Antimicrobial resistance in the nasopharyngeal flora of children with acute otitis media and otitis media recurring after amoxicillin therapy

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The objective of this study was to investigate the antimicrobial susceptibility of the organisms isolated from the nasopharynx of children who presented with acute otitis media (AOM) or otitis media that recurred after amoxicillin therapy. Nasopharyngeal cultures obtained from 72 patients, 40 with AOM and 32 with recurrent otitis media (ROM), were analyzed. Thirty-six potentially pathogenic organisms were recovered in 34 (85%) of the children from the AOM group, and 42 were isolated from 29 (91%) of the children from the ROM group. The organisms isolated were Streptococcus pneumoniae (n = 26), Haemophilus influenzae non-type b (n = 22), Moraxella catarrhalis (n = 13), Streptococcus pyogenes (n = 8) and Staphylococcus aureus (n = 9). Resistance to the eight antimicrobial agents used was found in 37 instances in the AOM group as compared to 99 instances in the ROM group (P < 0.005). The difference between AOM and ROM was significant with Streptococcus pneumoniae resistance to amoxicillin (P < 0.005), to amoxicillin/clavulanate (P < 0.005), to trimethoprim/sulfamethoxazole (P < 0.01), to cefixime (P < 0.01) and to azithromycin (P < 0.01), and for H. influenzae resistance to amoxicillin (P < 0.025). These data illustrate the higher recovery rate of antimicrobial-resistant Streptococcus pneumoniae and H. influenzae from the nasopharynx of children who had otitis media that recurred after amoxicillin therapy than those with AOM.

Methods
The study was a retrospective analysis of clinical microbiology data of nasopharyngeal cultures that were obtained between 1 September 1999 and August 2001 in a community clinic setting. The population studied was middle class, residing in suburban locations in the vicinity of Washington, DC. Included were all children who presented with AOM or recurrent otitis media (ROM) following amoxicillin therapy. All presented with clinical signs of active infection (i.e. fever, irritability) including opacified red–grey or yellow bulging tympanic membranes. AOM was defined as the presence of irritability, ear tugging and the presence of middle-ear effusion determined by pneumatic otoscopy. ROM was defined as an AOM episode that followed a previous ear infection by an infection-free interval of 4–6 weeks. All patients with ROM were treated with amoxicillin (45 mg kg⁻¹ day⁻¹) given twice a day for 14 days for their previous infection. Excluded were children who had serous ear effusion, otorrhea, tympanostomy tubes, craniofacial anomalies and chronic medical problems. Also excluded from the AOM group were those who had received antimicrobials in the past 3 months.

A total of 72 patients was studied, 40 with AOM and 32 with recurrent otitis media that presented with AOM or otitis media that recurred after amoxicillin therapy.
ROM. Patients ages ranged from 8 months to 4 years 11 months (mean age 2 years and 3 months) and 41 were males. No differences were noted in the age, gender, ethnic background, breast-feeding, second-hand smoking and day-care attendance distribution between the two groups. No siblings were allowed to be included in the study.

Nasopharyngeal cultures were obtained prior to administration of any therapy, using sterile calcium alginate swabs, and were immediately plated on to media supportive of growth of aerobic and facultative anaerobes. Sheep blood agar, chocolate agar and MacConkey’s agar plates were inoculated. The plates were incubated at 37°C aerobically (MacConkey’s) or under 5 % CO₂ and examined at 24 and 48 h. Potentially pathogenic organisms (Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes and Staphylococcus aureus) were identified by techniques described previously (Murray et al., 1999). β-Lactamase activity was determined for all isolates by the nitrocefin method (O’Callaghan et al., 1972).

MICs were determined for eight antimicrobial agents (Table 1) by the agar-dilution method with Mueller–Hinton agar (BBL Microbiology Systems) supplemented with 5 % sheep blood. The NCCLS breakpoints were used for MIC determination. For MIC determinations, suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml Mueller–Hinton broth (BBL Microbiology Systems). Suspensions were further diluted 1 : 10 to obtain a final inoculum of 10⁴ c.f.u. per spot. Plates were inoculated with a Steer’s replicator and incubated overnight in ambient air at 37°C. Standard quality control strains were included in each run. In addition, MICs of azithromycin were read after an additional 24 h incubation. Statistical analyses were done using the t-test and the chi-square test with continuity correction.

**Results**

Thirty-six potentially pathogenic organisms were recovered in 34 (85 %) of the children from the AOM group, and 42 were isolated from 29 (91 %) of the children from the ROM group. The organisms isolated were Streptococcus pneumoniae (26 isolates; 12 in the AOM group and 14 in the ROM group), H. influenzae non-type b (22 isolates; 10 in the AOM group and 12 in the ROM group), M. catarrhalis (13 isolates; 7 in the AOM group and 6 in the ROM group), Streptococcus pyogenes (8 isolates; 3 in the AOM group and 5 in the ROM group) and Staphylococcus aureus (9 isolates, 4 in the AOM group and 5 in the ROM group) (Table 1). All amoxicillin-resistant H. influenzae and M. catarrhalis strains produced β-lactamase.

Resistance to the eight antimicrobial agents used was found in 37 instances (of a total of 320 possibilities) in the AOM group as compared to 99 instances (of a total of 256 possibilities) in the ROM group (P, 0.005) (Table 1). The difference between AOM and ROM was significant with Streptococcus pneumoniae resistance to amoxicillin (P, 0.005), to amoxicillin/clavulanate (P, 0.005), to trimethoprim/sulfamethoxazole (P, 0.01), to cefixime (P, 0.01) and to azithromycin (P, 0.01), and for H. influenzae resistance to amoxicillin (P, 0.025) (Table 1).

**Table 1. Antimicrobial resistance of organisms isolated from nasopharynx of 40 children with AOM and 32 with ROM**

<table>
<thead>
<tr>
<th>Patient diagnosis</th>
<th>Streptococcus pneumoniae</th>
<th>Haemophilus influenzae</th>
<th>Moraxella catarrhalis</th>
<th>Streptococcus pyogenes</th>
<th>Staphylococcus aureus</th>
<th>TARI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of isolates</td>
<td>12 14</td>
<td>10 12</td>
<td>7 6</td>
<td>3 5</td>
<td>4 5</td>
<td>36 42</td>
</tr>
<tr>
<td>Antimicrobial resistance to:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>2 10†</td>
<td>3 9‡</td>
<td>7 6</td>
<td>0 0</td>
<td>0 3</td>
<td>12 28</td>
</tr>
<tr>
<td>Amoxicillin/clavulanate</td>
<td>2 10†</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 10</td>
</tr>
<tr>
<td>Trimethoprim/ sulfamethoxazole</td>
<td>3 7‡</td>
<td>2 4</td>
<td>1 2</td>
<td>2 3</td>
<td>1 1</td>
<td>9 17</td>
</tr>
<tr>
<td>Cefprozil</td>
<td>1 4</td>
<td>1 2</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 6</td>
</tr>
<tr>
<td>Cefuroxime/axetil</td>
<td>1 4</td>
<td>1 2</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>2 6</td>
</tr>
<tr>
<td>Cefdinir</td>
<td>2 6</td>
<td>0 1</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>1 2</td>
</tr>
<tr>
<td>Cefixime</td>
<td>2 9‡</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>3 4</td>
<td>5 13</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1 7‡</td>
<td>2 3</td>
<td>0 0</td>
<td>0 1</td>
<td>0 0</td>
<td>3 11</td>
</tr>
<tr>
<td>TARI*</td>
<td>14 57</td>
<td>9 21</td>
<td>8 8</td>
<td>2 4</td>
<td>4 9</td>
<td>37 99†</td>
</tr>
</tbody>
</table>

*TARI, Total antimicrobial resistance instances.
†P < 0.005 compared to AOM.
‡P < 0.01 compared to AOM.
§P < 0.025 compared to AOM.

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Discussion

These data illustrate the higher recovery rate of antimicrobial-resistant *Streptococcus pneumoniae* and *H. influenzae* from the nasopharynx of children who had otitis media that recurred after amoxicillin therapy as compared to those with AOM. The increased resistance is toward a broad range of antimicrobials that include amoxicillin, amoxicillin/clavulanate, trimethoprim/sulfamethoxazole, cefixime and azithromycin. Previous amoxicillin treatment might have selected these resistant strains and they could have persisted in the nasopharynx to re-emerge in the new ear infection.

These findings support previous reports where such a relationship was found in recovery of pathogens from middle-ear aspirates (Harrison et al., 1985; Teele et al., 1981; Dagan et al., 1996; Brook & Yocum, 1995; Brook & Gober, 1999). Harrison et al. (1985) illustrated a higher isolation of amoxicillin-resistant *H. influenzae* and *Staphylococcus aureus* in patients with recently treated or persistent otitis media compared to untreated AOM.

Brook & Gober (1999) evaluated the antimicrobial susceptibility of the pathogens isolated from the middle ear of 22 children with otitis media and from sinus aspirates of 20 patients with maxillary sinusitis who failed to respond to antimicrobial therapy, and correlated it with previous antimicrobial therapy. Resistance of at least two tube dilutions to the antimicrobial agents used was found in 23 of the 47 patients. These included 10 of 15 (67%) isolates of *Streptococcus pneumoniae*, 4 of 14 (29%) *H. influenzae*, 4 of 7 (57%) *Staphylococcus aureus* and 5 of 6 (83%) *M. catarrhalis*. A statistically significant higher recovery of resistant organisms was noted in those treated 2 to 6 months previously, and in those with sinusitis who smoked. The data illustrate the relationship between resistance to antimicrobials and failure of patients with otitis media and sinusitis to improve.

The major cause of decreased susceptibility to antimicrobials is the growing resistance of *Streptococcus pneumoniae*, *H. influenzae* and *M. catarrhalis* to a variety of antibiotics. *Streptococcus pneumoniae* has growing resistance to penicillin and other antimicrobials such as trimethoprim/sulfamethoxazole and macrolides (Ednie et al., 1997), and *H. influenzae* and *M. catarrhalis* are expressing their resistance mainly through the production of the enzyme β-lactamase (Ednie et al., 1997). Selection of antimicrobial agents can be improved by knowledge of the resistance patterns of the organisms in the community, and by consideration of the effect of previous antimicrobial therapy (Brook & Gober, 1984) or prophylaxis (Brook & Gober, 1996) that may select resistant strains. Increased resistance to several antimicrobials can be expected in children with ROM who have failed antimicrobial therapy. The selective use of tympanocentesis and aspiration of the middle-ear effusion for smear, culture and susceptibility studies, rather than the use of nasopharyngeal cultures, which are less specific, can be both diagnostic and therapeutic (Howie, 1993).

References


