Penicillin Susceptibility of Pneumococcal Isolates Causing Acute Otitis Media in Children
Seasonal Variation

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Background: During the past decade, the prevalence of nonsusceptible Streptococcus pneumoniae strains that cause acute otitis media (AOM) has increased to ~30%, and the proportion of fully resistant strains has also increased. The purpose of this study was to determine whether seasonal variation in resistance exists among pneumococcal isolates from children with AOM.

Methods: Between 1991 and 2003, children 2 months– 8 years of age diagnosed with AOM according to stringent criteria underwent tympanocentesis in various clinical trials.

Results: Cultures from 567 of 794 tympanocenteses (71.4%) performed between 1991 and 2003 yielded AOM pathogens. During 1991–1995, only 1 of 43 S. pneumoniae isolates recovered (2%) was nonsusceptible to penicillin. The present analysis focuses on the 691 cultures obtained during 1996 –2003; of these, 491 (71.1%) yielded AOM pathogens, of which 165 (33.6%) were S. pneumoniae. Of the pneumococcal isolates, 52 (31.5%) were nonsusceptible to penicillin. The proportion of nonsusceptible strains of S. pneumoniae increased over time: 0 of 3 (0%) in 1996; 2 of 11 (18%) in 1997; 14 of 40 (35%) in 1998; 3 of 34 (9%) in 1999; 11 of 25 (44%) in 2000; 11 of 22 (50%) in 2001; 4 of 18 (22%) in 2002; and 7 of 12 (58%) in 2003 (Cochran Armitage trend test, P = 0.03). AOM caused by nonsusceptible S. pneumoniae was more likely to occur as the winter progressed (P = 0.03); a similar trend was noted for the proportion of nonsusceptible strains that were fully resistant.

Conclusions: In children with AOM, an increase in the proportion of episodes caused by nonsusceptible S. pneumoniae as the winter months progress may serve as a potential factor in guiding antimicrobial therapy for such children.

Key Words: acute otitis media, Streptococcus pneumoniae, antimicrobial resistance

Streptococcus pneumoniae is one of the most common pathogens in children with acute otitis media (AOM), causing an estimated 7 million cases each year. Surveillance studies conducted in the late 1980s showed that fewer than 10% of pneumococcal strains were nonsusceptible to penicillin; however, through the mid-1990s, the prevalence of resistance grew to 20–30%. During this period, the overall degree of resistance also increased (based on the relative proportions of intermediate and resistant strains). A more recent outpatient surveillance study noted that 45% of pneumococcal isolates were nonsusceptible to penicillin [minimum inhibitory concentration (MIC), ≥0.1 μg/mL]; of these, ~16% were intermediate (MIC 0.1–1 μg/mL) and 29% were resistant (MIC ≥2 μg/mL).

The main concern regarding bacterial resistance is whether resistance increases the likelihood of treatment failure or complications. Clinical trials in children with AOM have shown that infection with resistant pneumococci does, in fact, increase the likelihood of clinical failure. Concern about pneumococcal resistance has resulted in a change in approach to the management of AOM, such that identification of children at risk for infection with resistant pneumococci has become an important determinant in the process of antimicrobial selection.

In 1999, the Drug-Resistant Streptococcus pneumoniae Therapeutic Working Group provided consensus recommendations for the management of AOM. These recommendations highlighted several factors that correlate with increased risk of infection with resistant S. pneumoniae, including young age (≤2 years), recent receipt of antimicrobial agents (within 90 days) and attendance at day care. The purpose of this study was to determine whether, in children with AOM,
seasonal variation in resistance exists among isolates of *S. pneumoniae*.

**MATERIALS AND METHODS**

As part of various clinical trials evaluating the efficacy of antimicrobials or vaccines in the management or prevention of AOM conducted at Children’s Hospital of Pittsburgh between 1991 and 2003, children 2 months–8 years of age (mean, 22.7 months) with AOM diagnosed according to stringent criteria underwent tympanocentesis for identification of the causative pathogen. An informed consent document approved by the Institutional Review Board was completed by parents of eligible children before enrollment in each study. In all studies, the same group of validated otoscopyists applied uniform, stringent criteria for diagnosing AOM. The criteria required: (1) the presence of middle ear effusion (evidenced by at least 2 of 3 tympanic membrane findings, namely, decreased or absent mobility, yellow or white discoloration and opacification not caused by scarring); and (2) at least 1 of 3 signs or symptoms of acute inflammation, namely, ear pain within the previous 24 hours, including unaccustomed tugging or rubbing of the ear; marked redness of the tympanic membrane; and distinct bulging of the tympanic membrane. Purulent otorrhea for less than 24 hours also constituted a sufficient criterion for diagnosing AOM. Children were further classified either as having “uncomplicated AOM” or as being “at risk for recurrent and/or persistent AOM.” The latter designation required that the child either had a history of recurrent AOM (at least 3 episodes in 6 months or 4 episodes in 12 months), had received an antimicrobial for AOM within the preceding 30 days, or was currently failing antimicrobial therapy.

Samples of middle ear fluid were obtained by diagnostic tympanocentesis before institution of antimicrobial therapy. Standard isolation procedures were used to identify *S. pneumoniae* in the microbiology laboratory of Children’s Hospital of Pittsburgh. Antimicrobial susceptibility values for *S. pneumoniae* were determined by broth microdilution methods following the guidelines of the National Committee for Clinical Laboratory Standards, using cation-adjusted Mueller-Hinton broth (Difco Laboratories, Detroit, MI) supplemented with 5% lysed horse blood. Definitions for susceptibility of isolates of *S. pneumoniae* were those recommended by National Committee for Clinical Laboratory Standards: penicillin MIC = 0.06 μg/mL for susceptible isolates and ≥0.1 μg/mL for nonsusceptible isolates. Nonsusceptible isolates were further classified as intermediate (penicillin MIC 0.1–1 μg/mL) or resistant (penicillin MIC ≥2 μg/mL). In May 2000, we began administering pneumococcal conjugate vaccine (PCV-7; Prevnar) to young children receiving primary care at the Children’s Hospital of Pittsburgh. Information on receipt of PCV-7 by individual children was obtained from a review of medical records.

We analyzed findings from all children diagnosed with AOM who underwent tympanocentesis during the survey period. For children who underwent >1 procedure, findings from succeeding procedures were included if at least 30 days had elapsed between procedures. When pneumococcal isolates were recovered from both ears in children undergoing bilateral tympanocentesis, only one of the isolates was included in the present analysis; when the susceptibilities of the 2 isolates differed, the more resistant isolate was selected for the analysis. We used the χ² test and Fisher’s exact test, when appropriate, to test for differences between proportions. We used the Cochran Armitage trend test to test for trends and logistic regression models to determine the effect of seasonality after adjustment for the remaining independent variables (age, gender, type of AOM episode, day-care attendance, failing antibiotic treatment and receipt of antibiotics within 30 days). We used 2-tailed tests for all analyses, and we set statistical significance at *P* < 0.05.

**RESULTS**

During the 1991–2003 study period, a total of 629 children with AOM underwent 794 unilateral or bilateral diagnostic tympanocenteses. Cultures of middle ear specimens yielded 1 or more AOM pathogens (*S. pneumoniae, Haemophilus influenzae, Moraxella catarrhalis or Streptococcus pyogenes*) in 567 (71.4%) of these instances. Pneumococcal isolates were recovered from 232 tympanocenteses in 208 children. During the period 1991 through 1995, 103 of the children underwent 103 tympanocenteses; only 1 of the 43 *S. pneumoniae* isolates recovered was nonsusceptible to penicillin. The remainder of the present analysis concerns the 528 children with AOM who underwent 691 diagnostic tympanocenteses between 1996 and 2003 (2 of whom had also undergone tympanocentesis during 1991–1995). Cultures of middle ear specimens yielded 1 or more AOM pathogens in 491 (71.1%) of these instances. A pneumococcal isolate was recovered in 165 of the children (31.3%); 52 (31.5%) of the 165 isolates were nonsusceptible to penicillin.

Overall ~60% of the episodes of AOM occurred during the late fall and winter months. The proportion of nonsusceptible strains of *S. pneumoniae* recovered from children with at risk for recurrent/persistent AOM episodes was significantly higher than the proportion recovered from children with uncomplicated AOM (*P* = 0.013) (Fig. 1). The proportion of episodes caused by nonsusceptible *S. pneumoniae* tended to increase over time, with variation attributable in part to the type(s) of study being conducted during a given period (ie, treatment of children with uncomplicated AOM or treatment of children at risk for recurrent/persistent AOM). The proportions of episodes caused by nonsusceptible *S. pneumoniae* were 1 of 43 (2.5%) during the period 1991–1995; 0 of 3 (0%) in 1996; 2 of 11 (18%) in 1997; 14 of 40 (35%) in 1998; 3 of 34 (9%) in 1999; 11 of 25 (44%) in 2000;
11 of 22 (50%) in 2001; 4 of 18 (22%) in 2002 and 7 of 12 (58%) in 2003 (Cochran Armitage trend test, \( P < 0.03 \)). In 1998, only a study evaluating children at risk for recurrent/persistent AOM was conducted.

When data were analyzed according to the months during which AOM episodes occurred, the proportion of episodes caused by strains of nonsusceptible pneumococci appeared to increase as the respiratory season progressed [Fig. 2A, May through December (12 of 66) versus January through April (40 of 99); \( P = 0.004 \)], both among children with pneumococcal isolates (Fig. 2A; test for trend, \( P = 0.03 \)) and in all children with AOM (Fig. 2B; test for trend \( P = 0.046 \)). Differences in the proportion of episodes caused by strains of nonsusceptible pneumococci between May through December and January through April remained significant when independent predictor variables [age, gender, day-care attendance, type of AOM episode, current or recent (within 3 months) receipt of antibiotics] were evaluated with the use of logistic regression models \( (P = 0.02) \). Furthermore the proportion of nonsusceptible pneumococcal strains that were resistant (as distinct from intermediate) was higher during the spring months (March–May) than during the winter months (December–February) \( (23 \text{ of } 26 \text{ versus } 11 \text{ of } 19; \ P = 0.03) \).

Comparisons of the proportion of children with AOM caused by nonsusceptible \( S.\ pneumoniae \) for the period May–December versus January–April within each individual year yielded numbers that were too small for meaningful analysis. The proportions of \( \beta \)-lactamase-producing isolates in children with AOM caused by \( H.\ influenzae \) and \( M.\ catarrhalis \) and in all children with AOM according to month of the year are presented in Figure 3. No significant trends for a seasonal variation in \( \beta \)-lactamase production were apparent \( (P = 0.69 \text{ and } P = 0.47, \text{ for Figs. 3A and 3B, respectively; Cochran Armitage trend test}) \.

There were no significant differences between the distribution of AOM pathogens \( (S.\ pneumoniae, H.\ influenzae, M.\ catarrhalis, S.\ pyogenes) \) isolated from children with AOM before (1996–2000), and the distribution after (2000–2003) the institution of routine immunization with PCV-7. Penicillin susceptibilities of \( S.\ pneumoniae \) isolates collected from children with AOM before (1996–2000) and after (2000–2003) the institution of routine immunization with PCV-7 are shown in Figure 4. A suggestive decrease in the proportion of susceptible strains of \( S.\ pneumoniae \) with a corresponding increase in resistant strains was observed after immunization was instituted \( (P = 0.07 \text{ for the comparison of susceptible versus intermediate versus resistant strains}) \.

There was no apparent relation between the number of doses of PCV-7 received and the likelihood of AOM caused by nonsusceptible strains, but the numbers of subjects involved were too small for meaningful analysis.
DISCUSSION

The apparently increasing prevalence worldwide of penicillin-resistant *S. pneumoniae* is an important public health problem. In the present analysis of data accumulated at Children’s Hospital of Pittsburgh during the past 12 years concerning the etiology of AOM, we also found a general trend for increasing proportions of AOM episodes to be caused by nonsusceptible strains of *S. pneumoniae*.

Temporal variations in the proportions of AOM episodes caused by nonsusceptible strains of *S. pneumoniae* have been noted in other clinical trials; however, the present analysis represents the first to our knowledge that specifically addressed seasonal variations in pneumococcal resistance in children with AOM. Our findings suggest that AOM is more likely to be caused by nonsusceptible strains of *S. pneumoniae* as the respiratory season progresses.

Seasonal variation in pneumococcal resistance may be related to day-care attendance. Children exposed to many other young children on a regular basis are more likely to experience viral respiratory infections than children not similarly exposed, and viral infections are well-established as predisposing factors for the development of AOM. Previous reports have documented a high degree of antimicrobial use during the winter months in children attending day-care

![Figure 3](image-url)
centers,11–13 and antimicrobial pressure in such settings has been shown to select for nonsusceptible strains of *S. pneumoniae*.14 It seems plausible that through the winter months, increasingly prevalent viral respiratory infections initiate a cycle of increasing numbers of AOM episodes and increasing antimicrobial treatment. This in turn may eventually select for resistant strains of *S. pneumoniae* that had not been prevalent earlier in the season but that then become more prevalent throughout the community.

The 7 serotypes of *S. pneumoniae* present in PCV-7 account for ~70% of all middle ear isolates of *S. pneumoniae* in children.15 An initial report by Black et al16 documented a 7% reduction in otitis media visits after the administration of PCV-7. Similarly Eskola et al17 reported reductions, after PCV-7 administration, of 6% in the number of AOM episodes overall, 34% in the number of pneumococcal episodes, 57% in the number of episodes caused by PCV-7 serotypes and 51% in the number of episodes caused by serotypes that are cross-reactive with those in the vaccine. At the same time, the number of episodes caused by all other pneumococcal serotypes increased by 33%, and the number caused by *H. influenzae* increased by 11%. Because most nonsusceptible strains of *S. pneumoniae* are serotypes contained in PCV-7, the expected effect of administration of the vaccine would be a modest reduction in AOM overall, with a potential shift in bacterial etiology toward relatively fewer episodes caused by nonsusceptible *S. pneumoniae* and more caused by *H. influenzae*. Our data suggest a slight increase in the proportion of AOM episodes caused by *H. influenzae* since introduction of the vaccine, but to date we have not documented a relative reduction in episodes caused by strains of nonsusceptible *S. pneumoniae*.

Intermittent shortages of PCV-7 have likely limited the full potential of the vaccine to reduce nasopharyngeal colonization with nonsusceptible vaccine-specific and vaccine-related strains and to reduce the occurrence of episodes of AOM caused by those strains. It seems likely that if increasing proportions of children receive the full schedule of PCV-7, one might observe a shift toward greater proportions of AOM episodes being caused by susceptible nonvaccine types. More judicious use of antimicrobials, as advocated in recent management guidelines, would be expected to have a similar effect.6,18

In summary, our data suggest that changes in pneumococcal susceptibility occur throughout the year, with resistance increasing as the respiratory season progresses. This phenomenon may constitute an additional factor that can help guide clinicians in their choices of antimicrobial therapy for children with AOM.

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