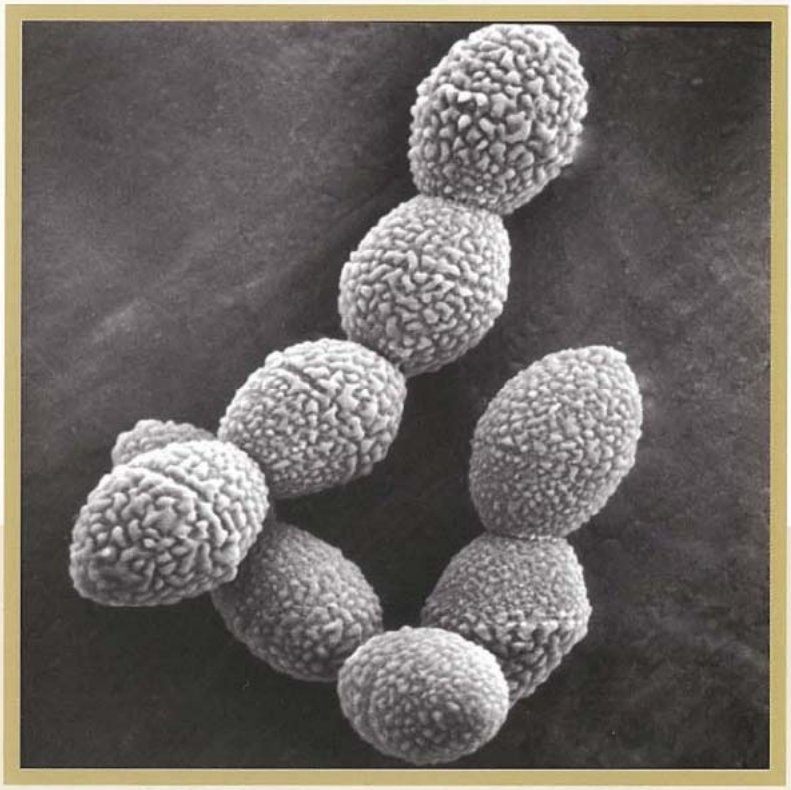
Pneumococcal Infections and Vaccines

Think Globally, Act Locally



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Greetings

At the “Joint Meeting of the 91st Meeting of the Japanese Association for Infectious Diseases and the 65th Meeting of the Japanese Society of Chemotherapy” held in April 2017, we had an opportunity to deliver a lecture titled **“The Latest Developments in Pneumococcal Vaccines - Think Globally, Act Locally -”** as a Luncheon Seminar 23. Owing to the extensive contents of the lecture, covering basics to clinical aspects, we compiled all the contents in a booklet for a thorough understanding of the contents and distributed it as a part of the enlightenment activities.

It was approximately 3 years after the **“7-valent pneumococcal conjugate vaccine (PCV7)”** was switched to the **“13-valent pneumococcal conjugate vaccine (PCV13)”** in children. Unfortunately, a part of the analysis of the basic data was not available at that time. After completion of the analysis of all strains collected during the period from FY2010 to FY2016, we prepared a research paper as the 7-year molecular epidemiology study and submitted it to Journal of “Emerging Infectious Diseases (EID),” which was fortunately accepted. Based on these complete data, we have revised the actual status of pneumococcal infections in Japan and why vaccines are needed.

After World War II, until the appearance of penicillin, an antibiotic, pneumococcal pneumonia that developed suddenly in the community was a highly fatal infection even in young population. Looking back, immense development of antimicrobial agents in Japan has offered many benefits. The organization of social infrastructure, together with easy medical access supported by Japan’s National Health Insurance system, led to the world’s unprecedented super-aging society.

However, today, we are facing issues that cannot be addressed by the proper use of antimicrobial agents alone.

In our aging society, some infections, such as pneumococcal infection and streptococcal infection, follow a rapid clinical course. If vaccines are available for the prevention of the onset of these infections, vaccinations should be performed per the principle of “**population prophylaxis of infections”**. Unlike in children, individual immunity level differs markedly among middle-aged and older adults depending on the presence or absence of underlying diseases. We must accept and respond to the fact that “**aging is not only associated with a decline in physical function but also with a decline in immune function at the cellular level**.”

We hope that this booklet, which summarizes our research achievements on the basics and clinical aspects of *Streptococcus pneumoniae* in an easy-to-understand manner, would help you understand “Pneumococcal Infections and Vaccines” better.

March 1, 2019 Ubukata (responsible for drafting this article), Iwata, Ishii, and Hanada.

December, 2019 Translation into English was performed with Wajima (PhD) as a contributor.

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I

Introduction

1 Prologue

1) Potency of one penicillin ampule

Penicillin, the first antimicrobial agent, was accidentally discovered by a UK bacteriologist, Sir Alexander Fleming, in 1928 as a blue mold that prevented the growth of *Staphylococcus aureus* in a Petri dish. In 1941, over 10 years after that, Howard Walter Florey (Baron Florey), a physiologist, and Ernst Boris Chain, a biochemist, successfully obtained stable penicillin, and its mass production started in 1944. In 1945, all 3 scientists, Sir Alexander Fleming, Howard Walter Florey, and Ernst Boris Chain, were awarded the Nobel Prize in Medicine and Physiology for their achievements.

In 1944, in the middle of World War II, domestic penicillin, named "Hekiso (blue element)" in Japanese, was developed by Japanese bacteriologists, based on a paper that was secretly brought to Japan. Although Hekiso was undoubtedly an important pharmaceutical product for the military services, the intelligence of the Japanese bacteriologists who successfully produced a usable antimicrobial agent based on a paper in a very short period of time (Fusako Tsunoda, Hekiso/Japanese Penicillin Story, SHINCHOSHA Publishing Co., Ltd., 1978) is commendable.

After the war, the development of antimicrobial agents flourished in Japanese companies equipped with fermentation technology because it was necessary for the extraction of 6-aminopenicillanic acid (6-APA), the core of penicillin, by massive culturing of blue mold. The ampoules of "Hekiso," which were clearly brown but had a low titer, were available to people in the black market.

Figure-1 presents a picture about the chaotic era after the war, which is unimaginable to those aged 70 years or younger, clearly showing how a life was saved with just one penicillin ampule, and how penicillin was effective against pneumococcal infection at that time.

2) Pneumonia that follows a rapid clinical course in the elderly

Figure-2A shows the photographs of the right lung obtained after the autopsy of an honorary professor of the First Department of Internal Medicine, Medical Director of the Faculty of Medicine (former), the Private University K, who died of pneumonia in February 2011 at the age of 78 years. Before dying, the patient ordered his "**autopsy to help the society**." His son, a doctor, sent all the data of his "**entire medical history and pathological findings**" to "Ubukata" for use in the research on *Streptococcus pneumoniae* (*S. pneumoniae*). At the time, we did not have an opportunity to publish these valuable data. We believe that these are very valuable materials for understanding the present status of adult pneumococcal infections, and by compiling this booklet; we present some of the information from the vast amount of data sent to us.

My curriculum vitae (4)
Yoji Ohashi

To Okayama

In the end of 1946, my mother and I returned to Japan on a ship that brought back Japanese people from China. For a while, we stayed in Azae town (present Maniwa city) in Okayama, which was my father's hometown. Then, we moved to Hagoromo, Osaka, the hometown of my mother for my recuperation.

In April 1947, we went back to Okayama and I entered the local Azae Elementary School. However, I went to the school for only one day. I remember reading a line on a text book, "Blooming, blooming, cherry blossoms are blooming". From the next day, I did not feel well and was forced to stay home for recuperation.

Probably, as a rebound from the very poor life in China, I think I had eaten anything I saw after returning to Japan. Because of that or not, I had persistent diarrhea. On the second day of school, I had a high fever and was immediately banned to go to school. In any case, my lungs hurt, it was difficult to breathe, and I could not speak. Sadly, there was nothing I could do.

One day, when I had been lying on the bed since morning as usual, I heard my mother and grandmother talking in a terrace. "Yoji may die..."

On the following day, my grandmother who astonished me by saying so somehow obtained penicillin and injected it to me. Then, surprisingly, I got well so fast and was able to walk on my own after only one month. I continued to get better and was able to return to the school as a first grade student in April 1948.

By the time we moved to Bicchu Takahashi in the second semester of the second grade of elementary school, I was a mischievous young boy. In the local Takahashi Kita Elementary School, I was lucky to meet my lifelong friends: Masao Kawabata, Junzo Takahashi, Jushi Ibuki, and Osamu Morimitsu.

During the third semester of the sixth grade of elementary school, I was transferred to the elementary school attached to Okayama University as my father strongly recommended it. However, we are still good friends. Before our honorable teacher, Minoru Okuda passed away, we used to get together and enjoyed Bara (Festival) Sushi, the famous dish of Okayama.

Figure-1 Excerpt from "My curriculum vitae" dated April 4, 2017, The Nikkei.
The description suggests that he definitely had pneumococcal pneumonia.

Briefly, the patient was a heavy smoker (20 cigarettes/day × 50 years) and had been treated for atrial fibrillation since 2007. In August 2010, chest X-ray revealed "a ground-glass opacity" in the right upper lung field; accordingly, prednisolone 60 mg/day was started from November the same year. In January 2011, as interstitial pneumonia relapsed, prednisolone was resumed, although it was not effective. In February 2011, the concomitant administration of azathioprine 100 mg/day was started. However, the patient also developed diabetes mellitus. In the early morning of February 18, 2011, his family found him collapsed in the washroom. He was immediately transferred to a general hospital by ambulance but died 1 hour later.

An autopsy was performed on the following day. The lung specimens obtained at that time showed lobar pneumonia (**Figure-2A,** yellow circles); staining these tissue specimens with hematoxylin and eosin revealed infiltration of inflammatory cells, filling the airspace (**Figure-2B,** low-power field). In the high-power field, a number of diplococci with capsules were found around neutrophils and macrophages filled in the alveolar space (**Figure-2C,** high-power field, arrows). The pathological diagnosis was "interstitial pneumonia + alveolar (bacterial) pneumonia + cardiomegaly."

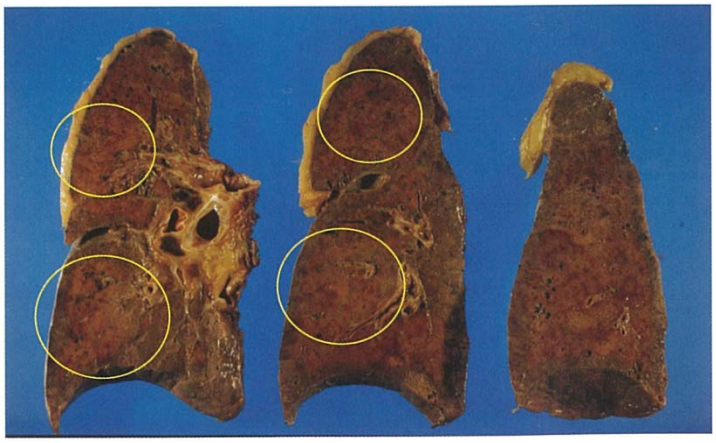


Figure-2A
Right lung of a case of death due to adult pneumococcal pneumonia
Courtesy of Professor Hiroshi Kamma, Department of Pathology, Faculty of Medicine, Kyorin University

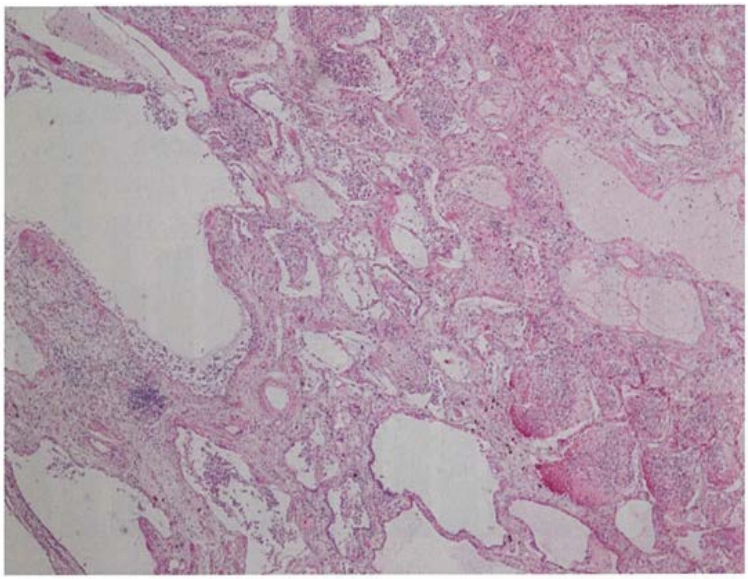


Figure-2B Low-power field view of the right lung at autopsy

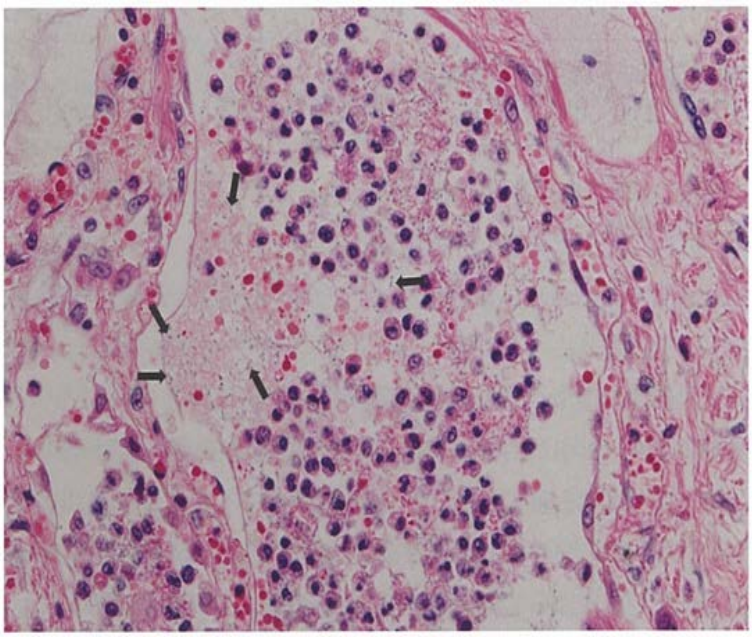


Figure-2C High-power field view of the right lung at autopsy

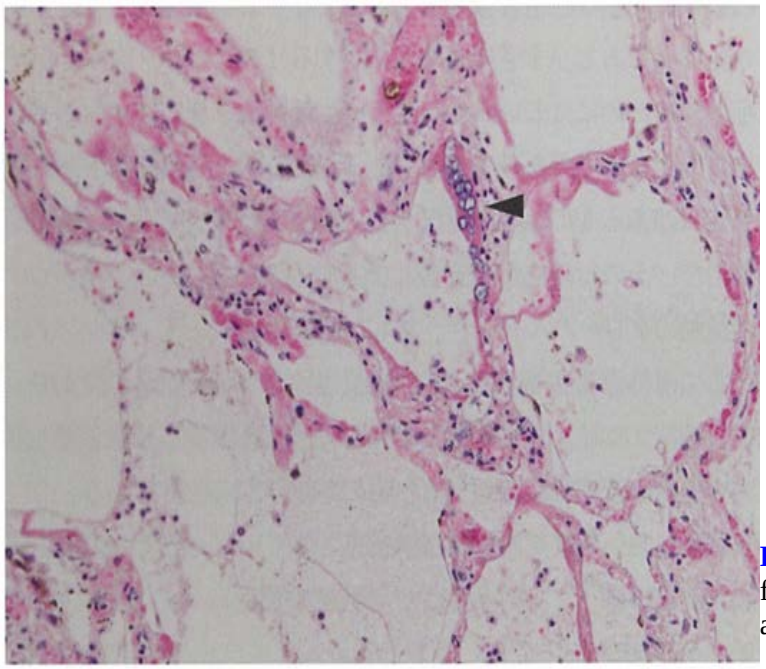


Figure-2D High-power field view of the right lung at autopsy

The case findings were suggestive of typical rapidly progressive (fulminant) pneumococcal infection. In a part of the view, multinucleated giant cell formation of the alveolar epithelium was observed (**Figure-2D** high-power field, arrow), suggesting super-infection with a virus such as respiratory syncytial virus.
Unfortunately, no pneumococcal strains were stored, and analysis such as capsular typing was not performed.

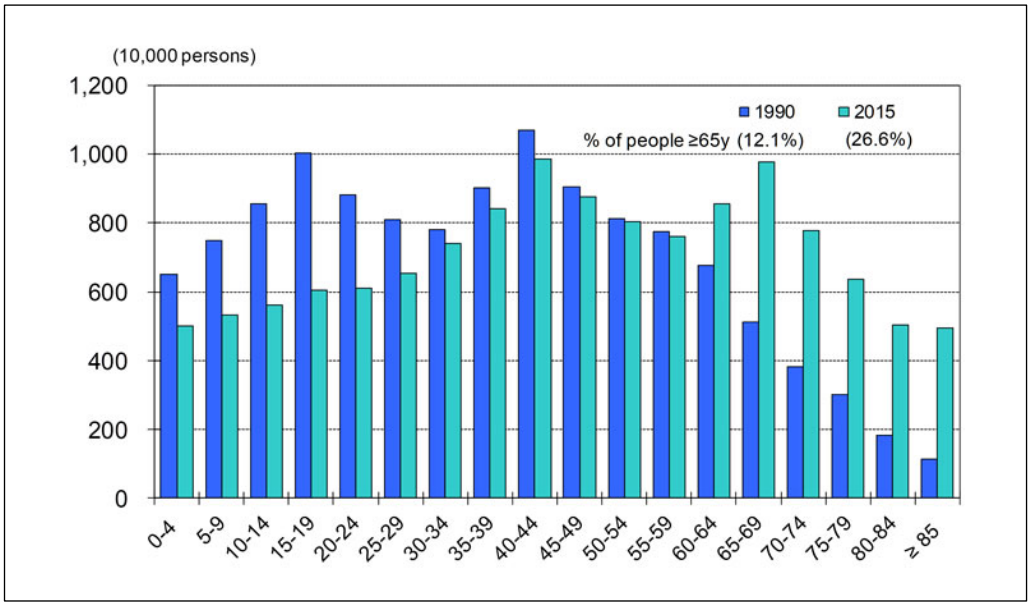


Figure-3 Rapid change in demographics in Japan

Demographics in Japan. Data obtained from the Statistics Bureau, Ministry of Internal Affairs and Communications. As of October 1, 2015, the total population was 127,095,000.
The percentage of people ≥65 years of age was 12.1% in 1990 and 26.6% in 2015.
Source: “Population Estimates” (Statistics Bureau, Ministry of Internal Affairs and Communications)

3) Social background of the shift from antimicrobial agents to vaccines

Figure-3 presents the comparison of age-stratified demographics in Japan between 1990 and 2015 based on "Population Estimates" by the Statistics Bureau, Ministry of Internal Affairs and Communications. Among the Organisation for Economic Co-operation and Development (OECD) member countries, the number of children is decreasing, and super-aging of the society is progressing rapidly in Japan. In 2018, the number of births in Japan decreased below a million to 921,000, and it was estimated that the population aged ≥65 years accounted for 28% of the total population.

A problem in the elderly is a rapidly increasing proportion of people with various underlying diseases, including lifestyle-related diseases. In other words, the number of people with risk factors for infection is increasing. Undeniably, the most important preventive measure against infection is the promotion of a healthy lifestyle from earlier ages. In addition, for the prevention of the onset of infections, individual/group "**vaccination**" is ideal for all if vaccines are available. From the standpoint of medical economy, vaccination is essential, and the government must take urgent measures for vaccination.

On the other hand, there are other issues related to considerable population traffic in association with globalization of economic activities, a significant increase in the number of inbound tourists, and political measures on the acceptance of overseas workers. The causative microorganisms of respiratory infections (bacteria and viruses) are repeatedly and rapidly transmitted and spread via humans. Besides, there are some inherent problems of causative microorganisms of intestinal infections, including parasites.

While development of drug resistance in these bacteria has been an issue, there is a concern that pharmaceutical companies are much less willing to develop antimicrobial agents in recent years.

2 Emergence of penicillin-resistant *S. pneumoniae*

1) EU and US (Figure-4)

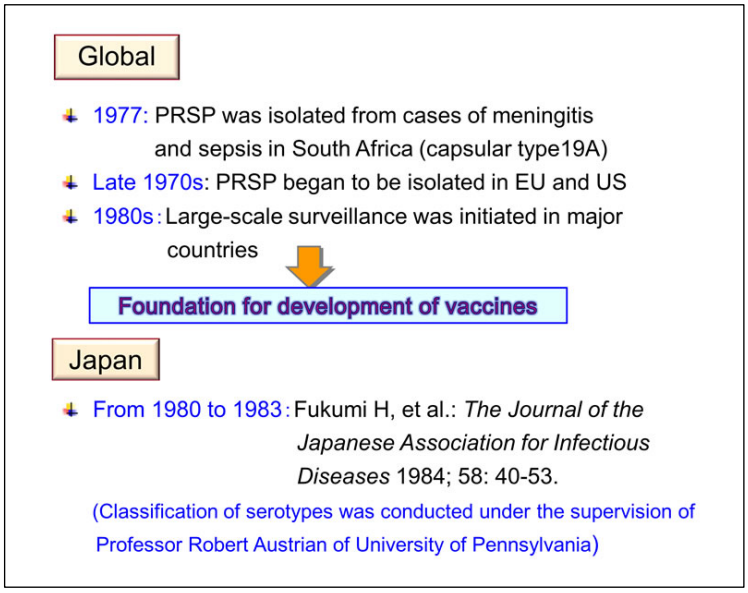


Figure-4
Comparison of pneumococcal research between EU/US and Japan

S. pneumoniae was known to have very high penicillin susceptibility. However, in 1977, in Durban, South Africa, penicillin-resistant *S. pneumoniae* (PRSP) was isolated from 3 cases of

purulent meningitis and 2 cases of sepsis. The penicillin susceptibility clearly reduced with the minimum inhibitory concentration (MIC) of 4 μg/mL; this finding caught attention after a shocking fact was reported that all cases of meningitis resulted in fatal outcomes. The capsular type (serotype) of the causative isolates was 19A (Appelbaum PC, et al., *Lancet*. 1977; ii: 995–997). Soon after that, PRSP began to be isolated more frequently in Spain; this trend gradually spread from France to other EU countries. By the late 1980s, PRSP was also isolated in South American countries and Southeast Asia.

In the EU and US where penicillin was the first-line drug, the appearance of resistant bacteria among the most significant causative pathogen of respiratory infections became a major problem. Thus, national continuous surveillance was started in some countries; through these initiatives, results were accumulated by capsular types, which are now used as the basis for the development of vaccines. In particular, the change in the incidence per 100,000 persons derived from the US CDC pneumococcal surveillance of 54 hospitals in 26 states conducted since the 1980s is essential for the evaluation of vaccine efficacy.

2) Japan

In Japan, no large-scale pneumococcal surveillance system existed at that time. A study primarily conducted by Dr. Hideo Fukumi (the then laboratory director) of the (former) National Institute of Health was the only study. However, collected strains were sent to the US for the analysis of capsular types because it was not possible to obtain capsular typing antisera in Japan.

In Japan, we started storing *S. pneumoniae* isolated from test specimens by freeze-drying in the late 1970s to 1980. Although the resistance of 60 obtained strains was retrospectively investigated at the molecular level of *pbp genes*, none had genetic mutations. The major capsular types were 6, 15, 18, 19, and 23.

In 1988, we first encountered and reported a case of purulent meningitis due to *S. pneumoniae* with reduced penicillin susceptibility (Arimasu O, et al., *The Journal of the Japanese Association for Infectious Diseases*. 1988; 62: 682–683). In addition, as shown in **Figure-5**, penicillin-intermediate *S. pneumoniae* (PISP) strains with gene mutations had already been reported around 1987 among those isolated at the Central Laboratory of the University of Tokyo Hospital; their capsular types were 6B, 19F, and 23F, which were assumed to be the origin of PRSP that gradually spread in Japan.

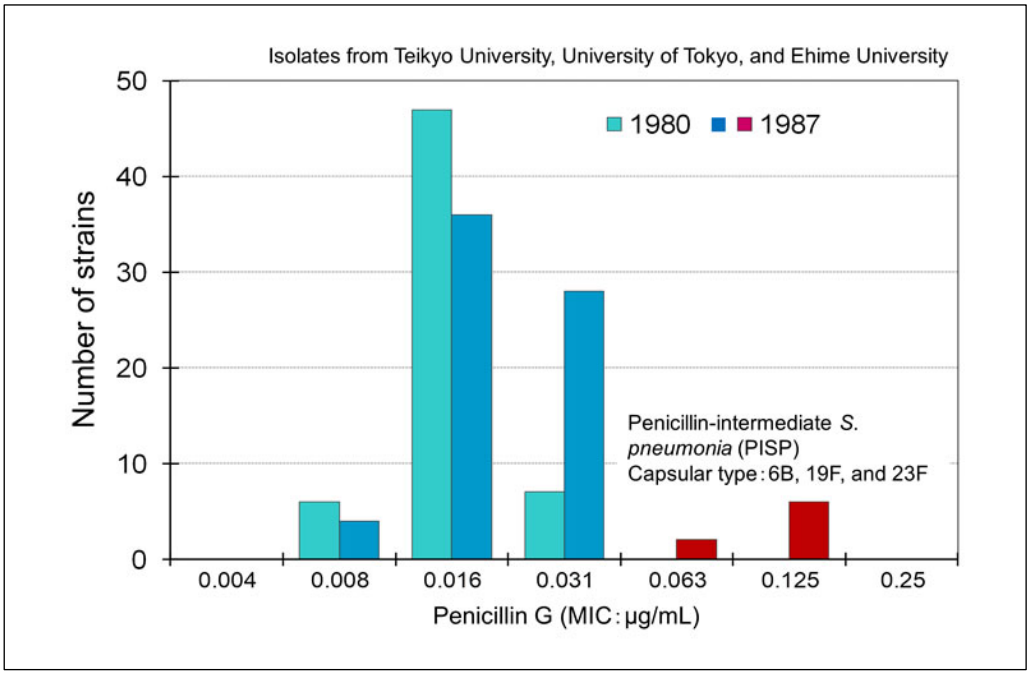


Figure-5 Timing of appearance of penicillin-intermediate *S. pneumoniae* with *pbp* gene mutations

(Ubukata K and Konno M, Revised penicillin-resistant *Streptococcus pneumoniae*, 1999)

3 Organization of "Penicillin-resistant *Streptococcus pneumoniae* Study Group"

1) Aiming at world-level surveillance studies

As described above, we organized a nationwide "**Penicillin-resistant *Streptococcus pneumoniae* Study Group**" following our first experience with a case of pediatric purulent meningitis caused by PISP in Japan. Obviously, such a report had to include not only accurate drug susceptibility test results but also a description of serotype of the bacterium involved in pathogenesis. Notably, the capsule is the most essential pathogenic factor of *S. pneumoniae*, and bacteria evolve by changing capsular genes to maintain its pathogenicity and escape phagocytosis by human white blood cells. Hence, capsules are highly diverse.

The study group was started in 1993 with the cooperation of laboratory technicians working at 48 medical institutions around Japan (Figure-6). In 3 years, 4,255 strains were collected from children and adults, including 75 strains derived from the cerebrospinal fluid and 95 strains derived from blood. For all strains, antisera were imported from Statens Serum Institut (SSI) in Denmark and each strain was examined by the Quellung reaction under a light microscope. We examined a total of 90,000 slides at the minimum. Although not detailed here, this analysis revealed that serotypes considered to be dominant in the US and South Africa were considerably different from those of Japanese isolates.

Later, this study group developed to the "National Surveillance of Invasive Pneumococcal Disease," which was limited to strains derived from sterile test materials, excluding sputum, nasopharyngeal swab, and otorrhea, for which the presence of indigenous bacteria could not be ruled out.

2) Accurate association between serotypes and resistant bacteria

Before conducting epidemiological research, we analyzed the mechanism of resistance at the genetic level as the basic research. We found that mutations of at least 3 types of cell-wall synthetases, i.e., penicillin-binding proteins (PBPs), were involved in the development of resistance. The **development of resistance due to changes in PBPs** is a mechanism "**common among bacteria living in the respiratory system**" such as *S. pneumoniae* and *Haemophilus influenzae* (*H. influenzae*).

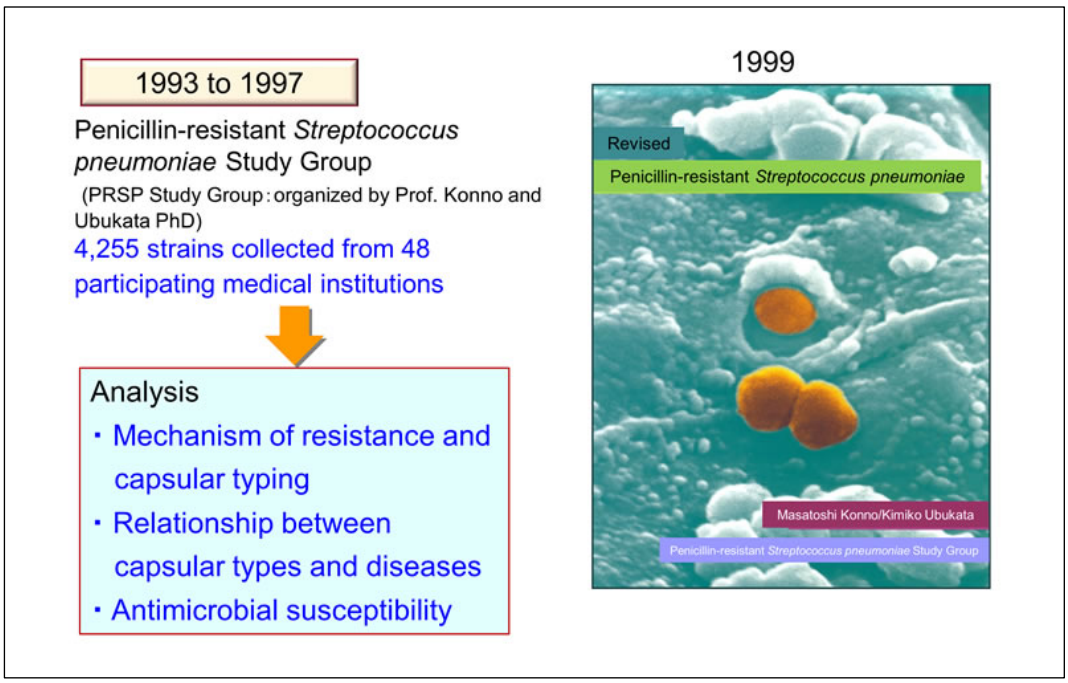


Figure-6 Necessity of a large-scale epidemiological research: Activities by the PRSP Study Group

In other words, **the essence of resistance development** is to survive by changing enzymes that are essential for bacterial survival slightly, such that it would not interfere with their survival, and by increasing the saturation concentration for binding of β-lactam drugs to PBPs to a level higher than that in the spinal fluid or tissue. The slight changes mentioned above make them definitively different from those living in the intestinal system that produce β-lactamases, enabling them to survive even at high concentrations (Ubukata K, *J Infect Chemother*. 2003; 9: 285–291).

In addition to the development of resistance by slight changes, we realized that analysis of resistance at the gene level was essential because these bacteria produce autolytic enzymes and the measurement of drug susceptibility highly varied among laboratories. In the real world, indeed, results reported from laboratories to clinical practice may not always be correct.

The relationship between mutations of genes encoding PBPs and susceptibility is described in a separate section. We feel proud that the establishment of the analysis method for these data put our research on *S. pneumoniae* at a global level, although it had been at least 10 years behind the EU and US.

Of note, the achievements of the PRSP Study Group, including both basic and clinical analyses, are summarized in a book.

II

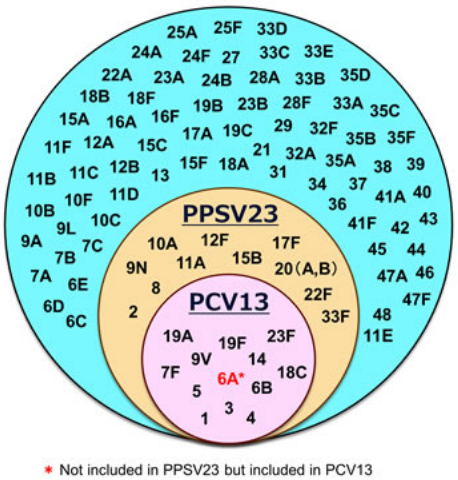
Nature of *S. pneumoniae*

1 Capsules and β-lactams target, PBPs

1) Diversity of capsules

For capsules, the key pathogenic factor in *S. pneumoniae*, 97 types have been reported, to date, as shown in **Figure-7** (Geno KA, et al., *Clin Microbiol Rev*. 2015: 28: 871–899). Based on the differences in their antigenicity, capsules types are classified into 24 single types and group types with more than one type. For example, the type 6 group comprises five types (6A, 6B, 6C, 6D, and 6E). Of note, all types must be precisely differentiated because some types show "**cross-immunity**," and the effect of vaccine could be expected even if they are not included in PCV13. For example, 6B shows cross-immunity with 6A, and 6A with 6C; however, no cross-immunity is observed between 19F and 19A. As of 2018, 92 types can be classified using antisera available from Statens Serum Institut (SSI).

Various capsular types are differentiated by the method using typing antisera described above. The best protocol for identification of capsule type uses the typing antisera based on analysis of *pbp* genes by real-time PCR methods (**Figure-11**).



Capsular types	Red letters indicate types included in PCV13, and green letters indicate types included in PPSV23
Single	1, 2, 3, 4, 5, 8, 14, 20
6	6A, 6B, 6C, 6D, 6E
7	7F, 7A, 7B, 7C
9	9A, 9L, 9N, 9V
18	18F, 18A, 18B, 18C
19	19F, 19A, 19B, 19C
23	23F, 23A, 23B
10	10F, 10A, 10B, 10C
11	11F, 11A, 11B, 11C, 11D, 11E
12	12F, 12A, 12B
15	15F, 15A, 15B, 15C
17	17F, 17A
22	22F, 22A
33	33F, 33A, 33B, 33C, 33D, 33E

Figure-7 Diversity of capsules (97 types) and vaccines.

Statens Serum Institut, 4th Edition, 2017.
Geno KA, et al., *Clin Microbiol Rev*. 2015;28:871-899.
(Drawing figure by Ubukata K)

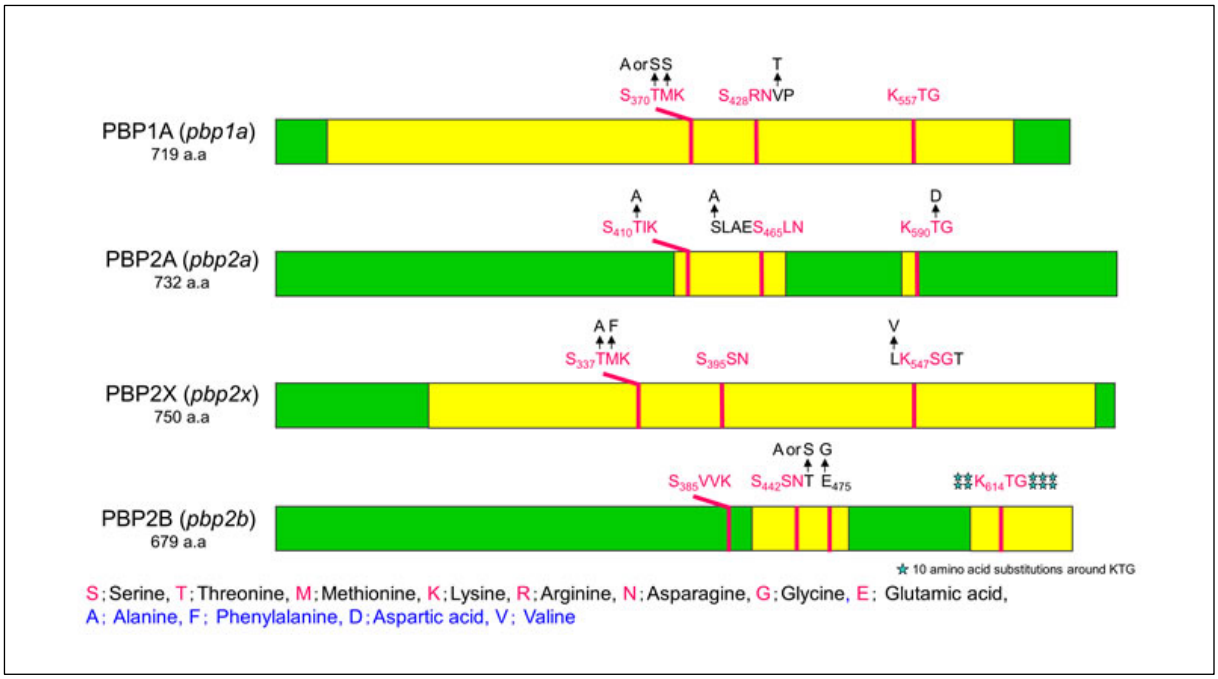


Figure-8 Amino acid (AA) substitutions of PBP enzymes related β-lactam-resistance.

Regions with several AA substitutions are shown in yellow; among these, the substitution of threonine within the conserved amino acid sequences (red line) by alanine the highest impact on the resistance development. However, AA substitutions around KTG motif are essential in PBP2B.

Asahi Y, et al., *Antimicriob Agents Chemother.* 1999;43:1252-1255.

As antisera are used, capsular typing is also referred to as serotyping.

As stated earlier, **the diversity of capsules** in *S. pneumoniae* **is it's intrinsically system to escape human immunity to invade the human body. The diversity of capsules, which could be considered the evolution of bacteria, indicates that *S. pneumoniae* can skillfully incorporate leaked DNA into strains of different capsular types, thereby changing itself and surviving with humans, despite a characteristic that it dies easily.**

Probably, the essential properties of *S. pneumoniae* will not change in the future.

2) Characteristics of *pbp* gene mutations

The development of resistance of *S. pneumoniae* cannot be elucidated without referring to the antibiotic concentration in the nasopharyngeal environment in which it lives as indigenous bacteria; this is true not only for β-lactam agents but also for macrolides and new quinolones. Notably, the blood or tissue concentrations obtained with usual doses of oral penicillin, ampicillin and amoxicillin or oral cephems, are low (see the package insert of each agent).

Thus, even if PBPs (Penicillin-Binding Proteins) involved in resistance are mutated (amino acid (AA) substitution, to be precise), the decline in susceptibility is subtle and often indistinguishable by bioassay.

For pneumococcal strains, we sequenced each *pbp* gene encoding four enzymes mediating peptidoglycan synthesis, as shown in **Figure-8**, namely PBP1A (*pbp1a*), PBP2B (*pbp2b*), PBP2X (*pbp2x*), and PBP2A (*pbp2a*) genes. Then, we drew conclusions based on the differences in morphological changes after *S. pneumoniae* was treated with β-lactams of different lineages.

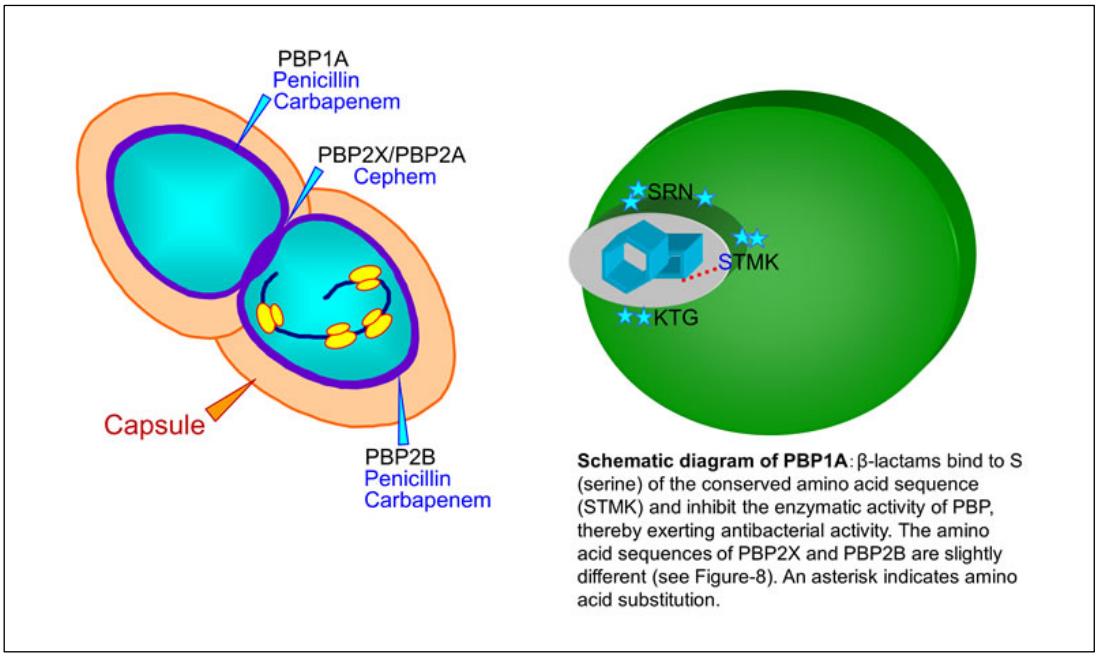


Figure-9 Relationship between β-lactams and PBP function of the targets.

Ubukata K, et al., *J Infect Chemother.* 1996;2:213-221.
Ubukata K, et al., *J Infect Chemother.* 1997;3:190-197.

Our conclusions were as follows:

- Each *pbp* gene encodes transglycosylase activity in the upstream region and transpeptidase activity in the downstream region.
- AA substitutions of, or in the proximity of, STM(V)K, SS(R)N, and KT(S)G in the conserved AA sequence of the transpeptidase domain are the most relevant for the onset of effect of β-lactams.
- PBP1A is a synthetic enzyme that extends the cell wall to the long axis during cell division. Penicillins and carbapenems, particularly panipenem, bind to (have an affinity to) PBP1A more than cephems, and strongly inhibit its enzymatic activity; in other words, the susceptibility to these antimicrobial agents is reduced in the presence of substitution in the conserved AA sequence of PBP1A.
- Enzymes PBP2X and PBP2A, which are assumed to be an alternative enzymes of PBP2X, synthesize septa during cell division and have a high affinity to cephems. When the enzymatic activities of PBP2X and PBP2A are inhibited, bacterial cell division is inhibited, resulting in significant bacterial cell elongation. When an AA substitution occurs in PBP2X and PBP2A, the susceptibility to cephems is markedly reduced.
- Enzyme PBP2B is involved in the synthesis of lancet form of cells, the final stage of cell division characteristic of *S. pneumoniae*; this gene has about 10 AA substitutions around the KTG motif of conserved amino acid sequence. Penicillins and carbapenems exhibit an excellent affinity to PBP2B. When an AA substitution occurs in this gene, the susceptibility to these antimicrobial agents is reduced (**Figure-9**).

Postscript: Subsequent three-dimensional analysis revealed that AA substitutions in, and in the proximity of, the conserved AA sequence are crucial (Asahi Y, et al., *Antimicrob Agents Chenmother*. 1999: 43: 1252–1255).

3) Relationship between *pbp* mutations and susceptibility

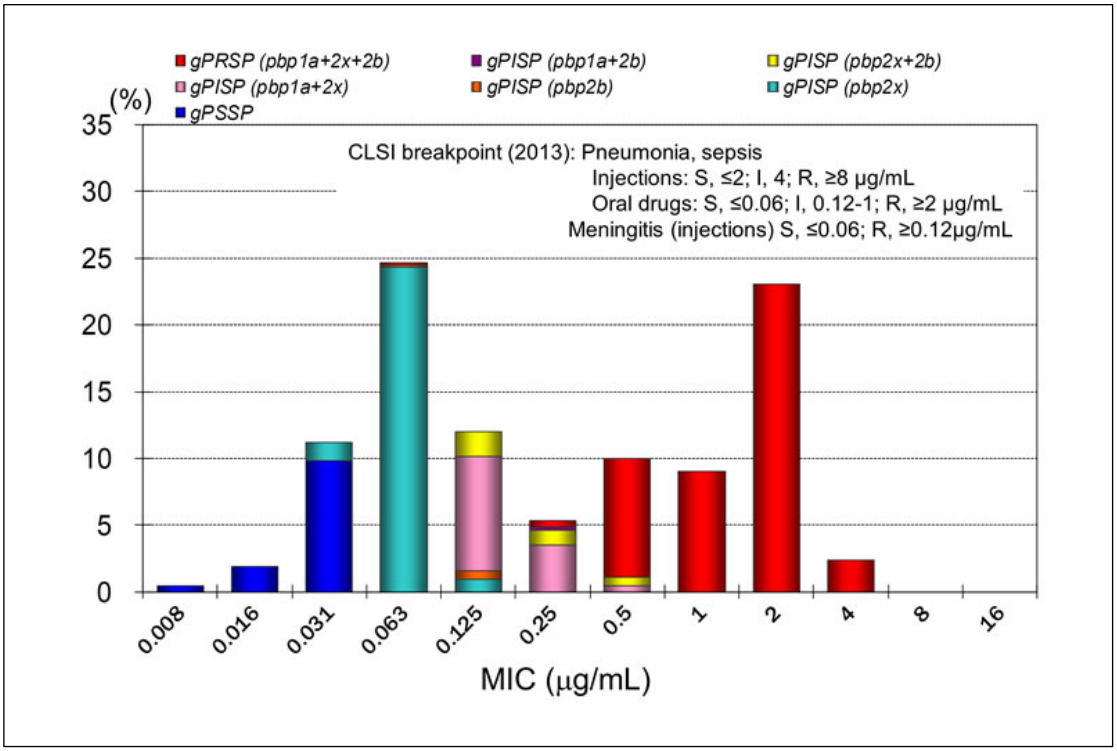


Figure-10 Abnormal 3 *pbp* genes and susceptibility to penicillin G (*n* = 633).
Chiba N, et al., *Emerg Infect Dis*. 2014;20:1132-1139.

Figure-10 shows the relationship between the results of the 3 *pbp* genes and susceptibility by bioassay. In addition, **Figure-10** presents the breakpoints by the US Clinical and Laboratory Standards Institute (CLSI) in 2013, as well as EUCAST (European Committee on Antimicrobial Susceptibility Testing) criteria. However, notably, the overseas identification criteria should not be applied to clinical practice in Japan where amount of doses differ in the first place. Moreover, the antibiotic concentration into location of inflammation fundamentally varies between oral and intravenous agents. As MICs determined by bioassay are highly variable and not absolute values, gene identification is more reproducible.

In **Figure-10**, genotypes (g) were represented as follows: penicillin-susceptible *S. pneumoniae* (gPSSP) without mutations; gPISP (*pbp2x*) with mutation in *pbp2x* gene; gPISP (*pbp1a* + *pbp2x*), gPISP (*pbp1a* + *pbp2b*), or gPISP (*pbp2x* + *pbp2b*) with mutations in two *pbp* genes; and gPRSP (*pbp1a* + *pbp2x* + *pbp2b*) with mutations in three *pbp* genes. Apparently, gene mutations differ based on the differences in one test tube; however, as bioassay could not accurately separate these differences, it was necessary to establish a rapid diagnostic method by polymerase chain reaction (PCR).

4) Rapid identification of serotypes and resistant genes

Genetic analysis of *S. pneumoniae* with various mutations in *pbp* genes needed an opposite approach. Specifically, primers were designed to search for *pbp* genes without any substitutions in the conserved AA sequence or proximal AA of each *pbp* essential for the development of β-lactam resistance. In the method described above, **clinical isolate was determined to be "without mutation" if DNA amplification was observed** with constructed primers**, or "with mutation" if amplification was not observed**.

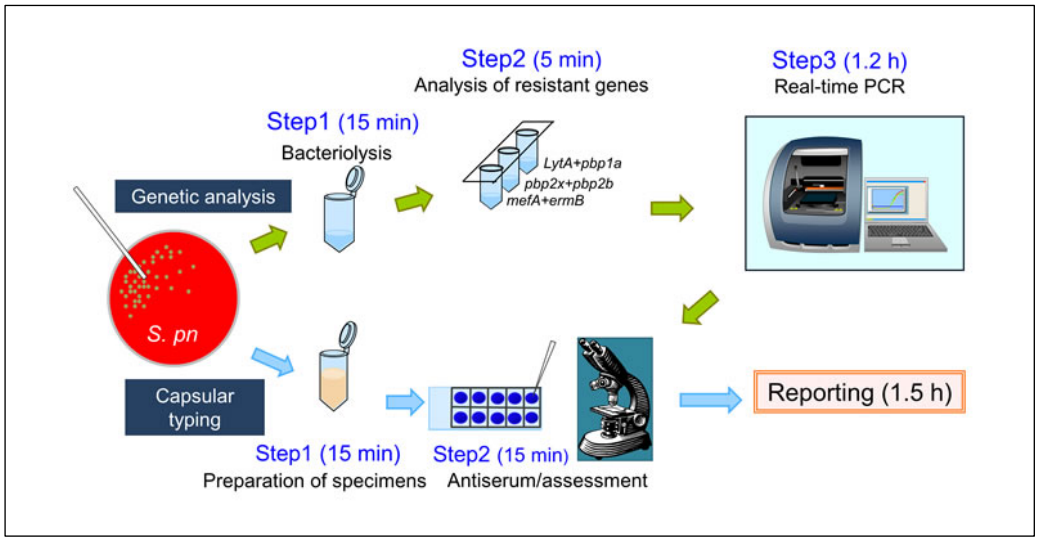


Figure-11 Protocol for simultaneous identification of resistant genes and capsular typing.

Ubukta K, et al., *J Infect Chemother.* 1997;3:190-197.
Chiba N, et al., *Microb Drug Resist*. 2012;18:149-156.

Currently, the *pbp* gene analysis method that we constructed has been improved by using a fluorescent probe (Cycleave probe) and has been developed into a kit and used as a research reagent (Takara Bio Inc.). The kit can search for not only *pbp* genes but also autolytic enzyme genes to identify *S. pneumoniae* (*lytA*) and macrolide-resistant genes (*mefA* and *ermB*). **Figure-11** shows the genetic analysis protocol. The PCR device is turned on after extracted bacteria are set. The data are displayed on the computer screen on a real-time basis, and the final results can be obtained within 1.5 h. Simultaneously, specimens are prepared for capsular typing. Based on the PCR results, serotyping is performed using antisera for serotyping. By determining the gene mutations first, we can limit the estimated serotypes of the test strain based on a vast amount of data collected. In the surveillance, the collected *S. pneumoniae* strains were analyzed as mentioned above, and the results were reported to each medical institution without delay.

2 Development of vaccines targeting capsules

1) Capsule as a vaccine target

As shown in **Figure-12,** *S. pneumoniae* has various pathogenic factors; hyaluronidase is involved in invasion, neuraminidase and choline-binding protein are involved in adhesion, surface layer proteins (PspA and PspC) avoid phagocytosis by neutrophil cells, pneumolysin and autolysin are pathogenic substances, and C-polysaccharide is related to C-reactive protein testing is essential for the diagnosis of infection.

Among the pathogenic factors mentioned above, the most important pathogenic factor is the capsule made of polysaccharides, covering the entire surface layer of the pneumococcal cells. The capsule plays a vital role in escaping phagocytosis by neutrophil cells. As described earlier, a total of 97 capsular types have been reported to date, and all are extremely diverse. Thus, the capsule is the definitive antigen, and the vaccines are developed by extracting and purifying the polysaccharides.

2) Differences in the production method between PCV13 and PPSV23

Currently, two types of pneumococcal vaccines, **"13-valent pneumococcal conjugate vaccine (PCV13)"** and **"23-valent pneumococcal polysaccharide vaccine (PPSV23),"** are available.

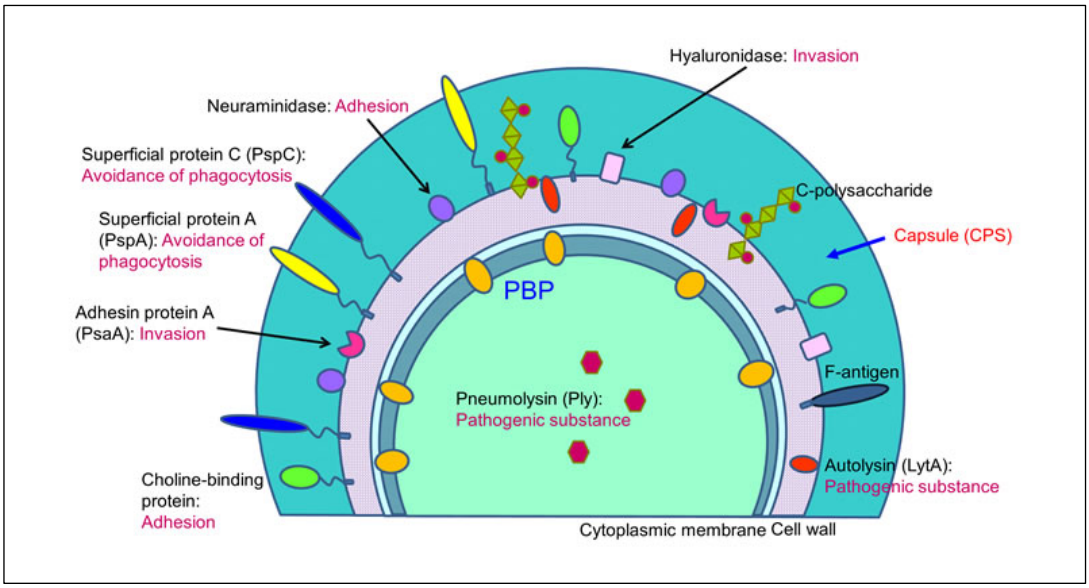


Figure-12 Various pathogenic factors produced by *S. pneumoniae*.

In addition, IgA1 proteases that break down IgA1, which accounts for ≥90% of IgA found in the human airway, are also produced extracellularly.

Tomasz A, *Streptococcus pneumoniae*: Molecular Biology & Mechanism of Disease, 2000. (modified)

PCV13 and PPSV23 are commercially available as Prevenar 13® and Pneumovax NP®, respectively (see the package inserts).

PCV13 contains 13 types of purified polysaccharides (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F), each conjugated to non-toxic diphtheria toxoid protein (CRM197), and is characterized by high immunogenicity (antigenicity) because of CRM197 binding. Currently, PCV13 is **"routinely vaccinated" in children aged ≥2 months and <5 years** (it can be administered to children aged 5–6 years as voluntary vaccination). The indication has been expanded for **those aged ≥65 years as "voluntary vaccination."**

PPSV23 is a vaccine containing 23 types of purified polysaccharides (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F). In October 2014, it was included in the routine vaccination program in Japan for those aged ≥65 years and high-risk individuals aged 60–65 years for 5 years until the end of March 2019 as a transitional measure. In addition, inoculation of PPSV23 to ≥65 years is now continued.

3) Differences in the induction of antibody production between PCV13 and PPSV23

A definitive difference exists in the postvaccination antibody production between **PCV13** and **PPSV23** (**Figure-13**).

After vaccination of CRM197-conjugated PCV13, antigens are first presented to dendritic cells to induce T-cell activation (T cell-dependent). Then, an interaction between activated T and B cells activates B cells. After that, in one pathway, B cells produce IgM, and maturation to plasma cells leads to the production of a large amount of IgG. Of note, IgG has a high affinity for antigens and is involved in opsonization, complement activation, and antigen neutralization. In another pathway, B cells mature to memory B cells, acquiring immunological memory. When a strain of capsular type in the memory invades the body, high IgG antibody production is rapidly induced (**booster effect**), resulting in increased immunity.

Conversely, PPSV23 only contains polysaccharides and, thus, does not result in the acquisition of immunological memory via T cells (T cell-independent).

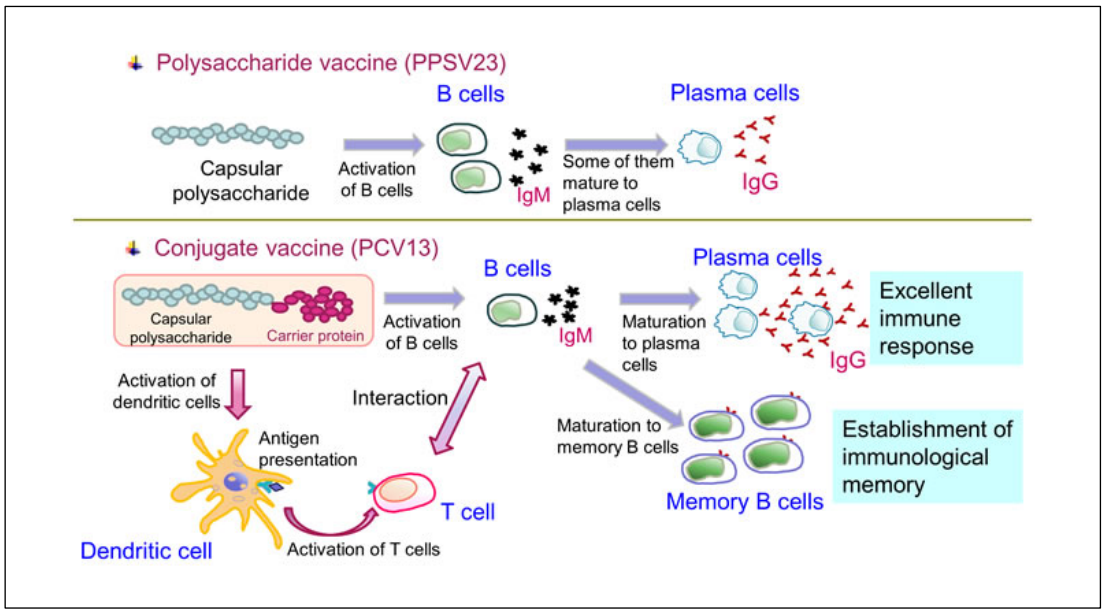


Figure-13 Differences in the immune induction between pneumococcal vaccines.

Figure derived from
Pollad AJ, et al., *Nat Rev Immunol*. 2009;9:213-220.
Schroeder HW Jr., et al., *J Allergy Clin Immunol*.2010;125(Supple2):S41-52.

After the activation of B cells, a part of B cells matures to plasma cells that produce IgG. Therefore, once obtained, the IgG antibody gradually weakens over time, following which the effect of the vaccine cannot be expected if infection occurs by a strain of capsular type contained in the PPSV23 vaccine. Hence, whether or not PPSV23 should be reinoculated 5 years after the vaccination remains debatable.

When scientific consideration is given to the use of these two vaccines, it is reasonable to administer PCV13 first because it can establish excellent immune response and immunological memory even in adults.

S. pneumoniae frequently colonizes the nasal cavity and nasopharyngeal mucosa of infants and has an aspect as indigenous bacterial flora, serving as the reservoirs for the establishment of infections such as otitis media, sinusitis, acute bronchitis, and pneumonia. In fact, the pneumococcal carriers have also been reported in adults. While PCV13 also induces immunity in the mucosa to prevent the colonization of *S. pneumoniae*, such prophylactic effect has not been observed with PPSV23.

III

Epidemiology of pneumococcal isolates

1 Introduction of pneumococcal vaccines (Table-1)

In Japan, the introduction of pneumococcal vaccines in children was started in full scale by "**public subsidy**" for children <5 years of age based on the "**Urgent Promotion of Vaccination Against Cervical Cancer**" in "Notification of the Health Service Bureau and the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare" on November 26, 2010. Although pneumococcal vaccines were not included in the routine vaccination program, the expectations were high. The vaccination rate increased rapidly, and it was estimated that more than 95% of the target children received vaccination within 2 years after the introduction. Subsequently, PCV7 was included in the routine vaccination program in 2013 and was switched to PCV13 by the end of 2013. Since then, the vaccination rate has been maintained at 90% or higher.

On the other hand, with regard to the introduction of pneumococcal vaccines for adults, PPSV23 was introduced in those ≥65 years of age and high-risk individuals between 60 and <65 years of age in October 2014, as an irregular routine vaccination (Class B disorder) with "time-limited transitional measure." However, the vaccination rate has been around 40% to 50% of the target subjects. During this time, it became feasible to inoculate PCV13 to adults of ≥65 years of age; however, being a "voluntary vaccination," its vaccination rate has been extremely low.

Performing accurate epidemiological analyses reflecting complicated administrative measures on vaccines and the status of vaccination is challenging. **The precise measurement of the effect of a vaccine warrants an epidemiological study of the same scale conducted before the introduction of the vaccine as the reference.** As we conducted nationwide epidemiological surveillance before the introduction of PCV7, we were able to perform a multifaceted analysis of isolates from invasive pneumococcal disease (IPD). The periods were defined as follows: FY2010 immediately before the introduction of PCV7 as "**Period I: Before the introduction of vaccines**" (the reference); 3 years from FY2011 to FY2013 as "**Period II: After the introduction of PCV7**"; and 3 years from FY2014 to FY2016 as "**Period III: After the introduction of PCV13**."

Table-1 Status of introduction of pneumococcal vaccines in Japan

Analysis	Month and year	Children (<5 years of age)	Adults (≥65 years of age)
Period I	February 2010	Launch of PCV7	
	November 26, 2010	Start of public subsidy for PCV7, "Urgent Promotion of Vaccination Against Cervical Cancer"	
Period II	2011	PCV7	
	2012	PCV7	
	April 1, 2013	Start of routine vaccination of PCV7	
	November 1, 2013	Switching from PCV7 to PCV13	
Period III	2014	PCV13	Introduction of PPSV23*
	2015	PCV13	
	2016	PCV13	

Period I (FY2010): Before the introduction of PCV7
Period II (FY2011-2013): Period of vaccination with PCV7
Period III (FY2014-2016): Period of vaccination with PCV13
* From October 2014, it is inoculated to those ≥ 65 years of age and high-risk individuals between 60 and <65 years of age as a routine vaccination.

2 Invasive pneumococcal disease (IPD)

1) Medical institutions and departments from which strains were sent

Figure-14 presents a list of medical institutions from which *S. pneumoniae* from IPD were sent in 7 years. A total of 2,856 strains of *S. pneumoniae* isolated from initially sterile clinical samples, such as blood, pleural effusion, cerebrospinal fluid, and synovial fluid, were collected. Strains were most frequently sent from foundation hospitals with bacterial laboratory (laborers' hospitals and mutual aid hospitals, etc., 37%), followed by community hospitals in each region (<25%) and private medical university hospitals, clearly reflecting the fact that IPD is the most commonly treated infections at core hospitals that are responsible for local healthcare, and that "**pneumococcal infection is an acute infectious disease that develops in the community**."

Regarding clinical departments where adult patients were first visiting, excluding children, the most common department was emergency department (32%), as shown in **Figure-15**. In contrast, 53.9% of patients visited the department of internal medicine, while one-third of them visited the department out-of-office hours, suggesting that the clinical course of IPD become progress rapidly to worsening after the onset. However, based on the collected questionnaire, awareness for the severity of pneumococcal infection seemingly differed considerably among departments.

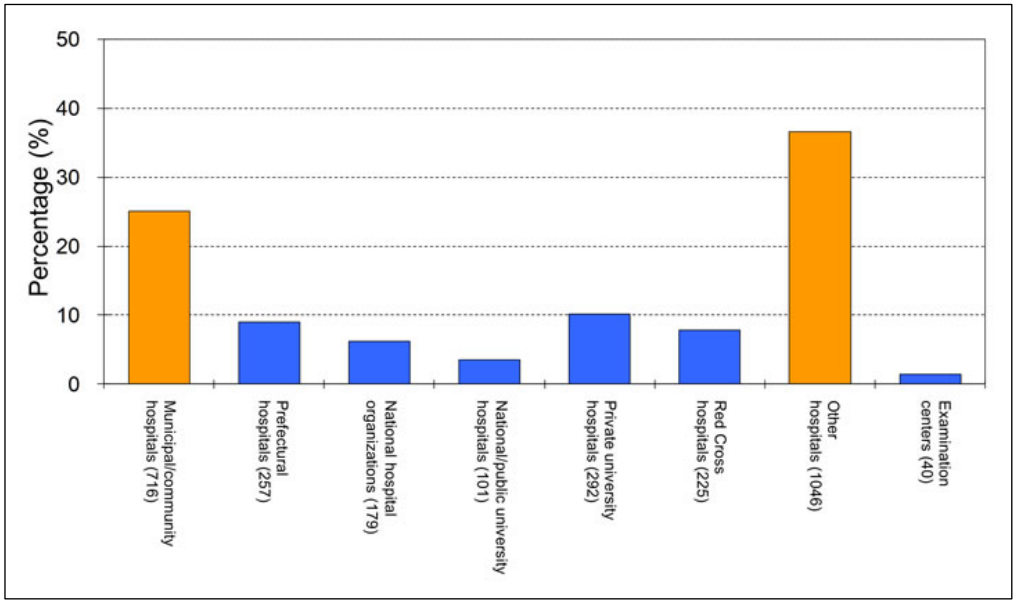


Figure-14 Medical institutions from which *S. pneumoniae* from IPD (n = 2,856) were sent (FY2010–FY2016)

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

2) Age distribution

Figure-16 shows the distribution of IPD cases by age. The proportion of adults was 64.8%, which was higher than that of children. Although not shown here, the proportion of adults and children was similar around 2006. Therefore, it can be asserted that the proportion of adults has been increasing in recent years.

In children, the peak was observed at the age of 1 year. Children ≤1 year of age accounted for 60.6% of the cases. **Among adults, 80.1% were ≥60 years of age, and the median age was 71 years.**

As described below, the number of pediatric IPD cases reduced after the introduction of PCV7 and PCV13; in adults, however, the rate seemed to be increasing with the increasing number of elderly because the post-war baby-boomer generation reached ≥65 years of age. Thus, controlling pneumococcal infections in the adult population is an urgent issue. Of note, hereafter, both PCV7 and PCV13 are referred to as PCVs in the document.

3) Age and Disease

Figure-17 shows the results of an investigation on differences in IPD among age groups. In children ≤5 years of age who received PCVs, 60% of the cases were of sepsis or bacteremia. On the other hand, the proportion of sepsis/bacteremia cases was almost constant (~20% to 30%) in adults ≥18 years of age. The higher the age, the higher the proportion of pneumonia (only cases in which bacteria were isolated from blood culture). In fact, pneumonia accounted for 65% of the cases in the elderly aged ≥75 years.

Characteristically, the proportion of adult purulent meningitis was significantly higher in a younger age group and those ≤64 years of age.

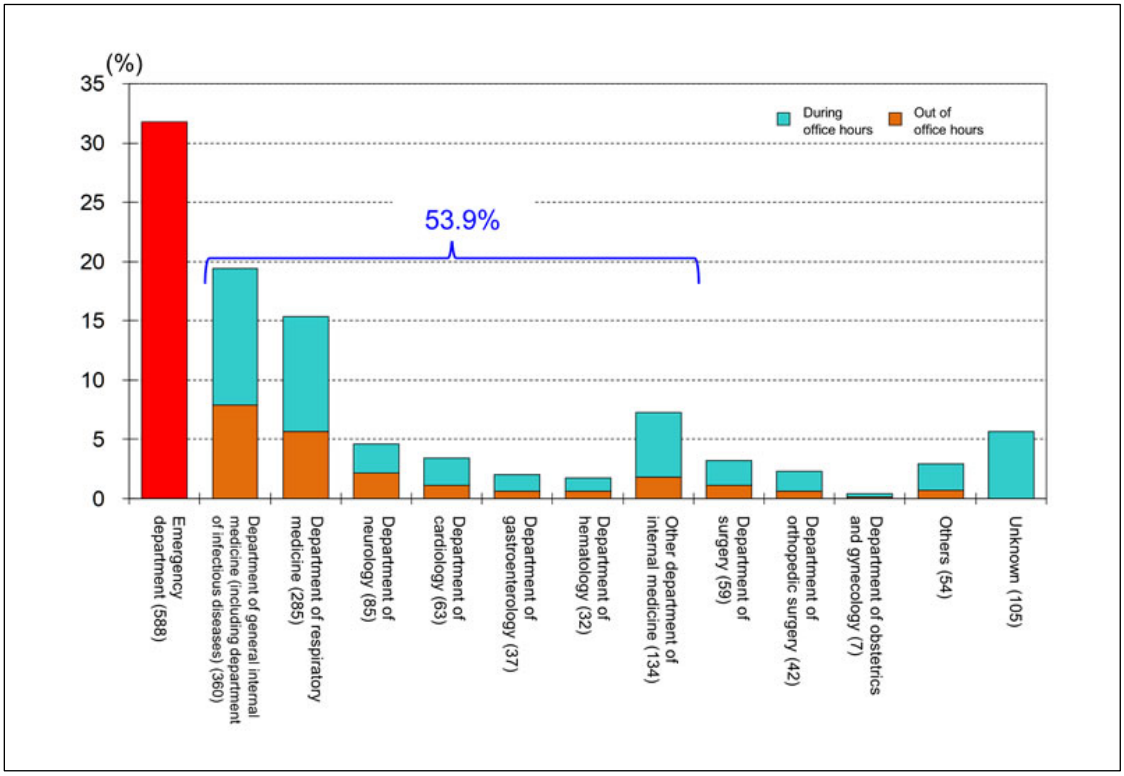


Figure-15 Consultation fee and status of consultation in adult IPD cases

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

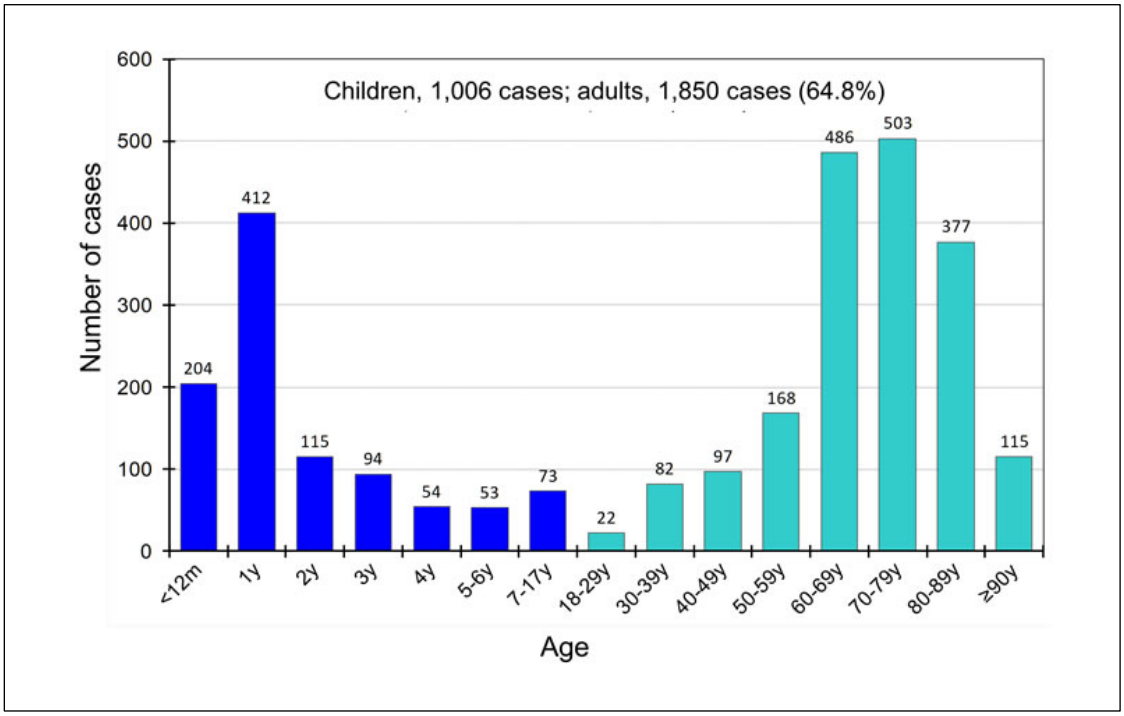


Figure-16 Age distribution of IPD cases (FY2010–FY2016)

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

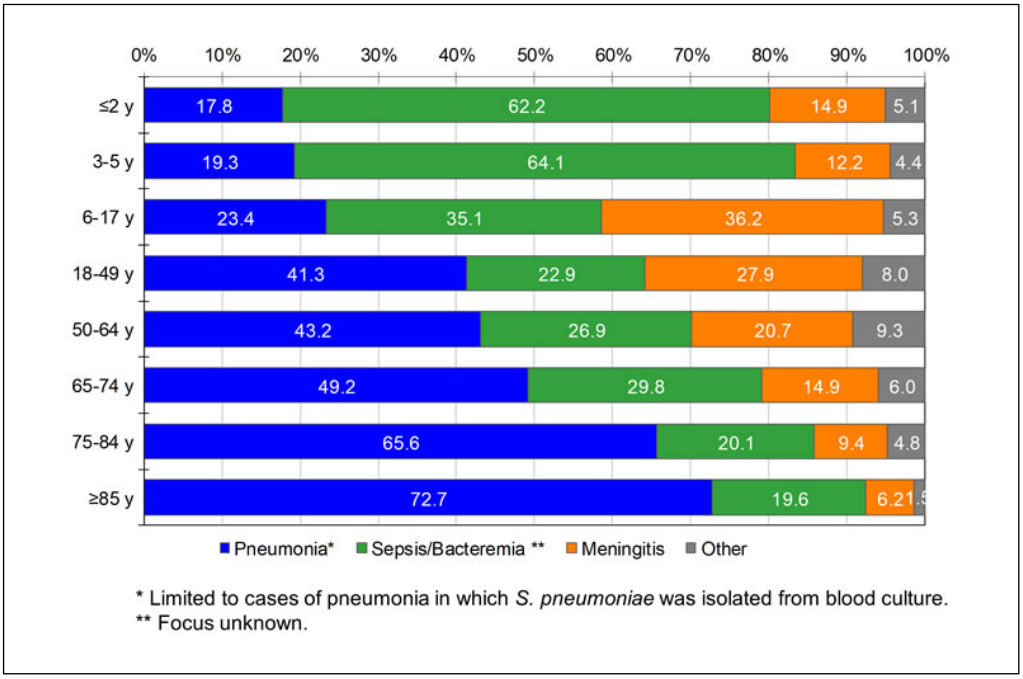


Figure-17 Relationship between age and IPD (n = 2,856)

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020. (plotted based on data)

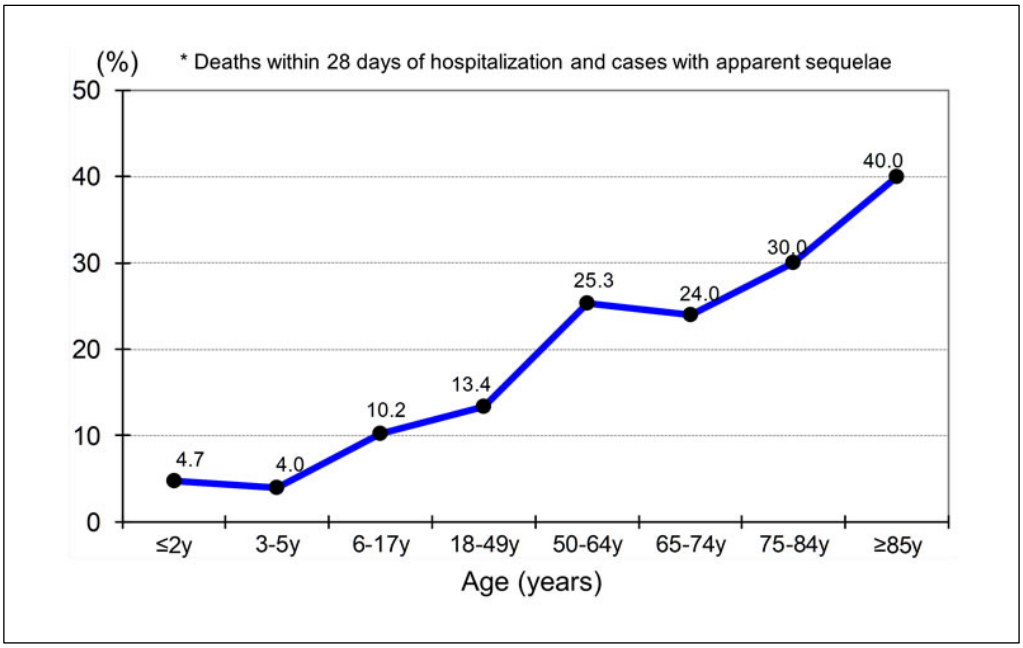


Figure-18 Relationship between age and rate of poor prognosis* among cases of IPD

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020. (plotted based on data)

4) Age and prognosis

Figure-18 shows the rate of poor prognosis in cases of IPD by age group. The proportion included deaths within 28 days of hospitalization and cases with apparent sequelae.

In children, the rate of poor prognosis was as low as around 4%, probably, because of a higher proportion of bacteremia. However, the rate increased with the increasing age group. In those in their 60s to 80s, the rate of poor prognosis was approximately 25% to 30%. Therefore, the prognosis was poor in one out of every 4 persons.

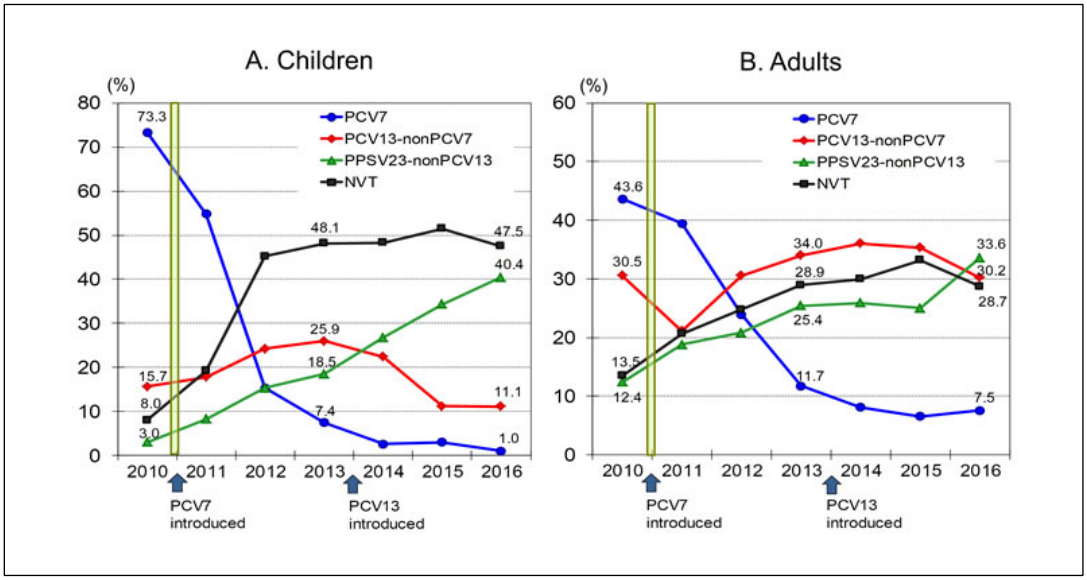


Figure-19 Changes in capsular types of strains from IPD after the introduction of PCVs in children

Children, <18 years; adults, ≥ 18 years
Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

3 Changes in capsular types and resistance genotypes

1) Introduction of conjugate vaccines (PCVs) and changes in capsular types

We compared the time courses of changes in capsular types as follows. The 7-year analysis (before the introduction of PCV7, after the introduction of PCV7, and after switching to PCV13 in children) was categorized into strains from children and strains from adults by fiscal year. Furthermore, strains were classified into the following categories: (i) PCV7: serotypes included in PCV7; (ii) PCV13: serotypes included in PCV13, excluding PCV7 serotypes; (iii) PPSV23: serotypes included in PPSV23, excluding PCV13 serotypes; and (iv) NVT (non-vaccine type); serotypes not included in either vaccine. The results are shown in **Figure-19.**

a) Strains from children (A): After the introduction of PCV7, PCV7 serotypes were drastically reduced from 73.3% in FY2010 before the introduction to 7.4% in FY2013. The vaccination rate in the year following the introduction of PCV7 (FY2011) was estimated to be 40% to 50%; even at these rates, a 20% reduction was observed. After PCV7 was switched to PCV13, additional serotypes included in PCV13 were also halved. On the other hand, the proportion of PPSV23 serotypes is increasing over time. PPSV23 serotypes and NVT accounted for 90% of strains from IPD. In recent years, the majority of *S. pneumoniae* from children that are sent for analysis are PPSV23 serotypes.

b) Strains from adults (B): After 3 years of the introduction of PCV7 in children (FY2013), the proportion of PCV7 serotypes drastically reduced from 43.6% to 11.7% in adults as well; it was a phenomenon called **herd immunity** in children and adults overall. We interpreted that *S. pneumoniae* of PCV7 serotypes which colonize in the nasopharynx of children were gradually reduced because of the acquisition of mucosal immunity by vaccination, resulting in a reduction of PCV7 serotypes of *S. pneumoniae* in the population. Subsequently, PCV13 serotypes tended to increase slightly, although these serotypes were also gradually reduced after the introduction of PCV13 in children.

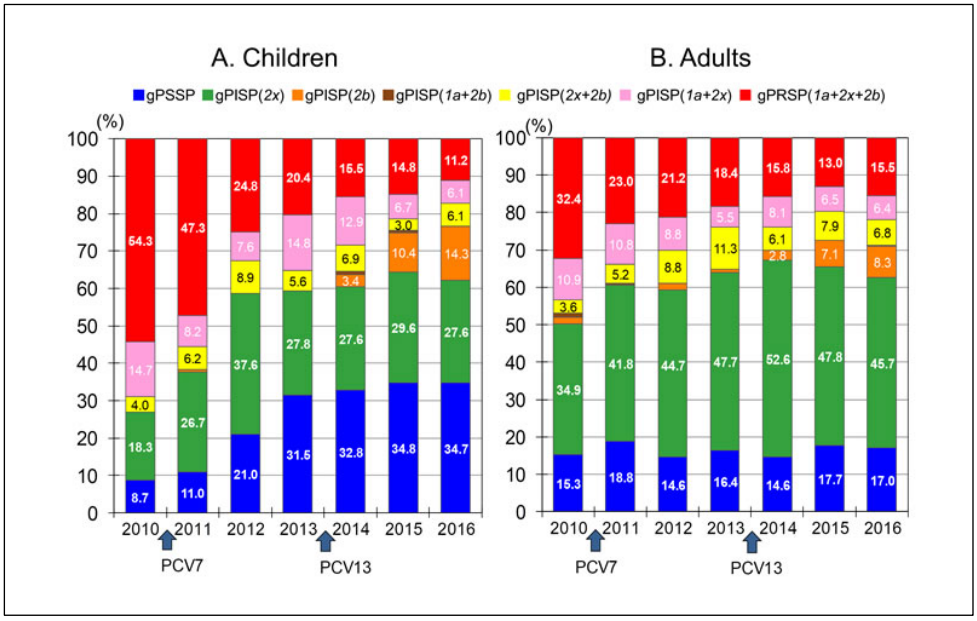


Figure-20 Changes of resistance genotype after the introduction of PCVs in children
Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

On the other hand, the vaccination rate of PPSV23 has been reported to be approximately 40%. In recent years, PPSV23 serotypes have been increasing, and future trends are attracting attention. As described earlier about the differences in immune induction based on the manufacturing method of these vaccines, PPSV23 has no effect of herd immunity.

2) Introduction of PCVs and changes of resistance genotypes

The introduction of PCVs in children resulted in significant changes in strains from IPD. Before the introduction of vaccines, half of the isolates were genotype (g) PRSP. To clarify how the proportion of resistant isolates, a major therapeutic issue changed, comparing data from genetic analysis is a more accurate method, as described earlier. **Figure-20** shows the yearly changes of resistance genotypes identified molecularly for *pbp* genes by PCR. Along with dramatic changes in serotypes caused by the introduction of vaccines, resistance isolates including gPRSP were also drastically reduced

a) Strains from children (A): In FY2012, when the vaccination rate of PCV7 was >90%, the proportion of gPRSP was halved compared with FY2010, while the proportion of gPSSP and gPISP (*pbp2x*) was increased. After switching to PCV13, the proportion of gPRSP was further reduced to 11.2%, while the proportion of gPSSP and gPISP (*pbp2b*) was increased. These results indicate a close association between serotypes and resistance genotypes in the isolates (detailed in the next section).

b) Strains from adults (B): With regard to the changes in the proportions of resistance isolates in adults, the proportion of gPRSP was clearly reduced from 32.4% in FY2010 to 18.4% in FY2013. In contrast, the proportion of gPISP (*pbp2x*) was increased. In and after FY2014, when the vaccine for children was switched to PCV13, the proportion of gPRSP continued to be reduced; it was only 15.5% in FY2016. However, little change occurred in the proportion of gPSSP and gPISP. As shown here, gPISP (*pbp2x*) continued to account for half of the isolates because the majority were mucoid, serotype 3 strains. Moreover, the proportion of gPISP (*pbp2b*), shown in orange, started to increase from around FY2015 as in children; this gPISP (*pbp2b*) of serotype 12F has been isolated from an earlier phase in the EU and US, where penicillins are frequently used.

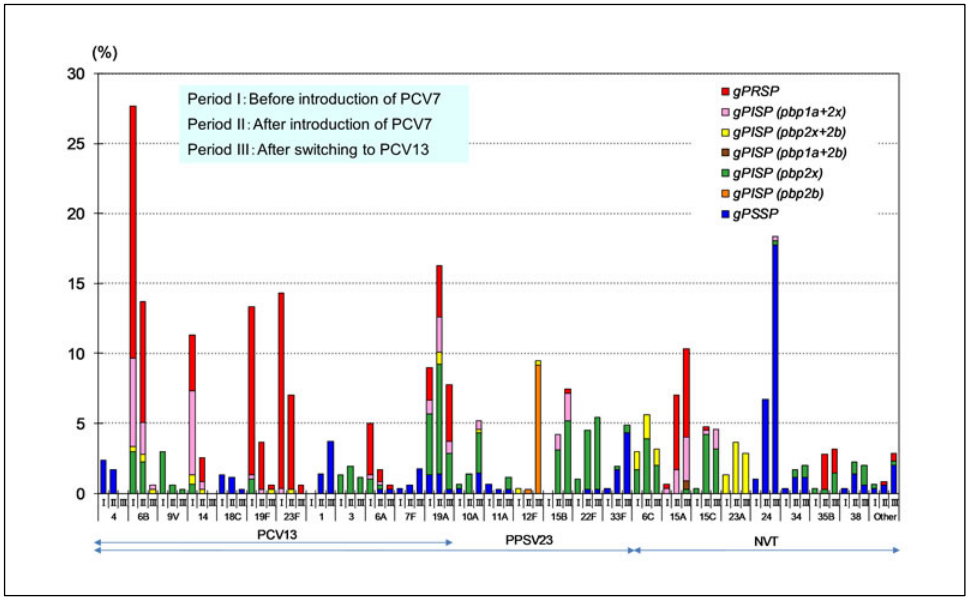


Figure-21 Changes in capsular types and resistance types in strains from children (n = 1,006)
Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

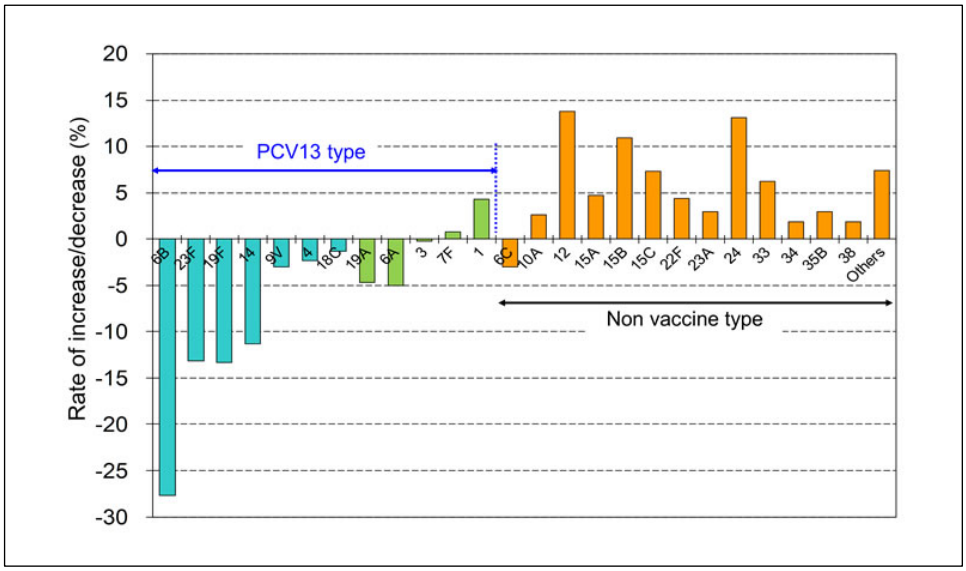


Figure-22 Children: Changes in each capsular type after the introduction of PCVs
- Comparison between FY2010 (n = 300) and FY2016 (n = 92)-
Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.(plotted based on data)

3) Relationship between capsular types and resistance genotypes

a) Strains from children: Consistent with the timing of introduction of PCVs in children, results were classified into the following periods: Period I (before introduction), Period II (after the introduction of PCV7), and Period III (after the switching to PCV13). The results of the changes in each serotype and resistance genotype are shown in **Figure-21**.

For gPRSP, which accounted for half of the isolates before the introduction of PCV7, serotypes 6B, 14, 19F, and 23F were dominant; however, the proportion of these serotypes decreased drastically after the introduction of PCV7, and they are hardly isolated at present. Among 6 serotypes added by PCV13, 19A has also been markedly reduced. For serotypes that are not included in PCV13, 6 serotypes (12F, 15B, 22F, 33F, 15A, and 24) tended to increase rapidly. In addition, changes in 15A and 35B, including gPRSP, demand considerable attention.

In order to present the isolates in FY2010 (before the introduction of vaccines) and FY2016 in an easy-to-understand manner, the trend of each serotype is shown as the rate of increase/decrease in **Figure-22**. PCV13 serotypes were markedly reduced, except for serotypes 1 and 7F. When cases with serotypes 1 and 7F were examined for the history of vaccination, all had received PCV7. Serotype 6C tended to decrease because it shows cross-immunity with 6A.

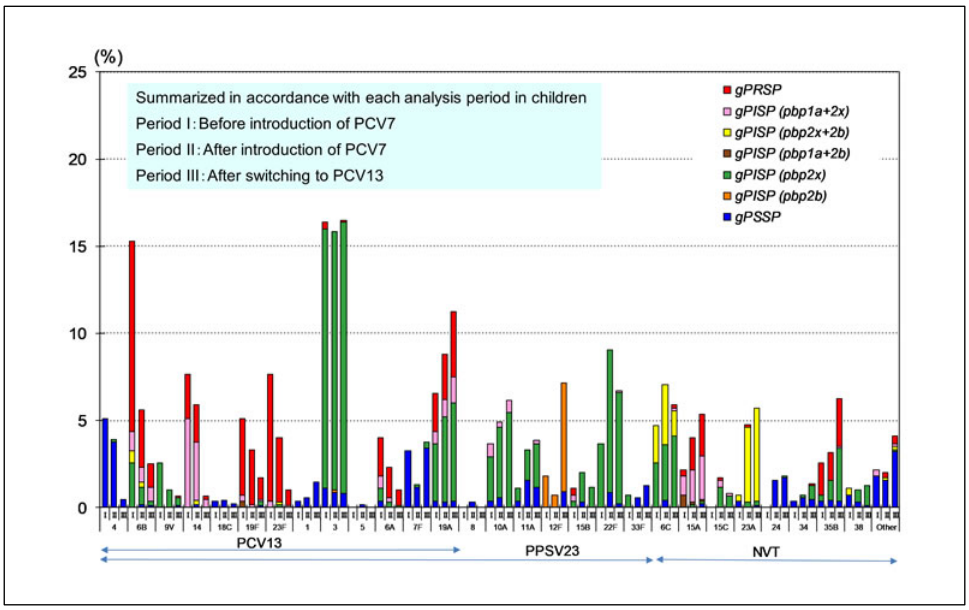


Figure-23 Changes in capsular types and resistance genotypes in strains from adults (n = 1,850)
Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

b) Strains from adults: The time courses of changes in each serotype and resistance genotypes of isolates from adults in Periods I, II, and III are shown in **Figure-23**. PCV7 serotypes were drastically reduced, including serotypes 6B, 14, 19F, and 23F, in which gPRSP had been dominant; this serotype change led to a decrease in gPRSP over time across cases of IPD in adults. For serotypes added by PCV13, 6A was decreased; however, the decrease was not observed in other serotypes. **The strains from adults were characterized by the fact that serotype 3 was always observed at a rate of 15%.** To control *S. pneumoniae* of serotype 3, vaccination of PCV13 directly to adults is considered necessary. In addition, the emergence of gPRSP in this serotype is a problem. The relevant mechanism will be discussed in a different section.

Among PPSV23 serotypes, excluding PCV13 serotypes, the proportions of 10A, 11A, 12F, and 22F were markedly increased. For NVT, the proportions of 15A, 23A, 24, 34, and 35B were also apparently increased. Among 15A and 35B, the proportion of gPRSP was increasing gradually as is the case in children.

Figure-24 shows the changes in serotypes in FY2016 compared with FY2010. Although all PCV7 serotypes introduced in children tended to decrease, serotypes added to PCV13 were not always clearly decreased. However, focus should be on the trends of PPSV23 serotypes, excluding PCV13 serotypes. Particularly, 10A, 12F, and 22F were increased approximately 5%. Notably, NVT was also increasing, except for 6C.

The prophylactic effect of PPSV23 against adult IPD and pneumonia cannot be accurately evaluated unless serotype data are consolidated to one research institution based on the nationwide surveillance, rather than data restricted to a specific region, besides strictly differentiating PCV13 serotypes from others.

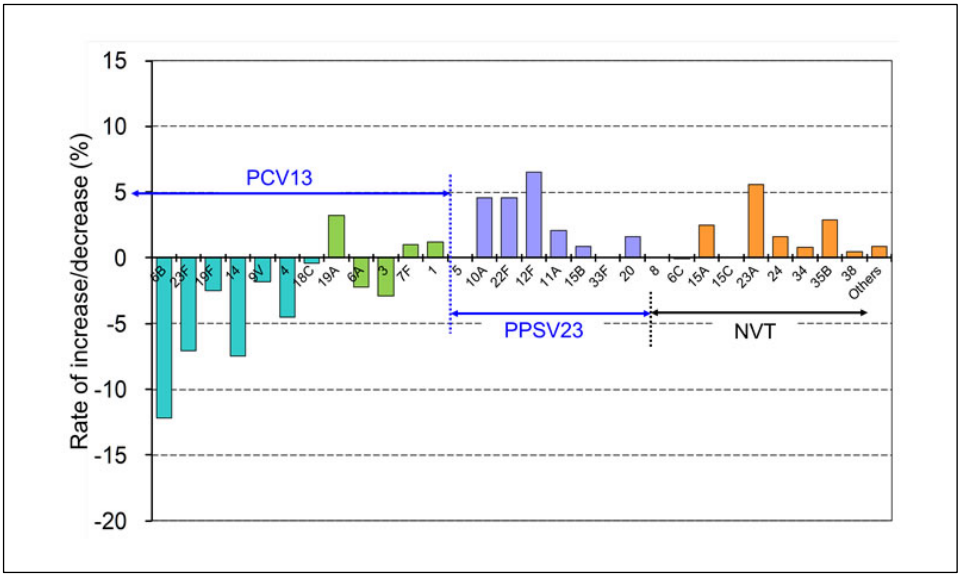


Figure-24 Adults: Changes in each capsular type of isolates from adults
-Comparison between FY2010 (n = 266) and FY2016 (n = 249) -
Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.(plotted based on data)

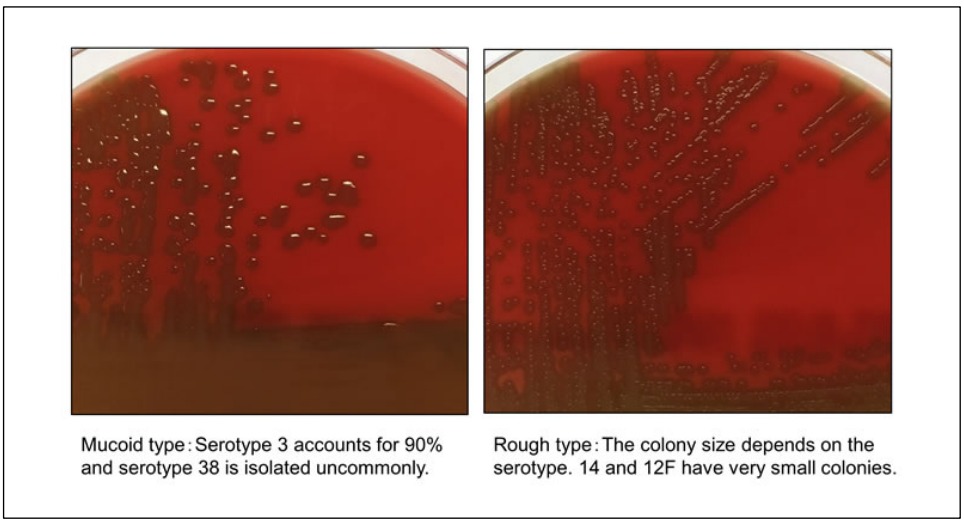


Figure-25 Mucoid and rough type colonies of *S. pneumoniae*
Photographed by Ubukata K. (Dep. of Infectious Diseases, Keio Univ. School of Medicine)

4) Capsular type 3 *S. pneumoniae*

The description of serotype 3 *S. pneumoniae* is as follows.

As stated earlier, serotype 3 *S. pneumoniae* has been continuously isolated (≥15%) in adults. Unlike other serotype strains, the serotype 3 strain forms characteristic, large, highly viscous, swollen colonies called mucoid on the blood agar plate, as shown in **Figure-25**; this serotype is known to induce severe otitis media (mucosus otitis media) in children. However, the serotype 3 strain is infrequently isolated from pediatric IPD but frequently isolated from adults. Moreover, serotype 3 is highly pathogenic, causing lobar pneumonia and pyothorax, which can rapidly progress to severe. However, strangely, serotype 3 only rarely occurs purulent meningitis.

Although the difference in the frequency of isolation of serotype 3 strains between adults and children remains unclear, it may be interpreted as follows. As children have a narrow airway, it can be presumed that serotype 3 strains invading and colonizing the upper airway are less

likely to fall into the lower airway, thereby failing to invade the lung and bloodstream. In adults, on the other hand, ciliated cells in the upper and lower airways become desquamated and their functions are reduced as the age increases, thereby making it easier for pneumococcal cells to attach to the platelet-activating factor (PAF) receptors in epithelial cells. In addition, as bacterial clearance function is reduced with aging, and serotype 3 strains have particularly viscous capsules, it is difficult to eliminate serotype 3 strains by wrapping them in mucus. Therefore, it may have a higher chance of invading the lung and bloodstream. Of note, special attention should be paid to secondary infections with *S. pneumoniae* after respiratory viral infection.

IV

Characteristics of Cases Based on Epidemiological Analysis

1 Children

1) IPD

The yearly changes in pediatric IPD cases are shown in **Figure-26**. IPD was broadly categorized as follows: (i) meningitis, (ii) sepsis and bacteremia with focus unknown, (iii) pneumonia in which *S. pneumoniae* were isolated by blood culture, and (iv) others.

Usually, on the global scale, the change in the number of cases is calculated as the change in the incidence per 100,000 people. Although we conducted our surveillance on the same scale, unfortunately, the morbidity could not be calculated because our activities were voluntary. Therefore, morbidity is expressed as the change in the number of cases. For accurate calculation of morbidity, all the following conditions must be fulfilled: (i) inadequate pretreatment with antimicrobial agents, especially intravenously, has not been administered, (ii) accuracy of the bacterial testing has been consistently high (it is difficult to demonstrate *S. pneumoniae* by the bioassay if antibiotic has been used even once), and (iii) blood culture has been performed upon hospitalization. However, given the medical situations in Japan, an accurate calculation of morbidity is highly challenging.

The most noteworthy trend in IPD was a drastic reduction in cases with meningitis after the introduction of PCV7 and PCV13 (87% reduction from before introduction). Meningitis mostly occurs in children <2 years of age; notably, the effect of vaccination in this age group was significant. Moreover, cases of sepsis and pneumonia were clearly reduced after the introduction of vaccines. However, some cases occurred due to pneumococcal strains of serotypes not included in the vaccines. Furthermore, other diseases, including septic arthritis, were also reduced.

2) Acute otitis media (AOM)

Figure-27 shows the results of surveillances on bacterial pathogens causing acute otitis media (AOM) in 2006 and 2016; these surveillances were conducted in cooperation with otolaryngologic clinics. The pathogens were identified by PCR in combination with the culture method (Ubukata K, et al., *Pediatr Infect Dis J*. 2018; 37: 598–604). Clinical samples were carefully collected by tympanocentesis and otorrhea.

Before the introduction of PCVs, AOM caused by *S. pneumoniae* and *H. influenzae* occurred at similar rates. However, after the PCVs introduction, AOM cases caused by *S. pneumoniae* halved, while those caused by *H. influenzae* relatively increased; **Figure-28** shows the changes in capsular types of *S. pneumoniae* associated with these changes. Before the introduction of vaccines, PCV13 serotypes were dominant (82.8%), which drastically reduced to 18.5% after 3 years of the introduction of PCV13. Instead, the proportion of NVT was relatively increased.

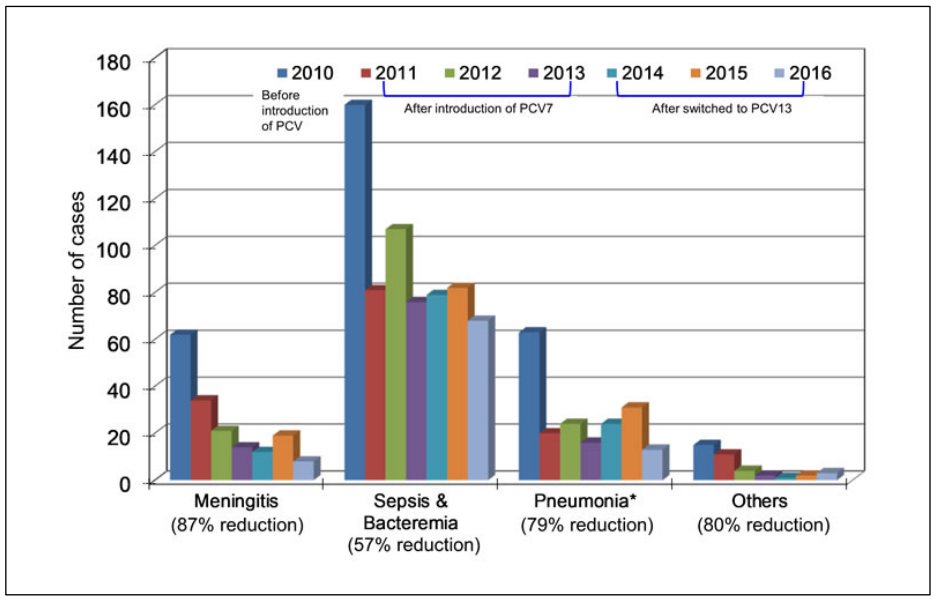


Figure-26 Reduction of IPD after the introduction of PCVs in children

* Only included isolates from the blood culture.

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020. (plotted based on data)

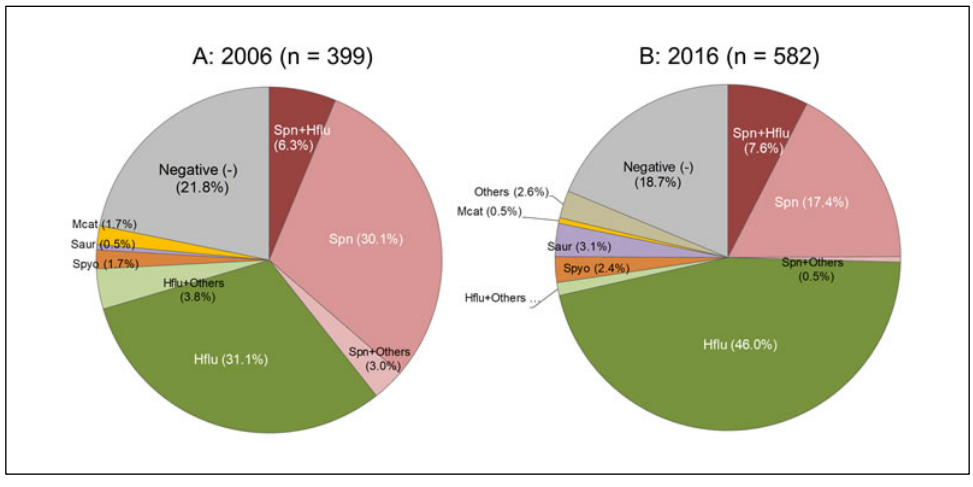


Figure-27 Changes in bacterial pathogens causing acute otitis media after the introduction of PCVs in children

Spn, *Streptococcus pneumoniae*; Hflu, *Haemophilus influenzae*; Spyo, group A hemolytic streptococci; Saur, *Staphylococcus aureus*; Mcat, *Moraxella catarrhalis*; Negative, cases in which pathogens could not be identified by PCR or culture.

Ubukata K, et al., Pediatr Infect Dis J. 2018;37:598-604. (plotted based on data)

In AOM, 2 facts garnered attention. One was that the proportion of serotype 3 for which the effect of the vaccine was considered to be low among PCV13 serotypes was nearly halved. The other was that the proportion of 15A and 35B, in which gPRSP was common among NVT, was increasing as in IPD. It is assumed that *S. pneumoniae* colonizing the nasopharynx is changing. The trends in AOM mentioned here warrant considerable attention in the future.

3) Summary

The relationship between pneumococcal infection in children and the introduction of PCVs can be summarized as follows:

- After the introduction of PCVs, *S. pneumoniae* of vaccine serotypes colonizing the

nasopharynx were drastically reduced.

- Consequently, the number of IPD cases, particularly cases with meningitis, was drastically reduced.
- Although it remains difficult to identify *S. pneumoniae* as pathogen in pediatric patients with pneumonia, the number of hospitalized patients with pneumonia was steadily reduced.
- The number of cases of AOM caused by *S. pneumoniae* was halved.
- Among strains from IPD, the proportion of gPRSP was drastically reduced from 54% before the introduction of PCVs to 11%.
- It is necessary to continuously conduct surveillance of trends in serotypes and resistance of pneumococcal isolates that cannot be covered by PCVs.

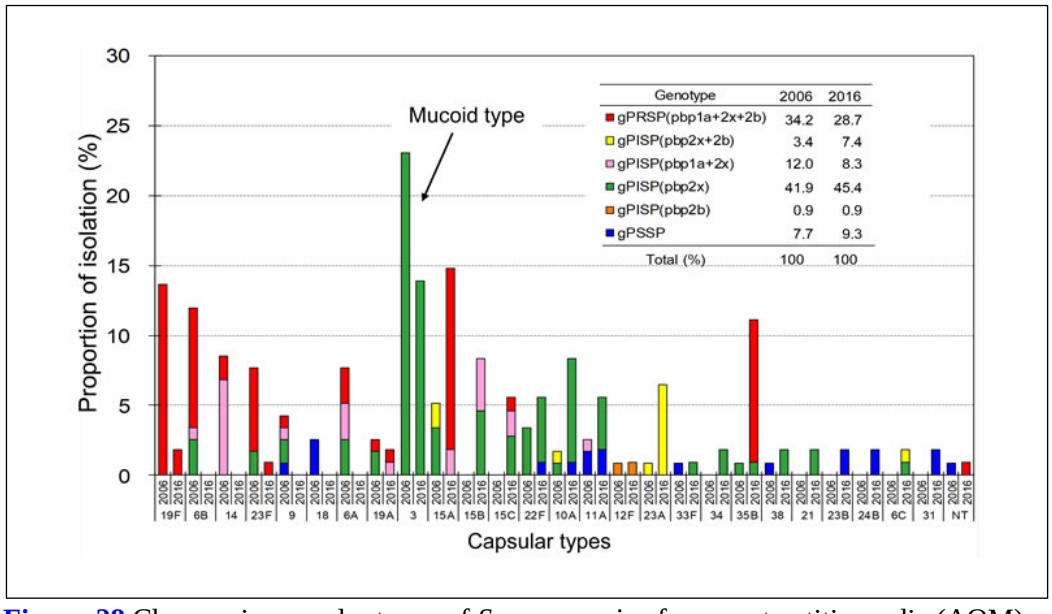


Figure-28 Changes in capsular types of *S. pneumoniae* from acute otitis media (AOM) - Comparison between 2006 (n = 116) and 2016 (n = 108)-

Ubukata K, et al., *Journal of Chemotherapy*. 2009;57(S-1):49-57.
Ubukata K, et al., *Pediatr Infect Dis J.* 2018;37:598-604. (plotted based on data)

2 Adults

1) Relationship between age and IPD

With regard to the relationship between age and IPD, cases were classified into the following 4 groups: meningitis, sepsis/bacteremia of focus unknown, pneumonia with blood culture-positive, and others. The results are shown in **Figure-29**.

The median age of patients with meningitis and pneumonia was 65 years and 74 years, respectively, showing a difference of nearly 10 years, while that of patients with sepsis/bacteremia was 69 years, which was just in between. In other words, a clear difference was noted in the age of onset among diseases. Furthermore, those <65 years of age accounted for 31.8% of all IPD cases.

Among these subjects, the outcome was reported in 1,652 cases (89%). The results are summarized in **Figure-30**. Sequelae (+) included neurological sequelae, and deaths were defined as deaths within 28 days after hospitalization that were judged by the attending physician to be due to this disease. In pneumonia and sepsis/bacteremia, in which the vast

majority of patients were ≥60 years of age, the mortality was as high as 21.3% and 24.1%, respectively. On the other hand, in meningitis, cases with neurological sequelae were overwhelmingly more common compared with cases of deaths. Statistically, the outcome of IPD differs significantly among diseases.

These data suggest that the systemic condition worsens in hours in cases of pneumonia with significant damage to lung function, and the outcome is largely dependent on the time from the onset to definitive diagnosis.

In contrast, cases of meningitis without damage to lung function were more likely to survive, also because of a relatively young age, but were left with serious sequelae.

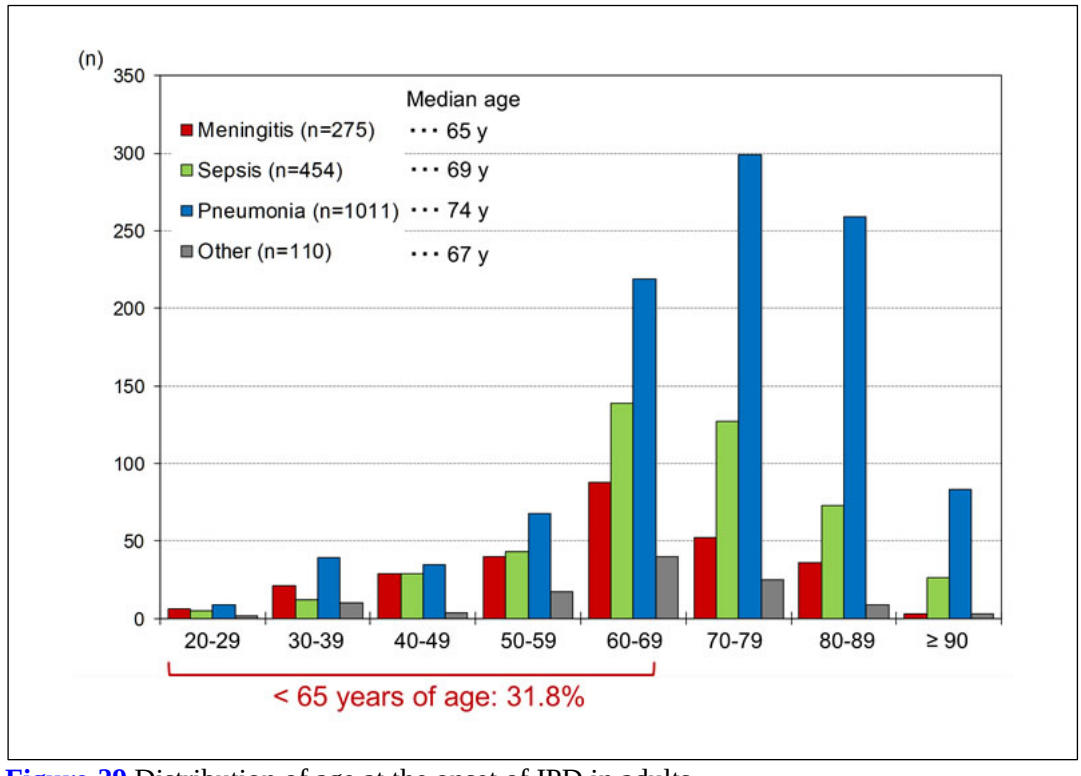


Figure-29 Distribution of age at the onset of IPD in adults

For pneumonia, only cases in which bacteria were isolated by blood culture were included.

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.

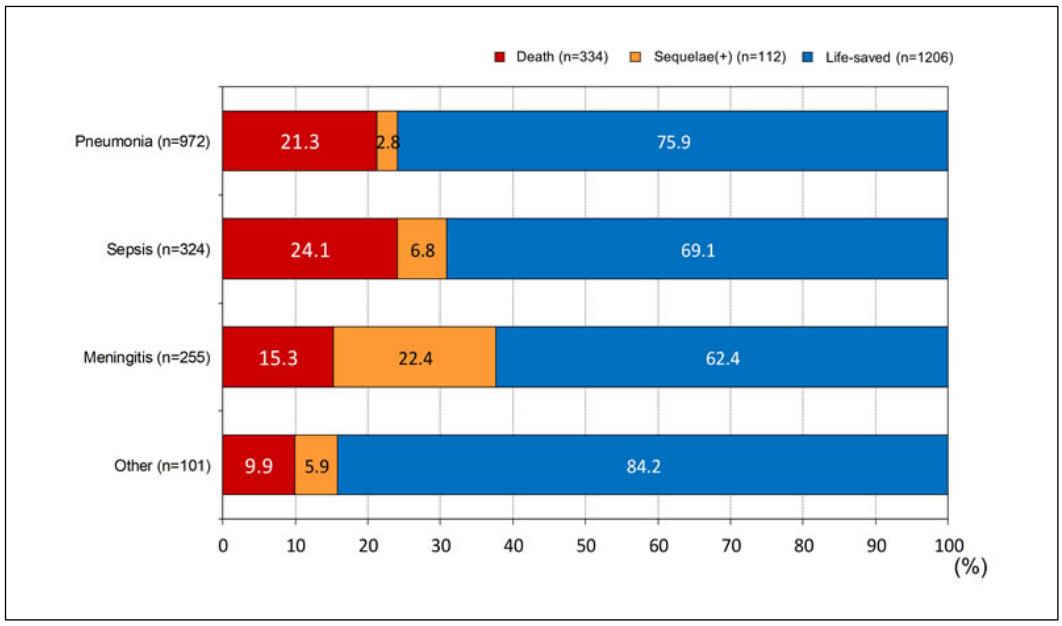


Figure-30 Outcome of IPD by disease in adults

Note: Only included cases in which the outcome was reported (89.3%).

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.(plotted based on data)

2) Relationship between underlying diseases and IPD

Many adult cases of IPD have various underlying diseases as risk factors.

The relationship between underlying diseases and outcome is shown in **Figure-31**; 85.4% of patients had some underlying diseases, such as malignant disease, diabetes mellitus, cardiovascular disease, immunosuppressive conditions, liver disease, kidney disease, and lung disease, including chronic obstructive pulmonary disease. The presence of various underlying diseases, as shown here, is itself a risk factor for onset. Among them, the rate of poor prognosis was particularly high in cardiovascular disease, liver disease, and kidney disease.

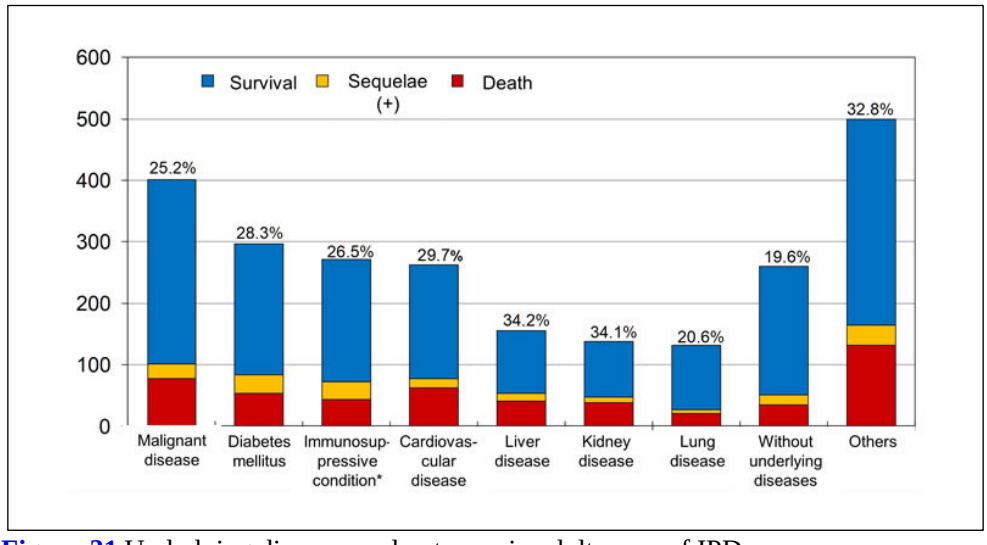


Figure-31 Underlying diseases and outcome in adult cases of IPD

Patients with underlying diseases:85.4%. * Including hematological diseases, connective tissue diseases, administration of anticancer drugs, and splenectomy.

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.(plotted based on data)

3) Risk factor analysis of cases of pneumonia

Based on the age characteristics described in Section (1), we considered it necessary to analyze poor prognostic factors in pneumonia and bacteremia separately from meningitis. Therefore, the analysis was performed only on cases of pneumonia and sepsis/bacteremia, excluding the confounding factors. Then, a multivariate analysis was performed with multiple factors as explanatory variables to identify poor prognostic factors.

The results are shown in **Table-2**.

The explanatory variables included (i) age, (ii) underlying disease, (iii) treatment performed at admission, (iv) various biomarker results at admission, and (v) capsular type and susceptibility to antibiotics of a pathogenic bacterium. **The risk factors most associated with death** were (i) white blood cell (WBC) <4,000/μL (odds ratio [OR], 6.9), (ii) age at onset of ≥80 years (OR, 6.5), (iii) abnormal serum creatinine (≥2.0 mg/dL; OR, 4.5), (iv) underlying liver disease (OR, 3.5), (v) mechanical ventilation (OR, 3.0), and (vi) high lactate dehydrogenase (≥300 IU/L; OR, 2.4).

For the capsular type, which was a bacterial factor, the OR was 1.5 for NVT, although no significant difference was observed. Although not shown here, types of drug resistance and types of first-line antibiotic therapy were not related to the outcome.

Table-2 Poor prognostic factors by multivariate analysis in adult IPD

Analysis items	No. of survivals (%)	No. of deaths (%)	Odds ratio (95% CI)	p value
Age group				
<50 y	45 (11.7)	5 (4.1)	1	-
50–64 y	87 (22.7)	18 (14.8)	1.6 (0.5–5.6)	NS
65–79 y	155 (40.4)	51 (41.8)	3.0 (0.9–9.6)	NS
≥80 y	97 (25.3)	48 (39.3)	6.5 (2.0–21.6)	.002
Underlying Diseases				
Liver dis.	33 (8.6)	17 (13.9)	3.5 (1.6–7.8)	.002
Lung dis.	34 (8.9)	4 (3.3)	0.4 (0.1–1.4)	NS
Mechanical ventilation	**52 (13.5)**	**40 (32.8)**	**3.0 (1.7–5.6)**	**< .001**
Laboratory findings				
WBC (<4000/μL)	39 (10.2)	41 (33.6)	6.9 (3.7–12.8)	< .001
Cr (≥2.0 mg/dL)	46 (12.1)	44 (36.4)	4.5 (2.5–8.1)	< .001
LDH (≥300 IU/L)	118 (32.1)	61 (53.0)	2.4 (1.4–4.0)	.001
Capsular types				
PCV7	139 (36.2)	44 (36.1)	1	-
PCV13 & PPSV23	182 (47.4)	50 (41.0)	0.8 (0.5–1.5)	NS
NVT	60 (15.6)	20 (23.0)	1.5 (0.8–3.0)	NS

NS, not significant ($p \geq .05$).

Note: Analyzed only in cases of pneumonia and bacteremia, excluding confounding factors.

Hanada S, et al., *PLoS One*. 2016;11:e0147877. (modified)

4) Relationship between WBC at admission and outcome

The relationship between WBC that had the highest OR in the multivariate analysis and duration of hospitalization in cases of deaths is shown in **Figure-32**. Deaths occurred most frequently from the day of admission to the day after. In fact, 82.9% of cases with WBC <4,000/μL died within 3 days. Even in cases with higher WBC, 53.1% had an unfavorable outcome within 3 days.

When the data mentioned above were fit into the Kaplan-Meier survival curves, significant differences were observed. In cases with WBC <4,000 μL, particularly, it became clear that the first 3 days after admission are extremely significant.

In other words, these findings indicate that the more severe the disease, the more essential is

the rapid diagnosis, and that the current culture-based bacterial test does not meet the clinical expectations.

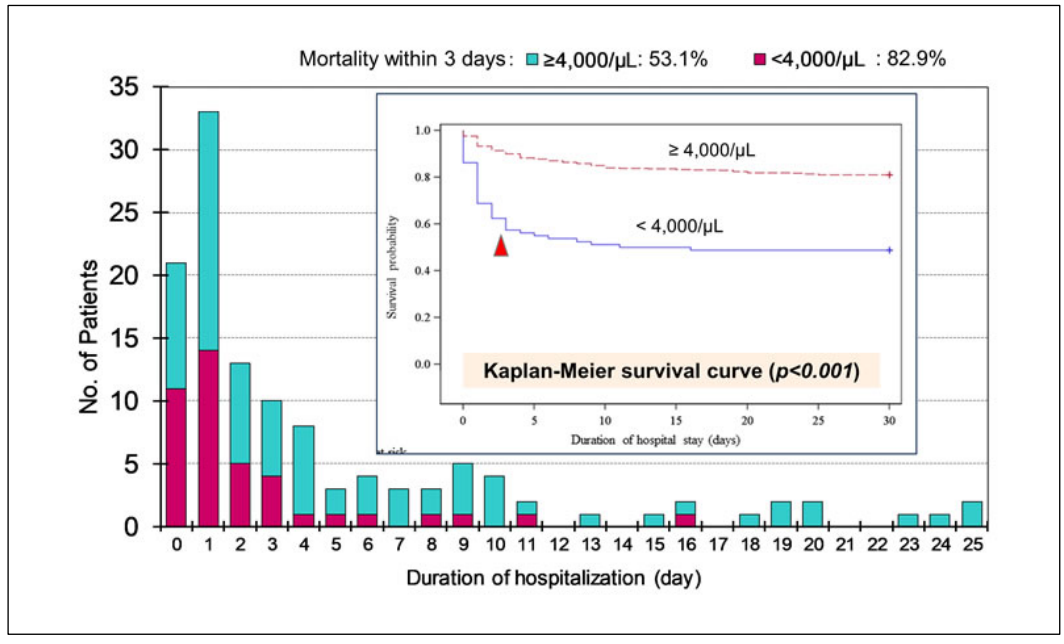


Figure-32 Relationship between death and WBC at admission in adult IPD

Hanada S, et al., *PLoS One*. 2016;11:e0147877. (modified)

5) Onset of IPD in those who received PPSV23

After the introduction of PCVs in children, we have not experienced the so-called "vaccine failures" among samples sent from those who received PCVs. However, the effect of PPSV23 in adults remains debatable. In particular, Japanese reports on the effect of PPSV23 are primarily based on the evaluation in facilities accommodating the elderly and in limited areas. These findings show that PPSV23 has a certain effect in preventing pneumonia in the elderly. However, it appears that the capsular type of *S. pneumoniae* that caused IPD after vaccination of PPSV23 has not been examined or formally reported. **Figure-33** summarizes the results of our surveillance from FY2014 to FY2016, including IPD 53 cases who received PPSV23, the majority of whom had pneumonia with bacteremia.

Among these 53 cases, 52.8% were caused by capsular types included in the 23-valent vaccine. Incidentally, 41.5% of cases could be covered by PCV13. Many of the cases involved the elderly, who had various chronic diseases at high proportions. As mentioned earlier, vaccination of PPSV23 alone is limited, especially for the prevention of IPD caused by capsular type 3 strains.

6) Differences in the rate of poor prognosis by capsule type

Figure-34 shows the differences in the rate of poor prognosis among capsular types. The overall rate of poor prognosis in adults was 27%, which varied considerably among capsular types from as high as 45% in 6A to as low as 9% in 7F. We noted that 11 types out of PCV13 serotypes were included. Moreover, half of 11 PPSV23 serotypes (excluding PCV13 serotypes) were included. These results indicate that it is critical to prevent the onset of disease by strains of capsular types that are expected to be highly pathogenic.

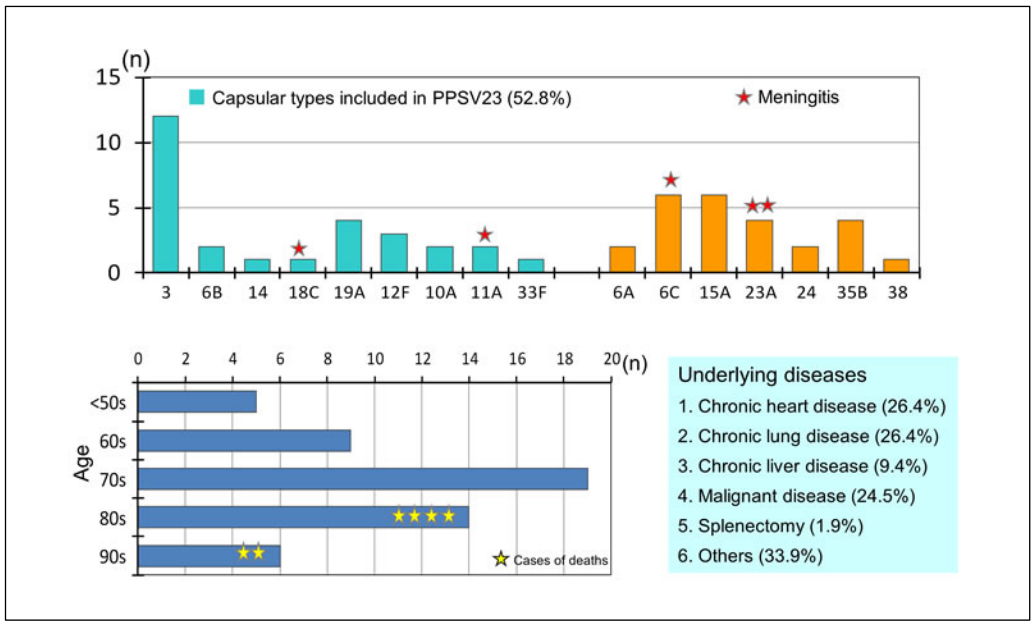


Figure-33 Onset of IPD in those who received PPSV23 (n = 53)

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.(plotted based on data)

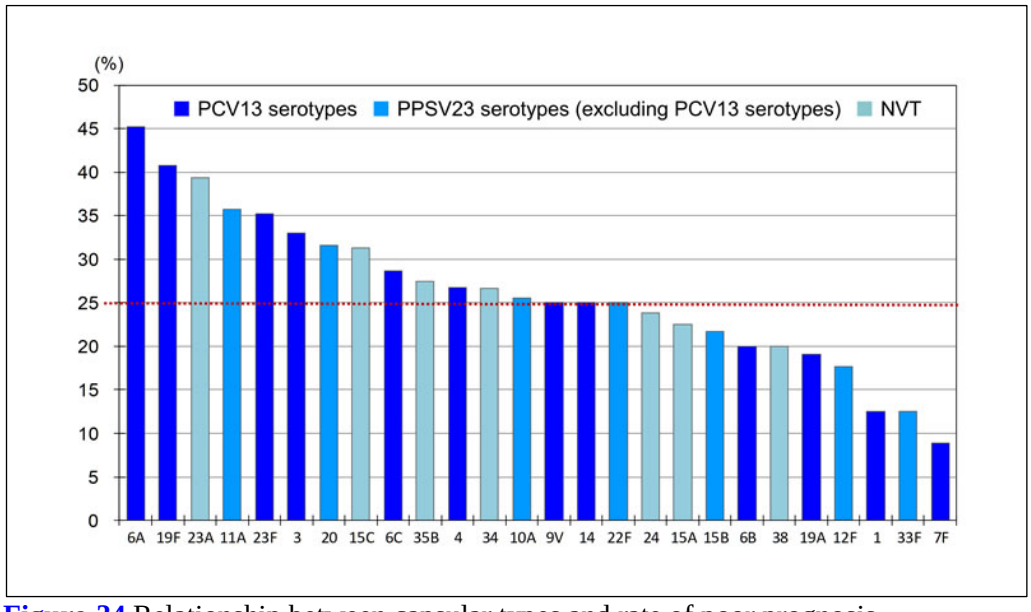


Figure-34 Relationship between capsular types and rate of poor prognosis

Note: 6C shows cross-immunity with 6A.

Ubukata K, et al., *Emerg Infect Dis.* 2018;24:2010-2020.(plotted based on data)

7) Summary

- The impact of the introduction of PCVs and routine vaccination in children has extended to adults, and the capsular types of IPD isolates are changing.
- Consistent with the above, gPRSP was significantly reduced from 32.4% to 15.5% in isolates from adults as well.
- However, it does not mean that adults have acquired antibodies against serotypes of *S. pneumoniae* included in PCVs.
- Adult cases of IPD have a higher prevalence of underlying diseases, and, thus, the rate of poor prognosis, including deaths and sequelae, is exceptionally high.

- A majority of deaths occurred within 3 days of hospitalization, and distinct characteristics were noted in results of serum biomarkers.
- In those ≥65 years of age with more than one risk factor, IPD was induced by serotypes included in the vaccine even in those who had received PPSV23.
- In adults, it is necessary to consider prevention of onset by mucoid, capsular type 3 strains.
- It is reasonable to start vaccination of PCV13 at ages between 60 and 65 years during which immunological senescence progresses gradually.

V

Evolution of *S. pneumoniae* revealed by Genomic Analysis

1 Genomic features

To date, genomic analysis of *S. pneumoniae* strain of various capsular types has been performed based on genomic information of the R6 strain (Accession No. NC_003098) as the reference.

Figure-35 shows the genomic position of each *pbp* gene and those of 7 genes for multilocus sequence typing (MLST) analysis, which has recently become an international standard method for comparing strains, in *S. pneumoniae*.

According to the genomic analyses, the *pbp2x* and *pbp1a* genes involved in resistance to β-lactams are located at 2 o'clock that they sandwich capsular polysaccharide synthesis (*cps*) genes. As the capsules consist of polysaccharides, several enzymes are involved in the synthesis, and >10 genes (group) encoding these enzymes form a 20- to 30-kb region; **this genomic nature leads to capsular switching, as well as the development of resistant bacteria with new capsular types.**

The genes used for MLST analysis, which can estimate the similarity of bacteria based on genomic homology, are selected from those that are relatively scattered in the genome; these selected genes among housekeeping genes which encode enzymes essential for survival and are less likely to have new mutations.

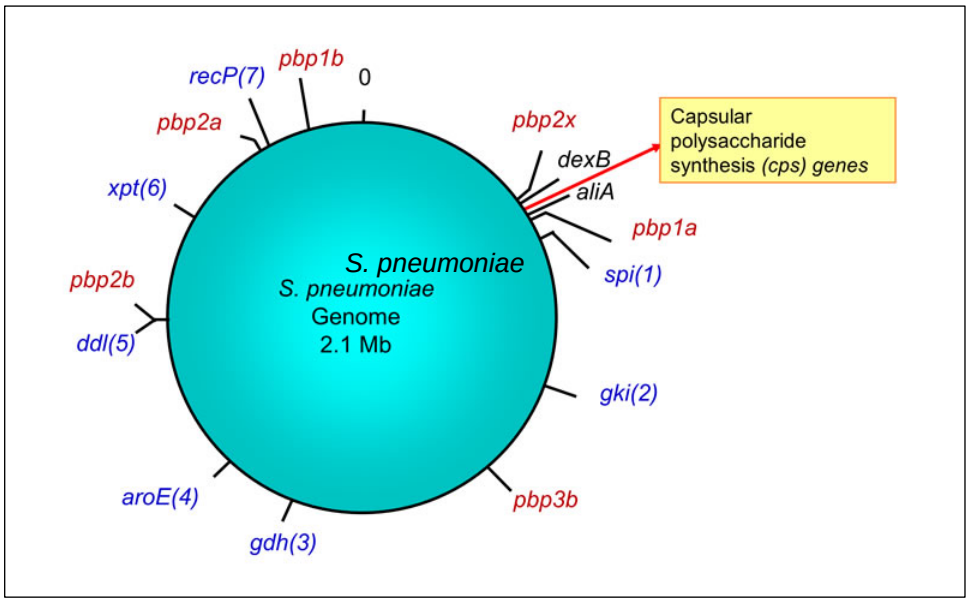


Figure-35 Genomic characteristics of *S. pneumoniae*

R6 strain: Accession No., NC_003098
Blue letters, housekeeping genes; red letters, *pbp* genes.
Hoskins J, et al., *J Bacteriol*. 2001;183:5709-5717.

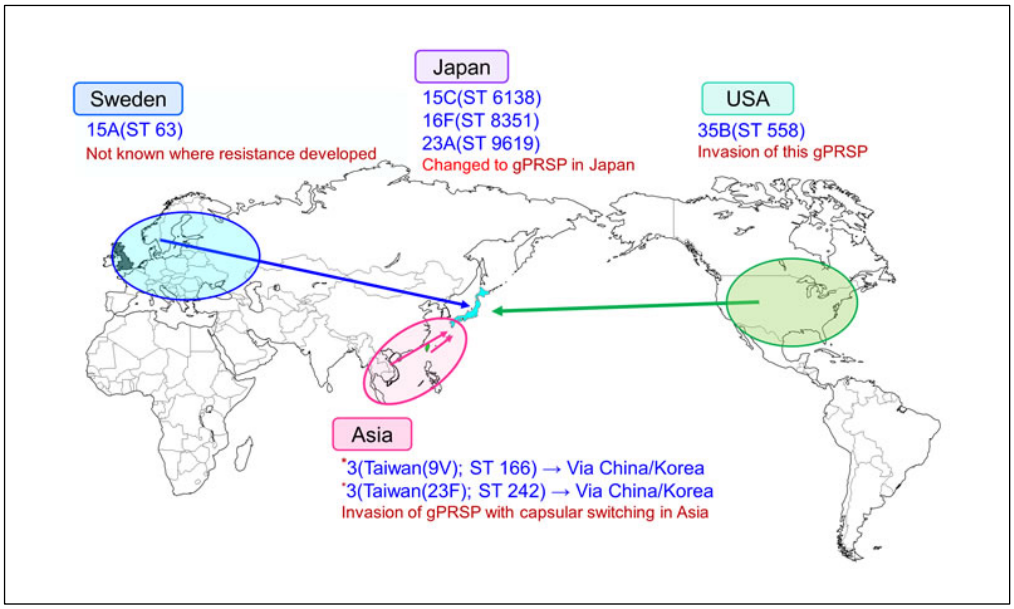


Figure-36 Presumed routes of invasion of gPRSP strains of new capsular types (based on the MLST website data and our data)

(Drafted based on MLST website and our data)

The MLST analysis is performed as follows: (i) first, seven genes are sequenced; (ii) then, it is compared with data of strains registered from all over the world through the MLST website (https://pubmlst.org/spneumoniae/), and the respective allele number is obtained; (iii) Based on the allele numbers of the seven genes, its profile number is determined; and (iv) a new profile number is assigned if the combination of allele numbers has not been reported. The profile number is referred as a sequence type (ST), and a population with similar STs is called a clonal complex (CC). STs and CCs of *S. pneumoniae* are closely related to the capsular type.

2 MLST analysis

Here, we omit the details of MLST analysis of all strains from IPD. In the website, 14,000 STs have been registered, which means that seven housekeeping genes have numerous mutations. As well as *S. pneumoniae*, bacteria that colonize the respiratory tract and have an aspect as indigenous bacteria, such as *H. influenzae*, also have many mutations. In contrast, the number of STs is small in group A hemolytic streptococci and *Mycoplasma pneumoniae*.

In the website, a lot of information regarding to strains was also registered, such as capsular type, countries, year of isolation, and antimicrobial susceptibility. When a new gPRSP is found, we can find out when it is registered and where it is from by searching for strains with the same ST in the website.

During our surveillance, several gPRSP belonging to certain capsular serotypes was found. Among these capsular-type strains, gPRSP had never been isolated until then. **Figure-36** shows the estimation where these gPRSP came from on the basis of information of STs. As representative examples, gPRSP of capsular type 35B would be from the US, gPRSP of capsular type 3 from Taiwan, and capsular type 15A originated from Sweden. In addition, gPRSP belonging to capsular types 15C, 16F, and 23A might have changed to gPRSP in Japan. These results suggest that pathogenic bacteria go around the world with humans **in association with globalization of economic activities and an increase in tourism-related inbound**. To predict future pneumococcal infections, it is essential to know which capsular types are becoming major types in the world and their genomic characteristics at all times.

3 Genetic recombination as a means of survival tool

As described earlier, the capsular polysaccharide synthesis genes form a specific gene group region sandwiched by the *pbp2x* and *pbp1a* genes. A study recently reported that *S. pneumoniae* can rearrange this 20- to 30-kb DNA chain at once (Golubchik T, et al., *Nat Genet*. 2012; 44: 352–355).

It has been considered that capsular type 3 strains accounting for 15% of strains from adult IPD and 12% of pediatric AOM were less likely to have new mutations or develop resistance because of a thick capsule. In fact, gPRSP has not been reported outside Japan.

However, we found mucoid, capsular type 3 gPRSP among our collected strains, probably for the first time in the world; **Figure-37** illustrates the emergence mechanism of gPRSP by genetic recombination which was elucidated by our whole genome analysis. Based on the results of genomic analysis, we concluded that the *cps* genes of Taiwan$^{23F\text{-}15}$/ST242 (gPRSP) went through rearrangement with that of the Netherlands$^{3\text{-}31}$/ST180 [gPISP (*pbp2x*)], resulting in the appearance of capsular type 3 gPRSP; this phenomenon is called "**capsular switching**."

The region of recombination is further illustrated in detail in **Figure-38**. The regions on the background of light orange are derived from type 23F gPRSP, and those on the gray background are derived from mucoid, capsular type 3. In addition, the sites of recombination are indicated by red arrows. The region is named *CPS* locus encodes >10 genes. A long 20-kb DNA chain completely including these genes underwent recombination.

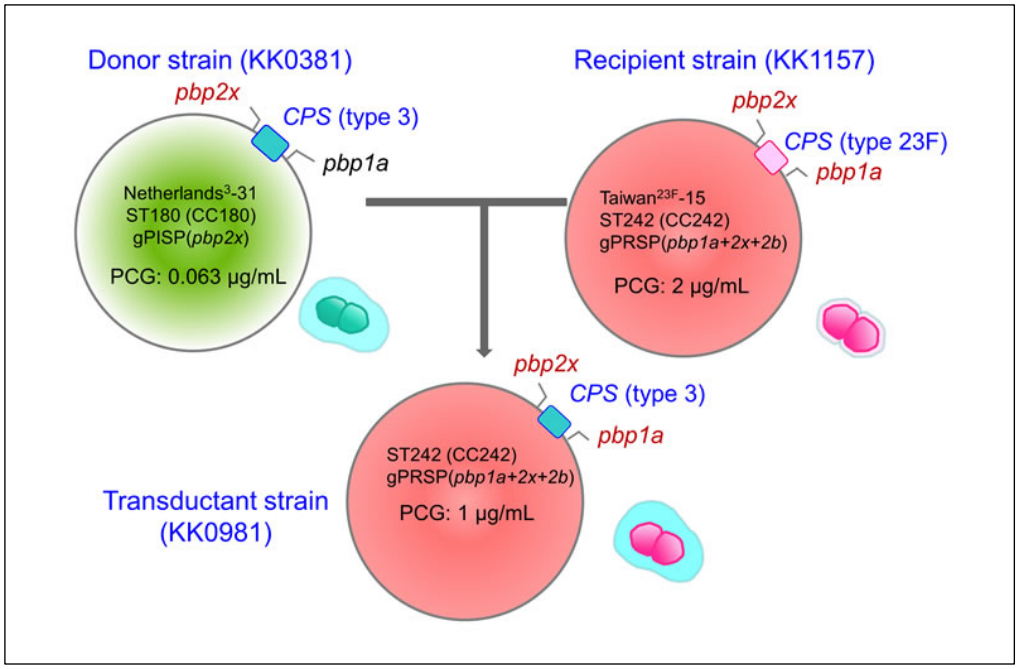


Figure-37 Development of β-lactam resistance by capsular switching of capsular type 3 strain (mucoid strain)

Chiba N, et al., *Antimicrob Agents Chemother*. 2017;61:e00478-17.

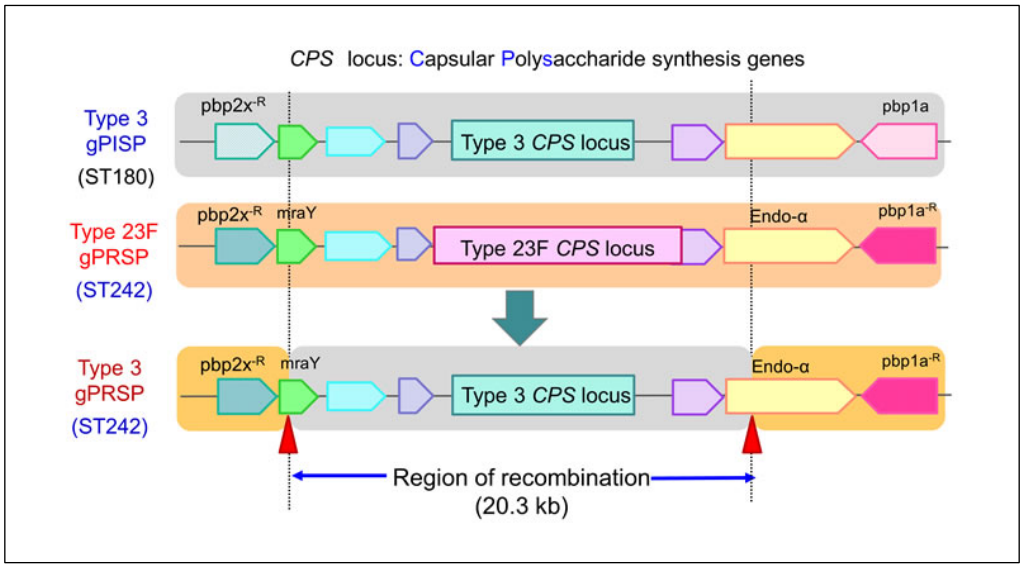


Figure-38 Appearance of capsular type 3 gPRSP by capsular switching

Chiba N, et al., *Antimicrob Agents Chemother*. 2017;61:e00478-17.

VI

Conclusions

S. pneumoniae is one of the representative bacteria that are very weak since *S. pneumoniae* produces autolytic enzymes. Therefore, it cannot be discussed in the same manner as other species such as *Staphylococcus aureus*. *S. pneumoniae* does not grow by the culture in most cases that antimicrobial agent has been given intravenously even once; the presence of *S. pneumoniae* cannot be demonstrated unless PCR is used. In addition, *S. pneumoniae* is a highly delicate bacterium that will die if the specimen is left for a long time before incubation. Unfortunately, these are why the importance of *S. pneumoniae* is underestimated in Japan.

Owing to the properties mentioned above, *S. pneumoniae* starts to die by autolysis after about 6 hours of incubation. At that time, DNAs are released. In the case that these DNAs are taken up by *S. pneumoniae* of a different type, genetic recombination can occur.

Thus, *S. pneumoniae*, which attaches and colonizes the deep nasopharynx of humans, can easily adapt to environmental changes by undergoing repeated mutations and genetic recombination, resulting in their survival and spread.

In order to control pneumococcal infections, three foundations must be established, as shown in **Figure-39.** First, vaccination is essential as a preventive measure against the onset of infections. The effects of both PCV13 and PPSV23 are as described throughout this booklet. For adults, in addition, enlightenment activities must be proactively performed to educate how to control underlying diseases and the risk factor related to severe diseases. For people with infections, therapeutic antibiotics must be selected appropriately and used properly. To achieve that, accurate and rapid PCR tests should be expected. However, as all medical institutions cannot perform these tests, we must establish the system to perform collecting strains and an accurate, continuous, large-scale epidemiological surveillance at one institution for understanding changes in bacteria on a real-time basis. The most important thing is the pathological analysis for clinical cases but it cannot be performed without a reliable relationship with the clinical side.

Finally, we emphasize that the cooperation of each medical institution in the surveillance cannot be obtained without "**prompt feedback to the clinical side**."

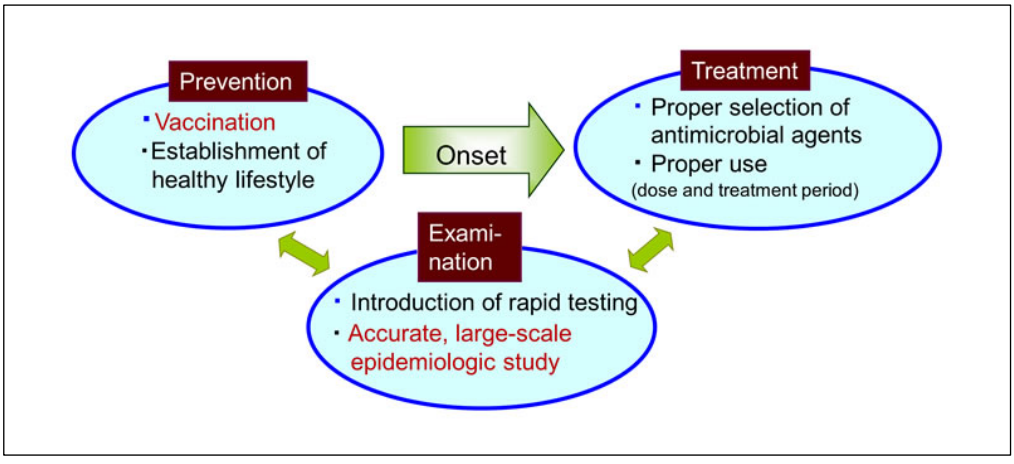


Figure-39 Three foundations for controlling pneumococcal infections

Our Literature on *S. pneumoniae*

1) **Ubukata K, Asahi Y, Okuzumi K, Konno M, The Working Group for Penicillin-Resistant *S. pneumoniae.*** Incidence of penicillin-resistant *Streptococcus pneumoniae* in Japan, 1993-1995. *J Infect Chemother*. 1996; 2: 177-184.

2) **Ubukata K, Asahi Y, Yamane A, Konno M.** Combinational detection of autolysin and penicillin-binding protein 2B genes of *Streptococcus pneumoniae* by PCR. *J Clin Microbiol.* 1996; 34: 592-596.

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6) **Asahi Y, Ubukata K**. Association of a Thr-371 substitution in conserved amino acid motif of penicillin-binding protein 1A with penicillin resistance of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother*.1998; 42: 2267-2273.

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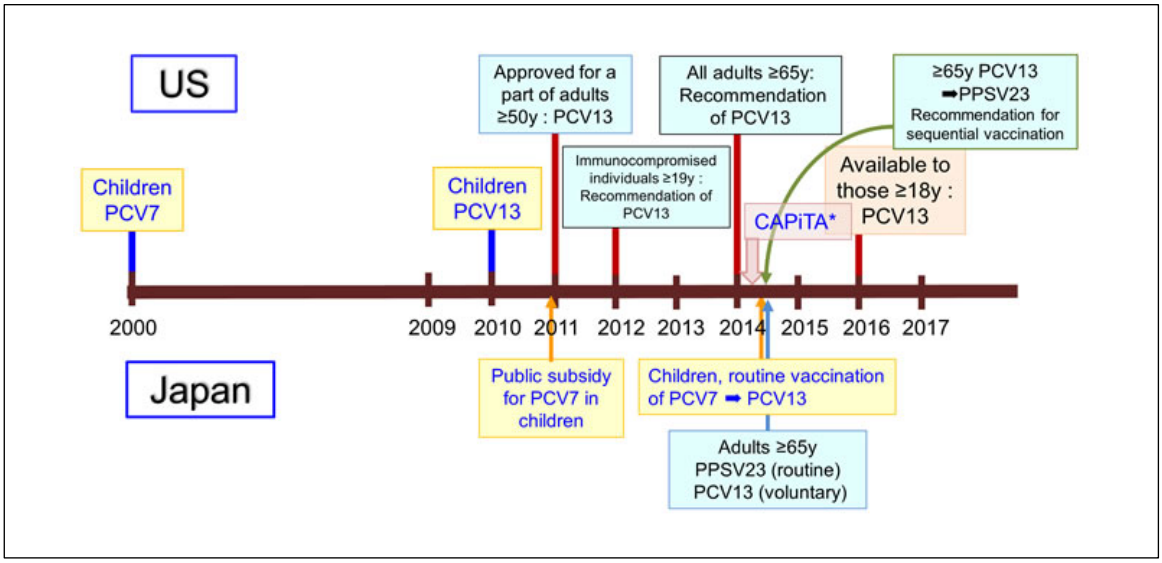
Complementary Materials

In Japan, 5 years have passed since the vaccine against *S. pneumoniae* was switched from PCV7 to PCV13 in children. The evaluation of PCVs for the prevention of pneumococcal infections in children, including 3 years with PCV7, has clearly been positive, and it is consistent with data reported from various countries.

On the other hand, only an irregular (in 5-year increment) routine vaccination with PPSV23 (public subsidy) is provided to the rapidly increasing adult population (≥65 years of age) in Japan. For clear evaluation of the direct and indirect preventive effects of vaccination, large-scale controlled clinical studies are necessary. However, owing to ethical issues, conducting such clinical studies is challenging, and it is not feasible in most countries. Likewise, it is, perhaps, not possible to perform such a study in Japan. Considering these circumstances, results that can be used as references are listed as complementary materials for better future administrative measures on pneumococcal vaccination for adults in Japan.

Appendix Figure-1 briefly shows the status of the introduction of conjugate vaccines in the US and Japan. In the US, the Advisory Committee on Immunization Practices (ACIP) is held 3 times a year, and administrative measures on vaccines are decided accordingly. In the US, where medical treatment is expensive, evidence for the effectiveness of a vaccine is reflected in medical administrative measures as soon as it is demonstrated. Unfortunately, in Japan, where medical access is excellent, it cannot be denied that beneficiaries of medical care had long been unmotivated to take preventive measures. In recent years, however, the perception may be changing.



Appendix Figure-1 Introduction of pneumococcal conjugate vaccines in the US and Japan

* CAPiTA: A large-scale clinical study conducted from September 2008 in Netherlands to clarify the preventive effect of PCV13 in CAP patients aged 65 years or older.
Bonten MJM, et al., *N Engl J Med*. 2015; 372:1114-1125.

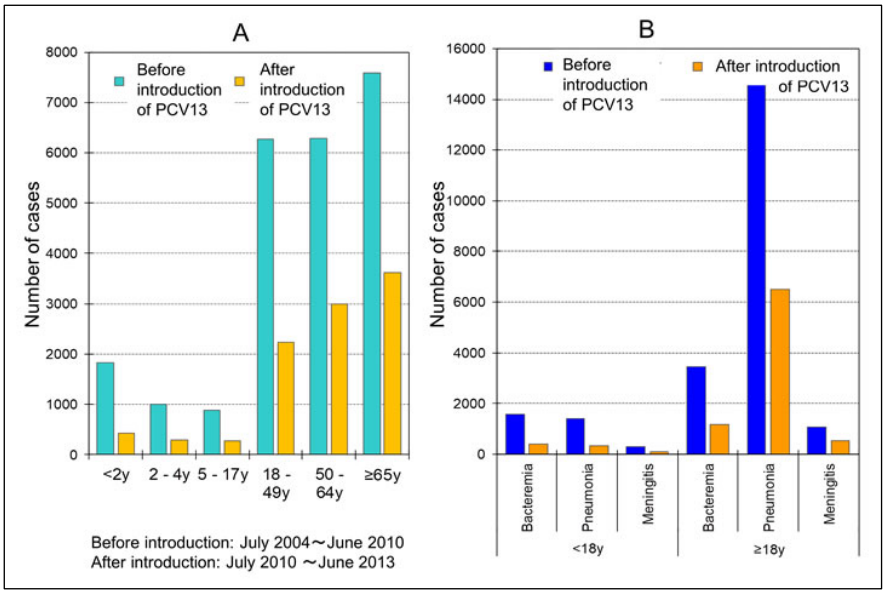
Sings HL, *Vaccine*. 2017;35:5406-5417 (modified)

1 Changes in IPD after the introduction of PCV13 in the US

In the US, PCV13 was included in the vaccination schedule for children in July 2010 and was formally introduced in adults in August 2014. Thus, **Appendix Figure-2**, which is an analysis of data up to 2013, shows the effect of the introduction of PCV13 in children on each age group. **Figure-2A** shows the change in the number of IPD cases in each age group before and after the introduction of PCV13 in children, while **Figure-2B** shows the change in the number of cases by major IPD. These results are based on the Active Bacterial Core Surveillance (ABCs surveillance) covering 10 states under the CDC. Owing to the preventive effect of PCV7, which has been administered to children since 2000, the number of pediatric cases of IPD was drastically reduced to 15% of all cases. Furthermore, the number of IPD cases was further reduced after PCV7 was switched to PCV13.

On the other hand, attention was paid to changes in the number of IPD cases in adults ≥18 years of age. In all age groups, the incidence was reduced by at least 50%, and the number of cases of IPD was halved, including pneumonia associated with bacteremia, bacteremia, and purulent meningitis. These results reconfirm the indirect effect of the vaccine for the prevention of IPD in adult age groups even after the vaccine was switched to PCV13 in children (Moore MR, et al., *Lancet Infect Dis*. 2015; 15: 301–309). Based on the background of IPD included in this tabulation, the percentage of cases with underlying diseases was reported to be 17% to 20% in children and 73% to 76% in adults, the percentage of hospitalization was 63% to 71% in children and 93% to 95% in adults, and the percentage of death was 2% to 3% in children and 12% in adults.

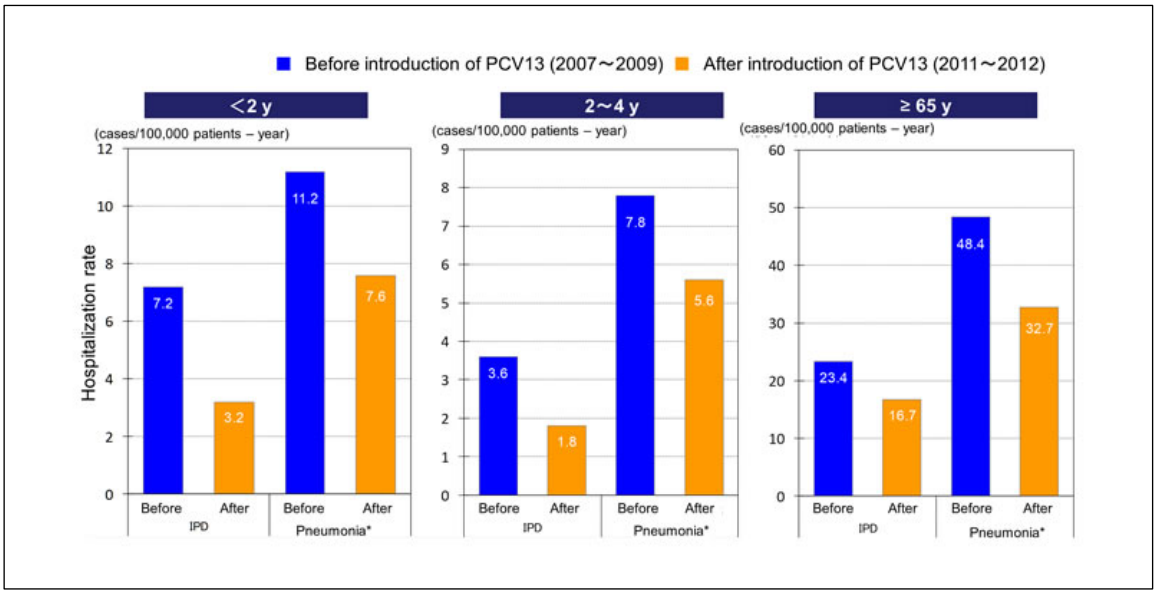
Appendix Figure-3 shows the changes in the annual number of hospitalizations per 100,000 cases of IPD and non-invasive pneumococcal pneumonia before and after the introduction of PCV13 in children, stratified by age <2, 2 to 4, and ≥65 years. In patients <2 years of age, hospitalization for IPD and non-invasive pneumonia was reduced by 56% and 32%, respectively. In contrast, hospitalization for IPD and pneumonia was reduced by 29% and 32%, respectively, in patients ≥65 years of age.



Appendix Figure-2 US: Characteristics of IPD in all age groups before and after the introduction of PCV13 in children.

Based on the 10-year data on IPD in CDC's ABCs surveillance (n = 33,688)

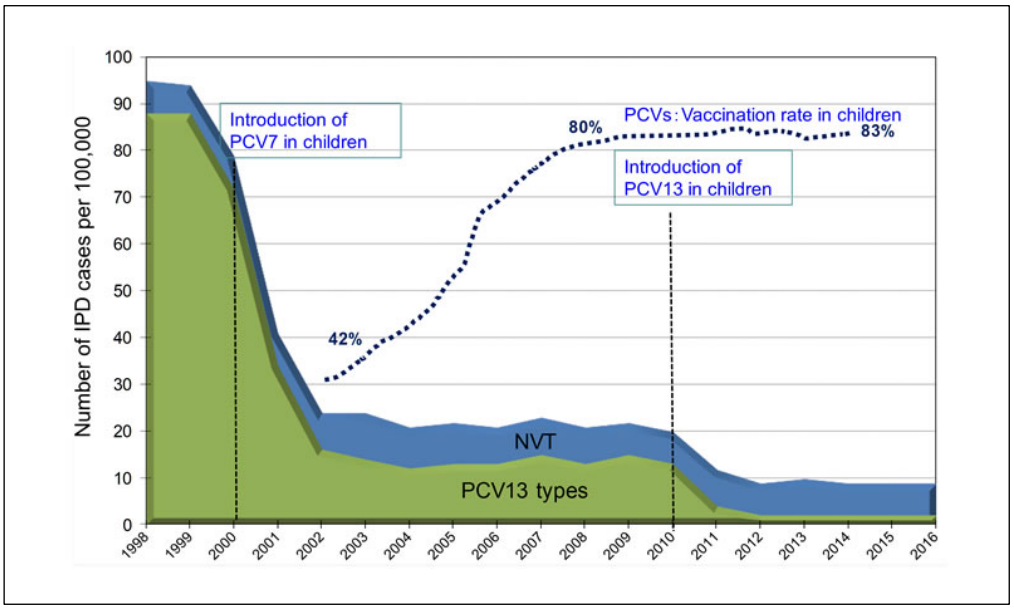
Moore MR, et al., *Lancet Infect Dis*. 2015;15:301-309. (figure derived from)



Appendix Figure-3 US: Changes in hospitalization rates for IPD and pneumococcal pneumonia before and after the introduction of PCV13 in children

* Noninvasive pneumococcal pneumonia.

Simonsen L, et al., *Lancet Respir Med*. 2014;2:387-394. (figure derived from)

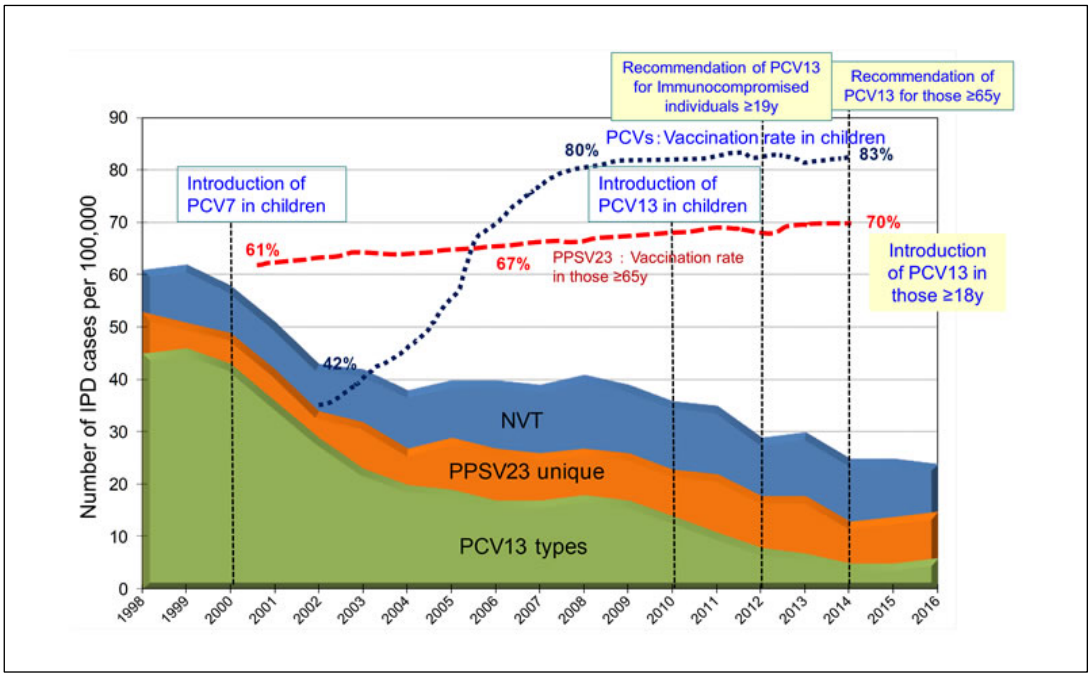


Appendix Figure-4 US: Annual changes in the number of IPD and capsular types in children <5 years of age according to the ABCs report

Note: NVT represents serotypes not included in PCV13.

From the data of ABCs surveillance, the annual changes in the number of IPD cases per 100,000 persons and the isolated *S. pneumoniae* by vaccine type are shown in **Appendix Figure-4** for children <5 years of age and **Appendix Figure-5** for adults ≥65 years of age. In children <5 years of age, it was evident that the number of IPD cases was drastically reduced by vaccination with PCV7. The effect of the vaccine was also apparent after it was switched to PCV13.

On the other hand, in the US, vaccination of PPSV23 for those ≥65 years of age was started since 2000, and the vaccination rate has been between 60% and 70%.



Appendix Figure-5 US: Annual changes in the number of IPD and capsular types in those ≥65 years of age according to the ABCs report

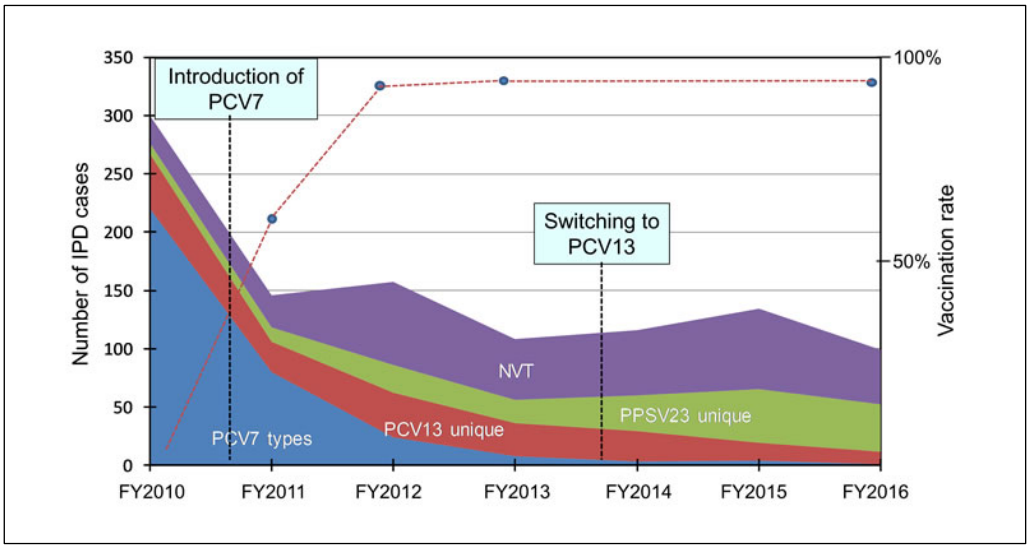
Note: PPSV23 unique indicates changes in PPSV23 serotypes, excluding PCV13 serotypes. NVT represents serotypes not included in either vaccine.

Vaccination of PCV13 for those ≥65 years of age was started in 2014. In the autumn of 2014, it was "recommended to perform sequential vaccination of PCV13 and PPSV23". The figure shows that IPD caused by PCV13 serotypes was also gradually decreasing in adults. When PCV7 was switched to PCV13 in children, it was further reduced, and the number of cases by PCV13 serotypes was reduced to approximately 5 per 100,000. However, the number of cases caused by PPSV23 serotypes, excluding PCV13 serotypes (11 serotypes) and NVT, was continuously 20 to 25 per 100,000, showing no changes. When these are converted into ratios, the proportions of PPSV23 and NVT are found to be relatively high.

In Japan, as shown in **Figure-26**, the number of IPD cases was markedly reduced after the introduction of PCVs in children. However, under the circumstances in our country, the accurate number of cases per 100,000 people cannot be calculated. For comparison with the US, the time courses of changes in the number of IPD cases by vaccine type are shown in **Appendix Figure-6** for children and **Appendix Figure-7** for adults; the figure shows that PCVs have been clearly effective in children. Regarding the indirect preventive effect in adults, the number of people ≥65 years of age is increasing every year, and, as a matter of fact, the number of cases is also increasing. Thus, it is not possible to calculate the accurate change by our method. Considering these facts, the figure shows that the number of IPD cases caused by serotypes included in PCVs, particularly *S. pneumoniae* of PCV7 serotypes is steadily decreasing.

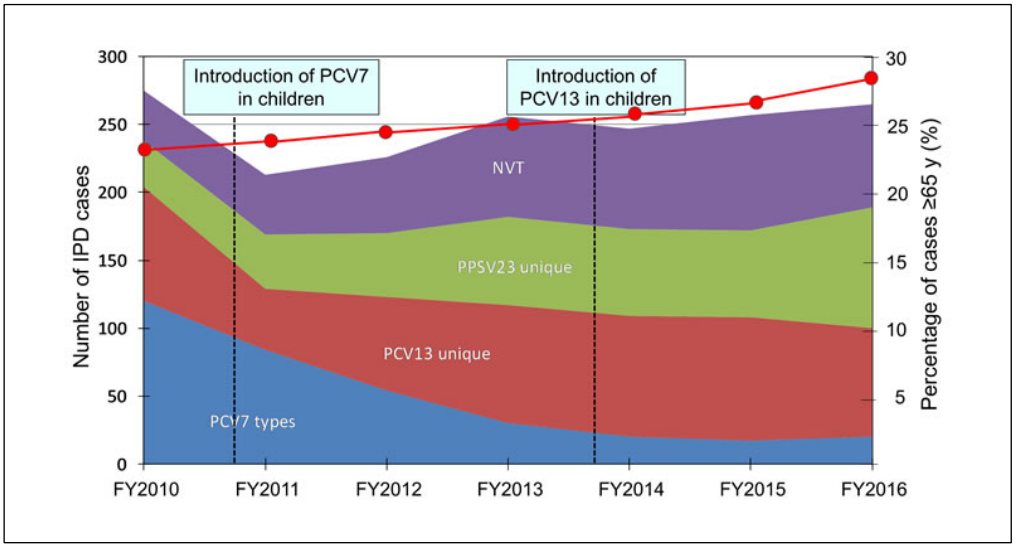
2 A randomized, double-blind, controlled study of PCV13 and placebo

As described earlier, accurate assessment of vaccines requires a large-scale controlled study, and a long-term follow-up survey is required for the analysis of the preventive effect. Under such circumstances, a randomized, double-blind, controlled study of PCV13 in adults ≥65 years of age (Phase IV study) was conducted in 84,496 subjects in the Netherlands in accordance with a strict protocol agreed in advance by the US Food and Drug Administration.



Appendix Figure-6 Japan: Annual changes in the number of IPD cases and capsular types in children (<18 years of age)

Note: NVT represents serotypes not included in either PCV13 or PPSV23.



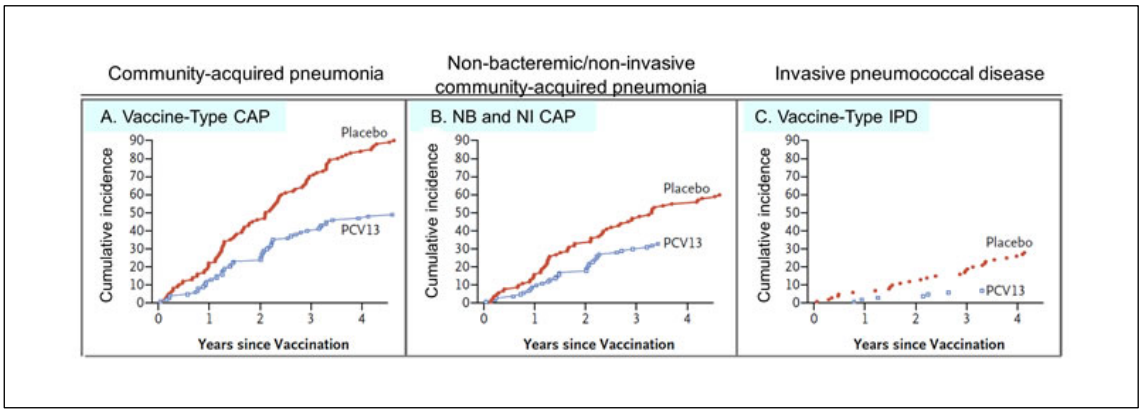
Appendix Figure-7 Japan: Annual changes in the number of IPD cases and capsular types in adults (≥18 years of age)

Note: NVT represents serotypes not included in either PCV13 or PPSV23.

This large-scale clinical study was called CAPiTA (Community-Acquired Pneumonia Immunization Trial in Adults). For approximately 16 months from September 2008, subjects were randomly assigned to receive either PCV13 or placebo. A 5-year follow-up survey was conducted to analyze the preventive effect of PCV13 against community-acquired pneumonia (CAP) and IPD caused by *S. pneumoniae* of capsular types included in PCV13.

Appendix Figure-8 shows the study results. This controlled study demonstrated the preventive effect of PCV13 against pneumococcal CAP in general adults ≥65 years of age for the first time in the world. Further detailed analysis with cases classified into community-acquired pneumonia, non-bacteremic/non-invasive community-acquired pneumonia, and invasive pneumococcal disease revealed that PCV13 was effective for the prevention of the respective disease caused by PCV13 serotypes. The study demonstrated that the effect was maintained throughout the follow-up survey period (median, 3.97 years) without diminishing. Eventually, the study was discontinued 5 years after the start because of an ethical issue that high efficacy of PCV13

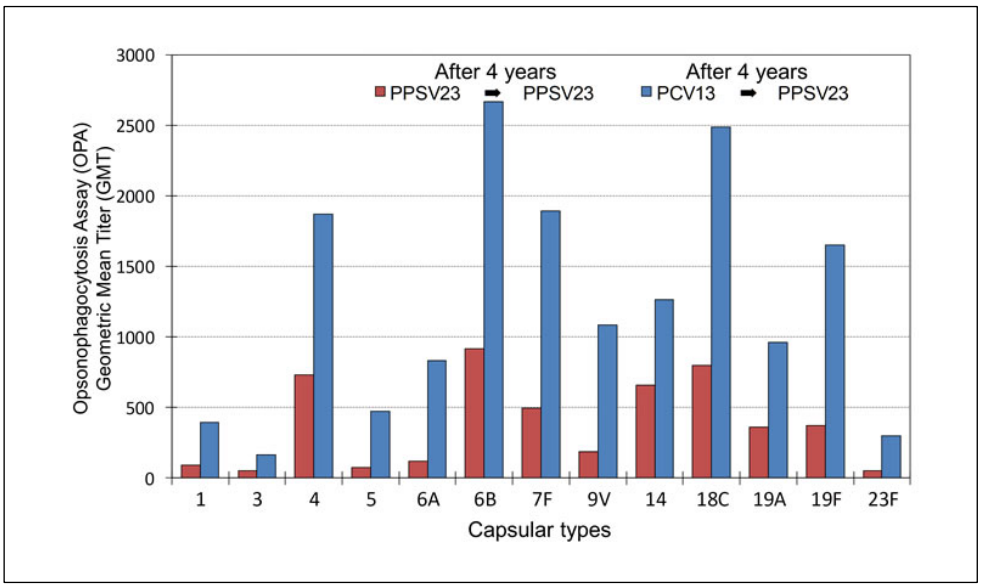
observed in this study was not provided to the placebo group, and PCV13 was also administered to the placebo group.



Appendix Figure-8 Phase IV clinical study of PCV13 in "pneumococcal vaccine-naïve subjects ≥65 years of age" (CAPiTA Study), n = 84,496

1) The preventive effect against CAP, non-bacteremic/non-invasive CAP, and IPD caused by PCV13 serotypes was maintained throughout the follow-up period (median, 3.97 years) without diminishing.
2) The study was discontinued 5 years after the start because of an ethical issue that high efficacy of PCV13 observed in this study was not provided to the placebo group, and PCV13 was also administered to the placebo group.

Bonten MJM, et al., *N Engl J Med*. 2015; 372:1114-1125.

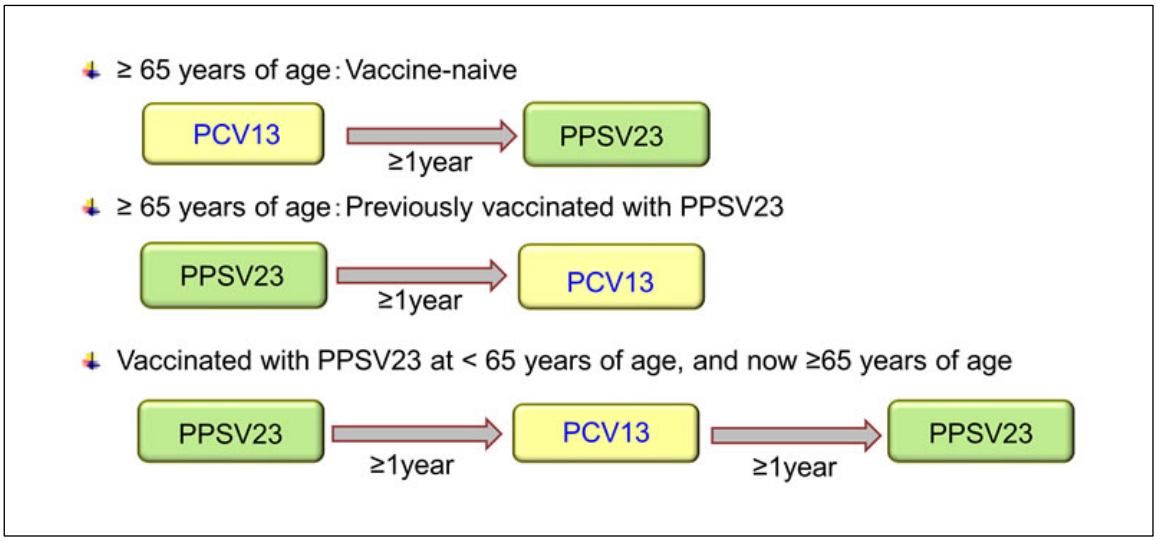


Appendix Figure-9 OPA activities after vaccination of PCV13 and PPSV23 in those between 60 and 64 years of age

Figure derived from Jackson LA, et al., *Vaccine*. 2013;31:3594-3602

3 OPA activity

Appendix Figure-9 shows the results of measurement of OPA (opsonophagocytosis assay)/geometric mean titer (GMT) activity at 6 months in subjects between 60 and 64 years of age who received PPSV23 approximately 4 years after PPSV23 vaccination or PPSV23 approximately 4 years after PCV13 vaccination. For all capsular types included in PCV13, the results showed that OPA activity was significantly higher when PCV13 was vaccinated first.



Appendix Figure-10 US: Pneumococcal vaccination for those ≥65 years of age
ACIP's recommendation *MMWR*. 2015; 64 (34): 944–947

For immunocompetent individuals ≥65 years of age who have received PPSV23 at <65 years of age and require a booster dose of PPSV23, the booster dose should be given at least a year after the vaccination of PCV13 and at least 5 years after the last vaccination of PPSV23. For immunocompromised individuals ≥65 years of age with functional/anatomic asplenia, cerebrospinal fluid leakage, or cochlear implant, PCV13 should be administered at least 8 weeks after the vaccination of PPSV23.

1) Tomczyk S, et al., *MMWR*. 2014;63(37):822
2) Kobayashi M, et al., *MMWR*. 2015;64(34):944

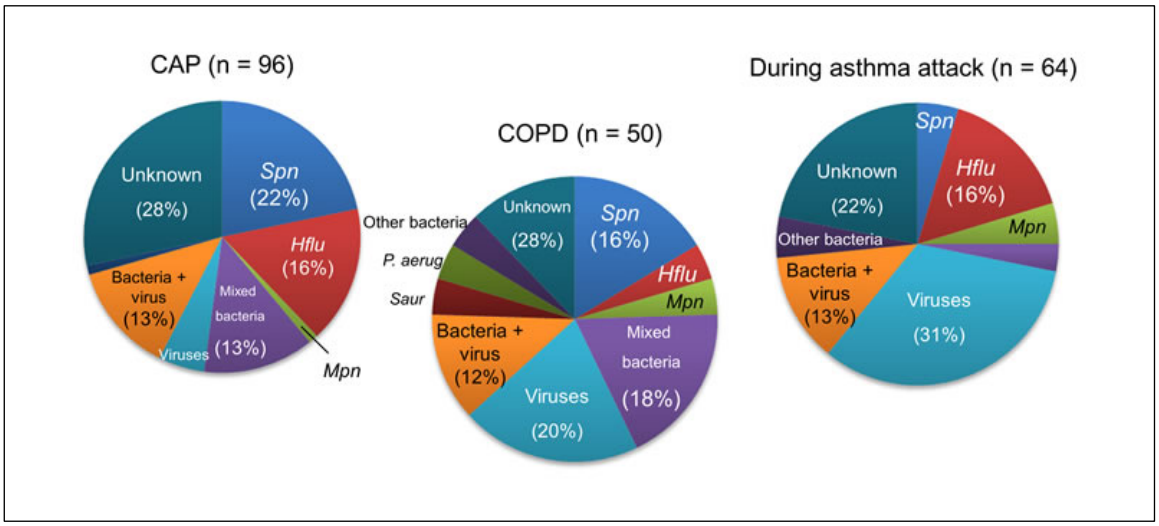
4 Recommendation of pneumococcal vaccination for adults in the US

Appendix Figure-10 shows the outline of pneumococcal vaccination for adults recommended by ACIP in 2015 based on the time course of change in the number of IPD cases per 100,000 persons, changes in capsular types, and evidence from a controlled study in pneumonia. Pneumococcal vaccination is basically recommended for those ≥65 years of age. It is recommended to administer 2 types of vaccines with a different manner of antibody acquisition. Importantly, it is recommended to start the vaccination with PCV13, which has higher immunogenicity, especially in those who have not received vaccines. For those who have received PPSV23, it is recommended to administer PCV13 at least a year after the vaccination of PPSV23 even if subjects are ≥65 years of age. In addition, for those who have received PPSV23 at <65 years of age and are now ≥65 years of age, PCV13 should be administered first. Furthermore, it is recommended to perform booster administration of PPSV23 at least a year after that and at least 5 years after the last vaccination of PPSV23.

In the recommendation of PCV13 for adults in other countries, as of 2018, PCV13 is indicated for elderly + people with risks + people with high risks in approximately 30 countries, and for people with risks + people with high risks in approximately 10 countries. In these cases, people with risks include those with chronic heart disease, chronic lung disease, chronic liver disease, diabetes mellitus, alcohol dependence, and people with high risks include those with HIV, asplenia, cochlear implant, spinal leakage, organ transplant, bone marrow transplant, sickle cell disease, congenital/acquired immunodeficiency, blood cancer, or receiving immunosuppressive therapy. **As demonstrated by our results in Japan, shown in Figure-31, it is important to note that 85% of adult IPD cases had these risks.**

5 Proportion of *S. pneumoniae* in adult respiratory diseases in Japan

In Japan, several relatively large-scale studies have already been conducted on the proportion of *S. pneumoniae* as the pathogenic bacteria in adult patients with pneumonia. However, there are few results of comprehensively examining the causative microorganisms, including viruses.



Appendix Figure-11 Japan: Differences in pathogenic microorganisms in various respiratory diseases in adults

1) Yoshii Y, Shimizu K, et al., *Infect Dis*. 2016;48:782-788.
2) Shimizu K, et al., *Int J Chron Obstruct Pulmon* Dis. 2015;10:2009-2016.
3) Yoshii Y, et al., *BMC Pulm Med*. 2017;17:150.

We constructed a real-time PCR method to comprehensively detect for bacterial and respiratory viruses, assuming community-acquired infections (Morozumi M, et al., *J Clin Microbiol.* 2006; 44: 1440–1446. Hamano-Hasegawa K, et al., *J Infect Chemother.* 2008; 14: 424–432). In the joint research with the Department of Respiratory Medicine, The Jikei University School of Medicine, the constructed PCR method was combined with the culture method, and the pathogens were identified taking account of clinical symptoms. The results are shown in **Appendix Figure-11.**

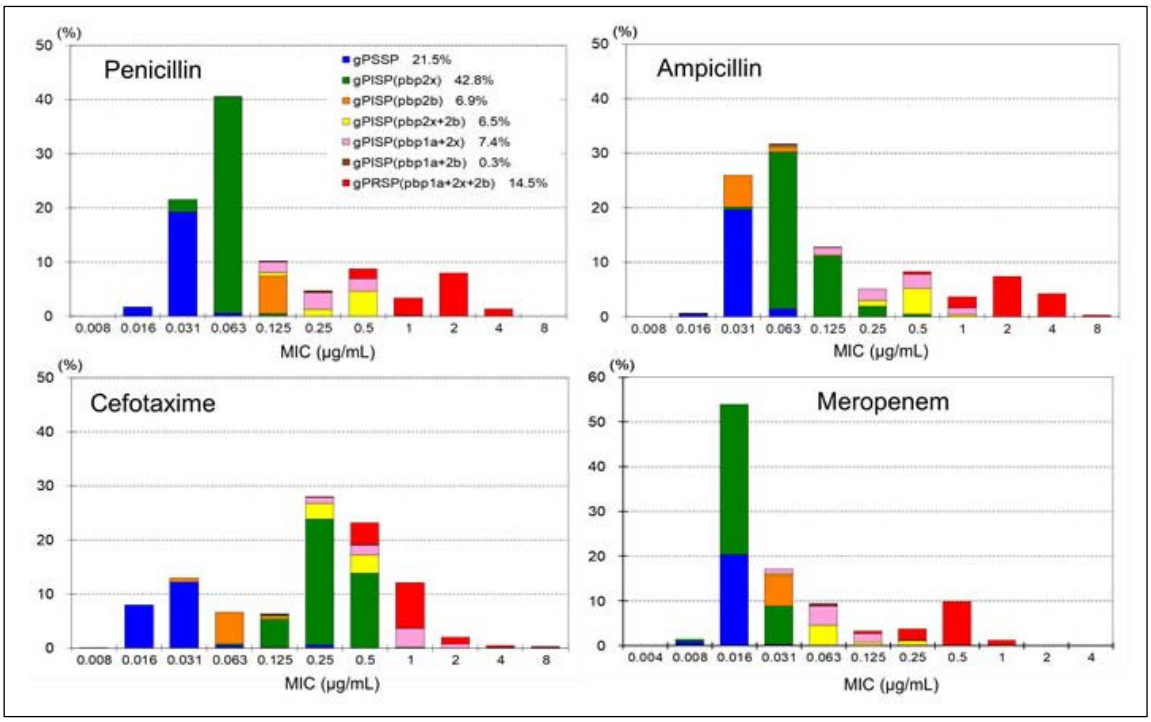
In CAP, *S. pneumoniae* was the most common, accounting for 38% of all cases; it was 24% in exacerbated cases of COPD and approximately 10% during an asthma attack. Furthermore, viruses were more likely to be involved in COPD and during an asthma attack, such as parainfluenza virus, influenza virus, RSV, and rhino virus.

6 Antimicrobial susceptibility

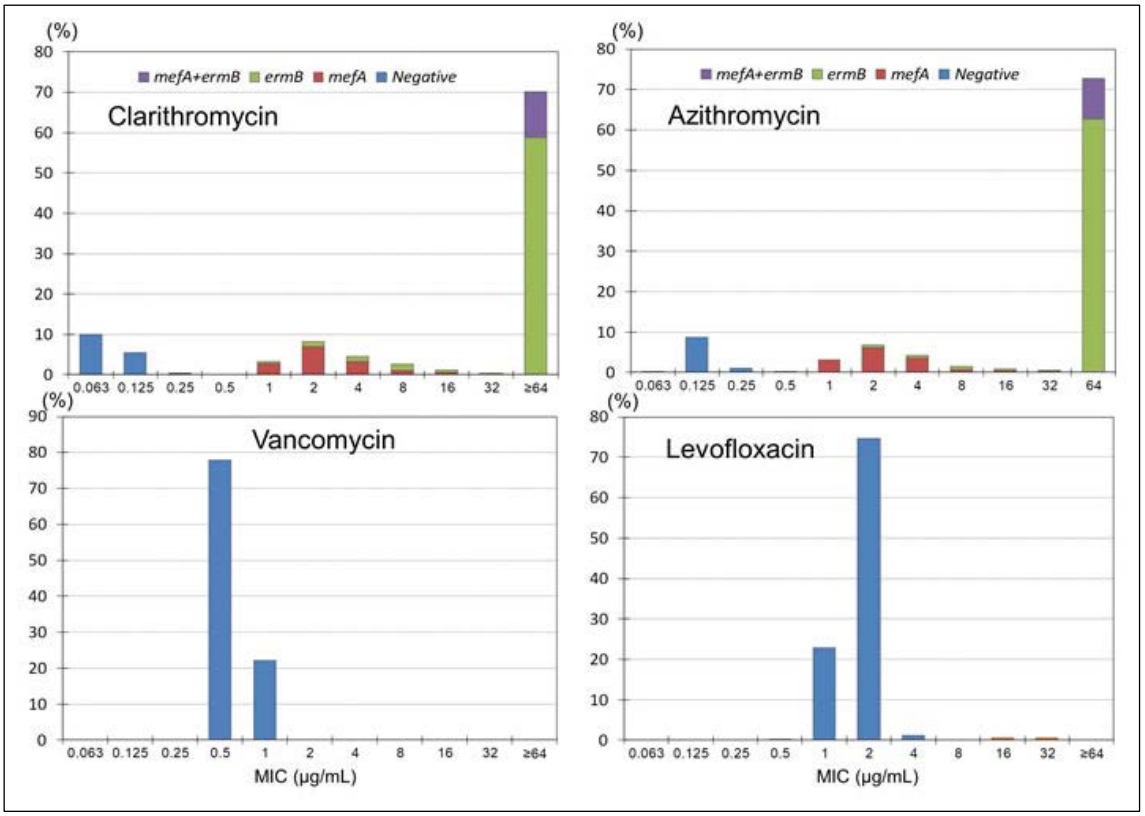
Finally, for antimicrobial agents used for the treatment of pneumococcal infection, the relationship between the accurate susceptibility and gene mutations in 1,229 strains from IPD between 2014 and 2017 is shown in **Appendix Figure-12** and **Appendix Figure-13**. For β-lactam agents, in limited cases of pneumonia and sepsis (excluding purulent meningitis), ineffective cases of these agents declined gradually because gPRSP has been drastically reduced in recent years. In adults, particularly, the proportion of gPISP (*pbp2x*) with mucoid capsular type 3 strains is high; this type has the highest susceptibility to panipenem, superior to meropenem by at least 4 times. Ceftriaxone is similar to cefotaxime. In the treatment of meningitis, the agent must be transferrable to the cerebrospinal fluid at an adequate concentration and must have excellent bactericidal activity.

For macrolides, 70% of strains have *ermB gene* that provide high resistance against clarithromycin and azithromycin. Although the susceptibility of vancomycin is not very high (0.5 to 1 μg/mL), there are no resistant strains. Vancomycin is indicated when consideration is given to tissue penetration. It is the same for levofloxacin. However, resistant strains that have mutations in genes encoding DNA gyrase and topoisomerase IV, the targets of quinolones have already been found at a rate of approximately 1%.

- See references for details -



Appendix Figure-12 Relationship between susceptibility to major β-lactam agents and *pbp* genes



Appendix Figure-13 Relationship between susceptibility to macrolides and new quinolone and resistance genes

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Ernst Liebenauer (1884–1970), *In Entrenchment, World War I* (c. 1915). Watercolor, pencil, and gouache on paper, 5.6 in x 4 in/14.2 cm x 10.1 cm. Digital image from private collection, Atlanta, Georgia, USA.

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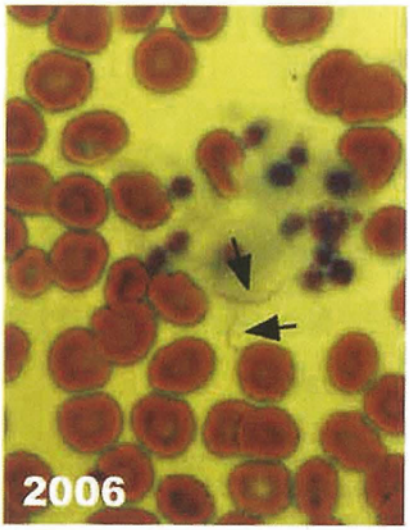

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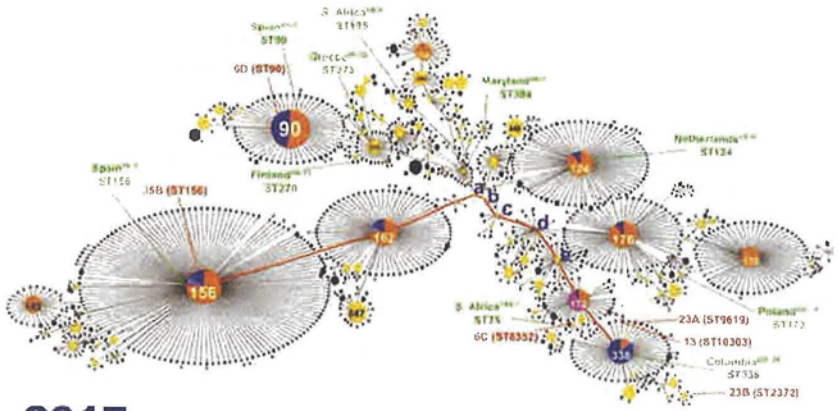

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Research Letters

Effects of Pneumococcal Conjugate Vaccine on Genotypic Penicillin Resistance and Serotype Changes, Japan, 2010–2017

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To clarify year-to-year changes in capsular serotypes, resistance genotypes, and multilocus sequence types of *Streptococcus pneumoniae*, we compared isolates collected from patients with invasive pneumococcal disease before and after introductions of 7- and 13-valent pneumococcal conjugate vaccines (PCV7 and PVC13, respectively). From April 2010 through March 2017, we collected 2,856 isolates from children and adults throughout Japan. Proportions of PCV13 serotypes among children decreased from 89.0% in fiscal year 2010 to 12.1% in fiscal year 2016 and among adults from 74.1% to 36.2%. Although nonvaccine serotypes increased after introduction of PCV13, genotypic penicillin resistance decreased from 54.3% in 2010 to 11.2% in 2016 among children and from 32.4% to 15.5% among adults. However, genotypic penicillin resistance emerged in 9 nonvaccine serotypes, but not 15A and 35B. Multilocus sequence typing suggested that resistant strains among nonvaccine serotypes may have evolved from clonal complexes 156 and 81. A more broadly effective vaccine is needed.

Among persons in all age groups, but particularly infants and elderly persons, *Streptococcus pneumoniae* remains a major cause of invasive pneumococcal disease (IPD) (e.g., pneumonia, meningitis, and sepsis), although generally effective antimicrobial agents are available (*1*). In the United States, 7-valent pneumococcal conjugate vaccine (PCV7) has been administered to children since 2000, resulting in both individual and herd immunity, with declines in pneumococcal infection among children and elderly persons (*2–6*). Unfortunately, introduction of PCV7 was followed by an increase in serotype 19A showing penicillin resistance and often multidrug resistance (*5–8*). In 2010, vaccination for children was upgraded to 13-valent pneumococcal conjugate vaccine (PCV13), which covers 6 additional serotypes: 1, 3, 5, 6A, 7F, and 19A (*9*). Introduction of PCV13 contributed to decreases in IPD (*10,11*), pneumonia (including community-acquired pneumonia without bacteremia) (*12,13*), and acute otitis media (*14–16*) caused by *S. pneumoniae* belonging to vaccine serotypes, especially 6A and 19A. As an indirect effect of wide administration of PCVs to children, pneumococcal infections in adults have also decreased, representing herd immunity (*11,12,17–23*).

Despite these benefits, in countries where PCV7 or PCV13 was introduced, proportions of disease preventable by PCVs gradually decreased because vaccine-serotype pneumococci were replaced by nonvaccine serotypes (NVTs). Increases in NVTs such as 6C, 15A/B/C, 23A, and 35B have been reported in the United States (*24–28*); 15A and 23B in Norway (*18*) and Germany (*29*); and 12F, 15A, 24F, and 35B in France (*30*).

In November 2010 in Japan, PCV7 vaccination use among children <5 years of age was introduced voluntarily by the Provisional Special Fund for the Urgent Promotion of Vaccination. In April 2013, PCV7 was officially incorporated into the vaccination program as public administration; in November of that year, PCV7 was replaced by PCV13. Promotion of PCV7 vaccination for children rapidly halved the number of IPD cases caused by vaccine-serotype pneumococci among children (*31*) and also produced a herd effect benefiting elderly persons (*32*). After PCV7 introduction, however, among persons of all ages, IPD caused by non-PCV7 serotypes such as 19A, 15A, 15B, 15C, 22F, and 24F showed relative increases in 2013. In November 2014, the Japanese Ministry of Health, Labour and Welfare began promoting vaccination of adults ≥65 years of age with 23-valent pneumococcal polysaccharide vaccine

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(PPSV23). In this study, we aimed to clarify year-to-year changes in capsular serotypes, genotypes of penicillin and macrolide resistance, and diversity of sequence types (STs) in all pneumococcal isolates collected throughout Japan during April 2010–March 2017.

Methods

Patients and Pneumococcal Strains

We included all specimens from patients of any age with IPD. Pneumococcal isolates from normally sterile clinical samples were collected from clinical laboratories at 341 hospitals participating in this IPD surveillance study. Each hospital had a microbiology laboratory as described previously (*31*), and participating hospitals were distributed nearly uniformly throughout Japan. These hospitals took part in the surveillance project after written permission was granted by the laboratory director or hospital director. This study was approved by the Keio University School of Medicine Ethics Committee (approval no. 20140432).

A total of 2,856 pneumococcal strains were collected from April 2010 through March 2017 (online Technical Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/24/11/18-0326-Techapp1.pdf). The first surveillance interval, April 2010–March 2011 (designated 2010), represented the pre-PCV7 period. The second surveillance interval, April 2011–March 2014 (designated 2011–2013, the PCV7 period), showed effects of PCV7 vaccination for children <5 years of age. The third surveillance interval, April 2014–March 2017 (designated 2014–2016, the PCV13 period), reflected PCV13 vaccination for children <5 years of age.

During the pre-PCV7 period, the rate of voluntary PCV7 vaccination among children in Japan was <10%. The PCV7 period corresponded to the Urgent Promotion of PCV7, a vaccination incentive program for children. The PCV7 vaccination rate throughout Japan was estimated at 50%–60% in 2011, 80%–90% in 2012, and >95% in 2013. During the PCV13 period, corresponding to substitution of PCV13 for routine vaccination, coverage remained >95%. In elderly persons (≥65 years of age), the rate of vaccination with PPSV23, starting in 2014, has remained at ≈54% as of 2017 (Vaccine Medical Affairs of Merck Sharp and Dohme K.K., Tokyo, Japan, pers. comm., 2017 Apr 1).

Pneumococcal isolates were sent promptly from each clinical laboratory to the Department of Infectious Diseases, Keio University School of Medicine (Tokyo, Japan), accompanied by a survey form completed by the attending physician. In compliance with ethics guidelines for epidemiology in Japan, patients were not identified.

Serotype and Resistance Genotype

We determined serotypes by using the capsular quellung test with antiserum purchased from Statens Serum Institute (Copenhagen, Denmark). Alterations in 3 penicillin-binding protein genes that mediate β-lactam resistance in *S. pneumoniae* (*pbp1a*, *pbp2x*, and *pbp2b*) were identified by real-time PCR as described previously (*33*). The *mef*(A) and *erm*(B) genes, which mediate macrolide resistance, were also identified by real-time PCR (*33*). Quinolone resistance was analyzed by sequencing the quinolone resistance–determining region in the genes *gyrA*, *gyrB*, *parC*, and *parE* in strains showing MICs of levofloxacin exceeding 4 μg/mL.

Genotypes (g) based on gene analysis were represented as follows: penicillin-susceptible *S. pneumoniae* (gPSSP), possessing 3 normal *pbp* genes; penicillin-intermediate *S. pneumoniae* (gPISP), subclassified as gPISP (*pbp2x*), gPISP (*pbp2b*), gPISP (*pbp1a+pbp2x*), gPISP (*pbp1a+pbp2b*), or gPISP (*pbp2x+pbp2b*); and penicillin-resistant *S. pneumoniae* (gPRSP), which possessed 3 abnormal *pbp* genes (*31,33*). Serotype and resistance genotype results were promptly reported to laboratory staff at each referring hospital.

Susceptibility Testing

For all isolates, we redetermined the MICs of 6 antimicrobial agents by using agar-dilution methods with reference strains R6 and ATCC49619 (*34*). The agents tested were penicillin, ampicillin, cefotaxime, meropenem, vancomycin, and levofloxacin.

Multilocus Sequence Typing

We performed multilocus sequence typing (MLST) analysis for all 2,849 isolates that could be cultured. Primers used for MLST were based on sequences listed at https://pubmlst.org/spneumoniae/. Clusters of related STs were analyzed by using eBURST version 3 (http://eburst.mlst.net/).

Statistical Analyses

For statistical analyses, we used Ekuseru-Toukei 2015 software (Social Survey Research Information, Tokyo, Japan) and R software 3.5.0 (R Foundation of Computational Statistics, Vienna, Austria). We used the χ^2 and Fisher exact tests as appropriate. We considered $p<0.05$ to indicate statistical significance.

Results

Relationships between IPD Type and Patient Age

Relationships between IPD type and patient age are shown in Table 1. IPD types were classified into 4 categories: pneumonia with bacteremia (41.9%), including empyema and pleuritis; bacteremia with unknown focus (37.0%); meningitis (15.4%); and others (5.6%), including endocarditis, necrotizing fasciitis, cellulitis, arthritis, and spondylitis. Pneumonia with bacteremia was most common among adults, especially

Table 1. Invasive pneumococcal disease in all patients, by age group, Japan, April 2010–March 2017

Disease	Total, no. (%), n = 2,856	Age, y, no. (%) ≤2, n = 731	3–5, n = 181	6–17, n = 94	18–49, n = 201	50–64, n = 387	65–74, n = 530	75–84, n = 457	≥85, n = 275	p value
Pneumonia with bacteremia*	1,198 (41.9)	130 (17.8)	35 (19.3)	22 (23.4)	83 (41.3)	167 (43.2)	261 (49.2)	300 (65.6)	200 (72.7)	<0.001
Bacteremia with focus unknown	1,058 (37.0)	455 (62.2)	116 (64.1)	33 (35.1)	46 (22.9)	104 (26.9)	158 (29.8)	92 (20.1)	54 (19.6)	<0.001
Meningitis	440 (15.4)	109 (14.9)	22 (12.2)	34 (36.2)	56 (27.9)	80 (20.7)	79 (14.9)	43 (9.4)	17 (6.2)	<0.001
Other†	160 (5.6)	37 (5.1)	8 (4.4)	5 (5.3)	16 (8.0)	36 (9.3)	32 (6.0)	22 (4.8)	4(1.5)	0.002

*Includes empyema (n = 32) and pleuritis (n = 25).
†Includes endocarditis (n = 6), necrotizing fasciitis (n = 1), arthritis (n = 34), cellulitis (n = 20), and spondylitis (n = 7).

those ≥75 years of age; however, among children <5 years of age, bacteremia with unknown focus was most common (p<0.001 for each). Meningitis and other IPDs were represented in higher proportions among persons 6–64 years of age (p<0.001) than among those in other age groups (p = 0.002).

Changes in Serotypes

Figure 1 shows yearly changes in pneumococcal capsular serotypes among children and adults. Pneumococcal capsular serotypes were classified into 4 groups: PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) (PCV7); PCV13 serotypes not included in PCV7 (1, 3, 5, 6A, 7F, and 19A) (PCV13–nonPCV7); PPSV23 serotypes not included in PCV13 (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F) (PPSV23–nonPCV13); and NVTs not including serotypes in PPSV23 and not including 6A. Among children, the proportion of PCV7 serotypes that accounted for 73.3% of serotype strains isolated from IPD patients during the pre-PCV7 period decreased rapidly to 7.4% in 2013 after PCV7 introduction (Figure 1). In contrast, in 2013, PCV13–nonPCV7 serotypes increased from 15.7% to 25.9%, PPSV23–nonPCV13 serotypes increased from 3.0% to 18.5%, and NVT serotypes increased from 8.0% to 48.1%. During 2014, after PCV7 was replaced with PCV13, the proportion of PCV13–nonPCV7 serotypes decreased by approximately half to 11.1% in 2016, while PPSV23–nonPCV13 serotypes increased to 40.4%, in contrast to the PCV7 period.

Among adults, the proportions of PCV7 serotypes, which accounted for 43.6% of isolates during the pre-PCV7 period, decreased to 11.7% in 2013, when children were vaccinated with PCV7. However, proportions of the PPSV23–nonPCV13 doubled from 12.4% to 25.4% and NVTs doubled from 13.5% to 28.9%. PCV13–nonPCV7 serotypes decreased slightly after replacement by PCV13 in 2014, but PPSV23–nonPCV13 serotypes continued to increase.

Serotype Changes during the Pre-PCV7, PCV7, and PCV13 Periods

Changes in serotypes of pneumococcal isolates collected between the pre-PCV7, PCV7, and PCV13 periods are shown in Table 2 for children and in Table 3 for adults. Among children, proportions of PCV7 serotypes decreased rapidly from 73.3% to 30.3% during the PCV7 period and decreased further to 2.3% during the PCV13 period (p<0.001). Among PCV13–nonPCV7 serotypes, serotype 19A apparently increased during the PCV7 period, but later it decreased significantly during the PCV13 period. PCV13–nonPCV7 serotypes decreased from 21.8% during the PCV7 period to 14.9% during the PCV13 period (p = 0.031). Although serotypes 1 and 7F showed relative increases during the PCV13 period, most were isolated from patients ≥3 years of

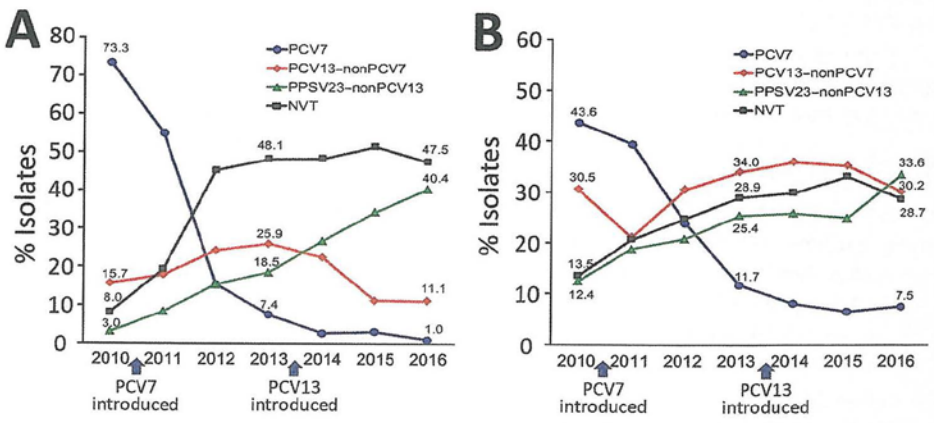

Figure 1. Yearly changes in pneumococcal serotypes of isolates from A) 1,006 children and B) 1,850 adults with invasive pneumococcal disease in Japan, April 2010–March 2017. Specific percentages are indicated at points along data lines. Fiscal years extend from April 1 through March 31 of the following year. PCV13–nonPCV7 covers 6 serotypes (1, 3, 5, 6A, 7F, and 19A). PPSV23–nonPCV13 covers 11 serotypes (2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F), but 2, 9N, and 17F were not isolated in this study. NVTs represent other serotypes not included in PPSV23 and 6A. NVT, nonvaccine serotype; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

Table 2. Distribution of pneumococcal serotypes among children before PCV7 and after introduction of PCV7 and PCV13, Japan, April 2010–March 2017*

Serotype	No. (%) Pre-PCV7 period, 2010, n = 300	PCV7 period 2011–2013, n = 357	PCV13 period, 2014–2016, n = 349	p value†
PCV7				
4	7 (2.3)	6 (1.7)	0	0.007
6B	83 (27.7)	49 (13.7)	2 (0.6)	<0.001
9V	9 (3.0)	2 (0.6)	1 (0.3)	0.005
14	34 (11.3)	9 (2.5)	0	<0.001
18C	4 (1.3)	4 (1.1)	1 (0.3)	0.336
19F	40 (13.3)	13 (3.6)	2 (0.6)	<0.001
23F	43 (14.3)	25 (7.0)	2 (0.6)	<0.001
Subtotal	220 (73.3)	108 (30.3)	8 (2.3)	<0.001
PCV13–nonPCV7‡				
1	0	5 (1.4)	13 (3.7)	**<0.001**
3	4 (1.3)	7 (2.0)	4 (1.1)	0.724
5	0	0	0	NA
6A	15 (5.0)	6 (1.7)	2 (0.6)	0.001
7F	1 (0.3)	2 (0.6)	6 (1.7)	0.153
19A	27 (9.0)	58 (16.2)	27 (7.7)	0.001
Subtotal	47 (15.7)	78 (21.8)	52 (14.9)	0.031
PPSV23–nonPCV13§				
8	0	0	0	NA
10A	2 (0.7)	5 (1.4)	18 (5.2)	**<0.001**
11A	2 (0.7)	1 (0.3)	4 (1.1)	0.356
12F	1 (0.3)	1 (0.3)	33 (9.5)	**<0.001**
15B	0	15 (4.2)	26 (7.4)	**<0.001**
20	0	1 (0.3)	0	NA
22F	3 (1.0)	16 (4.5)	19 (5.4)	**0.003**
33F	1 (0.3)	7 (2.0)	17 (4.9)	**0.001**
Subtotal	9 (3.0)	46 (12.9)	117 (33.5)	**<0.001**
NVT				
6C	9 (3.0)	20 (5.6)	11 (3.2)	0.159
15A	2 (0.7)	25 (7.0)	36 (10.3)	**<0.001**
15C	1 (0.3)	17 (4.8)	16 (4.6)	**<0.001**
23A	4 (1.3)	13 (3.6)	10 (2.9)	0.176
24F	1 (0.3)	20 (5.6)	52 (14.9)	**<0.001**
24B	2 (0.7)	4 (1.1)	12 (3.4)	0.197
34	1 (0.3)	6 (1.7)	7 (2.0)	0.126
35B	1 (0.3)	10 (2.8)	11 (3.2)	**0.016**
38	1 (0.3)	8 (2.2)	7 (2.0)	0.072
Other¶	2 (0.7)	2 (0.6)	9 (2.6)	**0.041**
Subtotal	24 (8.0)	125 (35.0)	171 (49.0)#	**<0.001**

*Years run from April 1 through March 31 of the following year. NA, not applicable; NVT, nonvaccine serotype; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.
†p values compare the 3 surveillance periods; boldface indicates significant increase.
‡Serotypes added to PCV7.
§Serotypes contained in PPSV23 but not PCV13.
¶Includes 7C (n = 2), 16F (n = 2), 21 (n = 2), 23B (n = 3), 28A (n = 1), 37 (n = 1), and 31 (n = 2).
#One strain identified as nontypeable was excluded from the table.

age who had received PCV7 or a single dose of PCV13. To the contrary, proportions of PPSV23–nonPCV13 serotypes and NVTs increased significantly between the pre-PCV7 period and the PCV7 period, continuing to increase up to the PCV13 period ($p<0.001$ for each). In particular, 9 serotypes (10A, 12F, 15A, 15B, 15C, 22F, 24F, 33F, and 35B) increased significantly after introduction of PCV7 and PCV13.

Among adults, proportions of PCV7 serotypes decreased sharply, from 43.6% during the pre-PCV7 period to 24.2% during the PCV7 period and 7.3% during the PCV13 period, particularly for serotypes 4, 6B, 9V, 14, 19F, and 23F. PCV13–nonPCV7 serotypes increased in serotypes 7F and 19A, whereas 6A showed a significant decrease because of cross-immunity with 6B (Table 3). PPSV23–nonPCV13 serotypes and NVTs increased respectively from 12.4% and 13.5% during the pre-PCV7 period to 21.9% and 24.9% during the PCV7 period and further to 27.8% and 30.9% during the PCV13 period ($p<0.001$ for each). In particular, significant increases were noted for serotypes 12F, 15C, 22F, 23A, 24F, and 35B. Tendencies to increase did not attain significance for serotypes 11A and 15A.

Changes in Penicillin and Other Resistance Genotypes

Figure 2 shows yearly changes of penicillin resistance genotypes among children and adults. Changes are shown from

Table 3. Distribution of pneumococcal serotypes in adults before PCV7 and after introduction of PCV7 and PCV13 administration to children, Japan, April 2010–March 2017*

Serotype	No. (%) Pre-PCV7 period, 2010, n = 275	PCV7 period, 2011–2013, n = 695	PCV13 period, 2014–2016, n = 880	p value†
PCV7				
4	14 (5.1)	27 (3.9)	4 (0.5)	<0.001
6B	42 (15.3)	39 (5.6)	22 (2.5)	<0.001
9V	7 (2.5)	7 (1.0)	6 (0.7)	0.042
14	21 (7.6)	41 (5.9)	6 (0.7)	<0.001
18C	1 (0.4)	3 (0.4)	2 (0.2)	0.642
19F	14 (5.1)	23 (3.3)	15 (1.7)	0.007
23F	21 (7.6)	28 (4.0)	9 (1.0)	<0.001
Subtotal	120 (43.6)	168 (24.2)	64 (7.3)	<0.001
PCV13–nonPCV7‡				
1	1 (0.4)	4 (0.6)	13 (1.5)	0.145
3	45 (16.4)	110 (15.8)	145 (16.5)	0.939
5	0	1 (0.1)	0	NA
6A	11 (4.0)	16 (2.3)	9 (1.0)	0.006
7F	9 (3.3)	9 (1.3)	33 (3.8)	**0.006**
19A	18 (6.5)	61 (8.8)	99 (11.3)	**0.045**
Subtotal	84 (30.5)	201 (28.9)	299 (34.0)	0.093
PPSV23–nonPCV13§				
8	0	2 (0.3)	0	NA
10A	10 (3.6)	34 (4.9)	54 (6.1)	0.244
11A	3 (1.1)	23 (3.3)	34 (3.9)	0.058
12F	5 (1.8)	5 (0.7)	63 (7.2)	**<0.001**
15B	3 (1.1)	14 (2.0)	10 (1.1)	0.356
20	1 (0.4)	7 (1.0)	14 (1.6)	0.261
22F	10 (3.6)	63 (9.1)	59 (6.7)	**0.008**
33F	2 (0.7)	4 (0.6)	11 (1.3)	0.352
Subtotal	34 (12.4)	152 (21.9)	245 (27.8)	**<0.001**
NVT				
6C	13 (4.7)	49 (7.1)	52 (5.9)	0.400
15A	6 (2.2)	28 (4.0)	47 (5.3)	0.068
15C	0	12 (1.7)	7 (0.8)	**0.034**
23A	2 (0.7)	33 (4.7)	50 (5.7)	**<0.001**
24F	0	11 (1.6)	16 (1.8)	**0.049**
34	1 (0.4)	5 (0.7)	12 (1.4)	0.301
35B	7 (2.5)	22 (3.2)	55 (6.3)	**0.004**
38	3 (1.1)	7 (1.0)	11 (1.3)	0.955
Other¶	5 (1.8)	6 (0.9)	22 (2.5)	**0.042**
Subtotal	37 (13.5)	173 (24.9)#	272 (30.9)	**<0.001**

*Years run April 1– March 31 of the following year. NA, not applicable; NVT, nonvaccine serotype; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.
†p values compare the 3 surveillance periods; boldface indicates significant increase.
‡Serotypes added to PCV7.
§Serotypes contained in PPSV23 but not PCV13.
¶Includes 6D (n = 2), 7C (n = 8), 13 (n = 1), 16F (n = 6), 18B (n = 1), 23B (n = 5), 31 (n = 3), and 37 (n = 7).
#One strain identified as nontypeable was excluded from the table.

the pre-PCV7 period to the PCV7 period and further to the PCV13 period.

Among children, the proportion of gPRSP declined sharply from 54.3% in 2010 during the pre-PCV7 period to 20.4% in 2013 during the PCV7 period; gPSSP and gPISP (*pbp2x*) increased (Figure 2). In 2016 during the PCV13 period, proportions of gPRSP and gPISP (*pbp1a+2x*) further declined to 11.2% and 6.1%, respectively. However, gPISP (*pbp2b*) rapidly increased.

Among isolates from adults during the pre-PCV7 period, gPISP (*pbp2x*) was most common (34.9%), followed by gPRSP (32.4%) (Figure 2). gPSSP accounted for only 15.3%. Similar to the trend for children during the PCV7 and PCV13 periods, gPRSP among adults continually decreased to 15.5% in 2016. However, also in 2016, gPISP (*pbp2b*) among adults increased to 8.3%, similar to the trend among children.

During the surveillance periods, macrolide-resistant isolates possessing *mef*(A) or *erm*(B) genes remained consistently high. Among children, proportions were 93.8% in 2010 and 91.8% in 2016; among adults, proportions were 87.2% in 2010 and 89.8% in 2016. Prevalence of resistance genes was 59.8% for the *erm*(B) gene mediating high macrolide resistance, 19.6% for the *mef*(A) gene mediating intermediate resistance, and 11.6% for both *erm*(B) and *mef*(A) genes (data not shown).

Isolates with mutations in both *gyrA* and *parC* genes, which are involved in resistance to quinolones, especially

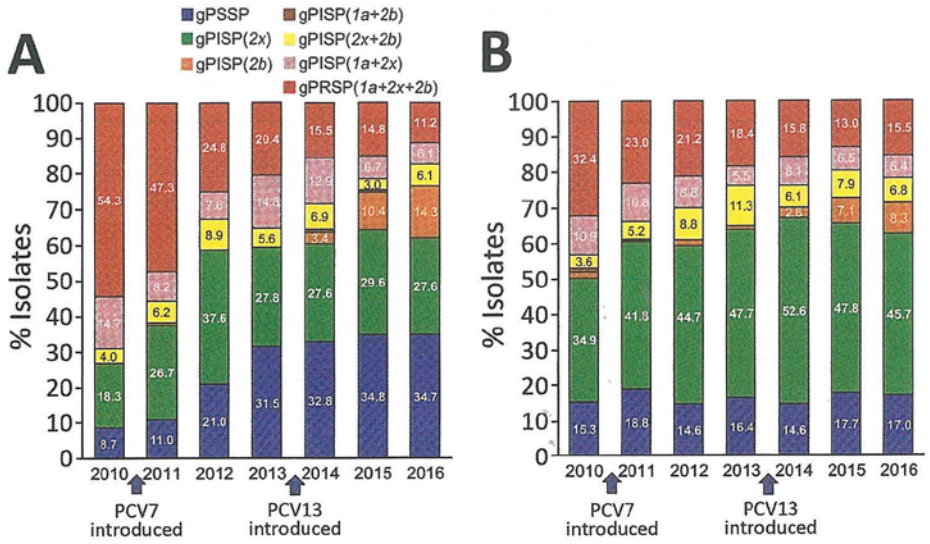

Figure 2. Yearly changes in genotypic penicillin resistance in isolates from A) 1,006 children and B) 1,850 adults with invasive pneumococcal disease in Japan, April 2010–March 2017. Fiscal years extend from April 1 through March 31 of the following year. Genotypes based on abnormal *pbp1a*, *pbp2x*, and *pbp2b* genes were identified by real-time PCR and are represented as gPRSP (*1a+2x+2b*), gPISP (*1a+2x*), gPISP (*1a+2b*), gPISP (*2x+2b*), gPISP (*2x*), gPISP (*2b*), and gPSSP. g, genotype; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PISP, penicillin-intermediate *Streptococcus pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*; PSSP, penicillin-susceptible *S. pneumoniae*.

levofloxacin, accounted for <1% of all isolates. These isolates showed no tendency to increase.

Relationships between Serotypes and Resistance Genotypes

Changes of serotypes and the penicillin resistance genotypes during the 3 periods (pre-PCV7, PCV7, and PCV13) are shown in online Technical Appendix Figures 2 (for children) and 3 (for adults). Decreases in gPRSP (*pbp1a+2x+2b*) and gPISP (*pbp1a+2x*) were closely related to reduction of serotypes 6B, 14, 19F, 23F, and 6A in children and adults during the PCV7 period, and this link became stronger during the PCV13 period. Serotype 19A, including several gPRSP, decreased by half among children during the PCV13 period, but this change has not yet become evident among adults.

The proportions of PPSV23–nonPCV13 and NVT serotypes generally increased among children and adults during the PCV13 period. gPRSPs were newly identified in serotypes 15B (n = 1), 15C (n = 1), and 16F (n = 2) in isolates from children and in serotypes 6C (n = 2), 6D (n = 2), 13 (n = 1), 15B (n = 1), 15C (n = 1), 16F (n = 2), 23A (n = 1), 23B (n = 1), and 34 (n = 1) in isolates from adults.

Relationships between genotypic macrolide and penicillin resistances and serotypes are shown in online Technical Appendix Table 1. Strains possessing *mef*(A), *erm*(B), or both were identified in most of the serotypes, with the exception of serotypes 8, 18B, 28A, and 31. No relationship was observed between macrolide resistance and penicillin resistance.

Antimicrobial Susceptibility by Genotype

Susceptibilities (50% MIC, 90% MIC, and MIC range) of 6 parenteral agents (penicillin, ampicillin, cefotaxime, meropenem, vancomycin, and levofloxacin) for *S. pneumoniae* strains obtained from April 2014 through March 2017, corresponding to the PCV13 period (n = 1,229), are shown in online Technical Appendix Table 2. Relationships between 6 genotypes for penicillin resistance and MICs of penicillin, ampicillin, cefotaxime, and meropenem for the strains are shown in online Technical Appendix Figure 4.

Because prevalence of gPRSP was reduced by the PCV vaccinations, the distribution of susceptibilities was shifted in favor of greater susceptibility, especially after introduction of PCV7. For penicillin and ampicillin, 90% MICs were 2 μg/mL; for cefotaxime, 1 μg/mL; and for meropenem, 0.5 μg/mL. gPRSP isolates showing high resistance for penicillin (≥8 μg/mL) were not found.

STs by Serotypes and Resistance Genotypes

STs by eBURST analyses for 2,849 pneumococcal strains are shown in Figure 3. These data are distinguished by ST and vaccine serotype (PCV7, PCV13–nonPCV7, PPSV23–nonPCV13, and NVT). Details of relationships among clonal complexes (CCs), STs, serotypes, and resistance genotypes are listed in online Technical Appendix Table 3.

By MLST analysis, 273 different STs were identified. STs of gPRSP in 11 serotypes included in PPSV23–non-PCV13 and NVTs were noteworthy: 15B (n = 2), ST242 (belonging to CC242) and ST83 (derived from CC81); 6C (n = 2), ST8352 (CC156) and ST5832 (CC5832); 6D (n = 2), ST90 (CC156) and ST282 (CC81); 13 (n = 1), ST10303 (CC156); 15A (n = 77), ST63, ST2105, ST2771, ST8354, and ST12000 (all CC63); 15C (n = 2), ST83 and ST6138 (CC81); 16F (n = 4), ST8351 (CC3117); 23A (n = 1), ST9619 (CC156); 23B (n = 1), ST2372 (CC156); 34 (n = 1), ST9395 (CC15); 35B (n = 55), ST558, ST1204, and

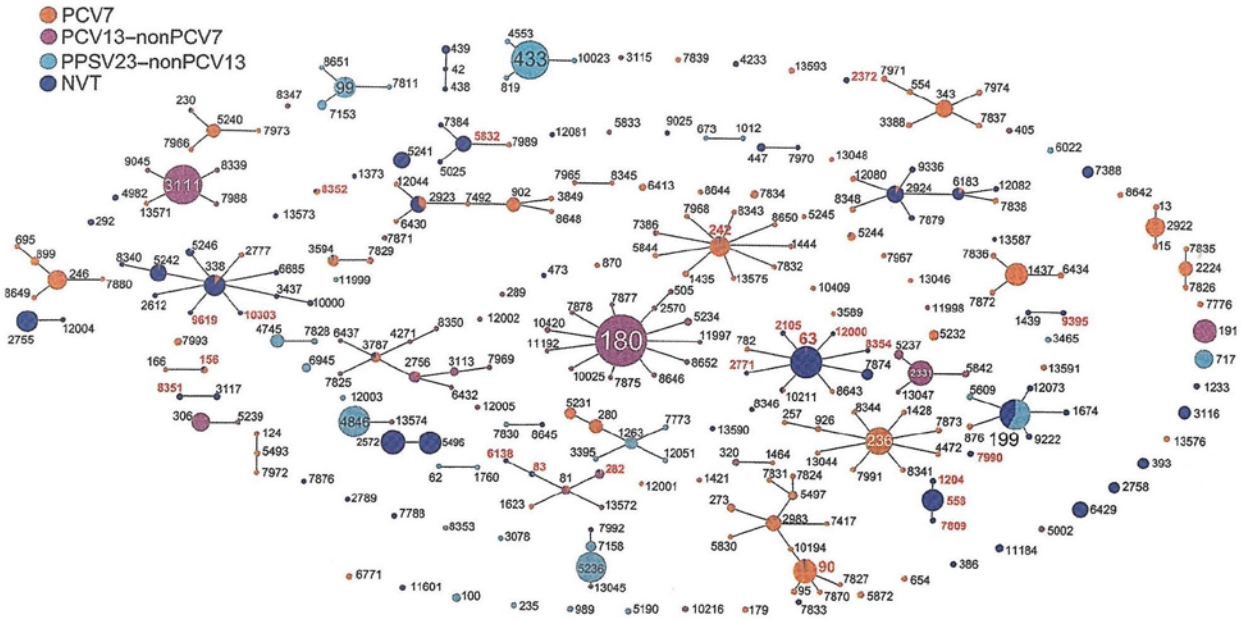

Figure 3. An eBURST (http://eburst.mlst.net/) diagram displaying pneumococcal sequence types (STs) causing invasive pneumococcal disease across patients of all age groups in Japan. All 2,849 strains are distinguished by colors to indicate PCV7, PCV13–nonPCV7, PPSV23–nonPCV13, and NVT. Size of each circle reflects the number of strains. ST numbers shown in red represent genotypes for penicillin-resistant *Streptococcus pneumoniae* confirmed among PPSV23–nonPCV13 and NVT as follows: 15B (n = 2), ST242 and ST83; 6C (n = 2), ST8352 and ST5832; 6D (n = 2), ST90 and ST282; 13 (n = 1), ST10303; 15A (n = 77), ST63 (n = 73), ST2105, ST2771, ST8354, and ST12000; 15C (n = 2), ST83 and ST6138; 16F (n = 4), ST8351; 23A (n = 1), ST9619; 23B (n = 1), ST2372; 34 (n = 1), ST9395; 35B (n = 55), ST558 (n = 49), ST1204, ST7809, ST7990, and ST156. NVT, nonvaccine serotype; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.

ST7809 (all CC558); and the remaining ST156 (CC156) and ST7990 (singleton).

A total of 6 STs identified as gPRSP belonged to the large CC156 (Figure 4). STs of 3 serotypes (13, 23A, and 23B) were derived from ST338, which includes the Colombia$^{23F\text{-}26}$ clone from the Pneumococcal Molecular Epidemiology Network (PMEN). Serotype 6C was derived from ST172, a neighbor of ST338. Of strains with serotypes 6D and 35B, each strain was distant from other gPRSPs. STs of serotypes 15B, 15C, and 6D among gPRSP belonged to CC81 (online Technical Appendix Figure 5).

In addition, STs of certain serotypes increasing in PPSV23–nonPCV13 and NVTs were noted. Serotype 12F was ST4846 (CC1527), 22F was ST433 (CC433), 23A included ST338 and ST5242 (CC156), and 24B/24F included ST2572 and ST5496 (CC2572).

Discussion

Wide use of PCVs among children in many countries has contributed to a dramatic reduction in incidence of IPD (*6,10,11,35–38*), pneumonia (*12,13,39*), and acute otitis media (*14,15*) caused by *S. pneumoniae*, while providing indirect herd immunity benefits for adults (*11,23,40,41*). Replacing PCV7 with PCV13 decidedly decreased serotype 19A isolates among causative pathogens, but in several countries, NVTs such as 15A and 35B increased. Gradual increases of NVTs, unfortunately, have blunted the effectiveness of conjugate vaccines (*42*).

In Japan, introduction of PCV7 in children <5 years of age began as an official government program in November 2010, continuing until it was replaced with PCV13 in November 2013. PPSV23 vaccination for adults ≥65 years of age was implemented in October 2014. We organized nationwide surveillance beginning in April 2010, with collection of pneumococcal strains from IPD patients in all age groups throughout Japan. In this article, we describe details of changes of serotypes, penicillin resistance genotypes, and MLST analyses that have followed implementation of PCV7 and PCV13 vaccination. As in other countries where PCV13 has been introduced, proportions of PCV13 serotypes among isolates from children and adults decreased significantly during the PCV13 period. In Japan, where population density is high, the decrease suggests early effectiveness of herd immunity not only among children but also among adults. However, serotypes 7F and 19A, included in PCV13, seem to be increasing among adults; for these serotypes, no indirect effect for adults is evident. These findings indicate a need for PCV13 vaccination of elderly

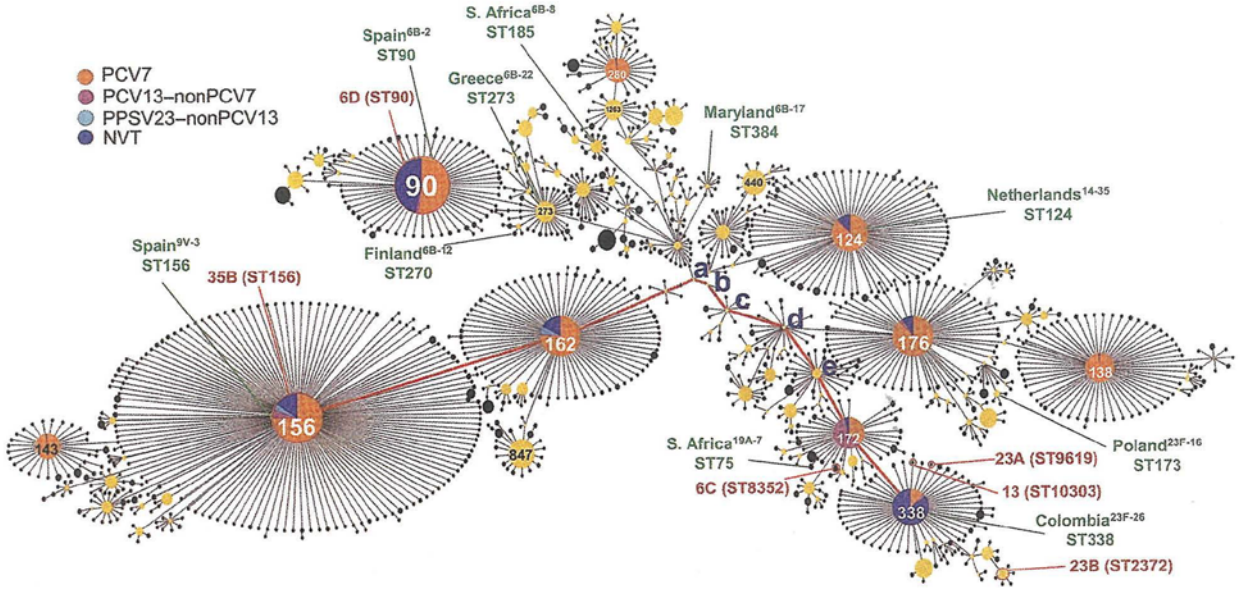

Figure 4. Details of *Streptococcus pneumoniae* clonal complex (CC) 156 (n = 4,736), including 1,308 sequence types obtained from the multilocus sequence typing website (https://pubmlst.org/spneumoniae/). Data include those from this study (n = 359). STs of 6 genotypes for penicillin-resistant *S. pneumoniae* identified in NVT serotypes belonged to CC156. STs of serotypes 6C, 13, 23A, and 23B were derived from ST338 and ST172 (shown in red). Serotypes 6D and 35B belonged to ST90 and ST156, respectively. The Pneumococcal Molecular Epidemiology Network clone identified in CC156 is also shown (in green). The red line indicates evolution from ST156 to ST338: a, ST8055; b, ST8618; c, ST4542; d, ST171; e, ST361. NVT, nonvaccine serotype; PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine; ST, sequence type.

and relatively immunocompromised persons, especially in Japan, where the population's average age is increasing. Of further concern is a test-negative design study conducted before introduction of PPSV23 to assess effectiveness of PPSV23 among elderly persons with community-acquired pneumonia in Japan. Effectiveness against community-acquired pneumonia caused by PPSV23 serotypes seemed low to moderate, depending on age group (*43*).

Proportions of many non-PCV13 serotypes during the PCV13 period have increased beyond proportions during the pre-PCV7 period. Nine serotypes (10A, 12F, 15A, 15B, 15C, 22F, 24F, 33F, and 35B) have increased significantly among children, and 5 serotypes (12F, 15C, 22F, 23A, and 35B) have increased significantly among adults, showing considerable overlap between age groups. Among these serotypes, 15A and 35B have increased rapidly since PCV13 introduction in Japan, as has occurred in other countries (*18,25,28–30*). The reason for increases in such serotypes is unclear; further epidemiologic surveillance may shed light on the matter.

Of note, gPRSP decreased sharply along with serotype replacements among children and adults. Highly penicillin-resistant strains with MICs ≥8 μg/mL, which sometimes were noted in serotypes 19F and 23F during the pre-PCV7 period (*33*), did not increase with introduction of PCVs. Susceptibilities of most gPISP (*pbp2b*) in serotype 12F and of gPISP(*pbp2x+2b*) in serotypes 23A and 6C for penicillin and ampicillin ranged from 0.125 to 0.5 μg/mL. Should mutation(s) occur in the regions encoding the conserved amino acids (STMK, SSN, and KTG) in the *pbp1a* gene, antimicrobial selection pressure could easily favor development from gPISP to gPRSP.

One concern is the evolution of gPRSP among isolates from 11 NVTs according to MLST analysis. Most (all but 2) serotype 35B isolates were found to belong to the same ST558 (CC558) that was reported from the United States in 1999 (*44*). Serotype 15A was identified as ST63, which belongs to CC63, as does the PMEN clone Sweden^{15A-25}. Each isolate of serotype 6D (ST282, CC81) and serotype 15B (ST83, CC81) was the same as those previously registered from South Korea (*45*) and Taiwan. These findings suggest that newly emerged resistant strains can spread rapidly between countries.

Among gPRSP identified in NVTs, STs of serotypes 6C, 13, 23A, 23B, and of both serotypes 6D and 35B, were noted to belong to CC156, which includes large numbers of isolates in ST156, ST90, ST162, ST124, ST176, and ST138; the PMEN clones Spain^{9V-3}, Netherlands$^{14-35}$, Spain^{6B-2}, Greece^{6B-22}, and S. Africa^{6B-8} are representative among these. STs of serotypes 6C, 13, 23A, and 23B were

derived from ST172 and ST338, which diverged from ST171 (Figure 4, letter d) and evolved further. Serotypes of many isolates registered as ST172 or ST338 were either NVTs or one of the serotypes of PCV13–nonPCV7. These findings suggested that wide use of PCVs led to a decrease in STs belonging to PCV7 and PCV13 serotypes, but some STs detected among NVTs escaped from the vaccine pressure and are increasing, such as ST338. However, whether the new gPRSPs emerged in Japan or originated in another country is unknown.

Capsular switching in *S. pneumoniae* can occur as a result of homologous recombination at a site outside the *cps* locus. Of note, *pbp1a* genes are located upstream and *pbp2x* genes are located downstream of the *cps* locus (*46–48*). Recombination including these 2 *pbp* genes, driven by antimicrobial pressure, can result in concomitant exchange of the *cps* locus. Such new ST strains arising from capsular switching can exhibit penicillin resistance and increase under antimicrobial selection pressure. The diversity of serotypes, resistant genotypes, and STs we describe reflects adaptability of *S. pneumoniae* to the human environment.

In conclusion, to assess whether gPRSP in NVTs will increase in the near future, sustained surveillance for IPD is needed. Control of pneumococcal infections, particularly in elderly and immunocompromised persons, requires development of further multivalent conjugate vaccines, new vaccines targeting a different microbial component, or both. Global consensus for appropriate use of antimicrobial drugs is also valuable for limiting spread of new resistant strains within and beyond national borders.

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Effects of Pneumococcal Conjugate Vaccine on Genotypic Penicillin Resistance and Serotype Changes, Japan, 2010–2017

Technical Appendix

Technical Appendix Table 1. Serotypes, penicillin-resistant and macrolide-resistant genotypes for all isolates (n = 2,849) from patients with invasive pneumococcal disease, Japan, April 2010–March 2017

		Macrolide resistance†			
	Penicillin resistance	Non	*erm*(B)	*mef*(A)	*mef*(A) and *erm*(B)
Serotype (n)	genotype*	n = 258	n = 1,704	n = 557	n = 330
4 (58)	gPISP (*pbp2x*)			1	
	gPSSP	7	2	48	
6B (237)	gPRSP		117	23	10
	gPISP (*pbp1a*+2*x*)	2	14	26	2
	gPISP (*pbp2x*+2*b*)		6	2	
	gPISP (*pbp2x*)		26	7	
	gPSSP	2			
9V (31)	gPRSP		1		
	gPISP (*pbp2x*)		5	24	
	gPSSP		1		
14 (111)	gPRSP	3	31	8	
	gPISP (*pbp1a*+2*x*)		54	6	1
	gPISP (*pbp2x*+2*b*)		4	1	
	gPISP (*pbp2x*)		2		
	gPSSP	1			
18C (15)	gPSSP	9	2	4	
19F (107)	gPRSP		10	80	4
	gPISP (*pbp1a*+2*x*)			3	2
	gPISP (*pbp1a*+2*b*)		1		
	gPISP (*pbp2x*+2*b*)			1	
	gPISP (*pbp2x*)		3	2	
	gPSSP	1			
23F (128)	gPRSP	2	41	22	57
	gPISP (*pbp1a*+2*x*)		3		
	gPISP (*pbp2x*+2*b*)		2		
	gPISP (*pbp2x*)			1	
1 (36)	gPSSP	11	9	16	
3 (314)	gPRSP		1		1
	gPISP (*pbp2x*)	15	279	2	
	gPISP (*pbp2b*)		1		
	gPSSP	11	3	1	
5 (1)	gPSSP		1		
6A (58)	gPRSP	3	25	6	7
	gPISP (*pbp1a*+2*x*)	2	3	1	
	gPISP (*pbp2x*)	1	6	2	
	gPSSP	2			
7F (60)	gPISP (*pbp2x*)		4		
	gPSSP	15	28	13	
19A (290)	gPRSP		1	1	89
	gPISP (*pbp1a*+2*x*)	1	7	1	28
	gPISP (*pbp2x*+2*b*)				3
	gPISP (*pbp2x*)	4	3	51	85
	gPSSP	4	7	4	1
8 (2)	gPSSP	2			
10A (123)	gPISP (*pbp1a*+2*x*)	8	3		1
	gPISP (*pbp2x*+2*b*)		1		
	gPISP (*pbp2x*)	9	86	4	
	gPSSP	2	9		

Serotype (n)	Penicillin resistance genotype*	Macrolide resistance†			
		Non	*erm*(B)	*mef*(A)	*mef*(A) and *erm*(B)
		n = 258	n = 1,704	n = 557	n = 330
11A (67)	gPISP (*pbp1a+2x*)			2	
	gPISP (*pbp2x*)	10		3	26
	gPSSP	2	1	23	
12F (108)	gPISP (*pbp2x+2b*)		2		
	gPISP (*pbp2b*)		98		
	gPSSP	4	4		
15B (68)	gPRSP		1	1	
	gPISP (*pbp1a+2x*)		12		
	gPISP (*pbp2x*)		50	1	1
	gPSSP	1		1	
20 (23)	gPSSP	3	19	1	
22F (169)	gPISP (*pbp1a+2x*)			1	
	gPISP (*pbp2x*)	43	85	30	
	gPSSP	7	3		
33F (42)	gPISP (*pbp2x*)			5	
	gPSSP	2	34	1	
6C (154)	gPRSP		1	1	
	gPISP (*pbp1a+2x*)		1		
	gPISP (*pbp2x+2b*)	16	22	18	1
	gPISP (*pbp2x*)	4	62	25	
	gPSSP	2	1		
6D (2)	gPRSP		1	1	
7C (10)	gPSSP		10		
13 (1)	gPRSP		1		
15A (144)	gPRSP		77		
	gPISP (*pbp1a+2x*)		56		
	gPISP (*pbp1a+2b*)		7		
	gPISP (*pbp2x*)		2		1
	gPSSP			1	
15C (53)	gPRSP		2		
	gPISP (*pbp1a+2x*)		9	1	
	gPISP (*pbp2x*)		40	1	
16F (8)	gPRSP			4	
	gPISP (*pbp1a+2x*)			2	
	gPSSP	2			
18B (1)	gPSSP	1			
21 (2)	gPISP(*pbp2x*)	1		1	
23A (112)	gPRSP		1		
	gPISP (*pbp2x+2b*)	1	104		
	gPISP (*pbp2x*)		1		3
	gPSSP	2			
23B (8)	gPRSP	1			
	gPISP (*pbp2x+2b*)	2			
	gPISP (*pbp2x*)	1			
	gPSSP	2	1	1	
24F (100)	gPISP (*pbp1a+2x*)		1		
	gPISP (*pbp2x*)		2		
	gPSSP		97		
24B (17)	gPSSP		17		
28A (1)	gPSSP	1			
31 (5)	gPSSP	5			
34 (32)	gPRSP		1		
	gPISP(*pbp2x*)	3	1	9	
	gPSSP	5	13		
35B (106)	gPRSP	6	9	33	7
	gPISP (*pbp1a+2x*)		1		
	gPISP (*pbp2x*)		42	1	
	gPSSP		7		
37 (8)	gPSSP	5	2	1	
38 (37)	gPISP (*pbp2x+2b*)		1		
	gPISP (*pbp2x*)	1		22	
	gPSSP	8		5	

*1a, 2x, and 2b in parenthesis indicate abnormal *pbp1a*, *pbp2x*, and *pbp2b* genes, respectively.
†Each gene mediates macrolide resistance such as clarithromycin (CLR) and azithromycin (AZM).

Technical Appendix Table 2. MIC_{50}, MIC_{90}, and MIC range of 6 intravenous antimicrobial agents for isolates from patients with invasive pneumococcal disease from April 2014 to March 2017 (n = 1229)

Antimicrobial agent	Concentration (μg/mL)		
	MIC_{50}	MIC_{90}	MIC range
Penicillin	0.063	2	0.016–4
Ampicillin	0.063	2	0.016–8
Cefotaxime	0.25	1	0.008–8
Meropenem	0.008	0.5	0.008–1
Levofloxacin	2	2	0.5–32
Vancomycin	0.5	1	0.5–1

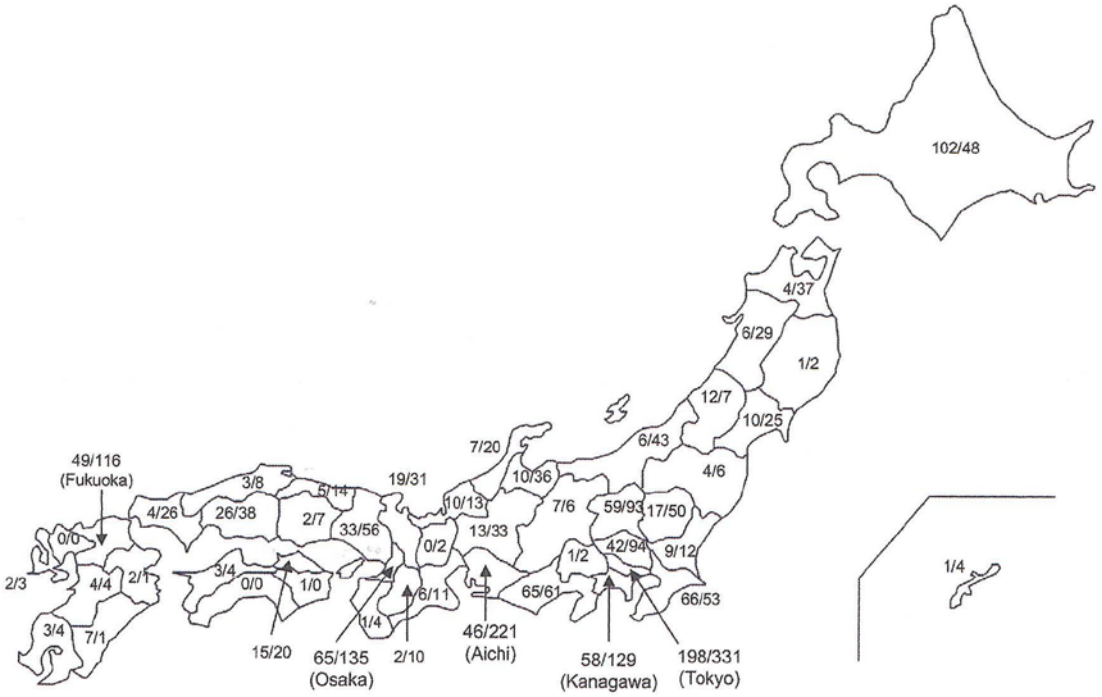
Technical Appendix 3. Serotypes, resistance genotypes, and multilocus sequence types for all isolates (n = 2,849) from patients with invasive pneumococcal disease, Japan, April 2010 - March 2017

Serotype	Resistance genotype†	Clonal complex (n)	Sequence type (n)
PCV13			
4	gPISP(*2x*)	81 (1)	899 (1)
	gPSSP	81 (51); 3784 (1); 5902 (1); none (4)	246 (43) 695 (1) 899 (5) 7880 (1) 8649 (1); 7776 (1); 13576 (1)*; 5872 (4)
6B	gPRSP	156 (84); 81 (28); none (11); 9789 (9); 2224 (8); 2924 (5); Singleton (3); 242 (2)	90 (66) 95 (3) 273 (5) 5497 (3) 7417 (1) 7827 (1) 7831 (2) 7870 (1) 13591 (1)* 13593 (1)*; 81 (1) 282 (1) 902 (19) 2923 (1) 3849 (1) 7492 (2) 8345 (1) 8644 (1) 8648(1); 5232 (10) 7967 (1); 2756 (3) 3787 (6); 2224 (7) 7835 (1); 6413 (5); 5244 (3); 242 (2)
	gPISP(*1a+2x*)	156 (23); 2224 (14); 7834 (4); 2924 (2); Singleton (1)	2983 (16) 5497 (5) 7824 (2); 2224 (13) 7826 (1); 7834 (4); 6183 (2); 13046 (1)
	gPISP(*2x+2b*)	81 (5); 156 (1); 2924 (1); 7834 (1)	902 (3) 7965(1) 12044 (1); 5830 (1); 8348 (1); 7834 (1)
	gPISP(*2x*)	81 (15); 156 (12); 2924 (4); 7834 (1); Singleton (1)	902 (1) 2923 (11) 5245 (1) 6430 (2); 273 (1) 2983 (7) 7839 (2) 10194 (2); 2924 (4); 7834 (1); 13048 (1)
	gPSSP	2224 (1); 7585 (1)	2224(1); 6771(1)
9V	gPRSP	156 (1)	166 (1)
	gPISP(*2x*)	156 (29)	280 (18) 5231 (11)
	gPSSP	156 (1)	280 (1)
14	gPRSP	343 (30); 156 (5); 15 (2); 230 (1); 63 (1); 199 (1); 320 (1); Singleton (1)	343 (23) 554 (2) 3388 (1) 7837 (1) 7971 (2) 7974 (1); 156 (2) 5493 (3); 2922 (2); 230 (1); 782 (1); 876(1); 236 (1); 8642 (1)
	gPISP(*1a+2x*)	15 (41); 230 (19); 3111 (1)	13 (3) 2922 (38); 5240 (17) 7966 (1) 7973 (1); 13571 (1)*
	gPISP(*2x+2b*)	343 (4); 15 (1)	343 (2); 554(2); 15 (1)
	gPISP(*2x*)	156 (2)	124 (1) 7972 (1)
	gPSSP	156 (1)	124 (1)
18C	gPSSP	3594 (14); 870 (1)	3594 (13) 7829 (1); 870 (1)
19F	gPRSP	320 (88); 156 (3); 63 (1); 81 (1); 242 (1)	236 (77) 926 (1) 1421 (1) 1428 (1) 1464(1) 4472 (1) 7873 (2) 7991 (1) 8341 (1) 8344 (1) 13044 (1); 7993 (1) 8352 (2); 10211 (1); 81 (1); 8650 (1)
	gPISP(1a+2x)	156 (2); 320 (1); 3111 (1); Singleton (1)	1263 (1) 7993 (1); 926 (1); 3111 (1); 12001(1)
	gPISP(*1a+2b*)	156 (1)	7993 (1)
	gPISP(*2x+2b*)	320 (1)	236 (1)
	gPISP(*2x*)	320 (3); 177 (1); 2924 (1)	236 (2) 257 (1); 179 (1); 6183 (1)
	gPSSP	251 (1)	654 (1)
23F	gPRSP	242 (59); 2924 (55); 156 (6); 81 (1); 5832 (1)	242 (48) 1435 (3) 1444 (1) 3589 (1) 7386 (1) 7832 (1) 7968 (1) 8343 (1) 10409 (1) 13575 (1)*; 1437 (51) 6434 (1) 7836 (1) 7872 (2); 338 (5) 2777 (1); 1623 (1); 7989 (1)
	gPISP(*1a+2x*)	63 (2); 242 (1)	63 (1) 8643 (1); 5844 (1)
	gPISP(*2x+2b*)	156 (2)	338 (1) 5242 (1)
	gPISP(*2x*)	242 (1)	242 (1)
1	gPSSP	306 (34); 217 (2)	306 (33) 5239 (1); 5002 (2)
3	gPRSP	156 (1); 242 (1)	166 (1); 242 (1)
	gPISP(*2x*)	180 (292); 99 (1); 113 (1); 156 (1); 242 (1)	180 (277) 5234 (5) 7875 (1) 7877 (1) 7878 (1) 8646 (1) 8652 (1) 10025 (1) 10420 (2) 11192 (1) 11997 (1); 99 (1); 13045 (1); 1263 (1); 7386 (1)
	gPISP(*2b*)	1527 (1)	13574 (1)*
	gPSSP	180 (13); Singleton (2)	180 (10) 505 (1) 2570 (1) 12002 (1); 10216 (1) 12005 (1)
5	gPSSP	289 (1)	289 (1)
6A	gPRSP	9789 (25); 81 (12); 156 (1); 3115 (1); Singleton (2)	2756 (17) 3113 (1) 6432 (2) 6437 (2) 7825 (2) 8350 (1); 81 (6) 282 (5) 13572 (1)*; 90 (1); 3115 (1); 5244 (1) 7871 (1)
	gPISP(1a+2x)	9789 (3); 156 (1); 2924 (1); Singleton (1)	3113 (2) 3787 (1); 5833 (1); 6183 (1); 11998 (1)

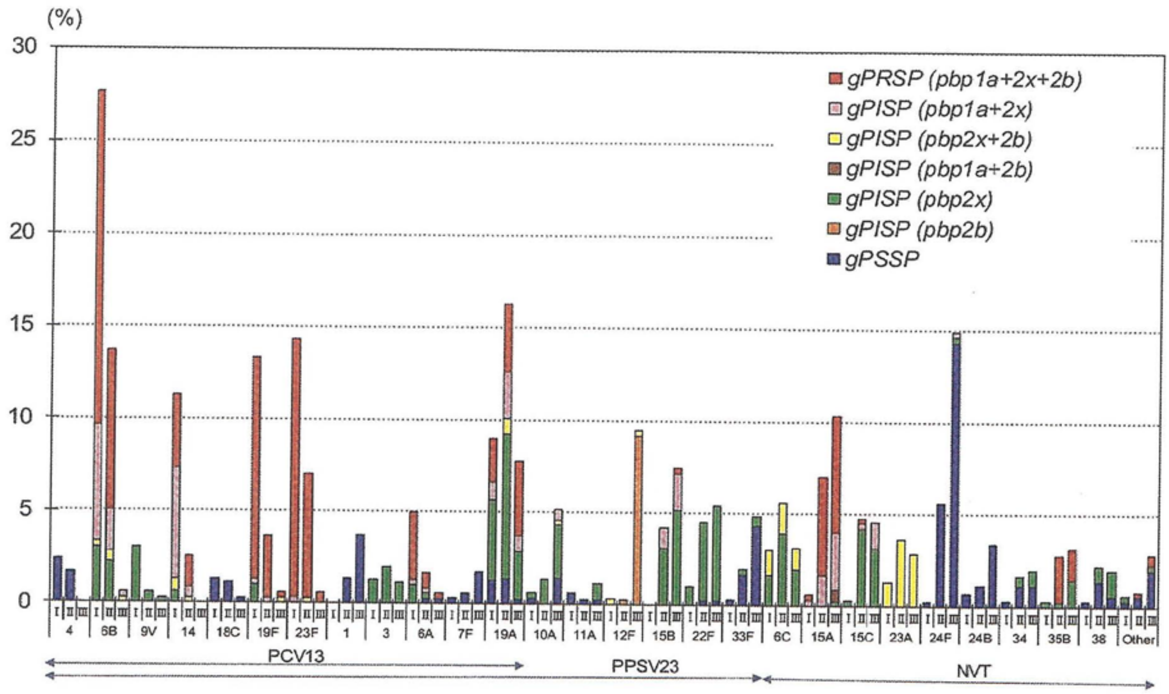
Serotype	Resistance genotype†	Clonal complex (n)	Sequence type (n)
	gPISP(*2x*)	9789 (7); 81 (2)	3113 (5) 3787 (1) 7969 (1); 2923 (2)
	gPSSP	9789 (1); Singleton (1)	4271 (1); 8347 (1)
7F	gPISP(*2x*)	191 (4)	191 (4)
	gPSSP	191 (54); 218 (2)	191 (54); 405 (2)
19A	gPRSP	3111 (86); 156 (1); 320 (4)	3111 (85) 9045 (1); 156 (1); 320 (4)
	gPISP(*1a+2x*)	3111 (28); 2331 (9)	3111 (27) 7988 (1); 2331 (1) 5237 (8)
	gPISP(*2x+2b*)	3111 (3)	3111 (3)
	gPISP(*2x*)	3111 (89); 2331 (54)	3111 (88) 7988 (1); 2331 (52) 13047 (2)
	gPSSP	2331 (13); 3111 (3)	2331 (9) 5842 (4); 3111 (2) 8339 (1)
NVT			
8	gPSSP	156 (1); none (1)	11999 (1); 6022 (1)
10A	gPISP(*1a+2x*)	156 (9); 113 (2); Singleton (1)	1263 (8) 7773 (1); 5236 (2); 3078 (1)
	gPISP(*2x+2b*)	156 (1)	5246 (1)
	gPISP(*2x*)	113 (84); 156 (15)	5236 (84); 1263 (11) 3395 (1) 7773 (1) 7830 (2)
	gPSSP	113 (9); 156 (2)	5236 (9); 1263 (1) 12051 (1)
11A	gPISP(*1a+2x*)	99 (2)	99 (2)
	gPISP(*2x*)	99 (38); 62 (1)	99 (30) 7153 (7) 7811 (1); 1760 (1)
	gPSSP	99 (23); 62 (3)	99 (21) 7153 (1) 8651 (1); 62 (2) 1012 (1)
12F	gPISP(*2x+2b*)	1527 (2)	4846 (2)
	gPISP(*2b*)	1527 (98)	4846 (98)
	gPSSP	1527 (7); 989 (1)	6945 (6) 12003 (1); 989 (1)
15B	gPRSP	81 (1); 242 (1)	83 (1); 242 (1)
	gPISP(*1a+2x*)	199 (12)	199 (12)
	gPISP(*2x*)	199 (52)	199 (51) 5609 (1)
	gPSSP	199 (2)	199 (2)
20	gPSSP	4745 (20); 99 (1); 235 (1); 5349 (1)	4745 (16) 7828 (4); 99 (1); 235 (1); 5190 (1)
22F	gPISP(*1a+2x*)	433 (1)	4553 (1)
	gPISP(*2x*)	433 (147); 113 (11)	433 (145) 819 (1) 10023 (1); 5236 (2) 7158 (9)
	gPSSP	433 (5); 113 (2); 2572 (1); 3594 (1); none (1)	433 (5); 7158 (1) 8353 (1); 5496 (1); 3594 (1); 3465 (1)
33F	gPISP(*2x*)	100 (5)	100 (5)
	gPSSP	717 (34); 62 (2); 100 (1)	717 (34); 673 (2); 100 (1)
6C	gPRSP	156 (1); 5832 (1)	8352 (1); 5832 (1)
	gPISP(*1a+2x*)	2924 (1)	12080 (1)
	gPISP(*2x+2b*)	156 (30); 5832 (24); 315 (2); Singleton (1)	5241 (29) 12081 (1); 5025 (2) 5832 (20) 7384 (2); 386 (2); 9025 (1)
	gPISP(*2x*)	2924 (59); 81 (23); 7781 (4); 156 (2); 9789 (2); 242 (1)	2924 (37) 6183 (16) 7838 (1)7879 (1) 9336 (2)12080 (1)12082 (1); 2923 (23); 7788 (2) 7876 (2); 4233 (1) 13587 (1)*; 3787 (2); 242 (1)
	gPSSP	156 (1); 473 (1); 7781 (1)	8645 (1); 473 (1); 2789 (1)
6D	gPRSP	81 (1); 156 (1)	282 (1); 90 (1)
7C	gPSSP	2758 (10)	2758 (10)
13	gPRSP	156 (1)	10303 (1)
15A	gPRSP	63 (77)	63 (73) 2105 (1) 2771 (1) 8354 (1) 12000 (1)
	gPISP(*1a+2x*)	63 (56)	63 (42) 7874 (13) 10211 (1)
	gPISP(*1a+2b*)	63 (6); Singleton (1)	63 (6); 8346 (1)
	gPISP(*2x*)	292 (2) 113 (1)	292 (2); 7992 (1)
	gPSSP	292 (1)	292 (1)
15C	gPRSP	81 (2)	83 (1) 6138 (1)
	gPISP(*1a+2x*)	199 (10)	199 (9) 1674 (1)
	gPISP(*2x*)	199 (41)	199 (39) 9222 (1) 12073 (1)
16F	gPRSP	3117 (4)	8351 (4)
	gPISP(*1a+2x*)	3117 (2)	3117 (2)
	gPSSP	3117 (2)	3117 (2)
18B	gPSSP	3594 (1)	3594 (1)
21	gPISP(*2x*)	1381 (2)	1233 (2)
23A	gPRSP	156 (1)	9619 (1)
	gPISP(*2x+2b*)	156 (105)	338 (57) 2612 (1) 3437 (1) 5242 (39) 5246 (5) 6685 (1) 8340 (1)
	gPISP(*2x*)	156 (4)	338 (1) 10000 (3)
	gPSSP	439 (2)	42 (1) 438 (1)
23B	gPRSP	156 (1)	2372 (1)
	gPISP(*2x+2b*)	156 (2)	1373 (2)
	gPISP(*2x*)	439 (1)	439 (1)
	gPSSP	439 (3); 63 (1)	439 (3); 63 (1)
24	gPISP(*1a+2x*)	230 (1)	230 (1)
	gPISP(*2x*)	2572 (2)	2572 (2)
	gPSSP	2572 (112); 4982 (2)	2572 (62)5496 (50); 4982 (2)

Serotype	Resistance genotype†	Clonal complex (n)	Sequence type (n)
28A	gPSSP	546 (1)	11601 (1)
31	gPSSP	7800 (5)	11184 (5)
34	gPRSP	15 (1)	9395 (1)
	gPISP(*2x*)	none (12); 15 (1)	3116 (12); 7388 (1)
	gPSSP	15 (13); none (5)	1439 (1) 7388 (12); 3116 (5)
35B	gPRSP	558 (53); 156 (1); Singleton (1)	558 (49) 1204 (2) 7809 (2); 156 (1); 7990 (1)
	gPISP(*1a+2x*)	1816 (1)	2755 (1)
	gPISP(*2x*)	1816 (43)	2755 (42) 12004 (1)
	gPSSP	1816 (7)	2755 (7)
37	gPSSP	447 (8)	447 (6) 7970 (2)
38	gPISP(*2x+2b*)	none (1)	7833 (1)
	gPISP(*2x*)	6429 (23)	6429 (23)
	gPSSP	393 (12); 6429 (1)	393 (12); 6429 (1)

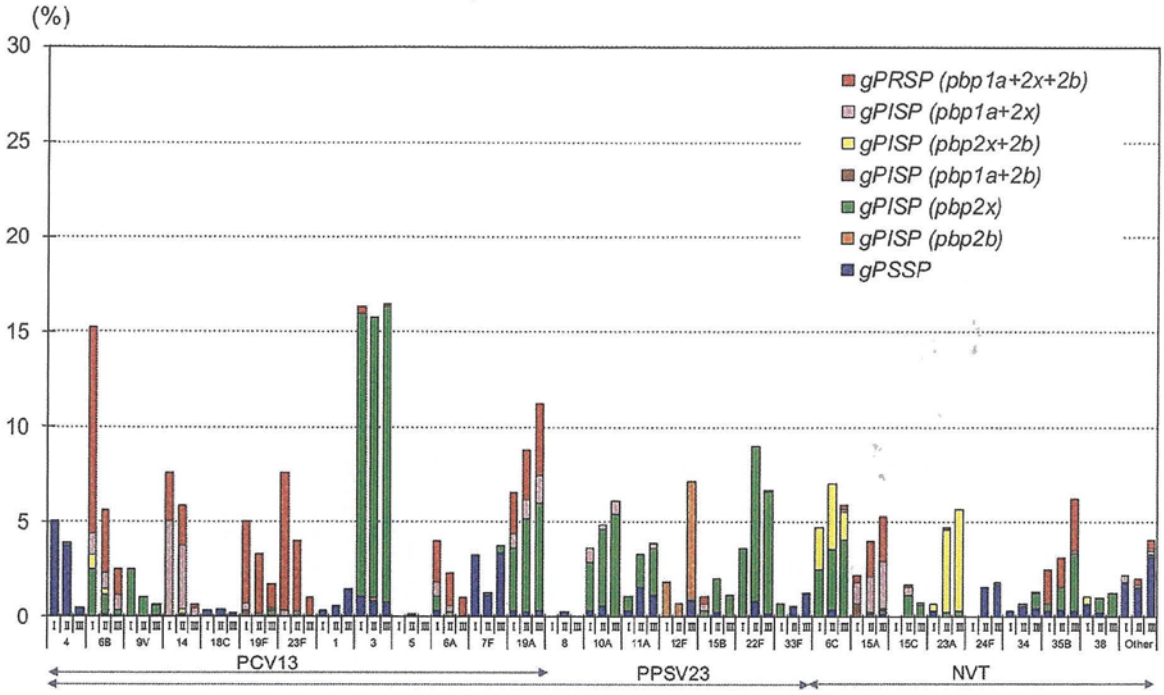
*STs newly identified in this study.
†*1a*, *2x*, and *2b* in the parenthesis indicate *pbp1a*, *pbp2x* and *pbp2b genes*, respectively. PBPs (PBP1A, PBP2X, and PBP2B) involved in peptidoglycan synthesis are encoded by these genes. Amino acid substitutions within or near each PBP's conserved amino acid motifs were identified in resistance strains with various combinations of abnormal genes.



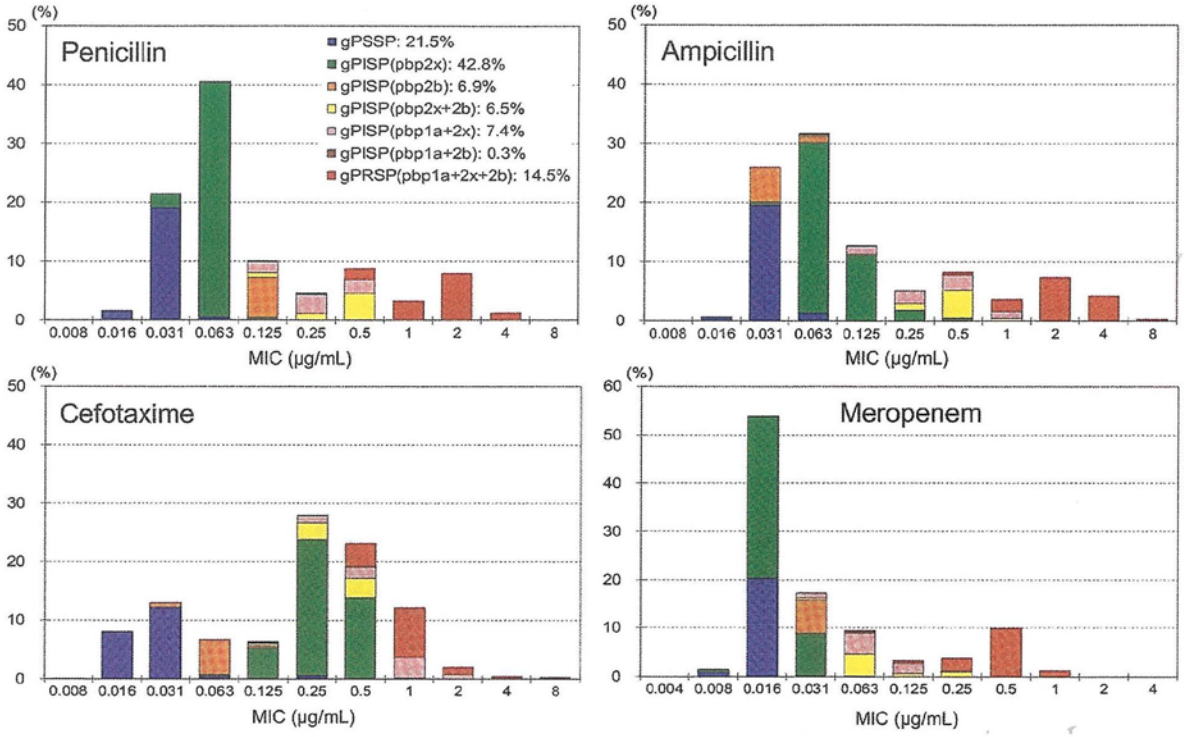
Technical Appendix Figure 1. Distribution of isolates collected by the surveillance for invasive pneumococcal disease in Japan from April 2010 to March 2017. Numbers of isolates obtained from children/adults are shown for each locality on the map.



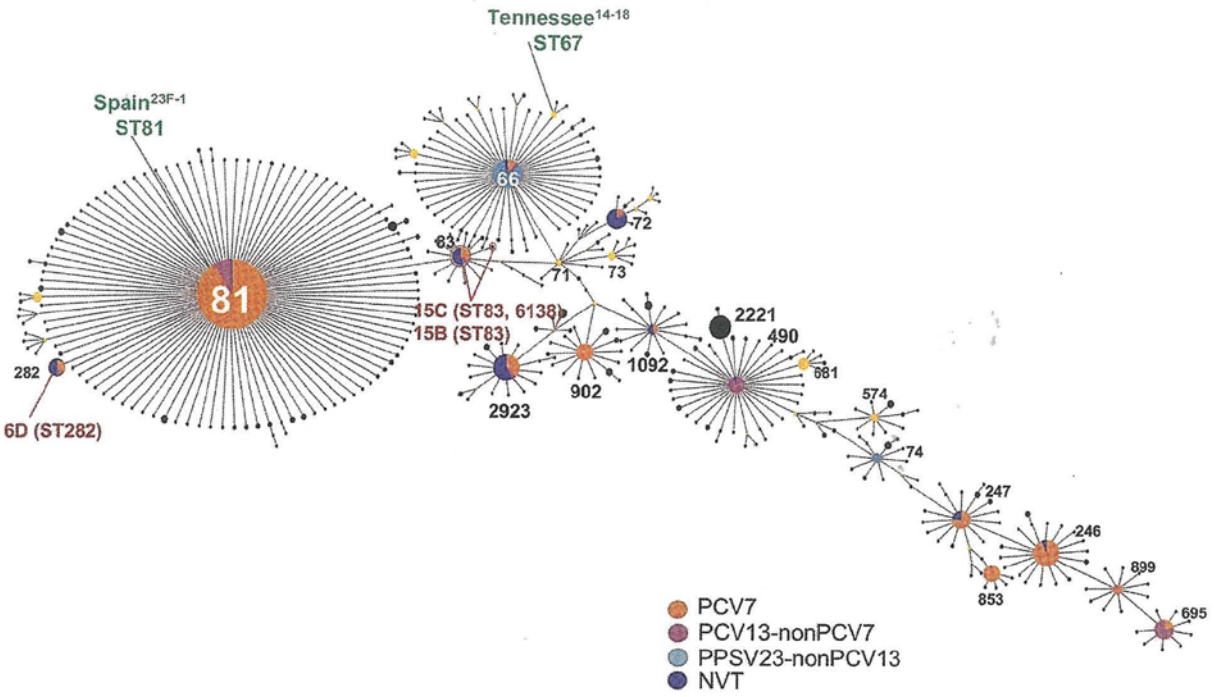
Technical Appendix Figure 2. Yearly changes of serotypes and penicillin-resistant genotypes among isolates from children with invasive pneumococcal disease in Japan from April 2010 to March 2017. I, II, and III on horizontal axis represent three surveillance periods as follows: pre-PCV7 period, from April 2010 through March 2011; PCV7 period, from April 2011 to March 2014; and PCV13 period, from April 2014 through March 2017. gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate resistant *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose mutated *pbp* genes, *pbp1a, pbp2x,* and/or *pbp2b,* that mediate penicillin resistance.



Technical Appendix Figure 3. Yearly changes of serotypes and penicillin-resistant genotypes among isolates from adults with invasive pneumococcal disease in Japan from April 2010 to March 2017. I, II, and III on horizontal axis represent three surveillance periods as follows: pre-PCV7 period, before vaccination to children, from April 2010 to March 2011; PCV7 period, after PCV7 vaccination to children, from April 2011 to March 2014; and PCV13 period, with PCV7 replaced by PCV13 vaccination in children, from April 2014 to March 2017. gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate resistant *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose mutated *pbp* genes, *pbp1a, pbp2x,* and/or *pbp2b,* that mediate penicillin resistance.



Technical Appendix Figure 4. Distribution of susceptibilities to 4 parenteral β-lactam antibiotics, penicillin, ampicillin, cefotaxime, and meropenem, by genotypes of isolates from patients with invasive pneumococcal disease in Japan from April 2014 to March 2017 (n = 1229). gPSSP indicates genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP indicates genotypic penicillin-intermediate-resistant *S. pneumoniae*; and gPRSP indicates genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose mutated *pbp* genes *pbp1a, pbp2x,* and/or *pbp2b,* which mediate penicillin resistance.



Technical Appendix Figure 5. Details of clonal complex (CC) 81 (n = 1858) included 420 sequence types (STs) from the Multilocus Sequence Typing Web site (https://pubmlst.org/spneumoniae/) (download October/02/2017). Data include our own from the present study (n = 143). STs of 3 gPRSPs identified in serotypes 6D, 15B, and 15C (in red) were derived from ST81, belonging to CC81. The Pneumococcal Molecular Epidemiology Network (PMEN) clones identified in CC81 also are shown (in green).

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- **History**

1968 Graduated from Japan Women's University
1968 Worked at the Bacterial Laboratory, Department of Pediatrics, The University of Tokyo Hospital
1985 Lecturer, Department of Clinical Pathology, Teikyo University School of Medicine
1990 Assistant Professor, Department of Clinical Pathology, Teikyo University School of Medicine
2002 Professor, Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences, Kitasato University
2011 Specially-appointed Professor, Kitasato Institute for Life Science, Kitasato University (Ministry of Health, Labour and Welfare, "Emerging and Re-emerging Infectious Diseases Including Novel Influenza" Research Project: Representative Researcher, FY2007 to FY2012)
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2000 Director, Department of Pediatrics, National Hospital Tokyo Medical Center
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Intestinal Microflora (President), Japanese Vaccine Action (President), The Japanese Society of Mycoplasmology (Executive Director), The Japanese Society of Parasitology (Executive Director), The Japanese Society for Clinical Microbiology (Supervisor), The Japanese Society for Infection Prevention and Control (Supervisor)

- **Serving as a public member of the following organizations**

The Expert Council on Promotion of Vaccination (Chairperson), Central Institutional Review Board of the National Hospital Organization (Deputy chairperson), Pharmaceuticals and Medical Devices Agency (Expert member), Ministry of Health, Labour and Welfare: Board member of the Expert Review Board on Medical Care Covered by Health Insurance, Drug Pricing Organization/Special Organization for Medical Materials Covered by Health Insurance, Ministry of Education, Culture, Sports, Science and Technology: Reviewer of Japan Initiative for Global Research Network on Infectious Diseases (J-GRID), member of Review Committee for Investigation of Desirable Infectious Disease Research in the Future

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- **History**

1996 Graduated from Keio University School of Medicine
1996 Resident, Keio University Hospital (Department of Internal Medicine)
1998 Senior Resident, Keio University Hospital (Department of Internal Medicine)
1998 Department of Internal Medicine, Kitasato Institute Medical Center Hospital (Current Kitasato University Medical Center)
1999 Department of Internal Medicine, Saiseikai Utsunomiya Hospital
2000 Assistant, Department of Internal Medicine, Keio University School of Medicine (Department of Pulmonary Medicine)
2004 Department of Internal Medicine, Saitama City Hospital (Department of Pulmonary Medicine)
2006 Immunological Program, Department of Pathology, University of Michigan Medical School, USA
Postdoctoral Fellow, Professor Steven Kunkel
2009 Assistant Professor, Department of Internal Medicine, Keio University School of Medicine (Department of Pulmonary Medicine)
2012 Full-time Lecturer, Department of Internal Medicine, Keio University School of Medicine (Department of Pulmonary Medicine) (in the faculty)
2018 Full-time Lecturer, Department of Internal Medicine, Keio University School of Medicine (Department of Pulmonary Medicine)
2019 to present Assistant Professor, Department of Internal Medicine, Keio University School of Medicine (Department of Pulmonary Medicine)

- **Certified by the following academic societies**

Japanese Society of Internal Medicine (certified physician, specialist, medical advisor, chairperson of Management Committee for Internal Medicine Training Program, Keio University), Japanese Respiratory Society (specialist, medical advisor, representative), Japanese Society of Infectious Diseases (specialist, councilor), Japanese Society of Tuberculosis (specialist, medical advisor), Japanese Society of Chemotherapy (certified physician, medical advisor), ICD, Japanese Society of Allergology (specialist, medical advisor), Japanese Respiratory Endoscopy Society (bronchoscopist, medical advisor), American Thoracic Society

- **Serving as a public member of the following organizations**

Editor, Japanese Journal of Japanese Respiratory Society (2014 to present), Delegate (since 2018) Member of Clinical Research Promotion Committee, Japanese Association for Infectious Diseases (2017 to present), Councilor (2015 to present)

Member of Primary Review Committee for Scientific Research Expenses of the Japan Society for the Promotion of Science (2015 to 2016)
Member of Tokyo Review Board, National Insurance (public interest representative) (2013 to present)
Executive Director, Non-tuberculous Mycobacteriosis Research Consortium (NTM-JRC) (2016 to present)

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- **History**

2004 Graduated from Kagawa University School of Medicine
2004 Junior Resident, Tokyo Metropolitan Hiroo Hospital
2006 Resident, Department of Respiratory Medicine, National Center for Global Health and Medicine
2009 Physician, Department of Respiratory Medicine, Respiratory Center, Toranomon Hospital
2014 Joined the Department of Infectious Diseases, Keio University School of Medicine
2017 Studied abroad as a research fellow at Pulmonary and Critical Care Medicine, University of Michigan
2018 to present Physician, Department of Respiratory Medicine, Respiratory Center, Toranomon Hospital

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Japanese Respiratory Society, Japanese Association for Infectious Diseases, American Thoracic Society

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- **History**

2006 B.S. (Pharmacy), School of Pharmacy, Kitasato University
2008 M.Sc (Infection Control Science), Graduate School of Infection Control Sciences, Kitasato University
2012 Ph.D (Medicine), Graduate School of Comprehensive Human Sciences, University of Tsukuba
2012 Postdoctoral researcher, Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences
2013 Assistant professor, Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences
2016 Postdoctoral researcher, Center for Infectious Medicine, Karolinska Institutet
2018 Senior lecturer, Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences

- **Certified by the following academic societies**

The Japanese Association of Infectious Diseases (councilor), Japanese Society of Chemotherapy, The Pharmaceutical Society of Japan, Japanese Society of Bacteriology, The Japanese Society for Clinical Microbiology

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