**Chlamydia pneumoniae** in Children with Otitis Media

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In this study the polymerase chain reaction was used to test for the presence of *Chlamydia pneumoniae* DNA in 118 middle–ear aspirates from 20 children with acute otitis media (AOM) and 53 children with otitis media with effusion (OME). *C. pneumoniae* was detected in 8 samples obtained from 5 children with OME and, together with *Streptococcus pneumoniae*, in a sample from 1 child with AOM. The mean age of these five children (6.6 ± 1.4 years) was significantly higher than that of the 48 children with OME in whom *C. pneumoniae* could not be detected (4.3 ± 1.9 years). The presence of *C. pneumoniae* in 9.4% of the examined children with OME suggests that *C. pneumoniae* might be a significant supplementary factor in the etiology of this common children’s disease.

Otitis media is one of the most common recurrent infectious diseases in children. By the age of 3 years ~75% of all children will have experienced at least one episode of acute otitis media (AOM), and more than one-third of these children will have recurrent infections. A bacterial pathogen can be isolated from the middle ear fluids of approximately two-thirds of all children with AOM and one-fifth of the children with otitis media with effusion (OME) [1]. Pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and * Branhamella catarrhalis* found in both AOM and OME are common in upper respiratory tract infections [1].

*Chlamydia pneumoniae* has been recognized as a pathogen of respiratory tract infections. It causes 5%–10% of all cases of community-acquired pneumonia and bronchitis [2], and has also been reported as a pathogen in upper respiratory tract infections such as pharyngitis and sinusitis [3]. In a Japanese study, *C. pneumoniae* was isolated from the middle–ear aspirates obtained from two children with otitis media [4], and otitis has been reported in patients with pneumonia caused by *C. pneumoniae* [5, 6]. Thus, it is possible that *C. pneumoniae* may cause otitis media. Consequently, this study aimed to assess the frequency of *C. pneumoniae* in middle–ear aspirates from children with AOM and OME.

**Materials and Methods**

**Subjects**

The study comprised 118 middle–ear aspirates, of which 26 were obtained from 20 children with AOM and 92 were obtained from 53 children with OME. Each child was included only once in the study, but some children were bilaterally infected. AOM and OME were defined by the presence of fluid in the middle ear, with and without signs or symptoms of acute infection, respectively [7]. The children were attending a county hospital clinic or a private ear–nose–throat clinic. Informed consent was given by the parents, and the study was approved by the local committee for ethics and science.

Characteristics of the patients are shown in table 1. None of the children studied had other chronic diseases or immunodeficiencies. Fifteen children had received an antibiotic within 14 days before the investigation, including amoxicillin (n = 8), phenoxymethylpenicillin (n = 3), erythromycin (n = 3), and amoxicillin/clavulanic acid (n = 1).

The samples were collected between June 1994 and August 1995. They were taken at the time of tympanocentesis in cases of AOM or placement of ventilation tubes in cases of OME. The middle–ear fluid was aspirated aseptically into a syringe.

**Culture of Bacteria**

Bacterial cultures were done on 5% horse blood agar, chocolate agar, MacConkey agar, and anaerobe agar from Statens Serum Institut (Copenhagen).

**C. pneumoniae PCR**

The first 85 samples were analyzed by method 1 (see below), and only the positive samples were retested, by method 2. The last 33 samples included in the study were analyzed by both methods in two different laboratories with use of different extraction procedures. Only samples positive by both methods were considered positive.
in at least one of two duplicates were considered positive. Inhibition of the PCR was detected by adding a known amount of Chlamydia trachomatis DNA (~100 inclusion-forming units) to the pretreated specimens, followed by analysis by a quantitative PCR-TRF for detection of C. trachomatis [10].

Method 2. The middle-ear secretion was suspended in 1.3 mL of sucrose/phosphate-buffered Chlamydia transport medium 2SP. DNA was released by boiling 100 μL of the suspension for 10 minutes in 300 μL of a 20% (w/v) Chelex 100 slurry (Bio-Rad, Hercules, CA) [11]. Twenty-five μL of the pretreated specimen was subjected to PCR using primers 1A/1B [12] and analogous mixed primers matching the 16S rRNA gene of other Chlamydia species. An internal processing control containing a 616-bp fragment of phage lambda DNA flanked by primers 1A/1B was used in order to detect inhibition of the PCR. A “touchdown” procedure [13] was used, with a 1°C decrement of the annealing temperature for the first 10 cycles. A total of 50 cycles were performed. Amplified material was labelled during PCR with digoxigenin-11-dUTP (Boehringer-Mannheim, Germany). Amplicons were visualized by staining with ethidium bromide after agarose gel electrophoresis.

In order to lower the detection limit and to differentiate between the three chlamydial species, a liquid hybridization assay using biotinylated species-specific probes was used. All C. pneumoniae PCR positive results were confirmed by repeated testing of an aliquot of the pretreated specimen in a C. pneumoniae–specific PCR, with primers CpnA/CpnB [14], which detected a 463-bp fragment of the 16S rRNA sequence. The PCR thermal cycling protocol was identical to the protocol used for the Chlamydia-specific PCR. Amplicons were detected only by agarose gel electrophoresis. Strict precautions were taken throughout the procedure in order to minimize the risk of PCR product carryover, as previously described [15].

Statistics

Significance of differences between children with and without C. pneumoniae infection was estimated with the Mann-Whitney rank-sum test.

Results

C. pneumoniae was detected in nine of the 118 middle-ear aspirates. All nine samples positive for C. pneumoniae DNA by method 1 were also found positive by method 2. The results were concordant for the 33 samples tested in parallel by both methods. Among these 33 samples, two were positive. Substances inhibitory to PCR method 1 were found in three samples.

C. pneumoniae was found in six of the 73 children. Five of the 53 children with OME (9.4%) and one of the 20 children with AOM (5%) had C. pneumoniae in their aspirates. Three of the six children with C. pneumoniae were boys, and
Table 2. Bacteriologic findings in middle-ear aspirates from children with otitis media. *Chlamydia pneumoniae* was detected by PCR, and the other bacteria were detected by culture.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute otitis media</td>
</tr>
<tr>
<td>Total no./no. with bilateral otitis media</td>
<td>20/6</td>
</tr>
<tr>
<td>Bacterium detected</td>
<td></td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>14 (70%)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>5*</td>
</tr>
<tr>
<td><em>Streptococcus group A</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>6*</td>
</tr>
<tr>
<td>* Branhamella catarrhalis*</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Serratia liquefaciens</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1</td>
</tr>
<tr>
<td>Mixed organisms</td>
<td>1†</td>
</tr>
</tbody>
</table>

* The patient was infected with *C. pneumoniae* and *S. pneumoniae*.
† One of the patients had bilateral middle-ear effusions containing *H. influenzae*.
‡ Three patients had bilateral middle-ear effusions containing *H. influenzae*.
§ *H. influenzae* and *S. aureus* were detected in one ear and *H. influenzae* in the other ear.
†† The patient had infection with *S. pneumoniae* and *H. influenzae*.

were girls. Five of the six children with *C. pneumoniae* had bilateral effusions, and *C. pneumoniae* was found in samples from both ears in three of these children.

The mean (± SD) age of the five children with OME and *C. pneumoniae* was 6.6 ± 1.4 years, which was significantly higher than the mean age of the remaining children with OME, which was 4.3 ± 1.9 years (Mann-Whitney rank-sum test; *P* = .01). The mean duration of the illness in children with OME and *C. pneumoniae* was 4.2 months, vs. 4.8 months in children without *C. pneumoniae*—negative OME, the age of the children from whom samples were selected for testing by PCR can influence the detection rate significantly.

In our study 5 of 6 children with *C. pneumoniae* had OME, and 1 of the 6 children had AOM. The child with AOM had a mixed infection with *S. pneumoniae*. It has been suggested that upper respiratory tract infection caused by *C. pneumoniae* may facilitate invasion by other bacteria such as *S. pneumoniae* [17]. The child with the mixed infection had upper respiratory tract symptoms, including signs of OME before the onset of high temperature and otalgia.

Isolation of *C. pneumoniae* from two children with OME has previously been reported [4]. These children were 5 and 7 years old, which corresponds to the findings in our study. This age level is in accordance with a seroepidemiological study showing that the age-specific incidence of *C. pneumoniae* infections is highest among children in the age group of 5–9 years [18].

Under normal conditions the middle ear is dry and sterile, and in otitis media the bacteria are believed to derive from the nasopharyngeal area. *C. pneumoniae* has been isolated from the nasopharynx of children with lower respiratory tract infections [19], as well as of children and adults who were clinically asymptomatic [20]. This has led to the hypothesis that such individuals may be a reservoir for this organism [21]. Further studies are needed, however, to decide whether children with *C. pneumoniae* otitis media have been newly infected or whether they are carriers of *C. pneumoniae* in their nasopharynx.

Persisting infection with *C. pneumoniae* following acute respiratory tract infection has been reported [22]. Our data may indicate that *C. pneumoniae* might cause chronic inflammation in the tympanic cavity, and this could explain the chronic exudate that is characteristic of OME. A slowly progressive inflammation in the tympanic cavity may also be involved in the pathogenesis of late sequelae of recurrent otitis media, such as fibrosis and tympanosclerosis.

Our knowledge about the importance of the various bacterial infections in OME is still limited. Treatment with insertion of ventilation tubes is merely an unspecific approach to reveal the
symptoms. The finding of *C. pneumoniae* in OME points toward a new understanding of the pathogenesis in some of the cases. Further studies are needed, however, to fully describe the role of *C. pneumoniae* infections in otitis media, the potential implications, and the possible effect of a specific antibiotic treatment.

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**References**