Serotype Distribution and Antimicrobial Susceptibilities of Nasopharyngeal Isolates of

*Streptococcus pneumoniae* from Children Hospitalized for

Acute Respiratory Illnesses in Hong Kong.

Margaret Ip*, MRCPath, FRCP(Glasg)

E Anthony S Nelson¹, MBChB, MD, FRCPCH

Edmund SC Cheuk, MPhil

Rita YT Sung¹, MD, FRCP

Albert Li¹, FRCPCH

Helen Ma, BSc

Paul KS Chan, MD, FRCPath.

Department of Microbiology and Dept of Paediatrics¹,

Chinese University of Hong Kong,

Prince of Wales Hospital,

Shatin, Hong Kong.

*Corresponding author

Dept of Microbiology,

Chinese University of Hong Kong,

Prince of Wales Hospital, Hong Kong

Tel: (852) 2632 1265

Fax: (852) 2647 3227

Email: margaretip@cuhk.edu.hk

**Running title:** *Serotypes and Antibiotic Susceptibilities of Streptococcus pneumoniae* in

Children
Abstract

Five hundred and nineteen *Streptococcus pneumoniae* isolates from nasopharyngeal aspirates of 3157 children <16 years from a respiratory surveillance study from Hong Kong in 2005/6 indicated penicillin and cefotaxime non-susceptibilities of 64.9% and 37.2%. Potential coverage by 7-valent conjugate vaccine was 72.3% and increased to 74.6% for serogroup-specific types.

Text

Penicillin-nonsusceptible *Streptococcus pneumoniae* (PNSP) has evolved to be a global problem in recent decades. The introduction of a 7-valent (types 4, 6B, 9V, 14, 18C, 19F and 23F) pneumococcal conjugate vaccine (PCV) in the United States and Europe has demonstrated its impact in reducing invasive disease [1] and otitis media [4]. Invasive disease caused by serotypes of PNSP clones have diminished significantly in the United States [15,18]. In addition, nasopharyngeal carriage of vaccine serotypes in children has declined, thus reducing transmission and infections caused by these serotypes [16].

Pneumococci are ‘inhabitants’ of the oral flora and colonize the nasopharynx, particularly in young children from birth and gradually declines with age. Although their isolation from the nasopharynx of children with respiratory illnesses does not necessarily represent pneumococcal disease, nasopharyngeal colonization is often the first step in the development of pneumococcal infections. Co-infection or secondary bacterial infection may result from organisms that had colonized in the nasopharynx. Invasive diseases with meningitis and bacteraemia remain ‘tip of the iceberg’ presentations for pneumococcal disease, but SPNE from CSF and blood are infrequently cultured, especially when prior antibiotics have been given. Besides, tympanic aspirates or sputa are rarely obtained from children. We thus sought to examine the serotypes and antibiotic susceptibilities of *S. pneumoniae* isolated from nasopharyngeal aspirates obtained in a prospective childhood acute respiratory illness
surveillance study. As PCV7 has been made available in Hong Kong since August 2006, the
serotypes and antibiotic susceptibilities of SPNE obtained from this study serve to represent
those prior to the introduction of PCV7.

**Pneumococcal Isolates.** All children aged >1 month old to 15 years who were hospitalized at
the Prince of Wales Hospital (PWH) during Mar 2005/06 for suspected respiratory or febrile
illness and had nasopharyngeal aspiration (NPA) performed were included. PWH is a 1350
bed teaching hospital that serves a population of approx.1,000,000 in the New Territories East
Region of Hong Kong. Nasopharyngeal aspirate in 2mls of transport medium (TM) containing
Hank’s BBS, 5% bovine albumin, and 7.5% NaHCO₃ without antibiotics, was obtained from
each child and 1µl was cultured onto blood agar with/without gentamicin (5mg/L). SPNE
were saved in 10% glycerol brain heart infusion broth at -70°C. Duplicate isolates from the
same child during the same hospital admission were excluded.

**Antibiotic Susceptibility Testing.** Minimum inhibitory concentrations (MIC) of penicillin G,
cefotaxime, chloramphenicol, tetracycline, erythromycin, lincomycin, ciprofloxacin,
levofloxacin, gatifloxacin, moxifloxacin, and linezolid were determined using microbroth
dilution method according to Clinical Laboratory Standards Institute [3].

**Capsular Typing.** Capsular typing was performed using Pneumotest antisera (Statens
Seruminstitut, Copenhagen) as according to the manufacturer’s instruction. Isolates that were
not typable by the Pneumotest were stated as NT. Typing of certain serogroups into serotypes
was done using a combination of factor sera and multiplex PCR that targeted serotypes of the
7-valent vaccine [13].

The isolation rate of SPNE from NPAs of 3157 children aged 1 month to 15 years of
age was 16.9%. 519 non-duplicate SPNE isolates were available for capsular typing and
antibiotic susceptibility testing. The median age of children isolating SPNE was 2.9 yrs
(25%IR 1.3yrs, 75%IR 4.5 yrs) and the male:female ratio was 1.3:1. The distribution of
serotypes in different age groups and the percentage penicillin non-susceptibility are listed in
Table 1. 82% of isolates were typable and the commonest serotypes/groups were 6, 19, 23F, 14, 15 and 18. The potential percentage serotype coverage by 7-valent, 9- and 11-valent vaccines were 72.3%, 72.4%, and 73.4% respectively. If serogroups were included, the PCV7 coverage increased to 74.4%, whilst the 9- and 11-valent vaccine coverage increased to 74.6 and 75.7% respectively.

The antibiotic susceptibilities of 519 SPNE isolates are listed (Table 2). The prevalence of PNSP was 64.9% whilst that for cefotaxime (MIC $\geq 1.0$) was 37.2%. High rates of non-susceptibility were obtained with erythromycin (75.7%) and tetracycline (61.8%). No resistance to respiratory fluoroquinolones was detected, except 2.3% of SPNE have ciprofloxacin MIC=4.0 $\mu$g/ml, suggesting that first step mutations at the QRDR region may already be present in some isolates. All isolates were susceptible to linezolid. The highest penicillin MIC remained at 4.0 $\mu$g/ml but high-level cefotaxime resistance (MIC $>$4 $\mu$g/mL) was identified. Previously, two fatal cases of children with pneumococcal bacteraemia; one also with meningitis, caused by strains with cefotaxime MIC of 4 $\mu$g/mL had been reported [17].

The antibiotic resistance profiles and the serotypes of these SPNE isolates reflect strains which may be associated with both carriage and with acute respiratory illness. Ho et al [7] compared both invasive and nasopharyngeal carriage isolates in children and did not yield significant difference in terms of resistance rates or to serotypes. Our potential 7-valent vaccine coverage rate fell between that based on invasive isolates (89.7%), and that on nasopharyngeal carriage (66.1%)[7]. Previous data concerning serotypes of <2 years age group was very limited and had been based on 33 invasive isolates saved over a six-years period [2,7]. However, a lower rate based on nasopharyngeal isolates would be expected and had similarly been shown in a study from Taiwan [12]. The application of DNA amplification by sequential multiplex PCR approach of the specific capsular genes [17] may further identify the serogroups/types presently nontypable by the Pneumotest antisera. Our previous findings
indicated that PNSP was significantly associated with children, particularly the 2–10 years age
group [8]. A rapid rise of PNSP was seen in Hong Kong since the early 1990s from the spread
of multidrug resistant clones of Spain 23F-1 and Spain 6B-2 [9-11]. Based on recent outcomes on
the use of PCV7 in populations with high prevalence of PNSP, PNSP is likely to remain
prevalent until such time when the vaccine could be introduced to effectively reduce the
incidence of invasive pneumococcal infections by these resistant strains.

The pneumococcal population is presently undergoing strong evolutionary pressure for
change as the conjugate vaccine is used more extensively in different parts of the world. This
study emphasizes the need for surveillance of the antibiotic resistance rates and serotypes of S.
pneumoniae with the changing scenes as vaccination programs and control of antibiotic usage
are being introduced.

Acknowledgement

The work described in this paper was supported by a grant from Wyeth (HK) Limited
for the study on Hospital Surveillance of Childhood Respiratory Infections in Hong Kong.

References

Elvin, K. M. Ensor, J. Hackell, G. Siber, F. Malinoski, D. Madore, I. H. Chang, R.
Permenente Vaccine Study Center Group. 2000. Efficacy, safety and immunogenicity of
195.

of antimicrobial-resistant Streptococcus pneumoniae among young children attending 79


Table 1. Distribution of Capsular Types of *S. pneumoniae* by Age (%) and Percentage

Penicillin non-susceptible (N=519 Isolates).

<table>
<thead>
<tr>
<th>Serotypes</th>
<th>Groups by Year, N</th>
<th>Total</th>
<th>PNSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;2</td>
<td>2-3</td>
<td>3-4</td>
</tr>
<tr>
<td>6B</td>
<td>36</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>19F</td>
<td>29</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>23F</td>
<td>17</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>18C</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Non-19F</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-6B(6A)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-23F</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18B</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9L</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NT</td>
<td>46</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Subtotal | 67   | 96   | 94   | 48  | 114   | 519 (100%) | 337 (64.9%) |

PNSP penicillin MIC ≥0.12 µg/ml
Table 2. Minimum Inhibitory Concentrations of *Streptococcus pneumoniae* to 11 Antibiotics (N=519 Isolates)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Range (µg/ml)</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>Non-susceptible*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.008-4.0</td>
<td>182</td>
<td>212</td>
<td>125</td>
<td>64.9%</td>
</tr>
<tr>
<td>Cefotaxime (non-meningitis)</td>
<td>0.015-≥4.0</td>
<td>451</td>
<td>41</td>
<td>27</td>
<td>13.0%</td>
</tr>
<tr>
<td>Cefotaxime (meningitis)</td>
<td>0.015-≥4.0</td>
<td>326</td>
<td>125</td>
<td>68</td>
<td>37.2%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.03-&gt;64.0</td>
<td>126</td>
<td>24</td>
<td>369</td>
<td>75.7%</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0.25 - &gt;32.0</td>
<td>-</td>
<td>-</td>
<td>225</td>
<td>43.4%</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.06- &gt;16.0</td>
<td>198</td>
<td>8</td>
<td>313</td>
<td>61.8%</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1.0 - 32</td>
<td>427</td>
<td>-</td>
<td>92</td>
<td>17.7%</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.12 -2.0</td>
<td>519</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25 – 4.0</td>
<td>-</td>
<td>-</td>
<td>12**</td>
<td>2.3%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.25 – 2.0</td>
<td>519</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0.06 – 0.5</td>
<td>519</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.03 – 0.25</td>
<td>519</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Intermediate + resistant isolates  *NA, no CLSI breakpoint available  **MIC ≥4.0 µg/ml.