Changes in the Distribution of Capsular Serotypes of *Streptococcus pneumoniae* Isolated from Adult Respiratory Specimens in Japan

Hisashi Shoji¹, Masayuki Maeda², Tetsuro Shirakura³, Takahiro Takuma¹,
Hideaki Hanaki⁴ and Yoshihito Niki¹

Abstract

Objective The objective of this study was to assess whether the distribution of pneumococcal capsular types has been changed, while also providing basic data on changes in the distribution after the introduction of Pneumococcal conjugated vaccine (PCV)13 in adult medical practice.

Methods We analyzed 431 *Streptococcus pneumoniae* strains (200 in 2006 and 231 in 2012) that had been isolated from respiratory infection specimens from adult patients. Capsular typing was performed by the Quellung reaction and multiplex polymerase chain reaction.

Results A comparison of the 2006 and 2012 strains revealed that the number and proportion of strains by serotype increased from 30 (15%) to 46 (20%) for serotype 3, from 4 (2%) to 14 (6%) for serotype 6A, and from 4 (2%) to 13 (6%) for serotype 6C, whereas the number and proportion of strains by serotype decreased from 8 (4%) to 0 (0%) for serotype 4 and from 24 (12%) to 17 (7%) for serotype 6B. From 2006 to 2012, the coverage rate significantly decreased from 39 to 28.1% for PCV7 ($p=0.017$).

Conclusion Our study showed a decrease in the vaccine coverage of PCV7. However, PCV13 covered serotypes 3 and 6A, which are prevalent, as well as penicillin-resistant *S. pneumoniae* strains. At present, PCV 13 in adult clinical practice seems to be highly significant. However, there is a possibility that the distribution has changed, and careful screening should be continued in the future.

Key words: *Streptococcus pneumoniae*, pneumococcal polysaccharide vaccine, pneumococcal conjugate vaccine, community-acquired pneumonia, invasive pneumococcal disease

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Introduction

Despite remarkable progress in the clinical practice for pneumonia, the disease remains the third leading cause of death in Japan. Mortality due to pneumonia is particularly increasing rapidly amongst the elderly. The most important factor related to this phenomenon is the aging of society. Other possible factors include inflation in medical costs, an increasing number of antimicrobial-resistant bacteria, and

slow development of new antimicrobial agents. There are no practical solutions to address these factors. Pneumococcus is the most important pathogen causing adult respiratory infections (1). *S. pneumoniae* is a gram-positive bacterial pathogen that causes respiratory and otolaryngological infections such as pneumonia, tympanitis and sinusitis. It also causes serious invasive diseases such as meningitis and septicemia in all age groups. Pneumococcal strains are covered by polysaccharide capsules, and the capsules are closely related to pathogenicity. These capsules serve as a barrier to prevent

¹Division of Clinical Infectious Diseases, Department of Medicine, School of Medicine, Showa University, Japan, ²Division of Infection Control Sciences, Department of Pharmacotherapeutics, School of Pharmacy, Showa University, Japan, ³Department of Microbiology, School of Medicine, Showa University, Japan and ⁴Research Center for Infections and Antimicrobials, Kitasato Institute for Life Science, Kitasato University, Japan

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Correspondence to Dr. Hisashi Shoji, itrshoji@med.showa-u.ac.jp

Table 1. The Detail of Specimens in this Study Each Year.

Characteristics	Year	
	2006	2012
Number of specimens	200	241
Participation number of facilities	35	39
Kind of specimens		
Sputum	190(95.0%)	199(86.1%)
Specimens of trans-tracheal aspiration	6(3.0%)	24(10.4%)
Specimens of bronchoscopy	2(1.0%)	6(2.6%)
Others	2(1.0%)	2(0.9%)
Patient backgrounds		
Age, Median (interquartile range)	72(62-80)	72(63-79)
Sex		
Men	142(71.0%)	153(66.2%)*
Female	58(29.0%)	76(32.9%)*
Diagnosis		
Community-acquired pneumonia	124(62.0%)	162(70.1%)
Hospital-acquired pneumonia	26(13.0%)	19(8.2%)
Acute Exacerbation of Chronic Bronchitis	27(13.5%)	24(10.4%)
Others	23(11.5%)	26(11.3%)
Susceptibility		
Penicillin-susceptible <i>S. pneumoniae</i>	122(61.0%)	146(63.2%)*
Penicillin-intermediate <i>S. pneumoniae</i>	70(35.0%)	73(31.6%)*
Penicillin-resistant <i>S. pneumoniae</i>	8(4.0%)	11(4.8%)*

* lack of data

human complement involved in the opsonization from attaching to the bacterial surface. This function gives the bacteria strong power to resist phagocytosis by human polymorphonuclear leukocytes. The capsule thus constitutes the principal cause of the strong pathogenicity of pneumococcal strains. At present, 93 different types of pneumococcal capsule have been identified (2).

Preventive measures against pneumococcal infections are important for carriers who are at a high risk for death due to an immunocompromised condition associated with anticancer agents, corticosteroids, and/or biologicals, human immunodeficiency virus infection or acquired immunodeficiency syndrome. Pneumococcal vaccination is an effective preventive method against pneumococcal infections. Pneumococcal vaccines are classified into two categories, namely, pneumococcal polysaccharide vaccine (PPV) and pneumococcal conjugate vaccine (PCV). Pneumococcal conjugate vaccine, showing higher immunogenicity than PPV, is mainly used in children (3). In the pediatric field, children have been immunized with PCV7, which contains seven types of capsular antigen, since 2010 and PCV13, which contains 13 types, since 2013. The efficacy of PCV7 has been demonstrated in the United States, as represented by a significant reduction in invasive pneumococcal diseases (IPDs) (4).

In Japan, where pneumococcal polysaccharide vaccine 23 (PPV23) containing 23 types of capsular antigen has been conventionally given to adults, PCV13 has become available for adult use since June 2014. In the pediatric field, it has been reported that vaccine coverage rates decreased in association with the changes in the distribution of capsular types after the introduction of the new vaccines (5). We herein report a study of the distribution of capsular serotypes of *Streptococcus pneumoniae* in the field of adult respiratory infections in Japan, including analysis at the two assessment

points of 2006 and 2012. The study is aimed to assess whether the distribution has been changed and also to provide basic data to assess changes in the distribution after the introduction of PCV13 in adult medical practice.

Materials and Methods

Streptococcus pneumoniae strains

In this study, we obtained 200 and 231 *Streptococcus pneumoniae* strains from a Japanese surveillance in 2006 and 2012, respectively. The surveillance was nationwide and conducted by the Japanese Society of Chemotherapy, the Japanese Association for Infectious Diseases and the Japanese Society of Clinical Microbiology (6). The strains of well-diagnosed adult respiratory tract infection were isolated from the sputum and specimens of the trans-tracheal aspiration or bronchoscopy (1, 6). Their causality was confirmed by quantitative culture, Gram staining, and observation of phagocytes (1, 6). Table 1 shows the participation number of institutes, patient backgrounds and kind of specimens. All strains were grown on Sheep Blood Agar[®] (Nippon Beckton-Dickinson, Tokyo, Japan) and maintained at 35°C with 5% CO₂. The strains were stored in a Microbank[™] system (IWAKI, Tokyo, Japan) at -80°C.

Susceptibility testing

The bacterial susceptibility to penicillin G (PCG) was studied using the broth microdilution method. The reference broth microdilution method was performed according to the Clinical Laboratory Standard Institution guidelines (7). The test medium was prepared using cation-adjusted Mueller-Hinton broth (Eikenkagaku, Tokyo, Japan) with lysed horse blood (Nippon Biotest Laboratory, Tokyo, Japan). Microtiter plates were then inoculated to produce a final inoculum density of approximately 5×10⁵ colony forming unit (CFU)/mL, which was regularly controlled by counting the colonies. The inoculated plates were incubated at 36°C for 20-24 h before interpreting the results. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotics that inhibited bacterial growth. Strains with PCG MIC ≤0.06 µg/mL were classified as penicillin-susceptible *S. pneumoniae* (PSSP), those with PCG MIC of 0.12 to 1 µg/mL as penicillin-intermediate *S. pneumoniae* (PISP), and those with PCG MIC ≥2 µg/mL as penicillin-resistant *S. pneumoniae* (PRSP).

Capsular typing

Capsular typing was performed by the Quellung reaction and multiplex polymerase chain reaction (PCR). The Quellung reaction was performed using a set of antisera obtained from Statens Serum Institute (Copenhagen, Denmark). The typing procedure was performed as described in the previous report (8). The multiplex PCR was performed based on the procedure of the Streptococcus Laboratory in the Center for Disease Control and Prevention (9). The PCRs were per-

Figure 1. Distribution of serogroups/types amongst 200 *Streptococcus pneumoniae* isolated from adult patients with respiratory infections in 2006. PSSP: penicillin-susceptible *S. pneumoniae*, PISP: penicillin-intermediate *S. pneumoniae*, PRSP: penicillin-resistant *S. pneumoniae*

Figure 2. Distribution of serogroups/types amongst 231 *Streptococcus pneumoniae* isolated from adult patients with respiratory infections in 2012. PSSP: penicillin-susceptible *S. pneumoniae*, PISP: penicillin-intermediate *S. pneumoniae*, PRSP: penicillin-resistant *S. pneumoniae*

formed in 25 μ L volumes, and each reaction mixture contained the following: 2 $\times$ PCR buffer (QIAGEN Multiplex PCR Kit, Hilden, Germany) 12.5 μ L, clinical sample of DNA 5 μ L, and primers with concentrations as specified in the protocol published by the Centers for Disease Control and Prevention.

Results

Figs. 1 and 2 show the distribution of the serotypes of the analyzed isolates. Among all 431 strains pooled from the 2006 and 2012 surveys, serotype 3 was the most common and was identified from the 76 strains, followed by sero-

types 6B, 19F, 23F, and 14. A comparison of the data of 2006 with those of 2012 revealed that the number and proportion of the strains by serotype increased from 30 (15%) to 46 (20%) for serotype 3, from 4 (2%) to 14 (6%) for serotype 6A, and from 4 (2%) to 13 (6%) for serotype 6C, whereas the number and proportion of strains by serotype decreased from 8 (4%) to 0 (0%) for serotype 4 and from 24 (12%) to 17 (7%) for serotype 6B. Table 2 shows the vaccine coverage rates by vaccine type in 2006 versus 2012. From 2006 to 2012, the coverage rate significantly decreased from 39 to 28.1% for PCV7 ($p=0.017$), and tended to decrease from 62 to 58.4% for PCV13, and from 73 to 69.7% for PPV23.

Table 2. Vaccine Coverage Rates by Vaccine Type in 2006 Versus 2012.

Vaccine type	Year		p value*
	2006	2012	
PCV7	39.0%	28.1%	0.017
PCV13	62.0%	58.4%	0.452
PPV23	73.0%	69.7%	0.450

*Chi-square test

Regarding the number and proportion of strains by penicillin susceptibility, the 200 strains from the 2006 survey consisted of 122 PSSP strains (61%), 70 PISP strains (35%), and 8 PRSP strains (4%), while the 231 strains from the 2012 survey consisted of 147 PSSP strains (64%), 73 PISP strains (32%), and 11 PRSP strains (4%). No significant changes in penicillin susceptibility were found over the time course. The 16 incremental strains of serotype 3 that increased from 2006 to 2012 were 15 PSSP strains and 1 PISP strain. The 10 incremental strains of serotype 6A were 1 PSSP strain, 7 PISP strains, and 2 PRSP strains. As a result, the vaccine coverage rate of PRSP was 88% (7/8 strains) for all vaccine types of PCV7, PCV13, and PPV23 in 2006, compared with 73% (8/11 strains) for PCV7 and PPV23, and 91% (10/11 strains) for PCV13 in 2012.

Discussion

We studied changes in the distribution of the capsular serotypes of *Streptococcus pneumoniae* in the field of adult respiratory infections in Japan. The distribution partially differed from the distribution in the field of pediatric IPDs. In the field of adult respiratory infections, serotype 3 was the most common, followed by serotypes 6B, 14, 19, and 23F. In the field of pediatric IPDs, however, serotype 3 was not common, and other serotypes, namely, 6B, 14, 19, and 23F were frequently isolated, as in the field of adult respiratory infections (10). These findings suggest a relative close relationship in the origin of pneumococcal strains isolated from adults and from children. In the present study, the numbers of serotypes 6B and 4 strains markedly decreased from 2006 to 2012, thus resulting in a decrease in the vaccine coverage of PCV7. The serotype 3 is the most frequently isolated in Japan. Furthermore the serotype 3 is often isolated from empyema, necrotizing pneumonia, septic shock and meningitis, and leads to severe clinical outcomes (11). In the United States, changes in the capsular serotypes distribution (included 3) after the introduction of new vaccines in the pediatric field resulted in a reduced vaccine coverage (5, 12). In Japan, PCV7 was introduced in the pediatric field in 2010. Since our study was not designed to analyze the capsular serotypes in the pediatric field, it is unclear whether the distribution of the capsular serotypes in children has changed or not. However, a decrease in the vaccine coverage of PCV7 found in adult respiratory infections may be somehow related to the introduction of PCV7 in the pediatric field.

Pneumococcal polysaccharide vaccine 23, which was con-

ventionally given to adults, is prepared using capsular polysaccharides as antigens. A capsular polysaccharide acts as a T cell-independent antigen, which induces the B cells to produce antibodies without T cell assistance. Capsular serotype-specific immunoglobulin G antibodies produced by B cells bind to the bacteria to exert opsonic activity and thereby help the body eliminate the bacteria. However, immunity acquired against T cell-independent antigens diminishes within several years owing to the absence of established immunological memory and cannot be boosted by additional vaccination. In infants aged two years and younger, the clinical efficacy of PPV23 cannot be expected due to low immunogenicity (13). Children have been immunized with PCV7 containing 7 types of capsular antigens since 2010, and PCV13 containing 13 types since 2013. These new vaccines are prepared with capsular saccharides conjugated to cross-reacting material 197 (CRM 197) of non-pathogenic diphtheria as the carrier protein to convert them to T cell antigens. They reportedly induce an adequate immune response by their strong immunogenicity in infants aged two years and younger (3).

In Japan, PCV13 vaccination in adults became available in June 2014. Our study demonstrated that PCV13 covers serotypes 3 and 6A, which are increasing in prevalence, as well as PRSP strains. In addition, there is a report that the serotype 3 has high pathogenicity (11). At present, the introduction of PCV13 in adult clinical practice seems to be highly significant. However, changes in the distribution of the capsular serotypes have been reported after the introduction of PCV13, as well as PCV7 (14). This indicates that careful screening should be continued in the future.

In the present study, there was not a significant change in the coverage rate for PPV23, which was 73% in 2006 and 69.7% in 2012. It is not known which vaccine is the most appropriate for adults, either a vaccine that covers a broad range of capsular serotypes, or a vaccine with increased immunogenicity. Further studies should therefore be continued in the future.

Author's disclosure of potential Conflicts of Interest (COI).

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