Clinical Manifestations and Molecular Epidemiology of Necrotizing Pneumonia and Empyema Caused by *Streptococcus pneumoniae* in Children in Taiwan

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Recently, there have been increasing numbers of pneumococcal pneumonia cases, with their associated complications. We conducted a retrospective review to increase the understanding of childhood pneumococcal pneumonia. Seventy-one patients with pneumococcal pneumonia were identified. Forty (56.3%) of them developed complicated pneumonia. Multivariate analysis showed that presence of immature polymorphonuclear leukocytes in peripheral blood (odds ratio [OR], 3.67; 95% confidence interval [CI], 1.08–12.63), high C-reactive protein levels (≥12 mg/dL) (OR, 5.24; 95% CI, 1.10–24.93), and no underlying disease at presentation (OR, 5.48; 95% CI, 1.06–28.25) were independent predictors of the occurrence of necrosis or/and abscess. Fourteen isolates (35%), which were genotypically identical and had the same pulsed-field gel electrophoresis pattern (serogroup 14, with MICs of penicillin of 0.1–0.5 μg/mL), were significantly associated with complicated pneumonia (P = .047). Whether the virulence of antibiotic-resistant pneumococci is evolving deserves further investigation.

*Streptococcus pneumoniae* is the most common pathogen of pyogenic pneumonia in children [1]. The critical issues concerning *S. pneumoniae* in recent decades are the global emergence of multidrug-resistant pneumococci and the high carriage rate of penicillin-resistant *S. pneumoniae* (PNSP) among children [2–4]. There are many reports that address the impact of PNSP. The majority of these studies conclude that there is no difference in the clinical characteristics of and outcomes for pneumococcal patients infected with penicillin-susceptible *S. pneumoniae* or PNSP [5, 6]. Only 1 retrospective study, which was performed in Atlanta, demonstrated a significant risk of suppurative complications among adults with pneumococcal pneumonia who were infected with PNSP strains [7].

We know from experience that pneumonia caused by *S. pneumoniae* uniquely preserves the pulmonary architecture, no matter how severe it is at the peak stage of disease. Since the introduction of penicillin into the treatment regimen, pneumonia has seldom evolved into empyema [8]. However, an increase in the prevalence of necrotizing pneumococcal pneumonia and pneumococcal empyema was observed among children, beginning in the 1990s [9–11]. In Taiwan—a geographic region in which there is a high prevalence of PNSP [12]—we observed pulmonary infection with *S. pneumoniae* in children usually progressing to necrosis or empyema along with the increase of PNSP in recent years. As a result, we conducted a retrospective study to evaluate the clinical epidemiology, microbiology, and course of pneumococcal pneumonia.

**PATIENTS AND METHODS**

**Patients.** We retrospectively identified patients <15 years old who received a diagnosis of pneumococcal pneumonia at the National Taiwan University Hospital (NTUH) between May 1995 and April 2003. NTUH is a 2000-bed medical center in northern Taiwan. Pneumococcal pneumonia was established on the basis of...
the following 3 criteria: clinical symptoms, a consolidation pattern revealed by chest radiography, and a positive result of a blood or pleural fluid culture or the detection of antigens in the pleural fluid by latex agglutination testing.

Medical records of patients with pneumococcal pneumonia were reviewed. Information abstracted included demographic data, clinical characteristics, underlying diseases, laboratory data, antimicrobial treatment, and outcome. Necrotizing pneumonia was defined as multiple small lucencies or pneumatoceles on a chest radiograph or as cavities of nonenhancement on a contrast-enhanced CT image. Empyema was defined as the presence of 1 major criterion or 2 minor criteria. Major criteria included the presence of pus in the pleural space, the performance of pleural decortication, and a positive result of a pleural fluid culture. Minor criteria included a pleural fluid pH of 7.2, a glucose level of 40 mg/dL, a WBC count of 10,000 cells/dL, and a lactate dehydrogenase level of 1,000 U/L [10].

Bacterial isolates, antimicrobial susceptibility test, and serotype. Fifty-three isolates of S. pneumoniae were recovered from blood or pleural effusion samples (36 isolates from blood samples, 7 isolates from pleural effusion samples, and 10 isolates from both blood and pleural effusion samples) obtained from children who received a diagnosis of pneumococcal pneumonia. The antibiotic susceptibility of these strains was assayed using the disk diffusion method. An inhibition zone diameter > 20 mm (using a 1-μg oxacillin disk) indicated that the MIC of penicillin of the isolates was > 0.06 μg/mL. MICs of penicillin and ceftriaxone were determined using the Etest (AB Biodisk). Criteria to define susceptibility or nonsusceptibility were based on the NCCLS guidelines for meningitis [13]. All isolates were tested at the Statens Seruminstitut (Copenhagen, Denmark) using duplicate slide agglutination assays with capsular group-specific antisera to determine the serogroup of each strain [14].

PFGE analysis. PFGE was performed according to methodology described elsewhere [15]. The DNA was digested with Smal. Bands were stained with ethidium bromide and visualized with UV light. PFGE patterns differing by ≤ 3 bands were defined as 1 PFGE type; isolates with the same PFGE patterns indicated indistinguishable strains, and those with 2 or 3 different bands indicated closely related strains [16, 17].

Statistical analysis. The primary end point was the presence or absence of complicated pneumonia (necrotizing pneumonia and/or empyema). We used χ² analysis or Fisher’s exact test for categorical variables and the Wilcoxon rank sum test for continuous variables to test for significant differences between groups. We then performed multiple logistic regression analysis to identify independent predictors of complicated pneumonia and the annual incidence of complicated pneumonia. A P value < .05 was considered to be statistically significant. All probabilities were 2-tailed. Results of comparisons are presented with 95% CIs. Data are reported as mean values (± SD), unless otherwise indicated.

RESULTS

Seventy-one patients who received a diagnosis of pneumococcal pneumonia at NTUH were identified during the study period. The percentage of patients with complicated pneumonia significantly increased during this period (figure 1). According to data from the NTUH pediatric medical records department, the annual hospitalization rate associated with pneumonia was approximately 6.1%–6.3% during 1996–2000. Information obtained from this department also showed that

Figure 1. Annual number of cases (bars) and percentage of complicated cases of pneumonia (dots) caused by Streptococcus pneumoniae from May 1995 through April 2003. The annual percentages of penicillin-nonsusceptible S. pneumoniae (MIC > 0.06 μg/mL) among all available isolates were 50%, 85.7%, 100%, 100%, 100%, and 83.5%, respectively. A “study year” is defined as the 12-month interval from May through April (e.g., the study year 1995 is the interval from May 1995 through April 1996).
the referral rates for patients with pneumonia were stable during the study period. Forty of these patients had complicated pneumonia (1 case of necrotizing pneumonia, 27 cases of empyema, and 12 cases of empyema and necrotizing pneumonia; table 1). The mean age was 52.3 months (range, 9–144 months), and the female-to-male patient ratio was 6:5. Those with underlying diseases included 5 patients with asthma, 4 with heart disease, 2 with genetic disorders, 2 with malignancy, 1 with hemolytic anemia, and 1 with metabolic disease. No patient was receiving steroid or immunosuppressive therapy.

Univariate analysis revealed that no underlying disease, age >36 months, presence of immature polymorphonuclear leukocytes (PMNs) in the peripheral blood, thrombocytopenia, and C-reactive protein (CRP) levels >12 mg/dL were significantly associated with the occurrence of necrosis or empyema (table 1). In the multivariate analysis, no underlying disease (OR, 5.48; 95% CI, 1.06–28.25; \( P = .04 \)), immature PMNs in the peripheral blood (OR, 3.67; 95% CI, 1.08–12.63; \( P = .037 \)), and high CRP levels (>12 mg/dL) (OR, 5.24; 95% CI, 1.10–24.93; \( P = .037 \)) were independent predictors of complicated pneumonia. Children who were >36 months old accounted for 62% of all patients, and age was a significant predictor of pneumonia complications in the univariate analysis only (\( P = .038 \)). The occurrence of complicated pneumonia was associated with longer durations of fever and hospital stays (\( P < .001 \)). Twenty-four (60%) of the 40 patients with complicated pneumonia received a thoracotomy. The mortality rate in this group was 7.5% (3 of 40 patients), compared with a rate of 3.2% (1 of 31 patients) among those with lobar pneumonia (\( P = .627 \)).

All but 4 patients with pneumococcal pneumonia were empirically treated with parenteral second- or third-generation cephalosporins or with a combination of amoxicillin plus clavulanic acid. After culture results and antimicrobial susceptibility reports were made available, the antimicrobial regimen was adjusted according to the patients’ clinical response. Two patients were empirically treated with a first-generation cephalosporin; 1 had lobar pneumonia due to \( S. \) pneumoniae (MIC of penicillin, 2 \( \mu g/mL \)), and the other had empyema due to \( S. \) pneumoniae (MIC, 0.38 \( \mu g/mL \)). Two other patients were empirically treated with high-dose penicillin (400,000 U/kg per day); 1 had lobar pneumonia due to \( S. \) pneumoniae (MIC of penicillin, 2 \( \mu g/mL \)), and the other had empyema due to \( S. \) pneumoniae (MIC, 3 \( \mu g/mL \)).

MIC testing was performed for 50 bacterial isolates. The prevalences of infection with \( S. \) pneumoniae immediately susceptible or resistant to penicillin were higher and lower, respectively, among patients with complicated pneumonia than among patients with lobar pneumonia (table 2). Five isolates had an MIC of penicillin of 4 \( \mu g/mL \); 2 isolates were from patients with complicated pneumonia, and 3 isolates were from patients with lobar pneumonia. No isolate had an MIC of penicillin that was >4 \( \mu g/mL \). The prevalence of ceftriaxone-non-susceptible \( S. \) pneumoniae was 40% (10 of 25) among patients with complicated pneumonia and 64% (16 of 25) among patients with lobar pneumonia. There was no statistically significant difference between the prevalences of PNSP and ceftriaxone-non-susceptible \( S. \) pneumoniae in these 2 groups.

The serogroup of 38 bacterial isolates was determined. Infection with \( S. \) pneumoniae from serogroup 14 was most prevalent among children with pneumococcal pneumonia. Isolates from serogroups 23, 19, and 6 were also recovered from these

### Table 1. Univariate analysis of demographic data and clinical characteristics for children with complicated or lobar pneumonia caused by Streptococcus pneumoniae.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Complicated (n = 40)</th>
<th>Lobar (n = 31)</th>
<th>OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months (range)</td>
<td>52.3 ± 28.8 (9-144)</td>
<td>41.8 ± 21.7 (10-97)</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Age &gt;36 months</td>
<td>72.5</td>
<td>48.4</td>
<td>2.81 (1.05–7.56)</td>
<td>.038</td>
</tr>
<tr>
<td>Female sex</td>
<td>62.5</td>
<td>51.6</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>No underlying disease</td>
<td>92.5</td>
<td>61.3</td>
<td>7.79 (1.96–30.99)</td>
<td>.001</td>
</tr>
<tr>
<td>WBC count, ( \times 10^9 ) cells/( \mu L )</td>
<td>13.3 ± 8.7</td>
<td>17.4 ± 9.5</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Presence of immature PMNs in peripheral blood</td>
<td>60</td>
<td>22.6</td>
<td>5.14 (1.79–14.74)</td>
<td>.002</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;10,000 platelets/mm(^3))</td>
<td>22.5</td>
<td>3.2</td>
<td>8.71 (1.64–20.70)</td>
<td>.004</td>
</tr>
<tr>
<td>C-reactive protein level &gt;12 mg/dL</td>
<td>87.5</td>
<td>60</td>
<td>5.83 (1.64–20.70)</td>
<td>.004</td>
</tr>
<tr>
<td>Time to fever clearance after hospital admission, days</td>
<td>13.5 ± 8.2</td>
<td>5.0 ± 4.8</td>
<td>...</td>
<td>.000</td>
</tr>
<tr>
<td>Thoracotomy</td>
<td>60</td>
<td>0</td>
<td>...</td>
<td>.000</td>
</tr>
<tr>
<td>Duration of hospital stay, days</td>
<td>25.2 ± 12.0</td>
<td>12.6 ± 6.8</td>
<td>...</td>
<td>.000</td>
</tr>
<tr>
<td>Mortality</td>
<td>7.5</td>
<td>3.2</td>
<td>...</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** Data are % of patients or mean values ± SD. NS, not significant.

\( ^a \) Defined as presence of bands, myelocytes, metamyelocytes, or promyelocytes.
patients (table 2). The distribution of serogroups was not statistically significantly different among patients with complicated pneumonia and those with lobar pneumonia.

PFGE analysis of 40 invasive isolates revealed 17 PFGE types (figure 2). There were 8 PFGE types among the 20 isolates associated with complicated pneumonia and 11 PFGE types among the 20 isolates associated with lobar pneumonia. Fourteen (35%) of 40 isolates had a major PFGE type (serogroup 14, with an MIC of penicillin of 0.1–0.5 \( \mu \text{g/mL} \)). These 14 strains were indistinguishable; 10 were from a group of 20 isolates associated with complicated pneumonia, and 4 were from a group of 20 isolates associated with lobar pneumonia (\( P = .047 \)). Eight (20%) of 40 isolates had another PFGE type (serogroup 14, with an MIC of penicillin of 1 \( \mu \text{g/mL} \)) accounted for 20% (8 of 40). These 8 strains were closely related; 3 were from a group of 20 isolates associated with complicated pneumonia, and 5 were from a group of 20 isolates associated with lobar pneumonia. Two isolates collected consecutively from siblings with pneumococcal empyema were identical and belonged to the major PFGE type (serogroup 14, with penicillin MICs of 0.1–0.5 \( \mu \text{g/mL} \)). One serogroup 14 isolate and 1 serogroup 23 isolate were closely related, according to results of PFGE typing (figure 2).

**DISCUSSION**

This 8-year retrospective study in a university hospital setting revealed that the number of cases of complicated pneumococcal pneumonia has increased significantly. Although our study was limited to observations in a tertiary care hospital, we believe it reflects the increasing number of complicated pulmonary infections caused by \textit{S. pneumoniae} in the community at large, because our hospital has been a medical center for >17 years and serves a steady population of referred patients.

We showed that the absence of an underlying disease, the presence of immature PMNs in the peripheral blood, and a high CRP level (>12 mg/dL) were significant predictors for necrosis or empyema complications in the multivariate analysis. The host inflammatory response to the pneumococcal components is the major element that causes tissue injury [18]. This may explain why immunocompetent children without underlying disease are at an increased risk for complicated pneumonia. CRP, an acute-phase protein, plays an important role in innate immunity by binding to the \( \text{C-polysaccharide of pneumococci} \) to increase the rate in which the invading pneumococci are cleared [19]. PMNs, even in immature form, have been shown to mediate microbial killing during invasive pneumococcal infection [20]. Thus, the presence of immature PMNs and a high CRP level in children with complicated pneumonia may imply a higher bacterial burden. Taking note of these signs can help in predicting severe pneumococcal infection at an early stage.

Antibiotic resistance is frequently seen in serogroups 6, 14, 19, and 23, which have the good ability to colonize the nasopharynxes of children [21]. Some clinical and epidemiological investigations showed that invasive isolates were more likely to be susceptible to antibiotics, whereas less-invasive isolates were more likely to be resistant to antibiotics [22, 23]. In Taiwan, the nasopharyngeal carriage of PNSP was highly prevalent among children [3]. We confirmed that the common invasive

### Table 2. Results of penicillin-resistance testing and serogroup analysis of \textit{Streptococcus pneumoniae} isolates from children with complicated or lobar pneumonia.

<table>
<thead>
<tr>
<th>Analysis type</th>
<th>No. (%) of isolates, by type of pneumonia</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin resistance (MIC)(^{a})</td>
<td>Complicated</td>
<td>Lobar</td>
</tr>
<tr>
<td>Susceptible (0.06)</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Intermediately susceptible (0.1–1)</td>
<td>18 (72)</td>
<td>11 (44)</td>
</tr>
<tr>
<td>Resistant (&gt;2)</td>
<td>6 (24)</td>
<td>13 (52)</td>
</tr>
<tr>
<td>Serogroup(^{b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4 (21.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>9</td>
<td>0 (0)</td>
<td>1 (5.3)</td>
</tr>
<tr>
<td>14</td>
<td>13 (68.4)</td>
<td>12 (63.1)</td>
</tr>
<tr>
<td>19</td>
<td>0 (0)</td>
<td>2 (10.5)</td>
</tr>
<tr>
<td>23</td>
<td>2 (10.5)</td>
<td>4 (21.1)</td>
</tr>
</tbody>
</table>

**NOTE.** NS, not significant.

\(^{a}\) Penicillin-resistance testing was performed on 25 isolates from patients with complicated pneumonia and 25 isolates from patients with lobar pneumonia. MICs are expressed as \( \mu \text{g/mL} \).

\(^{b}\) Serogroup analysis was performed on 19 isolates from patients with complicated pneumonia and 19 isolates from patients with lobar pneumonia.

![Figure 2](image-url)
isolates in Taiwan were penicillin-resistant serogroups 6, 14, 19, and 23. The distribution of serogroups 6, 9, 14, 19, and 23 did not differ much between the group with and the group without complications.

All patients (in both groups) were empirically treated with β-lactam antimicrobial agents, which have been suggested to be appropriate therapies for pneumonia caused by PNSP [24]. Therefore, complicated pneumococcal pneumonia was not the result of ineffective antimicrobial treatment. A PFGE analysis revealed that 35% of patients with pneumococcal pneumonia were infected with S. pneumonia from 1 major PFGE type (serogroup 14, with an MIC of penicillin of 0.1–0.5 μg/mL). One-half of the cases of complicated pneumonia were caused by bacteria from this major PFGE type. These results suggest that spread of S. pneumonia from this major PFGE type caused pneumonia among children in Taiwan. Surprisingly, we found that 2 closely related strains belonging to 1 PFGE type were associated with different capsular serogroups (serogroups 14 and 23). It suggested the mechanism of capsular transformation among pneumococcal isolates and has been previously described [25].

International dissemination of a limited number of clones by human-to-human spread is thought to have contributed to the rapid emergence of PNSP throughout the world. Examples include the spread of Spain23F-1 to the United States, Spain9V-3 to France, Taiwan19F-14 and Taiwan 6B clones to Hong Kong from other reported international serogroup 14 clones that have undergone PFGE profiling [28, 29].

It is important to note that a multidrug-resistant PFGE type endemic in Taiwan appears to be spreading and is capable of inducing complicated pneumonia in children. In general, antibiotic-resistant bacteria are thought to have the phenomenon of fitness cost [30]. However, in a recent report, Rieux et al. [31] showed that an avirulent antibiotic-resistant pneumococcal strain acquired virulence after 1 passage in vivo. Compensatory mutation in resistant pneumococci might be one way to explain why there were increased incidences of invasive infection and pulmonary complication caused by PNSP [11, 32]. Active surveillance of the molecular epidemiology of PNSP in invasive disease will help us to understand whether the PNSP has acquired not only a high transmission rate, but also high clinical virulence. On the other hand, the currently licensed heptavalent pneumococcal conjugate vaccine is documented to be effective in reducing pneumococcal pneumonia in children [33]. In our study, this vaccine could cover at least 25 (66%) of 38 serogroups causing pneumonia. We believe the administration of conjugate vaccines will substantially reduce pneumococcal pneumonia among children in Taiwan.

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