Serotype distribution and antimicrobial susceptibility of \textit{S. pneumoniae} causing invasive disease in Thai children younger than 5 years old, 2000–2005

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Abstract

In order to predict the potential benefit of pneumococcal conjugate vaccines (PCV), we evaluated the serotype coverage of the 7-, 9-, 11- and 13-valent PCV over the isolates causing invasive pneumococcal disease (IPD) in Thai children. One hundred and fifteen \textit{Streptococcus pneumoniae} isolates from sterile sites in children younger than 5 years old between 2000 and 2005 were serotyped. The coverages of 7-, 9-, 11- and 13-valent PCV were 69%, 73.8%, 73.8% and 85.7% in children younger than 2 years, and 73.9%, 77.4%, 77.4% and 87.8% in children younger than 5 years of age, respectively.

69.6% and 22.6% of the isolates were non-susceptible to penicillin and cefotaxime. 7-valent PCV covered 89% and 100% of penicillin and cefotaxime non-susceptible isolates.

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1. Introduction

\textit{Streptococcus pneumoniae} is the most common cause of bacteremia, sepsis, meningitis, pneumonia, sinusitis and otitis media in children worldwide. In February 2000, heptavalent pneumococcal conjugate vaccine (PCV7; Prevnar; Wyeth, Radnor, PA) was recommended for all children younger than 24 months in the US [1]. The serotypes contained in PCV7 accounted for 80–97.7% of the isolates causing invasive pneumococcal disease (IPD) among children in the US [2–5]. Serotypes included in PCV7, were also the majority of pneumococcal isolates among children in Europe, although the proportion was somewhat lower [6,7]. Several studies have indicated that the PCV7 is immunogenic, safe, and highly effective against diseases caused by serotypes contained in the vaccine [8–11]. Postlicensure surveillance in the US revealed a substantial regional and national decrease in IPD [12–17].

There has been an increasing trend in the resistance of \textit{S. pneumoniae} to antimicrobial agents from all geographic regions [18–22]. Most of the isolates that were resistant to the antimicrobial agents tested were covered by the PCV7 [23]. Previous study has shown a reduction of antimicrobial resistance among the IPD isolates after the introduction of routine PCV7 vaccination [14].

The nanovalent (heptavalent serotypes plus 1 and 5; PCV9), 11-valent (nanovalent serotypes plus 3 and 7V; PCV11) and 13-valent (11-valent serotypes plus 6A and 19A; PCV13) pneumococcal conjugate vaccines (PCV) are now in clinical trials and have been demonstrated to be highly immunogenic. They reduced the incidence of radiologically confirmed pneumonia, and IPD caused by vaccine-serotypes and antimicrobial-resistant serotypes in healthy children.
[24–27]. Population-based studies have demonstrated wide geographic and temporal variation in the incidence of IPD, as well as serotype distribution [28]. Therefore, the expanded serotype coverage of vaccine will potentially provide broader benefit in the countries where the serotypes of IPD are different.

In most developing countries, the true incidence of IPD is mostly unknown. The incidence of IPD in Thai children was found not to be as high as reported in the US [29]. However, a large retrospective study of bacterial meningitis in Thai children during 1980–2001 demonstrated that S. pneumoniae was the second most common cause, accounting for 22% [30]. There is limited data on serotypes that cause IPD and those with drug resistance.

The aim of this study was to determine the serotype coverage of the PCV7, PCV9, PCV11, and PCV13 among the isolates causing IPD in Thai children younger than 5 years of age. The results of this study will help guide the decision for the use of PCV in Thai children.

2. Materials and methods

S. pneumoniae isolates from sterile sites in children younger than 5 years old between January 1, 2000 and December 31, 2005, from the network of four institutes in Bangkok (Siriraj Hospital, King Chulalongkorn Memorial Hospital, Bhumipol Aduljadej Hospital, Queen Sirikit National Institute of Child Health) and from the Thailand National Institute of Health, that received specimens countrywide, were sent to the microbiological laboratory, Department of Microbiology, Siriraj Hospital. Isolates were confirmed of S. pneumoniae by optochin test and bile solubility test. Multiple isolates from different sites of same episode of a patient were counted only once. Capsular typing was carried out by the Quellung reaction, using group and factor sera of Pneumotest® kit provided by Statens Serum Institute, Copenhagen, Denmark. Serotyping was performed for 13 vaccine serotypes (polysaccharide serotypes 1, 3, 4, 5, 6A, 6B, 7V, 9V, 14, 19A, 19F, and 23F and an oligosaccharide serotype 18C). The isolates that were not one of the 13 serotypes were labeled as non-vaccine type, and were not further serotyped.

Antimicrobial susceptibility testing for penicillin and cefotaxime was performed by standard microbroth dilution with cation-adjusted Mueller–Hinton broth supplemented with 3% lysed horse blood according to the method described by the 2005 Clinical and Laboratory Standards Institute [31].

For cefotaxime, susceptibility categories were based on the site of infection. Because these isolates were collected with limited clinical information, we were unable to exclude meningitis in most of the cases with pneumococcal bacteremia. In order to be most conservative, we used meningitis criteria for pneumococcal isolates obtained from blood and CSF.

Statistical analysis was performed using $\chi^2$ and Fisher’s exact test.

3. Results

A total of 115 pneumococcal isolates were available for the study. Ninety-three (80.9%) isolates were from blood, 9 (7.8%) from CSF, 5 (4.3%) from both blood and CSF, and 8 (7%) from other sterile sites including lung tissue, pleural fluid, ascitic fluid and joint fluid.

Eighty-four (73%) isolates were from children younger than 2 years of age, and 27% were isolated from children 2–5 years old. Geographic area was available in 99 children. Of these, 48 (48.5%), 3 (3%), 13 (13.1%), 7 (7.1%), 15 (15.2%), and 13 (13.1%) were from the central, northern, southern, eastern, western and northeastern part of Thailand, respectively.

The information of underlying diseases was available in 53 (46%) children. Of these, 21 (40%) children had underlying medical conditions at risk for IPD; including chronic liver disease (7), congenital heart disease and congestive heart failure (6), HIV infection (3), hematologic malignancy (2), thalassemia (1), nephrotic syndrome (1), and congenital asplenia (1). Thirty-two (60%) children had no underlying risk factors for IPD.

The most common serotypes isolated were 6B (27.8%), 23F (20.0%), 14 (10.4%), and 19F (9.6%). These four serotypes accounted for 68% of all isolates (Fig. 1). There was no notable change of serotype distribution from the year 2000 to 2005, except that serotype 14 seemed to increase in the year 2005 (Table 1).

The overall coverage of PCV7, PCV9, PCV11 and PCV13 for serotypes of pneumococcal isolates were 69%, 73.8%, 73.8% and 85.7% in children younger than 2 years of age and 73.9%, 77.4%, 77.4% and 87.8% in children younger than 5 years of age, respectively. There was a significant increase in coverage between PCV7 and PCV13 in children younger than 2 (from 69% to 85.7%, $p < 0.001$), and in 5 years of age (from 73.9% to 87.8%, $p < 0.001$), while there was only minimal increase coverage between PCV7 and PCV9 or PCV11.

![Fig. 1. Serotype distribution of pneumococcal isolates causing invasive infection in children younger than 5 years of age, 2000–2005 (N=115).](image-url)
Table 1
Frequency of *S. pneumoniae* and common serotypes isolated by year, 2000–2005

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>6B</th>
<th>19F</th>
<th>23F</th>
<th>14</th>
<th>1</th>
<th>5</th>
<th>6A</th>
<th>9V</th>
<th>18C</th>
<th>19A</th>
<th>Non-vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>12</td>
<td>6(50)</td>
<td>1(8)</td>
<td>2(17)</td>
<td>1(8)</td>
<td>–</td>
<td>–</td>
<td>1(8)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1(8)</td>
</tr>
<tr>
<td>2001</td>
<td>23</td>
<td>7(30)</td>
<td>3(13)</td>
<td>4(17)</td>
<td>3(13)</td>
<td>–</td>
<td>1(4)</td>
<td>–</td>
<td>1(4)</td>
<td>2(9)</td>
<td>1(4)</td>
<td>1(4)</td>
</tr>
<tr>
<td>2002</td>
<td>25</td>
<td>7(28)</td>
<td>3(12)</td>
<td>2(8)</td>
<td>2(8)</td>
<td>2(8)</td>
<td>–</td>
<td>2(8)</td>
<td>–</td>
<td>1(4)</td>
<td>2(8)</td>
<td>4(16)</td>
</tr>
<tr>
<td>2003</td>
<td>13</td>
<td>3(23)</td>
<td>–</td>
<td>4(31)</td>
<td>–</td>
<td>–</td>
<td>1(8)</td>
<td>2(15)</td>
<td>–</td>
<td>2(15)</td>
<td>1(8)</td>
<td>–</td>
</tr>
<tr>
<td>2004</td>
<td>25</td>
<td>6(24)</td>
<td>3(12)</td>
<td>5(20)</td>
<td>1(4)</td>
<td>1(4)</td>
<td>–</td>
<td>1(4)</td>
<td>1(4)</td>
<td>–</td>
<td>–</td>
<td>7(28)</td>
</tr>
<tr>
<td>2005</td>
<td>17</td>
<td>3(18)</td>
<td>1(6)</td>
<td>6(35)</td>
<td>5(29)</td>
<td>–</td>
<td>1(6)</td>
<td>–</td>
<td>1(6)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*a Numbers in parentheses, percent.

Fig. 2. Antimicrobial susceptibility of IPD isolates from children younger than 5 years of age, 2000–2005. S, susceptible; I, intermediate; R, resistant. (A) Penicillin; (B) Cefotaxime.

During the study period, 69.6% of pneumococcal isolates were non-susceptible to penicillin (26.1% were intermediately susceptible, and 43.5% were resistant) and 22.6% were non-susceptible to cefotaxime (20.9% were intermediately susceptible, and 1.7% were resistant). There was an increase of penicillin non-susceptibility from 58.3% in the year 2000 to 88.2% in the year 2005 (*p* = 0.06), however, the MIC50 and MIC90 did not change over the time. Cefotaxime non-susceptibility was stable around 16–30% in 2000–2004, and dropped to 5.9% in 2005 (Fig. 2). Serotypes 6B, 23F, 19F predominated among isolates that were non-susceptible to penicillin and cefotaxime.

PCV7 covered 89% and 100% of penicillin and cefotaxime non-susceptible pneumococcal isolates, respectively. PCV13 provided additional coverage for penicillin non-susceptible isolates to 95% (Tables 2 and 3). Isolates with serotypes included in the PCV7, PCV9, PCV11 and PCV13 were significantly less likely to be susceptible to penicillin and cefotaxime than those non-vaccine serotypes (25/101 versus 10/14, *p* < 0.001 for penicillin, and 75/101 versus 14/14, *p* = 0.037 for cefotaxime).

4. Discussion

The serotype distribution reported in this study has some difference from that reported in other geographic regions [6]. Our data demonstrated that serotype 6B, 23F, 14 and 19F were the most common serotypes isolated from Thai children younger than 5 years of age with IPD. Serogroup 23

Table 2
Coverage of PCV 7, 9, 11 and 13 of IPD isolates in children younger than 5 years of age in relationship of penicillin susceptibility

<table>
<thead>
<tr>
<th>Serotype of IPD covered by</th>
<th>n</th>
<th>Penicillin susceptibility (%)</th>
<th>Coverage of penicillin NS* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S^b</td>
<td>F^c</td>
</tr>
<tr>
<td>PCV7</td>
<td>85</td>
<td>14 (16.5)</td>
<td>22 (25.9)</td>
</tr>
<tr>
<td>PCV9</td>
<td>89</td>
<td>18 (20.2)</td>
<td>22 (24.7)</td>
</tr>
<tr>
<td>PCV11</td>
<td>89</td>
<td>18 (20.2)</td>
<td>22 (24.7)</td>
</tr>
<tr>
<td>PCV13</td>
<td>101</td>
<td>25 (24.8)</td>
<td>27 (26.7)</td>
</tr>
<tr>
<td>Non-vaccine serotypes</td>
<td>14</td>
<td>10 (71.4)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>All isolates</td>
<td>115</td>
<td>35 (30.4)</td>
<td>30 (26.1)</td>
</tr>
</tbody>
</table>

*a Non-susceptible.

*b Susceptible.

*c Intermediate susceptible.

d Resistant.
which was found in 4.2–8.1% (the 5th rank) in the US and Canada, Europe, Africa and Oceania, and in 5.3% (the 7th rank) in other Asian countries [6], accounted for 20.9% of all isolates in this study. We found serotype 1, the common isolates in some Asian and African countries [6], in only 2.6%. This information underscores the importance of local serotype data to evaluate the potential benefit of the pneumococcal vaccines in various geographic areas.

Serotypes contained in the PCV7 were responsible for >80% of the IPD in young children in the US and Canada, and at least 50% of IPD in all other regions except Asia, where PCV7 serotypes were thought to be responsible for only 30% [6]. Previous reports from Thailand have demonstrated that the specific and related serotypes coverage of PCV7 in nasopharyngeal colonization and IPD combined in children and adults patients was approximately, 63–92.5% [20,32–34]. The most recent study found 62% and 76% coverage of PCV7 and PCV11 in Thai children younger than 5 years of age with IPD [35]. Our present study, was the largest collection that looked in specific group of IPD in young Thai children, and found that PCV7 provided a much higher coverage than that reported from other countries in Asia [36–41]. Of note was that 78.6% of the isolates from CSF were covered by PCV7.

Cross-coverage is thought to extend to other related serotypes within the same serogroup. For serotypes 6B and 19F, several studies have shown a significant cross-immunity with serotypes 6A and 19A [42–44]. Therefore, PCV7 coverage in our study may extend to 81% and 84.3% in children younger than 2 and 5 years of age, respectively, if cross-immunity occurred.

The significant increase in coverage of PCV11 from PCV7 has been shown in the PROTEKT (Prospective Resistance Organism Tracking and Epidemiology for the Ketolide Telithromycin) global surveillance of S. pneumoniae serotypes isolated from pediatric patients with community-acquired respiratory tract infections during 1999–2000 [19]. Our data found minimal increase in coverage between PCV7 and PCV9 or PCV11. However, because 6A and 19A were our significant serotypes, PCV13 provided a significant additional coverage over PCV7; 16.7% and 13.9% in children younger than 2 and 5 years of age, respectively.

Data from the Northern California Kaiser Permanente before and after licensure of PCV7 indicated that the most (>90%) of IPD occurred in normal children without underlying risk factors [13]. In our study, although underlying diseases were available in only half of the children, only about 40% had underlying risk factors. This data indicated that young children are at risk for IPD regardless of underlying diseases and therefore, PCV7 should be recommended for all young children, and not only those with underlying illness.

With regard to antimicrobial resistance, it has been consistently observed that only a small subset of serogroups comprises the vast majority of penicillin non-susceptible serotypes [45–48], and antimicrobial resistance occurs commonly in serotypes that cause IPD. These serotypes are mostly in PCV7 [49,50]. In our study, PCV7 covered 89% of penicillin non-susceptible isolates and PCV13 provided coverage up to 95%. In addition, all of cefotaxime non-susceptible isolates were covered by all PCV. According to our data, we predict that the introduction of PCV7 in our region would reduce the incidence of IPD caused by antimicrobial resistant isolates. The prevalence of penicillin non-susceptible S. pneumoniae in Thailand during 1986–2001 has been reported to be between 30.4% to 57.9% [20,33,34,51,52]. The proportion of isolates that were penicillin non-susceptible increased over the study period and reached a high of 88.2% in 2005. This high prevalence of antimicrobial resistance is alarming and underscores the appropriate use of antimicrobial agents in our region.

In conclusion, this study had elucidated the serotype distribution of the S. pneumoniae obtained from the largest pool of isolates causing invasive infection in young children all over Thailand over 6-year period. Although these were stored isolates, and have missed some non-survival isolates, but the non-survival isolates were estimated to be less than 2%. Our data underscored the importance of local serotype data to evaluate the potential benefit of the use of PCV, and the prudent use of antimicrobial agents to prevent the increase

<table>
<thead>
<tr>
<th>Serotype of IPD covered by</th>
<th>n</th>
<th>Penicillin susceptibility (%)</th>
<th>Coverage of cefotaxime NS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S&lt;sup&gt;b&lt;/sup&gt;</td>
<td>I&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PCV7</td>
<td>85</td>
<td>59 (69.4)</td>
<td>24 (28.2)</td>
</tr>
<tr>
<td>PCV9</td>
<td>89</td>
<td>63 (70.8)</td>
<td>24 (27.0)</td>
</tr>
<tr>
<td>PCV11</td>
<td>89</td>
<td>63 (70.8)</td>
<td>24 (27.0)</td>
</tr>
<tr>
<td>PCV13</td>
<td>101</td>
<td>75 (74.2)</td>
<td>24 (23.8)</td>
</tr>
<tr>
<td>Non-vaccine serotypes</td>
<td>14</td>
<td>14 (100)</td>
<td>0 0</td>
</tr>
<tr>
<td>All isolates</td>
<td>115</td>
<td>89 (77.4)</td>
<td>24 (20.9)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Non-susceptible.<br>
<sup>b</sup> Susceptible.<br>
<sup>c</sup> Intermediate susceptible.<br>
<sup>d</sup> Resistant.

Table 3

Coverage of PCV 7, 9, 11 and 13 of IPD isolates in children younger than 5 years of age in relationship of cefotaxime susceptibility
of antimicrobial resistance. Currently, PVC7 is available in Thailand, but not included in Expanded Program of Immunization (EPI). The cost-effective study of this vaccine must be further studied to help the policy makers to adopt this vaccine in EPI. The impact of the vaccine after widely usage needs to be further studied.

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References


