Case Report

A mixed bacterial infection of a bronchogenic lung cyst diagnosed by PCR

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An unusual paediatric case of a bronchogenic cyst infected with both Haemophilus influenzae type b and Streptococcus pneumoniae is described, which was detected not by culture of the purulent cyst fluid, but by real-time PCR amplification for several potential pathogens of DNA extracted from the fluid.

Case report

A 6-year-old girl was referred to Chiba University Hospital because of fever and left epigastric pain. She had not been vaccinated against Haemophilus influenzae type b (HIB) or Streptococcus pneumoniae. She had been hospitalized at 3 years of age for treatment of a left lung abscess with parenteral antibiotic therapy and percutaneous drainage. Culture of the abscess fluid, collected prior to the administration of antibiotics, revealed the presence of S. pneumoniae. Although her symptoms resolved with treatment, a chest radiograph and computed tomography (CT) scan obtained just before discharge from the hospital revealed a residual cystic mass in the superior segment of the inferior lobe (S6) of the left lung. Although the cystic lesion persisted for 3 years, she remained asymptomatic until her second hospital admission.

On admission, the patient’s axillary temperature was 38.9 °C, breath sounds were diminished over the left lower lung field, and coarse crackles were heard upon auscultation. Her white blood cell count was elevated to 25 100 mm−3 with 90 % neutrophils, and her C-reactive protein (CRP) was 8.7 mg dl−1 (upper limit of normal, 0.3 mg dl−1). A chest radiograph and CT scan showed an air-fluid level within the cyst, which was 6 cm in diameter and circumscribed with a thick wall, in the S6 segment of the left lung (Fig. 1a).

Transthoracic aspiration of the cyst using ultrasonographic guidance failed to yield fluid for culture or other tests. Empiric antimicrobial therapy was started with panipenem-betamipron (60 mg kg−1 per day), which is a parenteral carbapenem available commercially only in Japan, and the patient’s symptoms and laboratory values improved within 2 days. Blood, sputum and nasopharyngeal culture performed on the day of admission was negative. Over the 10 days of antibiotic therapy, the patient was afebrile, and her serum CRP dropped to 0.5 mg dl−1, but the fluid volume in the cyst increased gradually (Fig. 1b).

The patient was referred for surgical treatment, and on hospital day 26, a left lower lobectomy was performed under general anaesthesia through a lateral thoracotomy (Fig. 2). The patient’s recovery was uneventful and she was discharged on postoperative day 10.

Histology of the specimen showed ciliated epithelium on the lumen of the cyst, and the pathologic diagnosis was bronchogenic cyst.

A white–brown purulent fluid was aspirated from inside the extracted cyst (Fig. 2). No micro-organism was observed by Gram or Ziehl–Neelsen staining. Cultures of the fluid on blood and chocolate agar, and in BacT/Alert (bioMérieux) blood-culture bottles incubated under aerobic and anaerobic conditions were negative. Culture for mycobacteria was negative.

An aliquot of the purulent fluid (stored at −20 °C) was sent to the Gifu University Department of Microbiology for molecular diagnosis by PCR. PCR assays for several pathogens associated with respiratory tract infections have been developed. The nucleotide sequences of the primers used in this study are listed in Table 1. DNA was extracted

Abbreviations: CRP, C-reactive protein; CT, computed tomography; HIB, Haemophilus influenzae type b.

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with the MORA-EXTRACT kit (Kyokuto) according to the manufacturer’s instructions. Conventional PCR performed on the extracted DNA was positive for *H. influenzae* (16S rRNA gene) and *S. pneumoniae* (lytA gene), but negative for *Bordetella pertussis*, *Chlamydia sp.*, *Legionella sp.*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae* and *Staphylococcus*. In addition, HIB cap genes were identified with primers that amplified HIB-specific DNA. The *S. pneumoniae* gene encoding pneumolysin (ply) was also detected by conventional PCR.

For detection and quantification of HIB- and *S. pneumoniae* (lytA)-specific DNA, real-time PCR assays were performed in an iCycler iQ system (Bio-Rad Laboratories) using SYBR green. The amplification protocol consisted of an initial incubation of 5 min at 95°C, followed by 40 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, with Ex *Taq* DNA Hot Start polymerase (TaKaRa Bio). The specificity of the assays was assessed by testing with nucleic acid extracts from related organisms and a variety of micro-organisms commonly isolated from respiratory specimens. No signal in the HIB or *S. pneumoniae* (lytA) real-time PCR was observed with any of the control organisms tested. For the preparation of each external standard DNA, *H. influenzae* ATCC 10211 (HIB) or *S. pneumoniae* ATCC 49619 was used. The bacteria were suspended in Tris/EDTA buffer to a density of 0–5 McFarland units. DNA was extracted as described above by means of the MORA-EXTRACT kit. Six dilutions from $10^8$ to $10^3$ organisms ml$^{-1}$ were prepared and run in duplicate as external standards in parallel.

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**Fig. 1.** Chest radiograph performed on the day of admission, showing a cystic lesion with air-fluid level in the left lower lobe (a), and chest radiograph following the 17 days of antibiotic therapy, showing increased fluid level within the cyst (b).

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**Fig. 2.** Resected left lower lobe including the cyst. The cyst was $8 \times 8 \times 6.5$ cm in size, situated in the S6 segment. Abundant white–brown purulent fluid was obtained by aspiration from the inside of the extracted cyst.
with the test DNA. To generate a standard curve, the
threshold cycle (Ct) of the standard dilutions was plotted
against the organism cell numbers. The linear correlation
\( r^2 \) coefficients between the Ct values and organism cell
numbers were 0.998 and 0.996 for the HIB and
\textit{S. pneumoniae} PCR targets, respectively. The slopes were
2.3 \( \times \) 506 and 2.3 \( \times \) 983 for HIB and
\textit{S. pneumoniae} PCR targets, respectively. The real-time quantitative measure-
ments indicated that the cyst fluid contained amounts of
DNA equivalent to 1 \( \times \) 1 \( \times \) 10^7 and 1 \( \times \) 2 \( \times \) 10^6 organisms ml^{-1}
of specimen for HIB and \textit{S. pneumoniae}, respectively. The
sizes and the melting curves of the PCR products were as
expected, based on the primer designs. In addition, the PCR
products were sequenced using the Big Dye-Terminator
Cycle Sequencing kit (Applied Biosystems). Samples were
analysed on an ABI Prism 3100 DNA genetic analyser
(Applied Biosystems). In each instance, the sequence of the
PCR product was identical to the sequence of the reference
strain and confirmed the identity of the pathogens present in
the cyst fluid.

**Discussion**

Bronchogenic cysts account for 14–22\% of congenital
cystic diseases of the lung, and often present with infectious
complications in older children and adults (Lierl, 1993;
Laberge \textit{et al.}, 2004). However, pathogens of the infected
cysts frequently remained unidentified. Ribet \textit{et al.} (1996)
reported that culture of the purulent contents of broncho-
genic cysts revealed \textit{Streptococcus} sp., pneumococcus and
\textit{Escherichia coli} in each of three cases, and there are
two reports of \textit{H. influenzae} infection of an existing cyst
(Klapper & Sherman, 1986; Wewers \textit{et al.}, 1982). Recently,
\textit{Mycobacterium} species and fungus have been identified
in infected cysts (Minami \textit{et al.}, 2004). Nevertheless, the
pathogenic organisms are often unidentified in the cases of
infected cysts, and the frequency of infection by specific
pathogens is not clear.

\textit{S. pneumoniae} and capsulated \textit{H. influenzae} are both impor-
tant causes of bacteraemia and meningitis. In Japan, HIB is
still responsible for serious disease because HIB vaccines
are not currently used. These organisms are usually found
colonizing the upper airway, and occasionally result in
disseminated bloodstream infection. In the case reported
here, culture of blood, sputum and nasopharyngeal speci-
mens was negative. On the basis of quantitative real-time
PCR analysis, however, we concluded that the inflamma-
tion was due to simultaneous infection with HIB and
\textit{S. pneumoniae}. We were careful to avoid cross-contamination
and sample carryover by taking standard precautions,
including performing pre- and post-PCR procedures in
separate rooms. DNA amplification was performed with
primers targeting two different genes, 16S rRNA and the
cap region for HIB, and \textit{lytA} and \textit{ply} for \textit{S. pneumoniae}. In
addition, the specificity of the PCR products was confirmed
by sequence analysis.

In cases of purulent lung cyst, the source of bacterial
infection is often difficult to identify by culture because

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**Table 1. Oligonucleotide PCR primers used in this study**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Gene</th>
<th>Primer name</th>
<th>Sequence (5′–3′)</th>
<th>Product size (bp)</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>\textit{Haemophilus influenzae}</td>
<td>16S rRNA</td>
<td>HI 16S-F</td>
<td>GGAATCTGGCTTATGGAG</td>
<td>336</td>
<td>This study</td>
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<td></td>
<td></td>
<td>HI 16S-R</td>
<td>CACATCAACCTTCTCACA</td>
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<td>\textit{H. influenzae} type b</td>
<td>cap</td>
<td>Hib cap-F</td>
<td>GATACCTTAATTCGCGGCTGCCTCAT</td>
<td>152</td>
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<td>Hib cap-R</td>
<td>ATATCTGCACATCGTGTCCGACAC</td>
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<td>\textit{Streptococcus pneumoniae}</td>
<td>lytA</td>
<td>Sp lytA-F</td>
<td>AACCATAATGCAAGTACAC</td>
<td>429</td>
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<tr>
<td></td>
<td></td>
<td>Sp lytA-R</td>
<td>ATCATGTAAACCTGCTCAC</td>
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<td></td>
</tr>
<tr>
<td>\textit{S. pneumoniae}</td>
<td>ply</td>
<td>Sp ply-F</td>
<td>TGCAGAGGCCCTTTTGTCTAT</td>
<td>81</td>
<td>Corless \textit{et al.} (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sp ply-R</td>
<td>CTCTTACTCGTGGTTCCAACTTGA</td>
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<tr>
<td>\textit{Bordetella pertussis}</td>
<td>prn</td>
<td>Bpt-F</td>
<td>CATCCGACCTGAAACAC</td>
<td>427</td>
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</tr>
<tr>
<td></td>
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<td>Bpt-R</td>
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<td>\textit{Chlamydia phila} sp.</td>
<td>16S rRNA</td>
<td>Chp-F</td>
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<td></td>
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<td>Chp-R</td>
<td>CAGTCCGACCTGACATTG</td>
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<tr>
<td>\textit{Coxiella burnetii}</td>
<td>groEL</td>
<td>Cob-F</td>
<td>CGTAAAGAATCGGTAATTG</td>
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<td>Cob-R</td>
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<tr>
<td>\textit{Legionella} sp.</td>
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<td>Leg-F</td>
<td>GAGTAACCGGCTAGAAATA</td>
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<td>Leg-R</td>
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<td>\textit{Mycoplasma pneumoniae}</td>
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<td>Mpn-R</td>
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<td>\textit{Staphylococcus} sp.</td>
<td>16S rRNA</td>
<td>Sta-F</td>
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<td></td>
<td></td>
<td>Sta-R</td>
<td>GGACCGTGTCTCAGTTCAA</td>
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</table>
of previous or concurrent administration of antibiotics. Therefore, PCR analysis for several likely pathogens should be considered for culture-negative specimens.

To our knowledge, this is the first reported case of a bronchogenic cyst coinfected with HIB and S. pneumoniae that has been diagnosed by quantitative real-time PCR.

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References


