujisawa T, Ito Y, Chang B, Suga S, Noguchi T, et al. Serotypes, antimicrobial susceptibility, and molecular epidemiology of invasive and non-invasive Streptococcus pneumoniae isolates in paediatric patients after the introduction of 13-valent conjugate vaccine in a nationwide surveillance study conducted in Japan in 2012-2014. Vaccine. 2016;34:67-76.

[12] Ishiwada N, Hishiki H, Nagasawa K, Naito S, Sato Y, Chang B, et al. The incidence of pediatric invasive Haemophilus influenzae and pneumococcal disease in Chiba prefecture, Japan before and after the introduction of conjugate vaccines. Vaccine. 2014;32:5425-5431.

[13] Ubukata K, Morozumi M, Sakuma M, Takata M, Mokuno E, Tajima T, et al. Etiology of Acute Otitis Media and Characterization of Pneumococcal Isolates After Introduction of 13-Valent Pneumococcal Conjugate Vaccine in Japanese Children. Pediatr Infect Dis J. 2018;37:598-604.

[14] Chiba N, Morozumi M, Shouji M, Wajima T, Iwata S, Ubukata K, et al. Changes in capsule and drug resistance of Pneumococci after introduction of PCV7, Japan, 2010-2013. Emerg Infect Dis. 2014;20:1132-1139.

[15] Emgard M, Msuya SE, Nyombi BM, Mosha D, Gonzales-Siles L, Norden R, et al. Carriage of penicillin-non-susceptible pneumococci among children in northern Tanzania in the 13-valent pneumococcal vaccine era. Int J Infect Dis. 2019;81:156-166.

Figure legends

Figure 1. Annual changes in the number and frequency of *S. pneumoniae* isolated from patients in different age groups at Jikei University Hospitals from 2009 to 2017.

Isolation of *S. pneumoniae* was performed using specimens from patients at 4 Jikei University Hospitals, and annual changes in the number (columns) and frequency (isolation rate; lines) of *S. pneumoniae* isolates were investigated in patients <5 years old (A), patients 5-14 years old (B), patients 15-64 years old (C), and patients ≥65 years old (D) from 2009 to 2017. The study period was divided into three phases: (1) in 2009 and 2010, (2) from 2011 to 2013, and (3) from 2014 to 2017. The differences in the isolation rate of *S. pneumoniae* between each phase were statistically evaluated using a chi-square test (see Supplementary Materials and Methods for details). *P <0.05, **P <0.01. N.S.: not significant.

Figure 2. Annual changes in the sources of specimens from which S. *pneumoniae* was isolated at Jikei University Hospitals from 2009 to 2017.

S. pneumoniae was isolated from patient specimens at Jikei University Hospitals, and the frequencies (%) of *S. pneumoniae* isolated from the different sources were examined in patients <5 years old (A), patients 5-14 years old (B), patients 15-64 years old (C), and patients ≥65 years old (D) from 2009 to 2017. The specimen sources used are listed below the panels.

Figure 3. Annual changes in the antimicrobial susceptibility of *S. pneumoniae* isolated from patients at Jikei University Hospitals from 2009 to 2017.

S. pneumoniae was isolated from patients at Jikei University Hospitals, and the frequency (%) of *S. pneumoniae* isolates responding to a given MIC of each antimicrobial agent (listed on the right side of

each panel) was examined from 2009 to 2017. Susceptibility testing was performed for the following eight antimicrobial agents: PCG (A), CTX (B), CTRX (C), CFPM (D), MEPM (E), EM (F), VCM (G), and LVFX (H). N.E.: not evaluated.

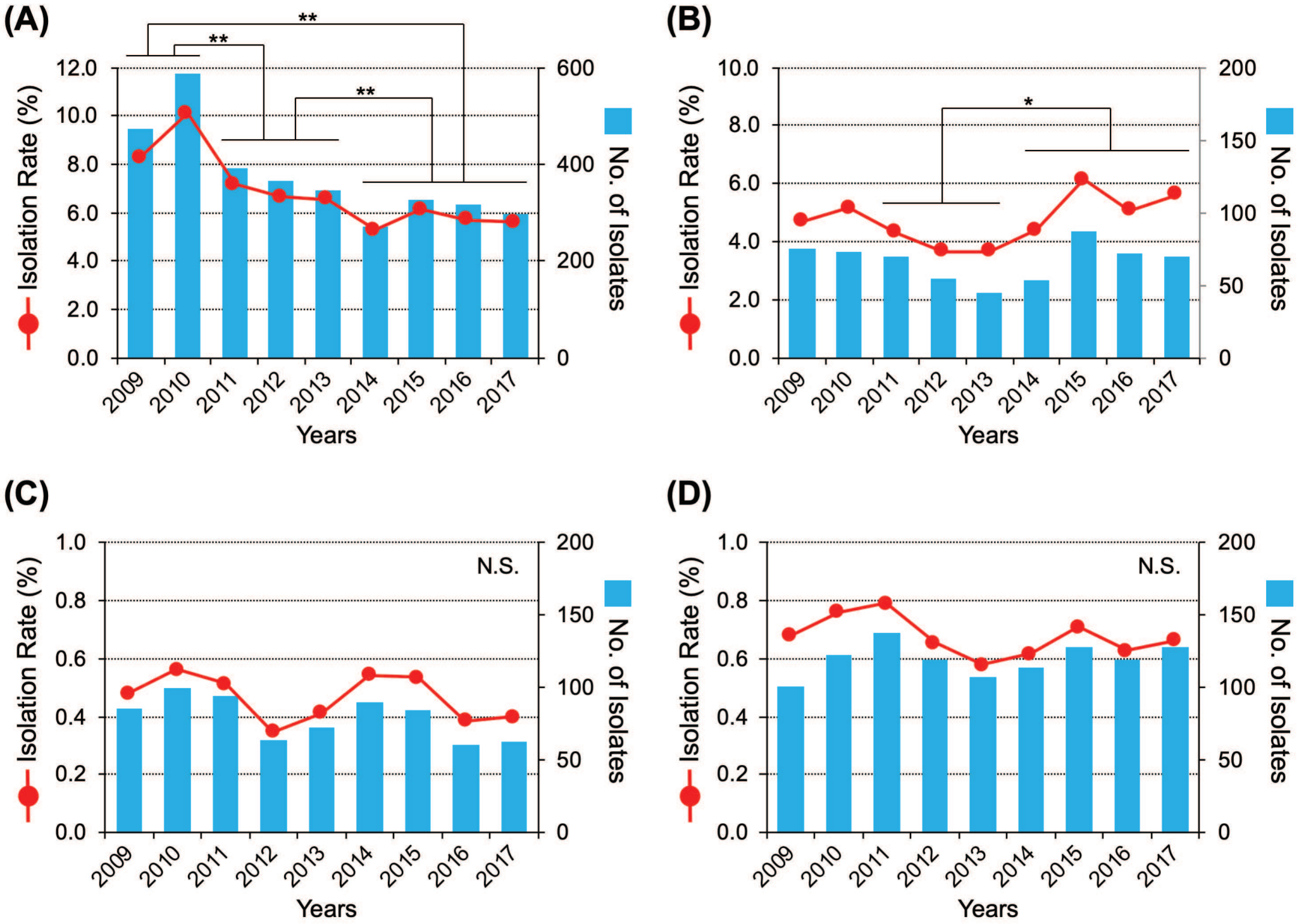

(A)
**
**
**
Isolation Rate (%)
No. of Isolates
12.0
10.0
8.0
6.0
4.0
2.0
0.0
600
400
200
0
2009
2010
2011
2012
2013
2014
2015
2016
2017
Years
(B)
*
Isolation Rate (%)
No. of Isolates
10.0
8.0
6.0
4.0
2.0
0.0
200
150
100
50
0
Years
(C)
N.S.
Isolation Rate (%)
No. of Isolates
1.0
0.8
0.6
0.4
0.2
0.0
200
150
100
50
0
Years
(D)
N.S.
Isolation Rate (%)
No. of Isolates
Years

Fig. 1

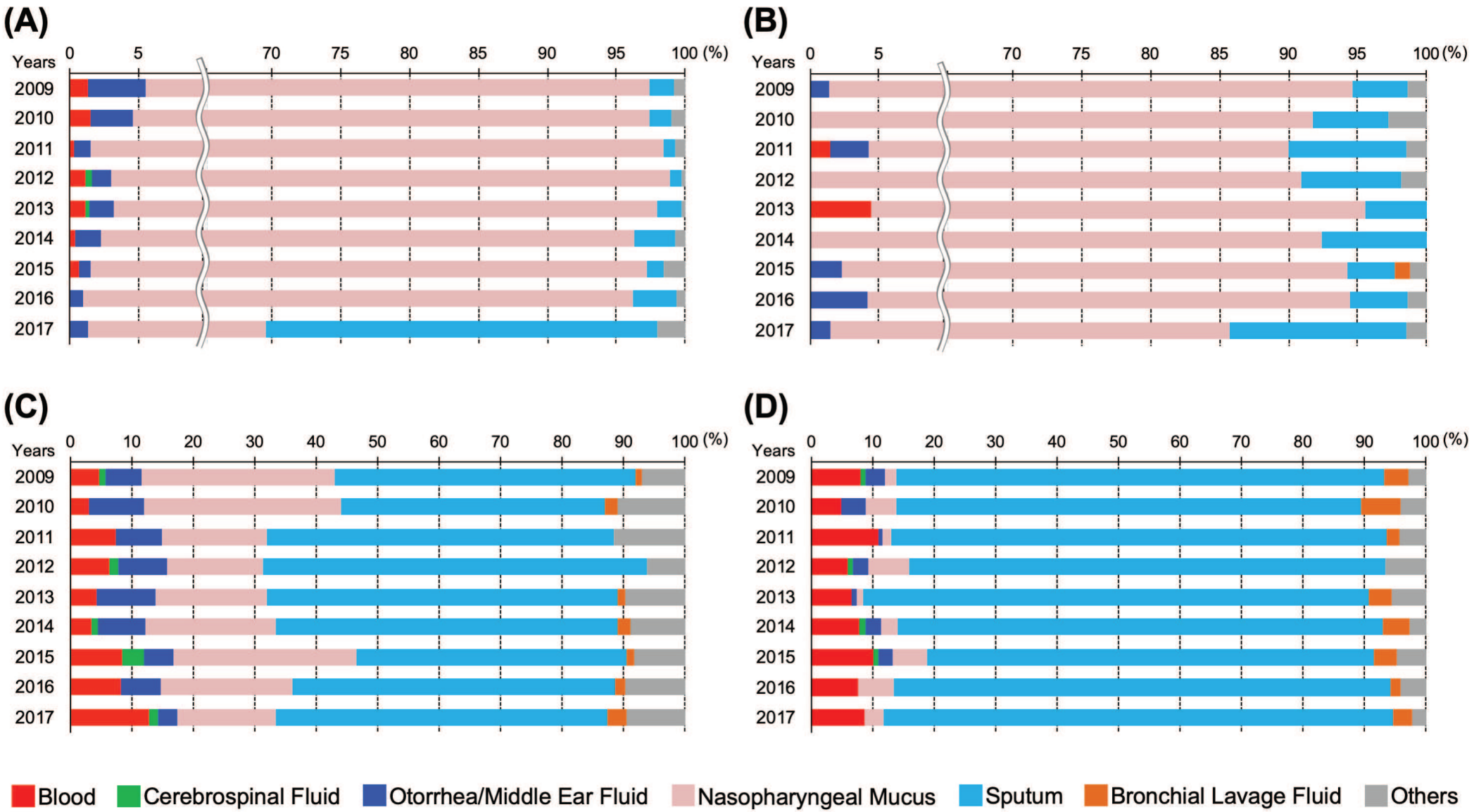

(A)
Years
0
5
70
75
80
85
90
95
100 (%)
2009
2010
2011
2012
2013
2014
2015
2016
2017
(B)
Years
0
5
70
75
80
85
90
95
100 (%)
2009
2010
2011
2012
2013
2014
2015
2016
2017
(C)
Years
0
10
20
30
40
50
60
70
80
90
100 (%)
2009
2010
2011
2012
2013
2014
2015
2016
2017
(D)
Years
0
10
20
30
40
50
60
70
80
90
100 (%)
2009
2010
2011
2012
2013
2014
2015
2016
2017
Blood
Cerebrospinal Fluid
Otorrhea/Middle Ear Fluid
Nasopharyngeal Mucus
Sputum
Bronchial Lavage Fluid
Others

Fig. 2

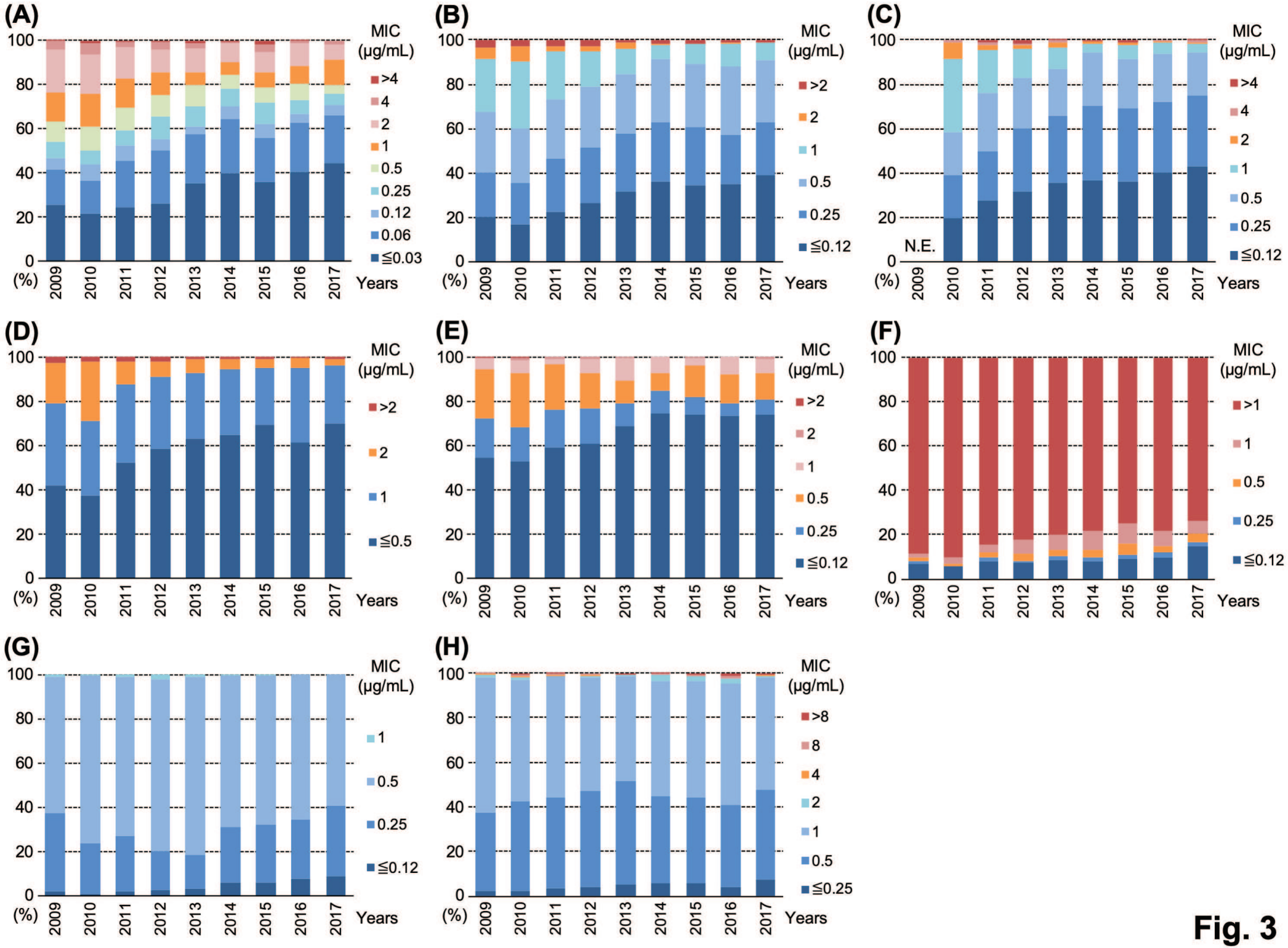
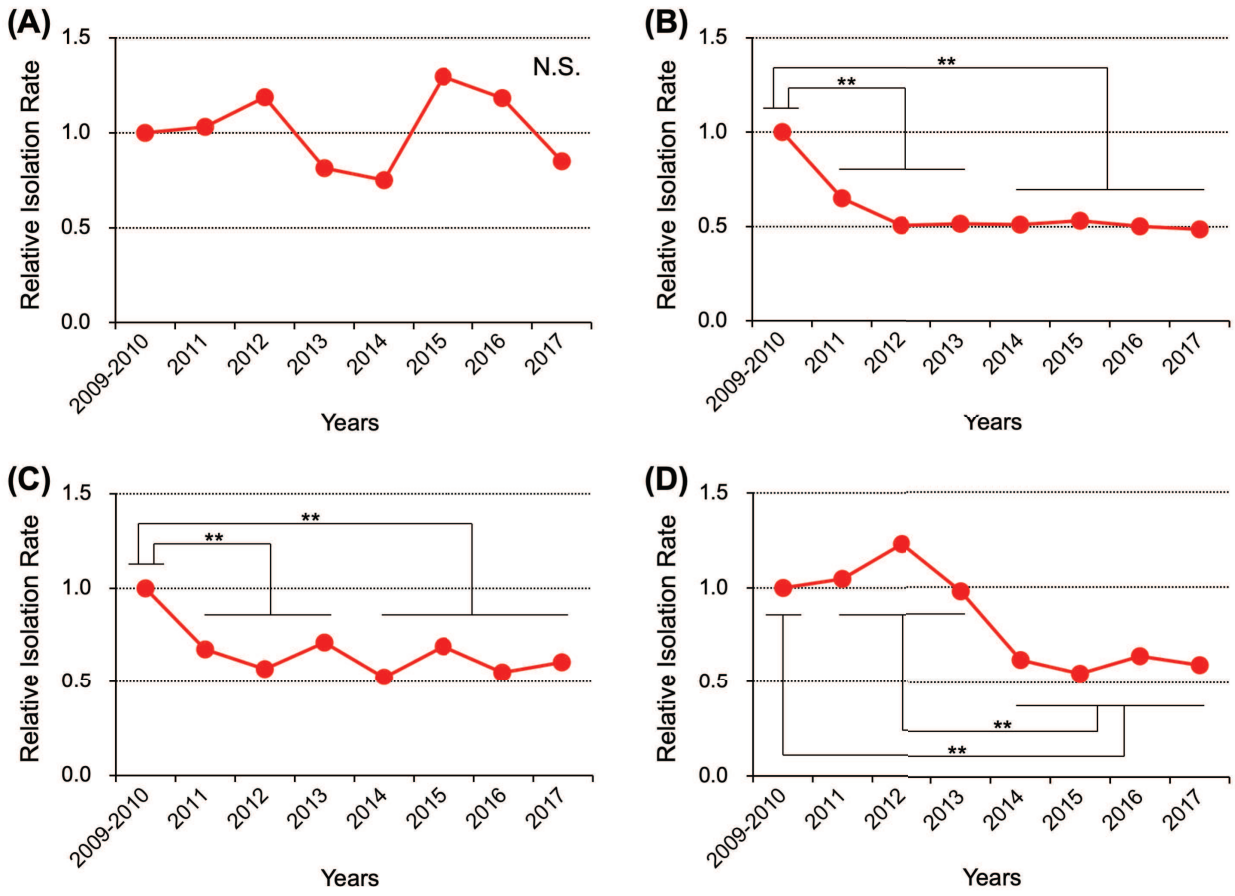

(A)
MIC (μg/mL)
>4
4
2
1
0.5
0.25
0.12
0.06
≦0.03
100
80
60
40
20
0
(%)
2009
2010
2011
2012
2013
2014
2015
2016
2017
Years
(B)
MIC (μg/mL)
>2
2
1
0.5
0.25
≦0.12
(C)
MIC (μg/mL)
>4
4
2
1
0.5
0.25
≦0.12
N.E.
(D)
MIC (μg/mL)
>2
2
1
≦0.5
(E)
MIC (μg/mL)
>2
2
1
0.5
0.25
≦0.12
(F)
MIC (μg/mL)
>1
1
0.5
0.25
≦0.12
(G)
MIC (μg/mL)
1
0.5
0.25
≦0.12
(H)
MIC (μg/mL)
>8
8
4
2
1
0.5
≦0.25

Fig. 3

Supplementary Data

"The prevalence and antimicrobial susceptibility of *Streptococcus pneumoniae* isolated from patients at Jikei University Hospitals after the implementation of the pneumococcal vaccination program in Japan" by T. Ando, *et al*.

1. Supplementary Figure and Legend



Supplementary Figure S1. Annual changes in the frequency of *S. pneumoniae* isolated from patients <5 years old at four Jikei University Hospitals from 2009 to 2017.

Isolation of *S. pneumoniae* was performed using specimens from patients at Jikei University Hospitals, and annual changes in the isolation rate of *S. pneumoniae* were investigated in patients <5 years old at the Main Hospital (A), Katsushika Medical Center (B), Daisan Hospital (C), and Kashiwa Hospital (D) from 2009 to 2017. The isolation rate of *S. pneumoniae* at each hospital in each year is represented as a value relative to the isolation rate in 2009 to 2010, which is set as 1.0. The study period was divided into three phases: (1) in 2009 and 2010, (2) from 2011 to 2013, and (3) from 2014 to 2017, and differences in the relative isolation rate of *S. pneumoniae* between each phase were statistically evaluated using a chi-square test (see Supplementary Materials and Methods for details). ***P* <0.01. N.S.: not significant.

2. Supplementary Tables

Supplementary Table S1. The number and frequency of *S. pneumoniae* isolated from patients in different age groups at four Jikei University Hospitals from 2009 to 2017.

Age (years)	<5	5-14	15-64	≥65	All
Hospitals	No. of isolates/ specimens (%)	No. of isolates/ specimens (%)	No. of isolates/ specimens (%)	No. of isolates/ specimens (%)	No. of isolates/ specimens (%)
Main Hospital	156/11,069 (1.41)	69/3,067 (2.25)	273/68,776 (0.40)	291/50,725 (0.57)	789/133,637 (0.59)
Katsushika Medical Center	1,327/11,229 (11.82)	168/3,294 (5.10)	144/26,014 (0.55)	273/29,528 (0.92)	1,912/70,065 (2.73)
Daisan Hospital	1,133/10,647 (10.64)	165/2,990 (5.52)	177/28,173 (0.63)	338/42,420 (0.80)	1,813/84,230 (2.15)
Kashiwa Hospital	756/16,087 (4.70)	198/3,294 (6.01)	120/30,751 (0.39)	175/37,371 (0.47)	1,249/87,503 (1.43)
Total	3,372/49,032 (6.88)	600/12,645 (4.74)	714/153,714 (0.46)	1,077/160,044 (0.67)	5,763/375,435 (1.54)

Supplementary Table S2. The sources of specimens from which *S. pneumoniae* was isolated in each patient group at Jikei University Hospitals from 2009 to 2017.

Age (years)	<5	5-14	15-64	≥65	All
Specimens	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)
Sputum	135 (4.0)	38 (6.3)	372 (52.1)	849 (78.8)	1,394 (24.2)
Nasopharyngeal mucus	3,108 (92.2)	541 (90.2)	165 (23.1)	40 (3.7)	3,854 (66.9)
Bronchial lavage fluid	0 (0.0)	1 (0.2)	10 (1.4)	35 (3.2)	46 (0.8)
Blood	27 (0.8)	3 (0.5)	44 (6.2)	85 (7.9)	159 (2.8)
Cerebrospinal fluid	3 (0.1)	0 (0.0)	7 (1.0)	4 (0.4)	14 (0.2)
Otorrhea and middle ear fluid	69 (2.0)	9 (1.5)	50 (7.0)	19 (1.8)	147 (2.6)
Others	30 (0.9)	8 (1.3)	66 (9.2)	45 (4.2)	149 (2.6)
Total	3,372 (100)	600 (100)	714 (100)	1,077 (100)	5,763 (100)

Supplementary Table S3. Antimicrobial susceptibilities of *S. pneumoniae* isolated from patients at Jikei University Hospitals from 2009 to 2017.

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)									$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	>4		
PCG	2009	25.7	15.8	5.1	7.3	9.3	13.1	19.6	3.8	0.3	0.25/2.0	95.7
	2010	21.3	15.3	7.4	6.2	10.5	14.9	18.1	5.0	1.3	0.25/2.0	93.7
	2011	24.6	21.0	6.7	7.2	10.1	12.9	14.4	2.6	0.5	0.12/2.0	96.9
	2012	26.2	24.1	5.2	10.3	9.6	10.3	10.0	3.6	0.8	0.06/2.0	95.2
	2013	35.5	21.9	3.5	9.1	9.9	5.6	10.7	2.3	1.4	0.06/2.0	96.3
	2014	39.8	24.3	6.2	7.8	6.2	6.0	8.4	1.0	0.4	0.06/1.0	98.4
	2015	35.8	20.3	6.1	9.4	7.2	6.5	9.4	3.1	2.1	0.06/2.0	94.6
	2016	40.6	22.4	3.8	6.5	6.9	8.1	10.5	1.0	0.2	0.06/2.0	98.8
	2017	44.6	21.5	4.5	5.3	3.9	11.8	6.9	1.6	0.0	0.06/1.0	98.4

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)						$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.12	0.25	0.5	1	2	>2		
CTX	2009	20.1	20.4	26.9	24.2	5.1	3.3	0.5/1.0	91.4
	2010	17.1	18.8	24.0	30.4	6.6	3.1	0.5/1.0	90.3
	2011	22.7	23.9	26.5	21.5	2.7	2.7	0.5/1.0	94.5
	2012	26.8	24.9	27.0	15.9	2.3	3.1	0.25/1.0	94.6
	2013	31.7	26.0	26.8	11.3	3.3	1.0	0.25/1.0	95.7
	2014	36.3	26.7	28.7	6.2	0.6	1.6	0.25/0.5	97.8
	2015	34.5	26.4	28.0	9.1	0.2	1.8	0.25/1.0	98.1
	2016	35.2	22.0	30.9	9.9	0.8	1.2	0.25/1.0	98.0
	2017	39.1	23.9	27.6	7.9	0.4	1.0	0.25/0.5	98.6

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)							$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.12	0.25	0.5	1	2	4	>4		
CTRX	2009	–	–	–	–	–	–	–	–/–	–
	2010	19.9	19.3	19.3	32.6	7.7	1.1	0.0	0.50/1.0	91.2
	2011	27.5	22.3	26.5	19.1	2.6	1.0	1.0	0.50/1.0	95.4
	2012	31.8	28.2	22.8	13.0	1.9	0.8	1.5	0.25/1.0	95.8
	2013	35.7	30.3	20.6	9.9	2.5	0.8	0.2	0.25/1.0	96.5
	2014	36.9	33.7	23.5	4.2	1.2	0.2	0.4	0.25/0.5	98.2
	2015	36.4	33.0	22.0	6.1	1.0	0.3	1.0	0.25/0.5	97.6
	2016	40.2	31.9	21.4	5.5	0.2	0.8	0.0	0.25/0.5	99.0
	2017	43.0	32.0	19.3	4.1	0.6	0.8	0.2	0.25/0.5	98.4

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)				$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.5	1	2	>2		
CFPM	2009	42.3	36.8	18.4	2.5	1.0/2.0	79.1
	2010	37.4	33.4	26.9	2.2	1.0/2.0	70.8
	2011	52.1	35.4	10.3	2.2	≤0.5/2.0	87.5
	2012	58.6	32.6	6.7	2.1	≤0.5/1.0	91.0
	2013	63.3	29.7	6.0	1.0	≤0.5/1.0	93.0
	2014	64.7	29.7	4.8	0.8	≤0.5/1.0	94.2
	2015	69.6	25.5	3.7	1.2	≤0.5/1.0	95.1
	2016	61.4	33.5	4.6	0.6	≤0.5/1.0	94.9
	2017	70.2	26.0	2.8	1.0	≤0.5/1.0	96.1

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)						$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.12	0.25	0.5	1	2	>2		
MEPM	2009	54.6	17.9	21.9	5.3	0.2	0.2	≤0.12/0.5	72.5
	2010	53.0	15.6	24.4	5.8	1.1	0.1	≤0.12/0.5	68.6
	2011	59.5	16.8	20.3	2.7	0.7	0.0	≤0.12/0.5	76.3
	2012	60.9	16.1	15.9	6.5	0.6	0.0	≤0.12/0.5	77.0
	2013	68.9	10.1	10.5	9.9	0.6	0.0	≤0.12/1.0	79.0
	2014	74.5	10.2	8.2	7.2	0.0	0.0	≤0.12/0.5	84.7
	2015	73.8	8.0	14.3	3.7	0.2	0.0	≤0.12/0.5	81.8
	2016	73.5	5.9	12.7	7.9	0.0	0.0	≤0.12/0.5	79.4
	2017	74.2	6.9	11.6	6.7	0.6	0.0	≤0.12/0.5	81.1

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)					$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.12	0.25	0.5	1	>1		
EM	2009	7.0	1.2	1.3	2.2	88.4	>1.0/>1.0	8.1
	2010	5.5	0.4	1.0	2.8	90.3	>1.0/>1.0	5.9
	2011	8.2	1.4	2.6	3.4	84.4	>1.0/>1.0	9.6
	2012	7.3	0.6	3.3	6.3	82.4	>1.0/>1.0	7.9
	2013	8.3	1.7	3.3	6.6	80.0	>1.0/>1.0	10.1
	2014	8.2	1.6	3.2	9.0	78.1	>1.0/>1.0	9.8
	2015	9.3	1.6	4.9	9.6	74.7	>1.0/>1.0	10.8
	2016	9.5	2.4	3.0	6.7	78.4	>1.0/>1.0	11.9
	2017	14.6	1.8	4.3	5.5	73.8	>1.0/>1.0	16.4

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)					$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.12	0.25	0.5	1	>1		
VCM	2009	2.2	35.5	61.4	1.0	0.0	0.5/0.5	100
	2010	1.0	22.9	75.5	0.7	0.0	0.5/0.5	100
	2011	2.1	25.4	71.5	1.0	0.0	0.5/0.5	100
	2012	2.7	17.6	77.4	2.3	0.0	0.5/0.5	100
	2013	3.5	15.1	80.4	1.0	0.0	0.5/0.5	100
	2014	6.0	25.5	68.1	0.4	0.0	0.5/0.5	100
	2015	6.5	25.7	67.1	0.7	0.0	0.5/0.5	100
	2016	7.9	26.9	65.0	0.2	0.0	0.5/0.5	100
	2017	8.9	31.8	59.0	0.2	0.0	0.5/0.5	100

Drug	Years	The percentage of isolates responding to a given MIC (μg/mL)							$MIC_{50/90}$ (μg/mL)	Susceptibility rate (%)
		≤0.25	0.5	1	2	4	8	>8		
LVFX	2009	2.3	35.0	60.9	1.0	0.5	0.3	0.0	1.0/1.0	99.2
	2010	2.2	40.1	54.6	1.4	0.3	0.6	0.8	1.0/1.0	98.3
	2011	3.6	40.5	54.3	0.3	0.3	0.5	0.3	1.0/1.0	98.8
	2012	4.2	43.1	50.6	1.0	0.4	0.6	0.0	1.0/1.0	99.0
	2013	5.0	46.8	46.6	0.8	0.0	0.0	0.8	0.5/1.0	99.2
	2014	6.0	38.8	51.6	3.0	0.0	0.2	0.4	1.0/1.0	99.4
	2015	5.6	38.5	52.4	2.3	0.2	0.3	0.7	1.0/1.0	98.8
	2016	4.4	36.8	54.3	2.4	0.0	0.8	1.4	1.0/1.0	97.8
	2017	7.7	40.2	50.5	0.4	0.2	0.2	0.8	1.0/1.0	98.8

PCG: penicillin G; CTX: cefotaxime; CTRX: ceftriaxone; CFPM: cefepime; MEPM: meropenem; EM: erythromycin; VCM: vancomycin; LVFX: levofloxacin; MIC: minimum inhibitory concentration

3. Supplementary Materials and Methods

3.1. Specimen collection

Specimens, including sputum, nasopharyngeal mucus, and blood, were collected from patients at four Jikei University Hospitals (Main Hospital, Katsushika Medical Center, Daisan Hospital, and Kashiwa Hospital) from 2009 to 2017. Specimens that were negative for *S. pneumoniae* repeatedly collected from the same source of a patient in a year were recognized as a single negative specimen. If *S. pneumoniae* was isolated multiple times from the same specimen source of a patient in a year, the first collected specimen positive for *S. pneumoniae* was used for further analyses.

This study was approved by the Ethics Committee of the Jikei University School of Medicine for Biomedical Research (Registration Number: 29-320 [8936]).

3.2. Isolation and identification of pneumococci

The samples were inoculated on sheep blood agar and incubated overnight at 35 °C in a 5% CO_2 atmosphere. The identification of *S. pneumoniae* was performed based on colony morphology and optochin sensitivity [1].

3.3. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of antibiotics against *S. pneumoniae* isolates were determined by the broth microdilution method using a MICroFAST plate (Beckman Coulter, Brea, CA, USA). Antimicrobial susceptibility was evaluated using the MIC breakpoints suggested by the Clinical and Laboratory Standards Institute guidelines [2]. The susceptibilities of meningeal pneumococcal isolates to penicillin G (PCG), cefotaxime (CTX), ceftriaxone (CTRX), and cefepime (CFPM) were evaluated using the breakpoints for meningitis, while the susceptibilities of non-

meningeal pneumococcal isolates were assayed using the breakpoints for non-meningitis and parenteral administration. Susceptibility testing was also performed for the following antimicrobial agents: meropenem (MEPM), erythromycin (EM), vancomycin (VCM), and levofloxacin (LVFX). Resistant and intermediate isolates were referred to as non-susceptible, and the susceptibility rate was defined by dividing the number of susceptible isolates by the total number of isolates.

3.4. Statistical analyses

To statistically study changes in the prevalence of *S. pneumoniae* isolated from patients after the introduction of pneumococcal vaccination, we divided the study period from 2009 to 2017 into three phases: (1) before the introduction of a public subsidy program for pneumococcal vaccination (in 2009 and 2010), (2) after the introduction of the public subsidy program (from 2011 to 2013), and (3) after the implementation of universal pneumococcal vaccination (from 2014 to 2017). A chi-square test was used to statistically evaluate differences in the isolation rate of *S. pneumoniae* between each phase. *P*-values of < 0.05 were considered to be statistically significant.

4. Supplementary References

[1] Spellerberg B, Brandt C, Sendi P. *Streptococcus*. In: Carroll KC, Pfaller MA, Landry ML, McAdam AJ, Patel R, Richter SS, et al., editors. Manual of Clinical Microbiology. 12th ed. Washington, DC: ASM Press; 2019. p. 399-417.

[2] CLSI. Performance standards for antimicrobial susceptibility testing; 27th informational supplement. CLSI document M100-S27. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.