

## Acute otitis media and respiratory viruses

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Received: 29 December 2005 / Revised: 13 June 2006 / Accepted: 20 June 2006 / Published online: 12 September 2006  
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**Abstract** The present study was performed to elucidate the clinical outcome, and etiology of acute otitis media (AOM) in children based on virologic and bacteriologic tests. The study group consisted of 120 children aged 6 to 144 months with AOM. Middle ear fluid (MEF) was tested for viral pathogens by reverse transcriptase polymerase chain reaction (RT-PCR) and for bacteria by gram-staining and culture. Clinical response was assessed on day 2 to 4, 11 to 13, 26 to 28. Respiratory viruses were isolated in 39 patients (32.5%). Respiratory syncytial virus (RSV) (46.5%) was the most common virus identified in MEF samples, followed by human rhinovirus (HRV) (25.6%), human coronavirus (HCV) (11.6%), influenza (IV) type A (9.3%), adenovirus type sub type A (AV) (4%), and parainfluenza (PIV) type -3 (2%) by RT-PCR. In total 69 bacterial species were isolated from 65 (54.8%) of 120

patients. *Streptococcus pneumoniae* (*S. pneumoniae*) was the most frequently isolated bacteria. Viral RNA was detected in 31 (56.3%) of 55 bacteria-negative specimens and in 8 (12.3%) of 65 bacteria-positive MEF samples. No significant differences were found between children representing viral infection alone, combined viral and bacterial infection, bacterial infection alone, and neither viral nor bacterial infection, regarding clinical cure, relapse and reinfection rates. A significantly higher rate of secretory otitis media (SOM) was observed in alone or combined RSV infection with *S. pneumoniae* or *Haemophilus influenzae* (*H. influenzae*) than in other viruses infection. **Conclusion.** This study provides information about etiologic agents and diagnosis of AOM in Turkish children. The findings highlight the importance of common respiratory viruses and bacterial pathogens, particularly RSV, HRV, *S. pneumoniae* and *H. influenzae*, in predisposing to and causing AOM in children.

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**Keywords** Acute otitis media · Tympanocentesis ·  
Viral etiology · Respiratory viruses

### Abbreviations

AOM	acute otitis media
MEF	middle ear fluid
RT-PCR	reverse transcriptase polymerase chain reaction
<i>S. pneumoniae</i>	<i>Streptococcus pneumoniae</i>
RSV	respiratory syncytial virus
HRV	human rhinovirus
PIV	parainfluenza virus
HCV	human coronavirus
IV	influenza virus
AV	adenovirus

<i>H. influenzae</i>	<i>Haemophilus influenzae</i>
<i>M. catarrhalis</i>	<i>Moraxella catarrhalis</i>
SOM	secretory otitis media

## Introduction

Acute otitis media is a major worldwide health care problem of childhood. The peak incidence of AOM occurs in children between 6 and 24 months of age [19]. By the age of 2 years, 70% of children have experienced at least one episode of AOM. According to a recent estimate, AOM accounts for 33% of all visits to physicians and approximately 40% of all antibiotic use in children younger than 5 years [4]. Acute otitis media is the most frequent reason for outpatient antibiotic therapy in children in the United States and most other developed countries [4].

Acute otitis media is generally considered a bacterial infection that is treated with antibiotics. Despite antibiotic treatment, one commonly encounters SOM, relapse, and reinfection that persist for weeks or months [10]. Antibiotic resistant bacteria is often considered to be the cause of refractory AOM. However, bacterial pathogens cannot be isolated from the middle-ear fluid in approximately 30% of AOM cases [6]. During the past 2 decades, researches in this area have produced strong evidence for the crucial role of viruses in the development of AOM. Respiratory viruses may be a copathogen and in some patients a true pathogen of AOM.

Little information is available about the role of bacterial and viral pathogens causing AOM in Turkish children. Therefore, we performed a clinical study to elucidate clinical outcomes, and etiology of AOM in children based on virological and bacteriological tests.

## Patients and methods

One hundred twenty children of ages ranging from 6 months to 144 months (median: 32.58 months) were enrolled in a study of AOM at the Department of Pediatrics and Otorhinolaryngology between March 2003 and December 2004. The Ethical Committee of Gaziosmanpasa University approved the study.

Children with complaints suggesting AOM (fever, irritability, and earache) were first examined by a pediatrician, and then referred to an otorhinolaryngology specialist for otomicroscopic examination. Middle ear fluid was revealed by tympanometry or pneumatic otoscopy. Patients who had MEF, two or more local signs such as erythema, fullness or bulging of the tympanic membrane, loss of tympanic membrane landmarks and acute perforation with purulent otorrhea were included in

the study. The following criteria were applied for the patients of the study:

1. Age ranging from 6 months to 12 years;
2. No antibiotic treatment given within 2 weeks;
3. No diagnosis of chronic otitis media or purulent otorrhea for more than 24 hours;
4. Absence of allergy in story of the drug used in study;
5. Absence of serious underlying disease that may impair response to treatment (immunodeficiency, renal or hepatic insufficiency);
6. Written consent from the parents.

Paracentesis was performed on all children with an intact tympanic membrane before the first dose of antibiotic treatment. Middle ear fluid samples were collected by paracentesis and by direct swab sample if the tympanic membrane ruptured. An aliquot of the sample was removed from the tip and immediately placed in a transport tube for identification of bacteria using standard laboratory methods. The remainder of the sample was stored at  $-70^{\circ}\text{C}$  for viral studies.

All children were treated with amoxicillin-clavulanate (amoxicillin 45 mg/kg/24 hr + clavulanate 6.4 mg/kg/24 hr, peroral, in two divided doses for 10 days). Three control otomicroscopic examinations were done on days 2 to 4, 11 to 13, and 26 to 28. Patients with SOM were followed-up for 3 months.

The criteria for success were defined as follows:

1. Clinical cure: Complete resolution of signs and symptoms due to AOM;
2. Clinical failure: Failure to clear signs and symptoms;
3. Improvement: Incomplete resolution of AOM signs and symptoms (SOM);
4. Relapse: After an initial period of improvement, recurrence of clinical and otoscopic findings on second control;
5. Reinfection: Recurrence of clinical and otoscopic findings in a patient during the 30-day follow-up period in whom cure or improvement had been detected on second control.

## Microbiology

The specimens were cultured on 5% sheep blood agar, eosine methylene blue agar, chocolate agar, sabouraud dextrose agar and thioglyconate agar (Oxoid) in aerobic conditions. All were incubated at  $35\text{--}37^{\circ}\text{C}$  for 24–48 h. The bacterias were classified according to standard methods of microbiology and the diagnosis were confirmed by sceptor. *H. influenzae* and *Moraxella catarrhalis* (*M. catarrhalis*) were tested for beta-lactamase production by the chromogenic nitrocefin test.

**Table 1** Age distribution of 120 children with AOM

Age (year)	Number (%) of children (n=120)
<1	32 (26.6)
1–2	18 (15)
2–4	47 (39.2)
4–6	13 (10.8)
>6	10 (8.3)

Total RNA was isolated from 50 to 100 µl of each specimen by an acid guanidinium-phenol-chloroform method [8]. Three microliters of RNA was suspended in 10 µl of sterile water, heated at 65°C for 5 min, and then cooled rapidly. The sample then was reverse-transcribed at 37°C for 60 min in a 10-µl volume containing a 0.5 mM of each dNTP (dATP, dCTP, dGTP, dTTP); 0.5 µg of random hexadenoxy-nucleotide primer; 40 U of RNase inhibitor (Roche Diagnostics, Tokyo, Japan); 20 U of M-MLV reverse transcriptase (Roche Diagnostics); and reverse transcriptase buffer (supplied with the enzyme). The reaction was stopped by a 10-min incubation at 80°C. Samples were diluted with sterile water to a final volume of 50 µl.

Multiplex nested RT-PCR was carried out to detect influenza A and B viruses; respiratory syncytial virus (RSV) types A and B; PIV types 1, 2, and 3; rhinovirus (HRV); coronavirus; and adenovirus. Primers used in this study were described previously [9, 10], as was the detailed protocol for multiplex nested PCR. PCR products were fractionated by size using electrophoresis on a 2.5% agarose gel and then visualized by ethidium bromide under

ultraviolet light. DNA size markers (1 kb plus DNA ladder; Promega, Madison, WI) were included in the gel.

**Statistical analysis**

Statistical comparisons were performed by using the Chi-square test, Spearman’s rank order correlation coefficient, and Fisher exact Chi-square test.

**Results**

The age range of the 120 children (60% boys) was from 6 months to 144 months (median: 32.58 months) (Table 1). Fifty children (41.6%) were under 2 years of age.

**Viral infections**

Respiratory viruses were isolated in 39 patients (32.5%). A total of 43 viral infections was identified (four specimens were infected by two viruses and eight specimens were infected by one bacterial pathogen). Respiratory viruses were not isolated in 81 (67.5%) of 120 patients with AOM. Of the 43 viral infections, RSV type A was detected in 20 (46.5%), HRV in eleven (25.6%), HCV in five (11.6), IV type A in four (9.3%), AV type sub type A in two (4%), and PIV type –3 in one (2%) (Table 2). There was a significant difference regarding frequency of the age groups 6–24 and 25–144 months (Table 3).

**Table 2** Distribution of pathogens in 120 children with AOM

Specific microorganisms	Specific microorganisms in the 120 samples of MEF that contained bacteria and viruses												
	Spn	Hi	Mca	Sa	Sp	Ec	Sf	RSV	HRV	HCV	PIV	IV	AV
Spn n=28	18	2	2	–	–	–	–	4	2	–	–	–	–
Hi n=16	2	12	–	–	–	–	–	1	1	–	–	–	–
Mca n=10	2	–	8	–	–	–	–	–	–	–	–	–	–
Sa n=9	–	–	–	9	–	–	–	–	–	–	–	–	–
Sp n=3	–	–	–	–	3	–	–	–	–	–	–	–	–
Ec n=2	–	–	–	–	–	2	–	–	–	–	–	–	–
Sf n=1	–	–	–	–	–	–	1	–	–	–	–	–	–
RSV n=20	4	1	–	–	–	–	–	12	2	1	–	–	–
HRV n=11	2	1	–	–	–	–	–	2	5	1	–	–	–
HCV n=5	–	–	–	–	–	–	–	1	1	3	–	–	–
PIV n=1	–	–	–	–	–	–	–	–	–	–	1	–	–
IV n=4	–	–	–	–	–	–	–	–	–	–	–	4	–
AV n=2	–	–	–	–	–	–	–	–	–	–	–	–	2
Total n=120	28	16	10	9	3	2	1	20	11	5	1	4	2

*S. pneumoniae*: Spn; *H. influenzae*: Hi; *M. catarrhalis*: Mca; *S. aureus*: Sa; *S. pyogenes*: Sp; *E. coli*: Ec; *S. faecalis*: Sf  
*Respiratory syncytial virus*: (RSV Type A, B); *Human rhinovirus*: HRV; *Human coronavirus*: HCV  
*Human parainfluenza virus*: PIV (PIV Type 1, 2, 3); *Influenza virus*: IV (IV Type A, B); *Adenovirus*: AV (AV Sub Type A, B, C, D, E, F)

**Table 3** Prevalence of viral pathogens in patients with AOM

Virus	Age (6–24 months)	Age (25–144 months)
	Number of cases (%)	Number of cases (%)
RSV	13 <sup>a</sup>	7 <sup>bc</sup>
HRV	6 <sup>a</sup>	5 <sup>bd</sup>
HCV	–	5 <sup>cd</sup>
IV	–	4
PIV	–	1
AV	–	2

<sup>a</sup> One MEF was positive for both RSV and HRV

<sup>b</sup> One MEF was positive for both RSV and HRV

<sup>c</sup> One MEF was positive for both RSV and HCV

<sup>d</sup> One MEF was positive for both HCV and HRV

### Bacterial infections

In total 69 species were isolated from 65 (54.2%) of 120 patients (Table 2). Bacteria were not isolated in 55 (45.8%) of 120 patients. *S. pneumoniae* was the most frequently isolated organism ( $n=28$ ; 40.5%) followed by *H. influenzae* ( $n=16$ ; 23.1%), *M. catarrhalis* ( $n=10$ ; 14.5%), *Staphylococcus aureus* ( $n=9$ ; 13%), group A beta-hemolytic streptococcus ( $n=3$ ; 4.3%), *Escherichia coli* ( $n=2$ ; 2.8%) and *Enterococcus faecalis* ( $n=1$ ; 1.4%) (Table 2). Multiple bacterias were isolated in four patients (Table 4). Two of 16 *H. influenzae* strains were b type (12.5%), and had beta-lactamase activity. Fifty percent of the *M. catarrhalis* strains (5 of 10) were beta-lactamase positive. There were significant differences in age of children between 6–24 months and 25–144 months for *S. pneumoniae*, *M. catarrhalis*, and *S. Aureus* (Table 4).

**Table 4** Prevalence of bacterial pathogens in patients with AOM

Bacteria	Age (6–24 months)	Age (25–144 months)
	Number of cases (%)	Number of cases (%)
<i>S. pneumoniae</i>	18 <sup>abc</sup>	10 <sup>cd</sup>
<i>H. influenzae</i>	8 <sup>ag</sup>	8 <sup>f</sup>
<i>M. catarrhalis</i>	1	9 <sup>d</sup>
<i>S. aureus</i>	2	7
<i>S. pyogenes</i>	–	3
<i>E. coli</i>	2	–
<i>E. faecalis</i>	–	1

<sup>a</sup> Two MEF were positive for both Spn and Hi; <sup>b</sup> Three MEF were positive for both Spn and RSV; <sup>c</sup> One MEF was positive for both Spn and RSV; <sup>d</sup> Two MEF were positive for both Spn and Mca; <sup>e</sup> Two MEF were positive for both Spn and HRV; <sup>f</sup> One MEF was positive for Hi and HRV; <sup>g</sup> One MEF was positive for Hi and RSV

### Viral and bacterial co-infections

Viral RNA was detected in 31 (56.3%) of 55 bacteria-negative specimens and in 8 (12.3%) of 65 bacteria-positive MEF samples ( $p<0.05$ ). *S. pneumoniae*-RSV was found in four cases, *S. pneumoniae*-HRV in two cases, *H. influenzae*-RSV in one case, *H. influenzae*-HRV in one case, RSV-HRV in two cases, RSV-HCV in one case, and HRV-HCV in one case (Table 2). Neither viruses nor bacteria were found in 24 MEF samples.

### Clinical outcomes

When clinical cure, relapse and reinfection rate were compared between children representing viral infection alone, combined viral and bacterial infection, bacterial infection alone, and neither viral nor bacterial infection, no significant differences were found (Table 5). Also, there were no significant differences in children with AOM younger than 2 years of age. At 3 months follow-up SOM was developed in 12 children (10%), of these 7 (58.3%) had viruses determined MEF samples (Table 5). A significantly higher rate of SOM was observed in combined RSV infection with *S. pneumoniae* or *H. influenzae* than in RSV infection alone. However, when analysis was limited to children less than 2 years of age, the rate of SOM was found to be unchanged.

### Discussion

The microbiological causes of AOM have been documented on the basis of culture results of MEF obtained by tympanocentesis. It has been reported that bacteria and viruses are the main causes of AOM [6, 16]. *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* have been reported to be the most frequent bacterial agents of AOM, respectively [6, 9]. In our study, the percentage of types of bacteria causing AOM has found to be in accordance with the literature for *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. However, *S. aureus* has been determined to be the fourth etiologic agent causing AOM in our study (13%), on the contrary to previous reports. A similar study in Turkey has stated that *S. pneumoniae*, *H. influenzae* and *S. aureus* are the most common isolated bacteria, respectively [12].

Acute otitis media is usually considered as a simple bacterial infection that is treated with antibiotics. However, ample evidence derived from studies ranging from animal experiments to extensive clinical trials support a crucial role for respiratory viruses in the etiology and pathogenesis of AOM [7]. Furthermore, respiratory viruses may be responsible for the difficulties in clearing bacterial pathogens from

**Table 5** Evaluation of clinical response according to culture results

Pathogens	Clinical cure <i>n</i> (%)	SOM <i>n</i> (%)	Failure <i>n</i> (%)	Relapse <i>n</i> (%)	Reinfection <i>n</i> (%)	Total <i>n</i> (%)
Culture negative	20 (80)	4 (20)				24 (20)
<i>S. pneumoniae</i>	18 (100)					18 (15)
<i>H. influenzae</i>	11 (92.7)	1 (8.3)				12 (10)
<i>M. catarrhalis</i>	7 (87.5)				1 (12.5)	8 (6.7)
Multiple bacteria*	2 (50)				2 (50)	4 (3.3)
Other bacteria	14 (93.3)			1 (6.7)		15 (12.5)
RSV	10 (83)	2 (17)				12 (10)
HRV	5 (100)					5 (4.2)
Other viruses	9 (90)	1 (10)				10 (8.3)
Multiple viruses	2 (50)	1 (25)			1 (25)	4 (3.3)
Viral+bacterial pathogens	5 (62.5)	3 <sup>a</sup> (37.5)				8 (6.7)
Total <i>n</i> (%)	103 (85.8)	12 (10)		1 (0.8)	4 (3.3)	120 (100)

Multiple bacteria\* were *S. pneumoniae* and *H. influenzae* in two cases, and *S. pneumoniae* and *M. catarrhalis* in two cases

Multiple viruses were RSV-HRV in two cases, RSV-HCV in one case, and HRV-HCV in one case

Viral+bacterial pathogens were *S. pneumoniae*-RSV in four cases, *S. pneumoniae*-HRV in two cases, *H. influenzae*-RSV in one case,

*H. influenzae*-HRV in one case

<sup>a</sup> *S. pneumoniae*-RSV in two cases and *H. influenzae*-RSV in one case

the middle ear and for the persistence of symptoms in some children with AOM who are receiving adequate antibiotic therapy. On the other hand, medical literature has shown an increase in the incidence of antibiotic-resistant bacterial strains [3]. No bacterial pathogens can be isolated in 30% of AOM cases. This can be attributed to technical inadequacies and the presence of fastidious bacterial organisms.

Heikkinen et al. [7] reported that respiratory viruses were found in 29% of the MEF specimens by viral culture; this rate was increased to 72% by use of RT-PCR. In the other studies in which PCR was used, the rates of respiratory viruses were described to be between 10 and 53% in children with AOM [10, 13, 15]. Such high rates of viral detection by PCR inevitably raises the question of the real clinical significance of these viruses in middle ear.

Respiratory syncytial virus has been reported to be the most common virus associated in children with AOM [6, 10], followed by IV type A, adenovirus, and PIV [11, 16]. We demonstrated respiratory virus RNA in 39 (32.5%) of 120 MEF samples. Our results clearly corroborate that the RSV and HRV are important causes of AOM in children. From the standpoint of age, there were significant differences in children between 6–24 months and 25–144 months for RSV (Table 3).

The clinical results of our study are in accordance with a recent study in which children with RSV infection alone or combined with *S. pneumoniae* or *H. influenzae* bacterial infection tended to have SOM [6]. However, when evaluation was limited to children less than 2 years of age, the rates of SOM was almost equal. It is reported that the presence of HRV infection combined with bacteria in

the MEF was associated with a higher bacteriologic failure rate in AOM compared with the presence of other respiratory viruses [1, 18]. We were not able to find any specific association between HRV infection and poor outcome.

Although many studies have been made, the exact mechanism of prolonged inflammatory responses in combined viral and bacterial infection remains unclear. These studies have shown that there are higher concentrations of some inflammatory mediators in MEF containing both bacteria and virus than in MEF containing bacteria alone [10, 14]. Also, a consensus has not been reached regarding the effect of viral infection on clinical outcome of AOM [1, 10, 15]. This probably results from including varied subjects and target diseases, since overall age distribution ranged from several months to 12 years and all respiratory viruses often were studied as a group [10]. It is reported that the incidence of treatment resistance or delayed cure in combined RSV infection is high in children under 2 years old. However, we didn't find any difference between children with AOM under 2 years old and children with AOM older than 2 years regarding treatment resistance or time for cure.

Clinical studies have associated respiratory virus infection with poor response to antibiotics or development of SOM [1, 14]. However, Pitkaranta et al. [15] did not find an association between virus infection and response to antimicrobial therapy or outcome of AOM. Although the conclusion that otitis media in children is largely the result of viral respiratory tract infection clearly has merit, it is not clear how this conclusion should affect management of patients, especially the decision to use antibiotics. Further



studies will be needed to help practitioners determine which patients do not need antibiotic treatment. Bacterial pathogens were frequently absent (45.8% of MEF samples) and viral RNA was detected in 56.4% of bacteria negative MEF samples in the present study. Previous studies have clearly demonstrated that prevention of particular virus infections by influenza vaccine [2, 5] and RSV immunoglobulin [17] has been associated with reduced frequencies of AOM.

These results indicate that the predominant pathogens including *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, RSV and HRV are responsible for about 80% of AOM. In addition to, combined or alone, RSV and HRV infections also carry a high risk for AOM. Our report provides information about bacterial and viral pathogens causing AOM in Turkish children.

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