Increased Rates of Intense Nasopharyngeal Bacterial Colonization of Vietnamese Children with Radiological Pneumonia

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ANH, D.D., HUONG, P.L.T., WATANABE, K., NGUYET, N.T., ANH, N.T.H, THI, N.T., DUNG, N.T., PHUONG, D.M., TANIMURA, S., OHKUSA, Y., NAGATAKE, T., WATANABE, H. and OISHI, K. Increased Rates of Intense Nasopharyngeal Bacterial Colonization of Vietnamese Children with Radiological Pneumonia. Tohoku J. Exp. Med., 2007, 213 (2), 167-172 — Acute lower respiratory infection (ALRI), primarily pneumonia, is the leading cause of death in children under the age of five. Bacterial ALRI is preceded by asymptomatic bacterial colonization. Bacterial colonization, therefore, may have an important role in the development of pneumonia in children. This case-control study was conducted in order to determine if intense bacterial colonization was increased in the nasopharynx of pediatric patients with ALRI. One hundred-sixty four pediatric patients with ALRI and 70 healthy children < 5 years of age were enrolled in Hanoi, Vietnam between 2001 and 2002. Bacterial pathogens were isolated from nasopharyngeal secretions and quantitatively cultured. Of 164 patients, 91 were diagnosed as having radiological pneumonia (PN group) and 73 as having acute bronchitis (AB group). Intense growth of any bacterial pathogen (≥ 10⁶ colony-forming units/ml) was highest in the PN group (49.4%), followed by the AB group (28.8%), with healthy children having the lowest (17.1%). Patients with intense bacterial growth were more likely to develop pneumonia, but not acute bronchitis, than were patients with light or no bacterial growth. The results of this case-control study suggest that the vertical spread of intense bacterial pathogens colonized in the nasopharynx to the lower airway leads to bacterial pneumonia in children under the age of five. ——— radiological pneumonia; children; bacterial colonization; Vietnam; Streptococcus pneumoniae

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Acute lower respiratory infection (ALRI), primarily pneumonia, is the leading cause of death in children under the age of five. A recent report indicated that worldwide 1.9 million children died from ALRI in 2000, world-wide and that 70% of these deaths occurred in Africa and Southeast Asia (Williams et al. 2000). The two most common bacterial pathogens associated with pneumonia are *Streptococcus pneumoniae* and *Haemophilus influenzae* (World Health Organization 1991; Factor et al. 2005; Watanabe et al. 2005).

In children, pneumococcal disease is preceded by asymptomatic bacterial colonization (Parry et al. 2002; Bogaert et al. 2004). Bacterial colonization, therefore, plays a central role in pneumococcal diseases, and may provide the basis for the vertical spread of pneumococci as well as other invasive diseases (Bogaert et al. 2004). A previous study reported a high positive rate of pneumococcal colonization during otitis media caused by pneumococci, and an increased tendency of pneumococcal colonization during viral respiratory infections (Syrjanen et al. 2001). Another study also reported a mildly increased rate of bacterial colonization in children with radiological pneumonia, compared with control children (Levine et al. 2000). To date, however, the association between intense bacterial colonization and the occurrence of pneumonia in children has not been examined. We hypothesized that an intense bacterial colonization rather than a light colonization in the nasopharynx might spread into the lower airway and develop bacterial pneumonia in children. Therefore, this case control-study was designed to examine if intense nasopharyngeal bacterial colonization was increased in pediatric patients with under 5 years of age with ALRI in Hanoi, Vietnam.

**Material and Methods**

The subjects for this study were chosen from among pediatric patients with ALRI enrolled at either the National Pediatric Hospital or the Bach Mai Hospital, which are the tertiary hospitals in Hanoi, Vietnam between January 2001 and December 2002. Inclusion criteria were: 1) age < 5 years of age and a diagnosis of ALRI made within 24 hrs of admission; 2) clinical symptoms of a productive cough, fast breathing and a fever (body temperature) > 37.5°C; and, 3) the finding of crackles in the lung fields by auscultation of the lung. The body weight on admission, preceding episodes of acute upper respiratory infections (AURI) and prior antibiotic use before the onset of ALRI were recorded. Exclusion criteria were: 1) age > 5 years, 2) illness of a non-infectious etiology; and, 3) failure to consent to study participation.

Laboratory examinations involved a chest radiograph (PA view). Currently, the best available method for diagnosing pneumonia is radiography. Chest radiographs showing recent infiltrations are indicative of pneumonia, while the absence of infiltrations warrants a diagnosis of acute bronchitis. The patients with ALRI were, therefore, classified into the pneumonia (PN) and the acute bronchitis (AB) groups. Radiological findings by the World Health Organization Radiology Working Group, such as “a dense or fluffy opacity that occupies a portion or the findings whole of a lobe or the entire lung that may or may not contain air-bronchogram” were used (Cherian et al. 2005). All of the radiological films were examined by three experts who were blind to the clinical presentation—a radiologist, a pediatrician and a pulmonary physician. Seventy healthy children, from a local nursery school in Hanoi, were enrolled as the control (CT) group. None of the study participants had previously received a pneumococcal conjugate vaccine or a *Haemophilus influenzae* type b (Hib) vaccine before being enrolled in this study. All study procedures were approved by the Institutional Review Boards of National Institute of Hygiene and Epidemiology. Parents or guardians of all patients and healthy children provided written informed consent.

A quantitative culture and Gram’s stain were performed simultaneously on nasopharyngeal secretions transnasally obtained from ALRI patients and from healthy children using two flexible swabs (Transwab®, Medical Wire & Equipment Co., Ltd., Wiltshire, England). This Rayon swab is non-toxic to microorganisms and can provide both good absorption and retrieval of specimen. After weighing the nasopharyngeal swab samples on a microbalance, the volume of the sample was determined to be approximately 0.01 ml. Samples were diluted in Brain Heart Infusion broth (BBL, Becton Dickinson, Cockeysville, MD, USA), and ten-fold dilution was then prepared in saline, as described previously (Utsunomiya et al. 1998; Yoshimine et al. 2001). The
quantitative bacterial culture was carried out on trypti-case soy agar (BBL, Becton Dickinson) containing 7% defibrinated rabbit blood and incubated in a 5% CO₂ incubator at 37°C overnight. The limitation of this bacterial quantitation method is \(10^3\) colony-forming units (CFU)/ml. The results of the quantitative culture of nasopharyngeal swab samples were classified into three categories: a) intense growth (\(\geq 10^6\) CFU/ml) of any bacterial pathogen; b) light growth (< \(10^6\) CFU/ml and \(\geq 10^3\) CFU/ml) of any bacterial pathogen; and, c) no bacterial growth (< \(10^3\) CFU/ml).

**Statistical analysis**

Age, body weight and the frequency of preceding episodes of AURI were compared between the cases (the PN group and the AB group) and the CT group or between the PN group and the AB group using the Mann-Whitney’s U-test. Differences in the isolation rates of bacterial pathogens were compared between the cases (PN vs AB group), and the CT group and cases (PN, AB) using the \(\chi^2\) statistic. Odds ratios (OR) were calculated by comparing the PN or AB groups with the CT group. ORs with 95% confidence intervals that do not include 1.00 were considered statistically significant. Data were considered to be statistically significant at \(p\)-value < 0.05. The initial target sample sizes were chosen to ensure that there would be at least an 80% chance of detecting a 20% difference (i.e., 40% vs 20%) in the rates of nasopharyngeal bacterial colonization between patients and controls, using a two-sided test at a \(p\)-value of 0.05, according to previously published studies (Bogaert et al. 2004). Thus, the target sample size was 82 patients (PN and AB groups) and 82 controls (CT group).

**RESULTS**

Among the 220 patients with ALRI, 160 patients were subsequently enrolled and classified as the PN group (\(n = 91\)) and the AB group (\(n = 73\)). Fifty-six patients could not be diagnosed because chest radiographs either were unavailable or of poor quality. Males comprised 62.5% of the PN group, 72.6% of the AB group and 51.4% of the CT group (\(n = 70\)). Sixty-two patients (68.1%) of the PN group and 53 patients (72.6%) of the AB group had prior antibiotic use, respectively. Most of these were oral antibiotics. The mean ages (± s.d., months) were: 14.6 ± 11.8 for the PN group, 12.4 ± 9.4 for the AB group, and 15.0 ± 9.4 for the CT group. No significant age differences were found between cases (PN and AB) and controls (CT) (\(p = 0.11\)) or between the PN and AB groups (\(p = 0.40\)). No significant difference was found between the PN and AB groups with respect to demographic characteristics on admission, the mean ± s.d. for body weight (8.8 ± 2.4 kg for PN, 8.5 ± 2.1 kg for AB; \(p = 0.917\)) or the frequency of preceding episodes of AURI (91.2% for PN, 93.2% for AB; \(p = 0.801\)).

Intense growth of any bacterial pathogen was highest in the PN group (49.4%), followed by the AB group (28.8%), with the CT group having the lowest (Table 1). A significant difference was found in the frequency of intense growth of potential pathogens between the cases (PN and AB) and the controls (CT) (\(p = 0.011\)) or between the PN group and AB groups (\(p = 0.001\)) and between the PN group and AB groups (\(p = 0.012\)).

### Table 1. Frequencies of intense, light and no growth of potential pathogens in the nasopharynx from patients with acute lower respiratory infections and healthy children.

<table>
<thead>
<tr>
<th>Bacterial colonization</th>
<th>Pneumonia ((n = 91))</th>
<th>Acute bronchitis ((n = 73))</th>
<th>Control ((n = 70))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. case (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any potential pathogen with a heavy growth</td>
<td>45 (49.4)</td>
<td>21 (28.8)*</td>
<td>12 (17.1)**</td>
</tr>
<tr>
<td>Any potential pathogens with a light growth</td>
<td>8 (8.8)</td>
<td>11 (15.1)</td>
<td>4 (5.8)</td>
</tr>
<tr>
<td>No growth</td>
<td>38 (41.8)</td>
<td>41 (56.1)</td>
<td>54 (77.1)</td>
</tr>
</tbody>
</table>

**\(*p = 0.001\) (vs Pneumonia and Acute bronchitis).**

**\(**\(*p = 0.012\) (vs Pneumonia).**
In contrast, no significant differences were found in the frequency of light growth or in the absence of bacterial growth among the three groups. Patients with intense bacterial growth were more likely to develop pneumonia, but not acute bronchitis, than were patients with light or no bacterial growth (Table 2). The pathogens most likely to exhibit intense growth in the PN group were *H. influenzae* and *S. pneumoniae*, followed by *Moraxella catarrhalis* (Table 3). No statistical significance was found in the frequency of a particular potential pathogen with intense growth among the three groups. In addition, isolates of *S. pneumoniae* and *H. influenzae* were highly resistant to β-lactam antibiotics. While most of pneumococci were genotypic penicillin-resistant *S. pneumoniae*, possessing altered *pbp 1a + 2x + 2b* genes (Watanabe et al., in press), most of *H. influenzae* strains had TEM-1 type β-lactamase gene (Watanabe et al. 2005).

### Table 2. Association between intense growth of potential bacterial pathogens and the group of pneumonia or acute bronchitis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>91</td>
<td>4.73</td>
<td>2.24, 9.96</td>
</tr>
<tr>
<td>Acute bronchitis</td>
<td>73</td>
<td>1.95</td>
<td>0.88, 4.35</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval.

### Table 3. Isolation rates of potential bacterial pathogens exhibiting intense nasopharyngeal growth in patients with acute lower respiratory infections and healthy children.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pneumonia (n = 91)</th>
<th>Acute bronchitis (n = 73)</th>
<th>Control (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heamophilus influenzae</strong></td>
<td>24 (26.3)</td>
<td>10 (13.7)</td>
<td>4 (5.7)</td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td>18 (19.8)</td>
<td>13 (17.8)</td>
<td>7 (10.0)</td>
</tr>
<tr>
<td><strong>Moraxella catarrhalis</strong></td>
<td>7 (7.7)</td>
<td>1 (1.7)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>0 (0)</td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Escherichiae coli</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.4)</td>
</tr>
<tr>
<td><strong>Enterobacter cloacae</strong></td>
<td>1 (1.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Klebsiella ozaenae</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.4)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this case-control study, a significant association between the increased frequency of intense bacterial growth and the PN group was found. Isolated bacterial pathogens, therefore, do not suggest the etiologies of ALRI. These data suggest that the vertical spread of bacteria pathogens from the nasopharynx to the lower respiratory tract subsequently might cause bacterial pneumonia. Furthermore, recent studies reported that strain-specific IgG found in sera plays a critical role in the recurrent exacerbation of chronic obstructive pulmonary diseases, and that the strain-specific protective immune response confers susceptibilities to infections by other strains of the same bacterial species (Sethi et al. 2002, 2004). Taken together, our present data suggest that a higher load of bacterial colonization, therefore, leads to bacterial pneumonia, especially in non-immune hosts to the certain bacterial strain found in the upper airway.

More than 90% of patients with ALRI had the preceding episodes of AURI in this study. Since the frequency of nasopharyngeal pneumococcal colonization was increased in children with viral respiratory tract infections (Syrjanen et al. 2001), the patients in the present study may have developed radiological pneumonia associated with viral upper respiratory infections. This possibility is supported by a scenario in which respiratory virus-infected epithelial cells facilitate
pneumococcal adherence, resulting in enhanced bacterial growth in the nasopharynx and the development of bacterial pneumonia in these children (Ishizuka et al. 2003; Madhi et al. 2004; Poltola et al. 2005).

A significant association between intense bacterial growth of a particular bacterial strain, such as *S. pneumoniae* or *H. influenzae*, and the development of pneumonia was not observed in the present study. No difference was also found in the isolation rates of pneumococci exhibiting an intense growth in between the groups of PN and AB. This may be, in part, explained by the underestimation of the burden of pneumococcal pneumonia diagnosed by the standardized definition of “radiologically-confirmed pneumonia” (Madhi and Klugman 2007). Therefore, additional studies are required to determine whether intense bacterial colonization of a particular pathogen in the nasopharynx is associated with progression to pneumonia in children. The influence of prior antibiotic use on results for bacterial growth in the nasopharynx of pediatric patients with ALRI may occur. Syrjanen et al. (2001) demonstrated that oral antibiotics prior to sampling reduced pneumococcal carriage only temporarily. Since most of patients with ALRI had oral antibiotics before admission in this study, the isolation rates of bacterial pathogens from the PN and AB groups, but not the CT group, may have been reduced by prior antibiotic use.

In summary, the novel finding of the present study was the association between intense nasopharyngeal bacterial colonization and the presence of radiological pneumonia in children. This result suggests that intense bacterial colonization plays a role in the development of pneumonia in children under the age of five.

## Acknowledgments

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