# PREVALENCE OF VARIOUS RESPIRATORY VIRUSES IN THE MIDDLE EAR DURING ACUTE OTITIS MEDIA

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## ABSTRACT

***Background*** Vaccines against respiratory viruses may be able to reduce the frequency of acute otitis media. Although the role of respiratory viruses in the pathogenesis of acute otitis media is well established, the relative importance of various viruses is unknown.

***Methods*** We determined the prevalence of various respiratory viruses in the middle-ear fluid in 456 children (age, two months to seven years) with acute otitis media. At enrollment and after two to five days of antibiotic therapy, specimens of middle-ear fluid and nasal-wash specimens were obtained for viral and bacterial cultures and the detection of viral antigens. The viral cause of the infections was also assessed by serologic studies of serum samples obtained during the acute illness and convalescence.

***Results*** A specific viral cause of the respiratory tract infections was identified in 186 of the 456 children (41 percent). Respiratory syncytial virus was the most common virus identified in middle-ear fluid: it was detected in the middle-ear fluid of 48 of the 65 children (74 percent) infected by this virus ($P \leq 0.04$ for the comparison with any other virus). Parainfluenza viruses (15 of 29 children [52 percent]) and influenzaviruses (10 of 24 children [42 percent]) were detected in the middle-ear fluid significantly more often than enteroviruses (3 of 27 children [11 percent]) or adenoviruses (1 of 23 children [4 percent]) ($P \leq 0.01$ for all comparisons).

***Conclusions*** Respiratory syncytial virus is the principal virus invading the middle ear during acute otitis media. An effective vaccine against upper respiratory tract infections caused by respiratory syncytial virus may reduce the incidence of acute otitis media in children. (N Engl J Med 1999;340:260-4.)



ACUTE otitis media is the most common bacterial infection among children and the most frequent reason for outpatient antibiotic therapy.[1] Despite proper antibiotic treatment, middle-ear effusion may persist for weeks or months,[2,3] often resulting in repeated courses of antibiotics and, eventually, surgical intervention.[4] In the United States, the annual costs of otitis media have been estimated to exceed $3.5 billion.[5] Although acute otitis media is generally considered a bacterial infection, there is ample evidence that respiratory viruses have a crucial role in the etiology and pathogenesis of this disease.[6,7] Moreover, viruses may also profoundly affect the outcome of acute otitis media.[7-10] A better understanding of the effect of viruses and the mechanisms of interactions between viruses and bacteria in otitis media is essential if major improvements are to be made in the management and prevention of this disease.

Several studies have documented the presence of viruses in the middle-ear fluid of children with acute otitis media,[8-16] but it is not known whether there are differences in the ability of various respiratory viruses to invade the middle ear. Knowledge of the relative frequencies of viral involvement in the middle ear would be important for the development of effective strategies to prevent otitis media. Vaccination against influenzavirus decreases the incidence of acute otitis media in infants and children,[17-19] and vaccines against other respiratory viruses, particularly respiratory syncytial virus, are being developed.[20-23] Vaccines against the viruses that are found most frequently in the middle ear will probably have the greatest potential for reducing the incidence of acute otitis media. We sought to determine the rates of middle-ear invasion by common respiratory viruses in children with acute otitis media and a concurrent, documented viral infection of the upper respiratory tract.

## METHODS

### Study Subjects

We studied 456 children (age, two months to seven years) with acute otitis media who were enrolled in various antibiotic trials between 1989 and 1993.[10,16] All the children were otherwise generally healthy and were seen at our primary care clinics. None of the children had received antibiotics during the week preceding the study. The diagnosis of acute otitis media was based on symptoms of fever, irritability, or earache; signs of inflammation of the tympanic membrane (red or yellow color or bulging of the membrane); and the presence of fluid in the middle ear on tympanocentesis. Informed consent was obtained from the parents or guardians of all the children, and all procedures conformed to the guidelines established by the Department of Health and Human Services and the institutional review board of the University of Texas Medical Branch.

### Specimens

At enrollment (visit 1), middle-ear fluid was obtained for bacterial and viral studies by needle tympanocentesis. After tympanocentesis, a nasal-wash specimen was collected for viral studies by flushing the nostril with 3 to 5 ml of phosphate-buffered saline solution in a 30-ml tapered rubber bulb.[24] Venous blood was also obtained for serologic studies during the acute illness.

Follow-up visits were scheduled two to five days after the initiation of therapy (visit 2), at the end of therapy (visit 3; days 9 to 12), and approximately one month after the initiation of therapy

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(visit 4). During the second visit, a second tympanocentesis was routinely performed and a second nasal-wash specimen was collected for viral studies. Venous blood was obtained during the third visit to assess antibody responses to viruses during convalescence.

### Processing of Specimens and Virologic Procedures

Immediately after collection, portions of the specimens of middle-ear fluid were inoculated onto blood-agar, chocolate-agar, and MacConkey-agar plates and into meat broth to test for aerobic bacteria. The remaining middle-ear fluid was diluted with 1.0 to 1.2 ml of phosphate-buffered saline solution and inoculated into cell cultures within two to three hours after collection. For viral culture of middle-ear fluid and nasal-wash specimens, each specimen was inoculated in two tubes each of primary monkey-kidney cells and human fibroblasts (MRC-5 cells). In addition, Hep-2 cells and Buffalo green-monkey–kidney cells were used during respiratory syncytial virus and enterovirus seasons, respectively. For each cell line, one tube was incubated at 33°C and the other at 36°C, and they were observed daily for 10 days for cytopathic effects. Hemadsorption was performed three times, and the final identification of viruses was made according to standard methods.

Rapid viral antigen testing was performed on specimens of middle-ear fluid and nasal-wash specimens. For middle-ear fluid, the presence of respiratory syncytial virus antigens was assessed by enzyme immunoassay (Respiratory Syncytial Virus Enzyme Immunoassay Diagnostic Kit, Abbott Laboratories, North Chicago, Ill., or Ortho Respiratory Syncytial Virus Antigen Enzyme-Linked Immunoassay Test, Ortho Diagnostic Systems, Raritan, N.J.). For nasal-wash specimens, the cell pellet from the centrifuged specimen was smeared onto microscope slides and subjected to an indirect fluorescence antibody assay with commercially available antiserum for respiratory syncytial virus, influenza A and B viruses, adenoviruses, and parainfluenza virus types 1, 2, and 3 (Bartels Viral Respiratory Panel, Baxter Healthcare, West Sacramento, Calif.). Serologic tests for the same respiratory viruses were also performed by the same indirect fluorescence antibody technique. Serum samples obtained from a patient during the acute illness and convalescence were tested at the same time.

### Definitions

The presence of a viral infection of the respiratory tract was considered to be confirmed if the results of viral culture or antigen-detection tests of either nasal-wash or middle-ear specimens obtained during the first or second visit were positive. An increase in viral titers by a factor of at least 4 from the time of acute illness to convalescence was also considered proof of a viral infection.

When we calculated the prevalence of the various viruses in the specimens of middle-ear fluid, we regarded the dual viral infections in nine children as separate infections. In 25 children with evidence of a viral infection on analysis of the middle-ear fluid, the specimens from the left and right ears had been combined before viral culture or antigen detection, and they were therefore excluded from the analysis of specific combinations of viruses and bacteria in the middle ear.

### Statistical Analysis

Comparison of proportions between the groups was done by the standard chi-square test and Fisher's exact test. The Mann–Whitney U test and Kruskal–Wallis one-way analysis of variance according to rank were used to compare nonparametric continuous data between the groups. A P value of less than 0.05 was considered to indicate statistical significance.

## RESULTS

Of the 456 children analyzed, the specific viral cause of the respiratory tract infections was determined in 186 (41 percent); these children had a total of 208 viral infections (18 children each were infected by two viruses, and 2 children each by three viruses). Of these 208 infections, 168 (81 percent) were caused by respiratory syncytial virus, parainfluenza virus type 1, 2, or 3, influenza A or B virus, enteroviruses, or adenoviruses (Table 1). There were no significant differences in sex, race or ethnic group, duration of respiratory symptoms, or age among the groups with specific viral illnesses or between these children and the group of children as a whole, except that children with influenzavirus infection were significantly older than the other children.

Respiratory syncytial virus was the most common virus identified in middle-ear fluid: the virus was detected in the middle-ear fluid of 74 percent of the children infected by this virus ($P=0.04$ for the comparison with parainfluenza viruses and $P\leq0.005$ for the comparison with any of the other viral groups) (Table 2). Also, parainfluenza viruses (52 percent) or influenzaviruses (42 percent) were found in the middle-ear fluid significantly more often than enteroviruses (11 percent) or adenoviruses (4 percent) ($P\leq0.01$ for all comparisons). The relative differences in the prevalences remained regardless of whether the rates were calculated per patient or per infected ear. There were no statistically significant differences in age, sex, race or ethnic group, or duration of respiratory symptoms between the children with viruses in middle-ear fluid and those without viruses.

The 456 children had a total of 815 ears with acute otitis media. *Streptococcus pneumoniae* was isolated from the middle-ear fluid of 203 of these ears (25 percent), *Haemophilus influenzae* from 190 ears (23 percent), and *Moraxella catarrhalis* from 122 ears (15 percent); 82 ears (10 percent) had either two or all three of these bacterial pathogens at the same time. Sixty-six ears from which viruses were detected in the middle-ear fluid could be analyzed for the presence of bacteria; both virus and bacteria were found in 43 of these ears (65 percent). In these combined viral and bacterial infections, *S. pneumoniae* was cultured significantly more often in middle-ear fluid containing influenzaviruses (100 percent) than in those containing respiratory syncytial virus (36 percent) or parainfluenza viruses (10 percent) (Table 3). No statistically significant differences were observed in the rates of detection of *H. influenzae* or *M. catarrhalis* in combination with any of these three viruses. Virus as the sole middle-ear pathogen (without bacteria) was found in 14 of 36 ears with respiratory syncytial virus (39 percent), 6 of 16 ears with parainfluenza viruses (38 percent), and 2 of 10 ears with influenzaviruses (20 percent) (no significant differences among the groups).

## DISCUSSION

We used tympanocentesis together with comprehensive viral and bacterial diagnostic methods in 456 children with acute otitis media to determine and

**TABLE 1.** CHARACTERISTICS OF THE GROUP OF CHILDREN AS A WHOLE AND ACCORDING TO THE SPECIFIC VIRAL INFECTIONS.*

| CHARACTERISTIC | RESPIRATORY SYNCYTIAL VIRUS (N=65) | PARAINFLUENZA VIRUSES (N=29) | INFLUENZAVIRUSES (N=24) | ENTEROVIRUSES (N=27) | ADENOVIRUSES (N=23) | ALL CHILDREN (N=456) |
|---|---|---|---|---|---|---|
| Age (mo) | | | | | | |
| Median | 14 | 13 | 27 | 10 | 12 | 14 |
| Range | 3–79 | 6–63 | 6–75 | 2–39 | 2–38 | 2–89 |
| Sex (no.) | | | | | | |
| Male | 38 | 13 | 15 | 15 | 12 | 247 |
| Female | 27 | 16 | 9 | 12 | 11 | 209 |
| Race or ethnic group (no.) | | | | | | |
| White | 29 | 12 | 8 | 9 | 9 | 172 |
| Black | 22 | 9 | 8 | 11 | 9 | 160 |
| Hispanic | 14 | 8 | 8 | 7 | 5 | 121 |
| Asian | 0 | 0 | 0 | 0 | 0 | 3 |
| Median duration of respiratory symptoms at enrollment (days) | 3 | 3 | 2 | 2 | 4 | 3 |
| Method of viral diagnosis (no.)† | | | | | | |
| Nasal-wash analysis | 29 | 20 | 19 | 26 | 19 | 138 |
| Culture | 18 | 20 | 18 | 26 | 15 | 122 |
| Antigen detection | 23 | 10 | 14 | NA | 9 | 56 |
| Analysis of middle-ear fluid‡ | 48 | 15 | 10 | 3 | 1 | 88 |
| Culture | 12 | 15 | 10 | 3 | 1 | 54 |
| Antigen detection | 41 | NA | NA | NA | NA | 41 |
| Serologic analysis | 26 | 10 | 11 | NA | 8 | 55 |

*A total of 168 infections by the five viruses shown were identified in 159 children. Other viruses detected were cytomegalovirus (32 infections), rhinovirus (4), herpes simplex virus (3), and an untyped hemadsorbing virus (1).

†The viral diagnosis could be based on positive findings in specimens from more than one site. NA denotes not applicable.

‡Virus was detected solely in the middle-ear fluid in 26 cases of respiratory syncytial virus infection, 2 cases of parainfluenza virus infection, and 1 case each of influenzavirus and enterovirus infections. Of the 77 children in whom one of the specified viruses was identified in the middle-ear fluid, 55 had positive results only for the specimens obtained at visit 1 (the first tympanocentesis), 11 had positive results only for the specimens obtained at visit 2 (the second tympanocentesis), and 11 had positive results for specimens obtained at both visits.

**TABLE 2.** PREVALENCE OF VARIOUS RESPIRATORY VIRUSES IN THE MIDDLE EAR IN CHILDREN WITH ACUTE OTITIS MEDIA.

| VIRUS | VIRAL INFECTION* | VIRUS IN MIDDLE-EAR FLUID |
|---|---|---|
| | no. of cases (%) | |
| Respiratory syncytial virus | 65 | 48 (74)† |
| Parainfluenza viruses | 29 | 15 (52)‡ |
| Influenzaviruses | 24 | 10 (42)‡ |
| Enteroviruses | 27 | 3 (11) |
| Adenoviruses | 23 | 1 (4) |

*Viral infection was diagnosed by one or more methods: viral culture or antigen detection in the nasal-wash specimen, viral culture or antigen detection in the middle-ear fluid, or on the basis of an increase in viral titers by at least a factor of 4 from the time of acute illness to convalescence.

†$P=0.04$ for the comparison with parainfluenza viruses and $P\leq 0.005$ for the comparison with any of the other viral groups.

‡$P\leq 0.01$ for the comparison with enteroviruses or adenoviruses.

compare the relative frequencies of respiratory viruses in the middle-ear fluid. Our results suggest that the rates of middle-ear invasion by the common respiratory viruses vary significantly during acute otitis media and that respiratory syncytial virus has a particularly strong ability to invade the middle ear. Our results also provide additional evidence of the active role of viruses in the pathogenesis of acute otitis media. The different prevalences of the viruses suggest that some viruses enter the middle ear passively along with nasal secretions, whereas other viruses actively invade the middle ear and contribute to the inflammatory process in the middle-ear mucosa. If the viruses found in the middle-ear fluid were only innocent bystanders with no active role, they should be detected in the middle-ear fluid at roughly equal rates during different viral infections.

Previous studies have demonstrated a wide array of viruses in the middle-ear fluids of infants and children with acute otitis media.[8-16,25,26] However, none of these studies have been able to determine the relative importance of the various viruses to otitis media. Obviously, the likelihood of detecting particular viruses in the middle-ear fluid during acute otitis media is dependent on the overall prevalence of those

**TABLE 3.** SPECIFIC MICROORGANISMS IN THE 43 SAMPLES OF MIDDLE-EAR FLUID THAT CONTAINED BOTH BACTERIA AND VIRUSES.*

| BACTERIAL SPECIES | RESPIRATORY SYNCYTIAL VIRUS (N=22) | PARAINFLUENZA VIRUSES (N=10) | INFLUENZAVIRUSES (N=8) | ENTEROVIRUSES (N=3)† |
|---|---|---|---|---|
| | | no. of cases (%) | | |
| *Streptococcus pneumoniae* | 8 (36) | 1 (10) | 8 (100)‡ | 1 (33) |
| *Haemophilus influenzae* | 10 (45) | 5 (50) | 2 (25) | 0 |
| *Moraxella catarrhalis* | 6 (27) | 5 (50) | 3 (38) | 1 (33) |

*More than one bacterial species was identified in samples from eight ears.

†*Pseudomonas aeruginosa* was cultured in two ears with enteroviruses.

‡P=0.003 for the comparison with respiratory syncytial virus, and P<0.001 for the comparison with parainfluenza viruses (by Fisher's exact test).

infections in the study population; therefore, the rates of viral detection in middle-ear fluid in different studies are not directly comparable. Furthermore, differences in the methods of viral detection used in earlier studies limit the comparability of the frequencies. The strength of the present study is that we consistently used similar methods of viral detection in a large number of children over several respiratory virus seasons. It could be argued, however, that we might have overestimated the differences in the rates of detection in middle-ear fluid between respiratory syncytial virus and the other viruses because we used antigen detection only to identify respiratory syncytial virus and not the other viruses in middle-ear fluid. The use of antigen detection for respiratory syncytial virus was essential, because unlike other respiratory viruses, this virus is relatively labile, and thus, the sensitivity of cell-culture technique for this virus is considerably lower than those of various rapid antigen techniques.[27] Although in theory the use of viral-antigen detection in nasopharyngeal specimens but not in middle-ear specimens could have resulted in falsely low rates of detection of influenzaviruses, parainfluenza viruses, and adenoviruses in the middle ear, this possibility did not affect our results or conclusions, because only one influenzavirus infection and two adenovirus infections were diagnosed solely on the basis of antigen detection in the nasopharyngeal specimens.

Our results are in accordance with those of previous epidemiologic studies of the association of viral infections with acute otitis media. Several reports have indicated that respiratory syncytial virus may be the type of virus that is most likely to predispose a child to acute otitis media.[28-31] The relative ability of different respiratory viruses to predispose a person to acute otitis media is, however, difficult to determine, because the development of acute otitis media depends on multiple factors. In particular, the age of the child is an important confounding factor that has not been adjusted for in many studies. Nonetheless, our results clearly corroborate the view that respiratory syncytial virus is an important cause of acute otitis media in children. We could not determine the relative importance of rhinoviruses in acute otitis media, because the incidence of rhinovirus infections was too low for any meaningful analysis. Rhinoviruses account for approximately one third of all infections of the upper respiratory tract,[32] and previous studies have demonstrated that rhinoviruses can also be found in middle-ear fluid in children with acute otitis media.[14,15] A recent study, which used a reverse-transcriptase–polymerase-chain-reaction assay to detect respiratory viruses, suggested that the prevalence of rhinoviruses in middle-ear fluid during acute otitis media may be similar to that of respiratory syncytial virus.[33] In general, the development of nucleic acid–based assays for a wide range of respiratory viruses may provide researchers with more sensitive methods for studying the role of respiratory viruses in acute otitis media.

The presence of viruses in the middle-ear fluid is important not only in regard to the etiology and pathogenesis of acute otitis media, but also in regard to the outcome of the disease.[7] Clinical studies have indicated that the presence of viruses in the middle ear may considerably worsen both the clinical and bacteriologic outcomes of otitis media.[8-10] The mechanisms by which viruses enhance or prolong the inflammation in the middle ear are still unclear, but studies have shown that there are higher concentrations of some inflammatory mediators in middle-ear fluid containing both bacteria and virus than in middle-ear fluid containing bacteria alone.[16,34,35]

The enormous impact of otitis media on society has made prevention of this disease a high priority

of research, and bacterial and viral vaccines are considered to hold the greatest promise for the ultimate prevention of acute otitis media.[36,37] Previous studies have clearly demonstrated that both inactivated and live, attenuated influenzavirus vaccines reduce the incidence of acute otitis media in infants and children.[17-19] Our results suggest that an effective vaccine against respiratory syncytial virus might have a major effect on the incidence of acute otitis media, together with the more serious manifestations of respiratory syncytial virus infection. It is apparent, however, that to prevent acute otitis media, such a vaccine should protect not only the lower respiratory tract but also the upper respiratory tract.

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