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Changes in the incidence of *Streptococcus pneumoniae* bacteremia and its serotypes over 10 years in one hospital in South Korea

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ABSTRACT

Here, we examined the distribution of pneumococcal serotypes and the antibiotic susceptibility of *Streptococcus pneumoniae* in clinical blood isolates. The serotypes of 91 *S. pneumoniae* blood isolates, collected from January 2003 to March 2014, were identified by multiplex PCR and sequencing. The most common serotypes were 19F, 19A, 3, 4, and 14, accounting for 53.8% of the total. The serotype coverage rates of pneumococcal conjugated vaccine (PCV) 7, PCV10, and PCV13 were different during three test periods: 38.7%, 70.9%, and 93.5% in period I (2003–2005), 46.8%, 50.0%, and 75.0% in period II (2006–2008), and 28.5%, 32.1%, and 64.2% in period III (2009–2014), respectively. By contrast, the number of non-PCV13 serotypes increased from 6.4% in period I to 25% and 35.7% in periods II and III, respectively. The susceptibility of non-PCV13 serotypes to antimicrobial agents (penicillin, erythromycin, cefotaxime, and meropenem) was higher than that of PCV serotypes. In particular, non-PCV13 serotypes showed 100% and 95% susceptibility to penicillin and cefotaxime, respectively. Serotypes 19A and 19F showed high prevalence (79.1%) among 24 multi-drug resistant (MDR) isolates. Notably, all serotype 19A isolates were MDR. From January 2003 to March 2014, the proportion of non-PCV13 serotype pneumococci in blood isolates increased whereas the coverage rate of PCV13 decreased. Effective pneumococcal vaccines are required to protect against MDR serotype 19A isolates and the increasing number of non-PCV13 serotypes.

1. Introduction

The Gram-positive bacterium *Streptococcus pneumoniae* is an important human pathogen that causes pneumonia, sepsis, and meningitis, all of which result in significant morbidity and mortality worldwide [1]. *S. pneumoniae* produces a capsule of which more than 90 serotypes exist, including newly identified serotypes 11E, 6C, 6D, and 20B [2–4]. The 90 serotypes are not equally pathogenic; a minority of serotypes is responsible for the majority of invasive pneumococcal diseases (IPD) [5]. All pathogenic pneumococci have a polysaccharide capsule, which shields the bacterium from the host's natural defenses. Different pneumococcal vaccines protect against different serotypes; therefore, serotyping pneumococcal isolates from patients is important to monitor the effectiveness of pneumococcal vaccines [6,7]. The seven-valent pneumococcal conjugate vaccine (PCV7), which protects against the seven most common serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F), was designed to elicit antibodies against the capsular polysaccharides. After the introduction of PCV7, the number of vaccine-targeted serotypes that cause IPD in patients declined [6–8]. In Korea, PCV7 was introduced in November 2003 but newly born children in Korea were not been vaccinated as a routine vaccination program [9]. From May 1 in 2014, the pneumococcal vaccine PCV10 or PCV13 have been provided to young children (2 months–5 years after birth) for free according to the national vaccine programs in South Korea, in which pneumococcal vaccines including PCV7, PCV10, PCV13 and PPVSV23 are available. However, the wide use of PCV7 has increased the prevalence of non-vaccine serotypes in many countries [10–12]. Also, a study examining serotype distribution in Korea revealed that the number of IPD-causing non-vaccine serotypes increased 1.5-fold from 1999 to 2002 [13].

Here, we analyzed pneumococcal serotypes isolated from the blood of patients with *S. pneumoniae* who were admitted to a hospital in South Korea during 2003–2014. The aim was to examine changes in serotype distribution over time. The experimental approach was based on a sequential PCR-based assay and sequence analysis techniques previously used to identify 40 serotypes [14,15].

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2. Materials and methods

2.1. Clinical specimens

Between January 2003 and March 2014, 91 *S. pneumoniae* blood isolates were collected from patients at a 600 bed university-affiliated hospital in Korea. *S. pneumoniae* strains were isolated from clinical blood specimens using the Bactec 9240 or Bactec Fx systems (BD diagnostics, Sparks, MD) and stored at −70 °C in 10% skim milk until required. Among the 91 isolates, six were collected in 2003, 15 in 2004, ten in 2005, ten in 2006, eight in 2007, 14 in 2008, eight in 2009, 11 in 2010, three in 2011, two in 2012, three in 2013, and one in 2014. In all, 14 strains (15.3%) were isolated from children aged <5 years, one strain (1.1%) was isolated from a child aged 10 years, 39 strains (42.8%) were isolated from adults aged 24–64 years, and 30 strains (32.9%) were isolated from adults aged >65 years. Seven strains (7.6%) were isolated from patients of unknown age. Thus, the age range of the patients was 10 months to 93 years (mean age, 50 years). Of these, 74% were male and 26% were female. To examine changes in serotypes over time, we divided the collection period into three: period I ($n = 31$; 2003–2005), period II ($n = 32$; 2006–2008), and period III ($n = 28$; 2009–2014).

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method and E-test strips (AB Biodisk, Solna, Sweden) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [16,17]. The interpretive criteria for susceptibility also adhered to CLSI guidelines [18]. *S. pneumoniae* ATCC 49619 was used as a control strain. Multi-drug resistance (MDR) was defined as resistance or intermediate resistance to more than three antimicrobial agents. The agents tested were penicillin (a penicillin), cefotaxime (a cephalosporin), meropenem (a carbapenem), and erythromycin (a macrolide).

2.3. DNA extraction

Pneumococcal isolates were retrieved from storage by subculture on blood agar plates and incubated overnight at 37 °C in 5% CO_2. Bacterial colonies were scraped and suspended in phosphate buffered saline (PBS). Total genomic DNA was isolated using the QIAamp DNA mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

2.4. Oligonucleotide primers

Forty-one primer pairs were designed to target serotypes 1, 2, 3, 4, 5, 6A/6B/6C/6D, 6C/6D, 7C/7B/40, 7F/7A, 8, 9N/9L, 9V/9A, 10A, 10F/10C/33C, 11A/11D, 12F/12A/12B/44/46, 13, 14, 15A/15F, 15B/15C, 16F, 17F, 18C/18F/18B/18A, 19A, 19F, 19Fvar, 20, 21, 22F/22A, 23A, 23B, 23F, 24F/24A/24B, 31, 33F/33A/37, 34, 35A/35C/42, 35B, 35F/47F, and 38/25F/25A, 39 [7,14,15,19,20]. An additional primer pair (*cpsA*-for and *cpsA*-rev, which targets the *cpsA* locus common to all pneumococci) was also included as an internal control [21]. The primers were designed to target the following genes: *wzy* (serotypes 1, 2, 4, 5, 7F/7A, 8, 9V/9A, 11A/11D, 14, 15A/15F, 15B/15C, 16F, 18C/18F/18B/18A, 19A, 19F, 19Fvar, 23A, 23F, 24F/24A/24B, 31, 33F/33A/37, 34, 35F/47F, 38/25F/25A, and 39), *gal*U (3), *wci*P (serotypes 6A/6B/6C/6D, and 17F), *wci*Nbeta (serotypes 6C/6D), *wcw*L (serotypes 7C/7B/40, 20), *wzx* (serotypes 9N/9L, 10F/10C/33C, 12F/12A/12B/44/46, 13, 21, 23B, and 35A/35C/42), *wcr*G (serotype 10A), *wcw*V (serotypes 22F/22A), and *wcw*H (serotype 35B).

2.5. Sequential multiplex PCR assay and serotype deduction

The primers were divided into nine multiplex reactions based on the serotype distribution among invasive pneumococci [22,23]. The serotypes of all pneumococci were determined using the multiplex PCR assay recommended by the CDC (www.cdc.gov/ncidod/biotech/strep/pcr.htm). Eight reactions (USA Reactions 1–8) were designed to include five primer pairs that target the serotype-specific regions of five different serotypes. Reaction 6C included two primer pairs targeting serogroups 6, 6A/6B/6C/6D and 6C/6D. All reactions also included an internal positive control targeting all known pneumococcal *cpsA* regions [7].

2.6. Sequencing assay

All isolates identified as serotype 6A/6B/6C/6D and 6C/6D in the multiplex PCR assay were confirmed by sequencing as previously described [3,24,25].

3. Results

3.1. Serotype distribution

The most common serotypes were 19F ($n = 11$, 12.0%), 19A ($n = 11$, 12.0%), 3 ($n = 11$, 12.0%), 4 ($n = 9$, 9.8%), and 14 ($n = 7$, 7.6%), which together accounted for 53.8% of all isolates identified (Fig. 1). Serotypes 19A and 19F were common in children (9/16, 56.2%) younger than 5 years-of-age. Overall, PCV7 covered 40.6% of all isolates, whereas a 10-valent conjugate vaccine (PCV10) covered 51.6%, and a 13-valent conjugate vaccine (PCV13) covered 78.0%.

Among the 91 isolates examined, 31 (34.0%) were obtained from 2003 to 2005, 32 (35.1%) from 2006 to 2008, 22 (24.1%) from 2009 to 2011, and six (6.5%) from January 2012 to March 2014 (Fig. 2). Of the 31 isolates from period I, the major serotypes were 19F ($n = 7$, 22.5%), 1 ($n = 4$, 12.9%), 3 ($n = 4$, 12.9%), 4 ($n = 3$, 9.6%), 5 ($n = 3$, 9.6%), and 19A ($n = 3$, 9.6%). Only two (6.4%) of the isolates from period I were non-PCV13 serotypes. However, the number of non-PCV13 serotypes increased markedly in period II: up from 6.4% in period I to 25% in Period II. The most common serotypes from period II were non-PCV13 serotypes ($n = 8$, 25%). Among the non-PCV13 serotypes identified, 11A/11D, 12F/12A/44/46, 33F/33A/37, and 35B newly emerged in period II. However, the proportion of serotype 19F isolates decreased over time: seven isolates (22.5%) in period I, four isolates (12.5%) in period II, and none in period III. PCV7 and PCV10 serotypes 19F, 23F, 18C/18F/18B/18A, 1, and 5 were not observed from 2009 to 2014 (period III); however, the number of detected 19A and 3 serotypes (PCV13) increased: from four (12.5%) and two isolates (6.2%), respectively in period II to four (14.2%) and five isolates (17.8%), respectively, in period III. In addition, the number of non-PCV13 serotypes increased from eight (25%) in period II to ten (35.7%) in period III.

3.2. Antimicrobial susceptibility

The antimicrobial susceptibility of the 91 *S. pneumoniae* isolates to penicillin, erythromycin, cefotaxime, and meropenem is shown in Table 1. According to the 2014 CLSI breakpoints, 71.4% ($n = 65$) of the 91 isolates were resistant to erythromycin, whereas 1.1% ($n = 1$) were resistant to penicillin (resistant: *R*, ≥8 μg/ml for non-meningitis), 8.7% ($n = 8$) were resistant to cefotaxime (resistant: *R*, ≥4 μg/ml for non-meningitis), and 19.7% ($n = 18$) were resistant to meropenem (resistant: *R*, ≥1 μg/ml). The non-susceptibility rates to penicillin, erythromycin, cefotaxime, and meropenem were 19.7% ($n = 18$), 72.5% ($n = 66$), 26.3% ($n = 24$), and 43.9% ($n = 40$), respectively. Compared with adults, most children aged <5 years harbored strains that were non-susceptible to erythromycin and