Microbiology

OUTLINE

Bacteriology
Geographic and temporal surveys
Early studies
1960s to the present
Surveys in school-aged children, adolescents and adults
Sites of culture
Nasopharyngeal cultures
Bacteremia with otitis media
Bacterial quantification in middle-ear fluids
Streptococcus pneumoniae
Serotypes responsible for acute otitis media
Adherence of pneumococci to respiratory mucosa
Susceptibility of pneumococci to antimicrobial agents
Haemophilus influenzae
Moraxella catarrhalis
Groups A and B streptococci
Staphylococcus aureus
Staphylococcus epidermidis and diphtheroids
Gram-negative bacilli
Anaerobic bacteria
Disparate bacterial cultures
Identification of bacterial antigens and DNA in middle-ear fluids
Sterile cultures
Viruses
Mycoplasma
Chlamydia
Uncommon Microorganisms
Corynebacterium diphtheriae
Mycobacterium tuberculosis
Non-tuberculous mycobacteria
Clostridium tetani
Parasitic infections
Fungi
Microbial Products
Biofilms
Endotoxin
Interferon
Neuraminidase
Bacteriology of Relapse and Recurrence
Bacteriologic Studies of Chronic Otitis Media with Effusion
Otitis Media in the Newborn Infant
Bacteriologic Studies of Acute Otorrhea in Children with Tympanostomy Tubes
Bacteriologic Studies of the External Ear Canal

The microbiologic causes of otitis media have been documented by appropriate cultures of middle-ear effusions obtained by needle aspiration. Many bacteriologic studies of acute otitis media (AOM) have been performed, and the results are consistent in demonstrating the importance of Streptococcus pneumoniae and Haemophilus influenzae and a minor role for Moraxella catarrhalis. Studies of children with otitis media with effusion indicate that bacterial pathogens are also present in these fluids, suggesting that bacteria may be a factor in the development and persistence of the effusion. Respiratory viruses, alone or combined with bacterial pathogens, have been identified in 17% of middle-ear fluids. New concepts in the pathogenesis of otitis media, such as the role of biofilms, and new techniques, such as polymerase chain reaction (PCR), are expanding our knowledge of the microbiology of otitis media. The introduction of conjugate pneumococcal vaccines is likely to reduce the proportion of AOM due to the pneumococcus and increase the proportion of disease due to nontypeable H. influenzae. Since we do not know why group A Streptococcus (GAS) diminished in importance after having been one of the leading causes of AOM in the first half of the 20th century, it is possible that new serotypes will arise (or old serotypes will reappear) and that it will once again make group A Streptococcus an organism of importance in middle-ear infections. This chapter reviews the results of these microbiologic studies, the in vitro antibiotic susceptibility patterns of the major
pathogens, and considers various aspects of the infectious process in the middle ear. The microbiology of chronic suppurative otitis media and of other suppurative complications of otitis media is discussed in Chapter 9, “Complications and Sequelae: Intratemporal” and Chapter 10 “Complications and Sequelae: Intracranial.”

**BACTERIOLOGY**

**Geographic and Temporal Surveys**

**Early Studies**

In his comprehensive text, *The Biology of the Pneumococcus*, Benjamin White describes the early studies of pneumococcal infections, including mention of studies of otitis media.¹ He attributes the first bacteriologic diagnoses of AOM to Netter who reported in 1887 finding cocci in the nasal fossae, sinuses and tympanic cavity, and to Zaufal in 1888, who identified diplococci in the middle ear cavity. As early as 1906, German investigators studied the bacteriology of AOM by aspiration of middle ear fluids.² During the following 50 years, similar studies were performed, most in European otolaryngologic centers. Through the 1950s, GAS was the leading bacterial pathogen isolated from middle ear fluids of children with AOM, followed by the pneumococcus. Isolates of *H. influenzae* and *Staphylococcus aureus* were uncommon, and *M. catarrhalis* was not identified.

**1960s to the Present**

It was not until the 1960s that studies of the bacteriology of AOM were performed in pediatric centers, including clinics and office practices.³⁴ From the 60s to the present, the results of bacteriologic studies of otitis media in children based on aspirates of middle-ear fluids have been similar from various pediatric and otolaryngologic centers (Table 1). The largest series of published data on the bacteriology of otitis media is from the Pittsburgh Otitis Media Study Group⁵; the results of bacterial cultures of middle-ear fluids from 2,807 patients with AOM and of fluids from 4,589 patients with otitis media with effusion are displayed in Figure 1.

At present, *Streptococcus pneumoniae* and *H. influenzae* remain the most frequent bacterial

<table>
<thead>
<tr>
<th>Author</th>
<th>Year of Publication</th>
<th># Patients</th>
<th>% Pneumo</th>
<th>% H. flu</th>
<th>% GAS</th>
<th>% M. Cat</th>
<th>% Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supfle⁶</td>
<td>1906</td>
<td>52</td>
<td>33</td>
<td>—</td>
<td>58</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>Kummel⁶</td>
<td>1907</td>
<td>144</td>
<td>28</td>
<td>—</td>
<td>66</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Neumann⁶</td>
<td>1909</td>
<td>97</td>
<td>19</td>
<td>—</td>
<td>58</td>
<td>—</td>
<td>11</td>
</tr>
<tr>
<td>Wirth⁶</td>
<td>1929</td>
<td>271</td>
<td>40</td>
<td>—</td>
<td>44</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Richardson¹⁹²</td>
<td>1942</td>
<td>665</td>
<td>17.1</td>
<td>2.1</td>
<td>25.4</td>
<td>0.6</td>
<td>20.3</td>
</tr>
<tr>
<td>Bjuggren and Tunevall¹⁹³</td>
<td>1952</td>
<td>131</td>
<td>50.0</td>
<td>17.0</td>
<td>21.0</td>
<td>—</td>
<td>4.0</td>
</tr>
<tr>
<td>Lahikainen EA²³</td>
<td>1953</td>
<td>734</td>
<td>38.4</td>
<td>15.3</td>
<td>24.4</td>
<td>—</td>
<td>1.7</td>
</tr>
<tr>
<td>Rudberg³</td>
<td>1954</td>
<td>1365</td>
<td>38.6</td>
<td>7.0</td>
<td>19.4</td>
<td>—</td>
<td>4.8</td>
</tr>
<tr>
<td>Halsted, et al³</td>
<td>1968</td>
<td>106</td>
<td>36.8</td>
<td>17.9</td>
<td>4.7</td>
<td>1.9</td>
<td>—</td>
</tr>
<tr>
<td>Howie, et al⁴¹</td>
<td>1970</td>
<td>858</td>
<td>37.7</td>
<td>24.0</td>
<td>2.6</td>
<td>7.8</td>
<td>—</td>
</tr>
<tr>
<td>Bluestone, et al⁸⁶</td>
<td>1992</td>
<td>2807</td>
<td>35</td>
<td>23</td>
<td>3</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Casey and Pichichero⁴¹</td>
<td>2004</td>
<td>195</td>
<td>29.2</td>
<td>22.6</td>
<td>1.5</td>
<td>2.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1995–1997</td>
<td>195</td>
<td>29.2</td>
<td>22.6</td>
<td>1.5</td>
<td>2.1</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1998–2000</td>
<td>204</td>
<td>30.0</td>
<td>26.5</td>
<td>1.5</td>
<td>3.9</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2001–2003</td>
<td>152</td>
<td>23.0</td>
<td>35.2</td>
<td>1.3</td>
<td>3.3</td>
<td>—</td>
</tr>
<tr>
<td>Block, et al³⁰</td>
<td>2004</td>
<td>152</td>
<td>23.0</td>
<td>35.2</td>
<td>1.3</td>
<td>3.3</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>2000–2003</td>
<td>83</td>
<td>31</td>
<td>56</td>
<td>2</td>
<td>11</td>
<td>—</td>
</tr>
</tbody>
</table>

¹ Data are reviewed in Lahikainen EA.²³
² Numbers do not add to 100% because sterile and uncommon pathogens are not included.
pathogens, followed by *M. catarrhalis*. GAS, *Staphylococcus aureus*, and gram-negative enteric bacilli are infrequent causes of otitis. No growth (or isolation of an organism considered to be a contaminant, such as *Staphylococcus epidermidis* or diphtheroids) occurs in approximately one-quarter to one-third of effusions obtained from patients with AOM dependent on selection of patients and the sensitivity of the microbiologic techniques. PCR may identify the presence of pathogens for which usual bacterial culture is negative. Since introduction of the conjugate pneumococcal vaccine (PCV 7) for routine immunization in the United States, there are preliminary data that suggest a decrease in incidence of pneumococcal AOM and a proportional increase in AOM due to non-typable *H. influenzae*.

**Surveys in School-aged Children, Adolescents and Adults**

The bacteriology of otitis media is similar in all age groups after the newborn period. Data from school-aged children and adolescents\(^6\)\(^7\) and adults\(^8\)\(^9\)\(^10\)\(^11\) identify similar patterns of dominance by *Streptococcus pneumoniae* and nontypeable *H. influenzae*. These microbiologic results indicate that initial therapy for AOM is the same in all age groups.

**Figure 1.** Comparison of distribution of isolates in 2,807 effusions from patients with acute otitis media (AOM) and 4,589 effusions from patients with otitis media with effusion (OME) at the Pittsburgh Otitis Media Research Center, 1980 to 1989. Total percentages are greater than 100% because of multiple organisms. Modified from Bluestone CD et al.\(^5\)

**Sites of Culture**

Aspiration of middle-ear fluids by tympanocentesis provides the most valid identification of organisms associated with otitis media. Bacteremia is infrequent. Nasopharyngeal and throat cultures are nonspecific and are inadequate to assess efficacy of antimicrobial agents and vaccines. Viruses are present in the nasopharynx, but less than half are identified in the middle-ear fluids of the same infants with AOM (see “Viruses”). Techniques for culture vary, and some, such as the use of broth subculture, may increase the yield of bacterial pathogens.\(^12\)

**Nasopharyngeal Cultures**

Bacteria with the potential to cause AOM are frequent colonizers of the nasopharynx but are rarely responsible for acute infection. The circumstances necessary for an organism colonizing the nasopharynx to gain access to the middle ear and cause inflammatory disease are uncertain but may include coinfection with viruses or strain virulence.

Faden and colleagues prospectively assessed children for nasopharyngeal carriage and for middle-ear isolates during episodes of AOM.\(^13\) Respiratory pathogens were usually present in the nasopharynx during an acute episode of otitis
media due to the homologous strain; thus, of cases of otitis media due to nontypeable *H. influenzae*, the homologous strain was present in the nasopharynx in 95% of cases, as were *Streptococcus pneumoniae* strains in 91% of cases, and *M. catarrhalis* strains in 86% of cases. However, 62.9% of the nasopharyngeal cultures concurrently yielded a second middle-ear pathogen. Thus, the absence of a middle-ear pathogen in the nasopharyngeal culture strongly suggested that the pathogen was not present in the middle-ear space (Table 2).

Similar results were obtained in a study of nasopharyngeal secretions and middle-ear aspirates in 354 children with AOM. During acute infections, the proportion of nasopharyngeal cultures with organisms not considered pathogens (viridans streptococci, diphtheroids, *Neisseria* species, and nonhemolytic streptococci) declined significantly compared with healthy control subjects. Groothuis and colleagues confirmed that nasopharyngeal cultures had a significant predictive clinical value—only when negative—in identifying children likely to have sterile middle-ear effusion. Long and colleagues performed semiquantitative middle-ear and nasopharyngeal cultures in children with AOM. The isolate from the middle-ear fluid was present in the nasopharyngeal culture, and it was either the predominant bacterial organism or responsible for at least 50% of bacterial growth.

In summary, nasopharyngeal cultures may provide useful information about the presence of a pathogen in the middle ear. The cultures are sensitive, but not specific, and do not provide the degree of documentation needed for investigative purposes or care of patients.

**Bacteremia with Otitis Media**

Bacteremia is uncommon in children with diagnoses of otitis media. Teele and coworkers reported that 1.5% of children younger than 2 years with temperatures above 38.9°C with AOM were bacteremic. In an office practice, Schwartz and Wientzen found that 5.8% of febrile infants with otitis media had bacteremia, and McCarthy and colleagues noted a similar incidence in a study of bacteremia in children managed in the outpatient setting. Schutzman and colleagues reviewed 2,982 patients managed on an outpatient basis and concluded that 3% of children with otitis media had bacteremia, a rate comparable to that reported in children with no focus of infection. As in other bacteremia studies, the incidence of bacteremia increases with higher temperatures.

**Bacterial Quantification in Middle-Ear Fluids**

Although animal experiments permit quantification of middle-ear fluids, few studies of bacterial colony counts in suppurative middle-ear fluids obtained from patients with AOM have revealed information of value to direct management. Raisanen and Stenfors did colony counts on middle-ear fluids obtained from children with AOM, otitis media with effusion, and chronic suppurative otitis media. Fluids from children with AOM contained bacteria with median counts per ml of $10^7$, fluids from mucoid effusion in otitis media with effusion had median counts of $10^4$, and fluids from children with chronic suppurative otitis media had median colony counts of $10^8$. Homogenization of thick mucoid or purulent materials presents technical problems

---

**Table 2. CORRELATION OF NASOPHARYNGEAL COLONIZATION AND CONCURRENT PATHOGENS ISOLATED FROM MIDDLE-EAR FLUIDS IN CHILDREN WITH ACUTE OTITIS MEDIA**

<table>
<thead>
<tr>
<th>Acute Otitis Media Due To</th>
<th>Nontypeable <em>H. influenzae</em> (n = 43)</th>
<th><em>Streptococcus pneumoniae</em> (n = 21)</th>
<th><em>M. catarrhalis</em> (n = 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95</td>
<td>43</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>91</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>48</td>
<td>86</td>
</tr>
</tbody>
</table>

Adapted from Faden H et al. 13
and may not allow adequate sampling to provide valid colony counts.

**Streptococcus pneumoniae**

*Streptococcus pneumoniae* is the most frequent bacterial agent associated with AOM and the most frequent cause of severe disease and supplicative complications. The increasing incidence of multidrug-resistant strains and the introduction of a safe and effective conjugate polysaccharide vaccine have focused interest on the role of the pneumococcus in systemic and local diseases, including AOM. Pneumococcal otitis media was first described in 1888, and much of the microbiology, pathogenicity, and immunology was already known at the beginning of the antimicrobial era in the 1930s. Interested readers are referred to *The Biology of Pneumococcus* for a comprehensive understanding of the history and microbiology of this organism.

**Serotypes Responsible for Acute Otitis Media**

There are now 90 antigenically distinct serotypes of *Streptococcus pneumoniae*. Distinctive types of pneumococci were described in studies as early as 1897. Types designated 1, 2, 3, and 4 were identified through the 1920s and expanded to 29 separable serotypes by 1932. Type 3 was the dominant serotype responsible for AOM through the 1950s. The dominance of type 3 in the various early studies of AOM may be real or it may be an artifact of selection based on the recognizability of type 3 on agar plates because of its large capsule.

Extensive studies of the pneumococcal serotypes responsible for AOM are available as early as the 1940s. The results of aspirates of middle-ear fluids in children with AOM in the United States and Europe in the past 35 years have indicated that relatively few types are responsible for most disease. In recent surveys, the most common types responsible for AOM in order of decreasing frequency were types 19F, 23, 14, 6B, and 3 (Table 3). In developing countries, the type distribution may be different. Serotypes isolated from Pakistani children during the winters of 1986 to 1989, in decreasing order, were types 19F, 31, 16, 18A, 9V, 6A, and 15C. Similar to local disease represented by AOM, six to eight serogroups are responsible for 75% of pneumococcal isolates recovered from blood and cerebrospinal fluid.

The serotypes responsible for sequential episodes of AOM were described by Austrian and colleagues. Of interest is the relative constancy of the four most frequently isolated types (19, 23, 6, and 14). The implications of these data are that children have recurrent disease due to the most common types. Other features of these studies included (1) simultaneous infection of the middle-ear fluid by two pneumococcal types, (2) isolation of the same type in consecutive episodes of AOM, and (3) isolation of the same type after an intercurrent episode of AOM due to another type.

The polysaccharide vaccine provides broad coverage but has limited efficacy for prevention of AOM in children under 2 years of age. PCV 7 (Prevnar, Wyeth Vaccines, Philadelphia, PA) includes the major serotypes but lacks coverage for types 1 and 3, which are present in the 11-type conjugate vaccine (GlaxoSmithKline Biologics, Rixensart, Belgium) and the investigational 13-type conjugate vaccine (Wyeth Vaccines), which also includes type 6A. The immunogenicity of the pneumococcal vaccines is described in Chapter 6, “Immunology,” and the clinical and microbiologic efficacies of the vaccines are described in Chapter 8, “Management.”

Pneumococci are frequent colonizers of infants and children, and the organisms present in the nasopharynx are frequently the types that result in AOM. The pneumococcal conjugate vaccine diminishes colonization due to types present in the vaccine. Data from South Africa and Israel indicate that colonization due to types present in the vaccine decreases, but colonization with vaccine serotypes remains constant over time because of replacement by non-vaccine serotypes.

Capsular polysaccharide antigens have been identified in most middle-ear fluids from which
Pneumococci can be cultured and also in some fluids that are sterile. Detection of bacterial antigens is discussed later.

**Adherence of Pneumococci to Respiratory Mucosa**

Microbial adherence identifies the binding of bacteria to components of the mucosal cell surface. Selective adherence to receptors on the mucosa of the nasopharynx and middle ear may contribute to the pathogenesis of AOM. Studies of adherence of pneumococci to mucosal surfaces suggest mechanisms of pathogenicity for respiratory infections. Pneumococci isolated from patients with recurrent episodes of AOM adhered in larger numbers to epithelial cells from the nasopharynx than did strains from cases of bacteremia or meningitis. More bacteria attached themselves to epithelial cells from patients with recurrent episodes of AOM than to cells obtained from control subjects. The availability of oligosaccharides that block the carbohydrate receptors on cells of the mucosa suggested an important mode of prevention of pneumococcal disease, but results of a large clinical trial did not confirm the promise of the oligosaccharides for prevention of AOM.

**Susceptibility of Pneumococci to Antimicrobial Agents**

Increased resistance of bacterial pathogens to available antimicrobial agents has been a constant concern since the introduction of
antimicrobial agents. Within a few years after introduction of the sulfonamides, previously susceptible group A streptococci and pneumococci became resistant; multidrug-resistant *Staphylococcus aureus* was a cause of pandemic disease in the 1950s and 1960s; gram-negative enteric bacteria that developed resistance to available antibiotics became a concern, particularly in hospitals, during the 1960s and 1970s; β-lactamase–producing *H. influenzae* and *M. catarrhalis* were identified in the 1970s; and multidrug-resistant pneumococci were identified in the 1970s and are now a major clinical concern throughout the world. In vitro antimicrobial susceptibility assays provide guidance for choice of effective drugs for treatment of AOM. The results of antimicrobial susceptibility tests and the clinical implications for choice of antimicrobial agents for treatment of AOM are discussed in Chapter 8, “Management.”

**Haemophilus influenzae**

Nontypeable strains of *H. influenzae* are commonly found in the nasopharynx of infants. Faden and colleagues studied the epidemiology of nasopharyngeal colonization in the first 2 years of life: 44% of children were colonized on one or more occasions; colonization with the initial strain persisted for 1 to 5 months (median, 2 months); and children carried one predominant strain at a time but were colonized with up to seven different strains.39 Otitis media due to *H. influenzae* is associated with non-typeable strains in most patients. When type b strains were prevalent, before the introduction of the polysaccharide vaccine, approximately 10% of the cases were type b, and about one-quarter of the cases of type b AOM had concomitant bacteremia or meningitis.40 Cases of otitis media due to types a, e, and f have been reported but are infrequent. For reasons that are unclear, *H. influenzae* was first identified as a pathogen of AOM in the 1940s; earlier studies identified the pathogen in few numbers or not at all. Subsequently *H. influenzae* emerged as second only to the pneumococcus as a cause of AOM and in recent years with the introduction of the pneumococcal conjugate vaccine, studies in Bardstown, KY, and Rochester, NY, suggest that nontypeable *H. influenzae* may replace the pneumococcus as the most frequently isolated pathogen of AOM.30,41

Studies by St. Geme and colleagues suggest that nontypeable strains of *H. influenzae* evolved from encapsulated organisms.42 The nonencapsulated types are heterogeneous and may be classified by biochemical and antigenic markers. The majority of strains of nontypeable *H. influenzae* isolated from middle-ear fluids belong to two biotypes based on assays of indole, urease, and ornithine decarboxylase.43 Current studies of outer membrane proteins also aim at a means of classifying the nonencapsulated strains.44 A serotyping system based on antigenic patterns of outer membranes has been suggested.45 The outer membrane protein profiles of paired nasopharyngeal and middle-ear isolates in children with AOM are similar.46 In addition, bacterial chromosomal deoxyribonucleic acid (DNA) has been used to determine the epidemiology and transmission of nontypeable *H. influenzae*.48

*H. influenzae* was considered to be restricted in importance to otitis media occurring in preschool children; however, the organism is a significant cause of otitis media in older children, adolescents, and adults. *H. influenzae* was isolated from middle-ear fluids of 36% of children aged 5 to 9 years with AOM.6 *H. influenzae* was the cause of otitis media in 33% of 18 children aged 8 to 17 years7 and was also isolated from 15 of 45 patients older than 16 years.9 In a survey of cases of AOM seen in primary care hospitals in metropolitan Tokyo for the year beginning July 1979, 28% of 31 bacteria isolated from middle-ear effusions of patients aged 10 to 15 years, and 15% of 76 bacteria found in patients aged 16 to 70 years, were *H. influenzae*.11 Thus, the proportion of AOM due to *H. influenzae* is approximately the same in all age groups.

*H. influenzae* appears to be the primary pathogen of the conjunctivitis–otitis-media syn-
drome. Bodor and colleagues obtained *H. influenzae* from simultaneous cultures of conjunctivae and middle-ear fluids in 18 of 20 episodes of the syndrome. Biotyping and outer-membrane protein analysis established that isolates obtained from the conjunctivae and middle ear were concordant.

Of the nontypeable strains of *H. influenzae* isolated from middle-ear effusions of children with AOM, 15 to 30% produce a β-lactamase that hydrolyzes ampicillin, amoxicillin, and penicillins G and V. The incidence of β-lactamase–producing strains has varied between 20% and 45% in Pittsburgh between 1981 and 1989, and was 41.6% in a 1997 US surveillance study. The antimicrobial susceptibility of *H. influenzae* strains recovered from patients with AOM is discussed in Chapter 8, “Management.”

### Moraxella catarrhalis

Nasopharyngeal colonization with *M. catarrhalis* is common throughout infancy; by the age of 2 years, three quarters of children have been colonized (see Table 2). In a study of children in the Buffalo, NY, area, colonization was more frequent in otitis-prone children than in children who did not have otitis media. Children tended to acquire and lose a number of different strains. Before the 1980s, *M. catarrhalis* was isolated infrequently from purulent middle-ear fluids, and many considered the organism a commensal with limited potential for causing disease. In 1983, reports from Pittsburgh and Cleveland noted a marked increase in incidence; the organism was isolated from middle-ear fluids of 22% and 27%, respectively, of a consecutive series of children enrolled in studies of AOM. In Dallas during a similar period, the incidence of *M. catarrhalis* in middle-ear fluids was lower: 6% of 150 children. The clinical course and epidemiology of AOM due to *M. catarrhalis* were described by Van Hare and colleagues. The pattern of outer membrane proteins appears to be homogeneous, including eight proteins with minimal variability among strains. Before 1970, almost all strains of *M. catarrhalis* were sensitive to penicillin and ampicillin. Today, a majority of strains of *M. catarrhalis* isolated from middle-ear fluids produce β-lactamase, and many of the patients fail to improve if they are treated with a β-lactamase–susceptible drug. The data from Pittsburgh are similar to those from other centers in the United States and Scandinavia and identify approximately 80% resistance in strains obtained from children with AOM, and close to 100% in fluids obtained from children with otitis media with effusion.

Leinonen and colleagues provided serologic evidence for a pathogenic role of *M. catarrhalis* in children with AOM. The presence of immunoglobulin G and immunoglobulin A antibodies to *M. catarrhalis* in serum or middle-ear fluid, or both, was correlated with isolation of the organism from the middle ear. An increase in titer of antibodies to the organism was found between acute and convalescent serums in 10 of 19 children with AOM whose middle-ear fluid yielded *M. catarrhalis* alone, and no increase was seen in 14 children with AOM whose middle-ear fluids yielded other pathogens.

### Groups A and B Streptococci

During the pre-antibiotic era, otitis media due to GAS was frequently associated with scarlet fever and was often of a destructive form. *Streptococcus hemolyticus* (presumably GAS) was the most prevalent organism in cultures taken at myringotomy for AOM and the most frequent cause of mastoid infection in patients undergoing mastoidectomy at the Manhattan Eye, Ear, and Throat Hospital during 1934. This experience was reflected in almost all studies of the bacteriology of AOM through the 1950s (see Table 1). In recent years, otitis media due to GAS is infrequent. A recent survey of the experience of the investigators in Beer-Sheva, Israel, identified GAS in 350 of 11,311 episodes (3.1%) of AOM during the years 1999 to 2003. The risk for mastoiditis was higher for patients with GAS AOM than for other pathogens.
almost complete disappearance of GAS as a cause of AOM since the 1950s is unexplained. We recognize the temporal changes in streptococcal M protein types that have been associated with the near disappearance of acute rheumatic fever in the United States, but there is a paucity of data about M types responsible for AOM in the past or present. Although the organism is still prevalent as a cause of tonsillo-pharyngitis, it appears to have lost its ability to replicate in the middle ear and cause suppurative otitis media.

Milder forms of AOM may occur with signs of common cold or pharyngitis in children younger than 3 years with culture and serologic evidence of streptococcal infection. In addition, Combs demonstrated that eustachian tube dysfunction is common in children with pharyngitis due to GAS. Rarely, acute necrotizing otitis media due to this *Streptococcus* occurs with rapid destruction of the tympanic membrane, which may be followed by mastoiditis and intracranial complications.

Group B streptococci have been isolated from various body fluids, including middle-ear fluid in neonates with otitis media. Bacteremia is frequently associated with otitis media in these infants.

**Staphylococcus aureus**

*Staphylococcus aureus* is an uncommon cause of AOM; the organism was isolated in less than 3% of samples of middle-ear fluids from children with acute infection (see Table 1). One study from Japan indicated a higher incidence of middle-ear infection (approximately 10%) due to *Staphylococcus aureus*, and a study of Italian children with human immunodeficiency virus (HIV) infection found a higher proportion of *Staphylococcus aureus* in middle-ear fluids of HIV-infected children (10%) contrasted with non–HIV-infected children (2.5%). *Staphylococcus aureus* was found in acute otorrhea that follows tympanostomy tube insertion. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as a concern in hospitalized patients for many years, but only recently has community-acquired MRSA appeared in patients without hospital experience. Community-acquired MRSA may result in AOM, and both MRSA and community-acquired MRSA have been identified in small series of patients with tympanostomy tubes who had AOM with otorrhea.

**Staphylococcus epidermidis and Diphtheroids**

The roles of coagulase-negative staphylococci and diphtheroids in AOM are uncertain. These organisms are considered commensals and are part of the skin flora of the external canal. Isolation of pure cultures of coagulase-negative staphylococci from cases of purulent middle-ear effusions after adequate cleansing of the external canal suggests a pathogenic role in a limited number of cases. Nine different species of coagulase-negative staphylococci have been isolated from middle-ear fluids; *Staphylococcus epidermidis* is the most common.

Specific antibody to diphtheroids was identified in middle-ear effusions and sera of children undergoing myringotomy for chronic otitis media with effusion. Bernstein and colleagues found antibody-coated *Staphylococcus epidermidis* and diphtheroids in the middle ears of children with otitis media with effusion. The fluids contained specific antibody, and in several cases of otitis media due to *Staphylococcus epidermidis*, antibody was present in middle-ear fluid but absent from serum. These data indicate that diphtheroids and *Staphylococcus epidermidis* may elicit an immune response in the middle ear. The role of these organisms in middle-ear disease, however, remains uncertain. It is possible that they are opportunistic bacteria that invade the middle ear only under certain circumstances, such as persistent effusion.

**Gram-Negative Bacilli**

Gram-negative bacilli are responsible for about 20% of cases of AOM in young infants, but these organisms are rarely present in the middle-ear effusions of older children with AOM.
A report from Israel described 33 patients of varying ages with AOM caused by gram-negative bacilli.76 *Pseudomonas aeruginosa* was isolated from middle-ear fluids of 23 patients, and an indole-positive *Proteus* species was isolated from the fluids in 6 patients. Of the patients, 7 were 3 months of age or younger, 16 were 4 to 24 months, and 10 were 2 to 80 years. Apart from 4 adult patients who had diabetes mellitus, there were no other patients with significant underlying diseases. However, the clinical patterns of the middle-ear infections were consistent with severe suppurative disease. Some of the patients were bacteremic, and others had complications, including mastoiditis and osteomyelitis of the base of the skull.

*P. aeruginosa* has a special role in chronic suppurative otitis media. The organism acts as an opportunistic pathogen, flourishes in moist environments such as the external ear canal, and may cause suppurative disease in contiguous sites. *P. aeruginosa* was the most frequent bacterial pathogen and was isolated from 38 of 40 Pittsburgh infants and children with chronic suppurative otitis media.77 The organism may also be found in acute otorrhea that follows tympanostomy tube insertion.69

**Anaerobic Bacteria**

Recent improvements in techniques to isolate and identify anaerobic bacteria have provided a better understanding of the anaerobic flora of humans and the roles of these organisms in disease. A workshop on the role of anaerobic bacteria in infections of the upper respiratory tract provided current information about the role of these organisms in AOM and otitis media with effusion. The consensus of the conference was that anaerobic bacteria played a minor role in acute and chronic otitis media.78 Of the studies that demonstrated a limited role for anaerobic bacteria in AOM, *Peptostreptococcus* species were the most frequent pathogen.79–82 In addition, *Fusobacterium* species and *Bacteroides* species have been implicated in chronic otitis media.83

**Disparate Bacterial Cultures**

Disparate results of cultures of middle-ear fluids occur when cultures of the two ears in bilateral disease yield different information: effusion from one ear is sterile, but a bacterial pathogen is isolated from the other ear; or a different bacterial pathogen is isolated from each of the two ears. Mixed cultures may also occur: two types or two species of bacteria are found in the same middle-ear fluid. Gronroos and colleagues reported 31.6% disparate results of cultures from children with bilateral otitis media.84 All children had either *Streptococcus pneumoniae*, *H. influenzae*, or group A streptococci in fluid from one middle ear and sterile fluid in the other. Van Dishoeck and colleagues found that 19% of cultures from children with bilateral otitis media yielded different results.85 The majority of children had a pathogen recovered from one ear and sterile fluid in the other. Also included were six cases in which cultures with middle-ear fluid yielded a single pathogen, but two pathogens were present in the opposite ear.

Austrian and colleagues recovered more than one serotype of *Streptococcus pneumoniae* from middle-ear fluids in 18 children, which represented 1.5% of the cases of bilateral pneumococcal otitis media.24 Pelton and coworkers cultured middle-ear fluid from both ears of 122 children with bilateral AOM.86 Disparate results were found in 31 (25%) of the children: in 25 children, a pathogen was present in one ear, and the fluid from the other ear was sterile or yielded a non-pathogen; in six children, different pathogens (*H. influenzae* and *Streptococcus pneumoniae* in each case) were isolated from the two fluids. Howard and colleagues noted *Streptococcus pneumoniae* and *H. influenzae* together in 20 effusions (5% of those studied).87

These data indicate that investigative microbiologic studies of bilateral otitis media must include aspiration of both ears to determine the efficacy of methods of treatment (ie, trials of antimicrobial agents) or prevention (ie, evaluation of vaccines or drugs). In addition, the complete bacteriologic assessment of the middle
Identification of Bacterial Antigens and DNA in Middle-Ear Fluids

Results of studies using techniques to identify bacterial antigens provide new insights into the infectious process and add to the number of cases of otitis media due to a bacterial pathogen. Countercurrent immunoelectrophoresis, latex agglutination, and enzyme-linked immunosorbent assay have been used to detect bacterial antigens such as capsular polysaccharides of *Streptococcus pneumoniae*, *H. influenzae* type b, *Neisseria meningitidis*, and group B streptococci in blood, urine, cerebrospinal fluid, and other body fluids. These methods are advantageous because of ease of performance, rapidity, specificity, sensitivity (as little as 0.2 ng of polysaccharide capsular antigens can be detected), and ability to identify bacteria that do not grow in culture media.

*Streptococcus pneumoniae* is identified by countercurrent immunoelectrophoresis in most middle-ear fluids in which the organism is cultured and in many specimens that have no bacterial growth. Luotonen and colleagues identified pneumococcal capsular polysaccharide antigen in 83% of middle-ear fluids from which *Streptococcus pneumoniae* was cultured and in about one-third of middle-ear effusions from which no bacteria were grown. Type-specific pneumococcal antigens may persist for periods in excess of 6 months. Palva and Lehtinen found pneumococcal capsular polysaccharide antigen in 16% of 108 middle-ear effusions from children who had otitis media with effusion; pneumococcus was isolated in only 1% of these samples.

Different serotypes have differing sensitivity of antigen detection. Thus, sensitivity for detection in culture-positive samples of types 1, 15, and 19 was high, whereas sensitivity for type 23 was low; the sensitivity for type 6A was higher than that for 6B. These methods used to detect bacterial antigen add information about the large number of patients who have negative results of bacterial cultures.

Use of PCR for bacterial and viral genome sequences adds an additional technique for identifying the role of microorganisms in AOM and otitis media with effusion. Post and colleagues identified DNA of *Streptococcus pneumoniae*, *H. influenzae*, and *M. catarrhalis* in 97 middle-ear fluids of patients with otitis media with effusion; 28.9% were both culture- and PCR-positive, but an additional 48% were PCR-positive and culture-negative for these bacterial species. Subsequent studies by the same group identified evidence of bacterial messenger ribonucleic acid (RNA) for *H. influenzae* in 11.8% of 93 specimens that were positive by culture and in 31.2% of specimens that were negative by culture. Middle-ear effusions from HIV-positive subjects were PCR-positive for HIV. Beswick and colleagues used a technique to extract DNA from middle-ear effusions of patients with otitis media with effusion and noted mixed bacterial populations, including organisms usually considered contaminants (*Staphylococcus* species and *Propionibacterium acnes*) or not previously identified with otitis media (*Shigella flexneri, Alloiooccus otitis*). Techniques that are far more sensitive than prior modes of microbiologic diagnosis raise questions about the importance of the new information in gaining insight into the role of various organisms in acute and chronic middle-ear infections.

Sterile Cultures

In all studies of AOM, a significant proportion (approximately one-third) of middle-ear fluids are sterile after appropriate and usual cultures for bacteria have been made. The cause of bacteriologic sterile cultures in these cases of otitis media may be one or more of the following:

- A nonbacterial organism, such as a virus, chlamydia, or mycoplasma.
- A fastidious bacterial organism, such as an anaerobic bacterium, that is not isolated by usual laboratory techniques.
• An immune response to a noninfectious agent, such as pollen or other antigen.
• Prior administration of an antimicrobial agent that would suppress growth of bacteria.
• Presence of antimicrobial enzymes, such as lysozymes, alone or combined with immunoglobulins in middle-ear fluid, that would suppress growth of bacteria.
• An acute illness in a child who has persistent middle-ear effusion from an episode of otitis media some time in the past.

Because children may have middle-ear effusion for weeks to months after the onset of AOM, an illness due to a subsequent non-otitic infectious episode during the time spent with middle-ear effusion persisting from a prior episode of otitis media, might be assumed by the physician to be a recurrence of AOM.95 Evidence of current or prior infection may be obtained by use of Gram stain, PCR, antigen detection, and culture for L-forms, which may increase the number of specific microbiologic diagnoses for AOM and otitis media with effusion. The Gram stain is of value in identifying fastidious bacterial organisms and may provide evidence of bacterial infection in spite of antibiotics or antimicrobial substances that inhibit growth of bacteria. PCR and antigen detection increase the sensitivity for identification of bacteria and viruses in various body fluids, including middle-ear fluids. L-forms are atypical morphologic forms of bacteria that have lost their rigidity because of defective cell walls. The L-forms require hypertonic media, such as hypertonic thioglycollate broth and hypertonic soft agar, and are undetectable by conventional culture methods. Ataoglu and colleagues identified L-forms in six of 40 specimens of middle-ear effusion obtained at the time of tympanostomy tube placement.96

VIRUSES

The clinical history suggests that viral infection is a frequent initiating event of AOM by producing congestion of the mucosa of the upper respiratory tract that may progress to obstruct the eustachian tube with pooling of middle-ear secretions behind the obstruction. Abnormalities of middle-ear pressure have been demonstrated during rhinovirus colds97 and after experimental rhinovirus,98 influenza virus,99 and respiratory syncytial virus (RSV) infections in adult volunteers.100 Epidemiologic data support the concept that viral infection is frequently an antecedent of AOM. In studies of Finnish children in day care, the highest incidence of AOM occurred on day 3 or 4 after the onset of an upper respiratory tract infection, and two-thirds or more of episodes of AOM occurred within a week of the onset of respiratory signs.101,102 Respiratory viruses have been isolated from the nasopharynx of between 30% and 50% of children with AOM.103 In a longitudinal study of respiratory illnesses and complications in children 6 weeks to 6 years old attending a day-care and school program, Henderson and colleagues demonstrated a correlation between isolation of viruses from the upper respiratory tract and clinical diagnosis of otitis media.104 Concurrent or antecedent (within 14 days) viral infection was identified in 26.3% of episodes of AOM in children younger than 3 years. Viral outbreaks coincided with epidemics of AOM. AOM increased in the 14 days after upper respiratory tract isolation of RSVs, adenoviruses (usually types 1, 2, and 5), influenza virus types A and B, parainfluenza and mumps viruses, and enteroviruses. Human metapneumovirus (hMPV) has been identified in children with acute respiratory illnesses ranging from lower respiratory tract diseases including bronchiolitis and pneumonia and upper respiratory tract infections105 and has been isolated from middle ear fluids of children with AOM.103 Human bocaviruses are recently described respiratory viruses identified by PCR techniques that have been identified in upper and lower respiratory tract infections including acute otitis media.105a

Further evidence for the role of viruses in the etiology of AOM has been obtained by isolating viruses or viral antigens from middle-ear fluids. The results of early studies indicated that virus isolation from middle-ear fluids of children with AOM was infrequent; a survey published in 1976...
indicated that virus was isolated from only 4.4% of 663 patients. The yield of virus in middle-ear fluids increased substantially in recent years with the use of more sophisticated techniques for virus isolation and identification of viral antigens. Ruuskanen and colleagues summarized eight studies published between 1982 and 1990 of the virology of AOM in 944 patients using immunoassay and isolation. Middle-ear fluid was positive for virus in 16%, and virus was identified as a single agent in middle-ear fluid in 6%; middle-ear fluid was found in combination with a bacterial pathogen in 11%; nasopharyngeal aspirates were positive in 39%; and RSV, rhinovirus, and adenovirus were most frequently isolated (Table 4). Similar data were presented by Heikkinen and colleagues in a study of middle-ear fluids of 456 children studied between 1989 and 1993. RSV was the dominant viral agent and was identified in 48 children (10.5%), followed by parainfluenza virus (3.3%) and influenza viruses (2.2%).

Reverse–transcriptase-PCR is a more sensitive tool than virus culture to identify viruses in middle-ear fluids. The use of PCR requires specific primers for each virus, and detection may be limited by the number of primers available and the small quantity of middle-ear fluid available from children with AOM. As more sensitive techniques are developed, and with the addition of new respiratory viruses such as hMPV, the yield of virus-associated AOM will likely increase. Pitkaranta and colleagues studied Finnish children with AOM and otitis media with effusion. Evidence of rhinovirus RNA was found in 22% and 19%, respectively; RSV in 18% and 8%, respectively; and coronavirus in 7% and 3%, respectively, in the acute and chronic infections. Pass noted that none of the studies to detect virus included a control group of healthy children, which would be necessary to distinguish a passive or active role for virus in the etiology of AOM.

Viruses may act in part by stimulating the release of inflammatory mediators, including tumor necrosis factor, interleukins (IL-1, IL-6, IL-8) and leukotrienes as well as histamine. In addition, respiratory viruses appear to activate epithelial cells, resulting in increased adherence of bacteria to the cells. Although increased adherence of bacteria to virus-infected respiratory mucosa has been demonstrated in vitro, in animal experiments, and in humans infected with influenza A virus, the mechanism of viral–infection-induced bacterial adherence in AOM remains obscure.

Most patients with coexisting viral and bacterial infections respond well to appropriate antibacterial agents, but persisting symptoms may be due to underlying virus infection. Chonmaitree and colleagues noted that a higher proportion of patients with virus and bacteria in middle-ear fluids failed to clear the bacteria 2 to 4 days after initiation of therapy, compared with the group who had bacteria alone. Rhinovirus was associated with a higher rate of bacteriologic failure than other respiratory viruses, including RSV, parainfluenza virus, and influenza viruses. The data suggest that the presence of a virus in the middle-ear infection may interfere with bacteriologic and clinical responses to antimicrobial agents.

Otitis media may accompany exanthematous viral infections, such as measles and infectious mononucleosis caused by Epstein-Barr virus. The isolation of cytomegalovirus (CMV) and herpes simplex virus in middle-ear fluids from 10 of 271 children (4%) with AOM by Chonmaitree and colleagues raised questions about the role of the organism in the clinical course of the disease. Eight of the children with CMV infection were 14 months of age or younger, which raised the possibility of persistent

<table>
<thead>
<tr>
<th>Virus</th>
<th>Middle-Ear Fluid (n = 5,810)</th>
<th>Nasopharyngeal (n = 773)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory syncytial</td>
<td>7</td>
<td>15</td>
</tr>
<tr>
<td>virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>39</td>
</tr>
</tbody>
</table>

Adapted from Ruuskanen O, et al.
congenital infection. CMV and herpes simplex virus were present in the middle-ear fluids alone or combined with bacteria or other viruses. Among other rare causes of viral otitis media, smallpox virus has been demonstrated by the presence of Guarnieri’s bodies in the tympanic membrane of a fatal case in a 3-month-old Indian child who died of smallpox.  

The key role of selected viruses suggests that viral vaccines may be critical to the efforts to reduce the incidence of AOM. Studies of influenza virus vaccines and RSV vaccines and immunoglobulins are described in Chapter 6, “Immunology”.

**MYCOPLASMA**

Isolation and identification of mycoplasma in secretions obtained from the upper respiratory tract is now readily accomplished in solid and liquid media. An initial report of a volunteer study suggested a role for these organisms in otitis media. Myringitis, associated with hemorrhage and bleb formation in the more severe cases, was observed in nonimmune volunteers inoculated with *Mycoplasma pneumoniae*.  

Bullous myringitis in children may result from various bacterial pathogens responsible for otitis media; its presence does not indicate mycoplasma infection. The middle-ear fluid of a large number of patients (771) has been studied, and *Mycoplasma pneumoniae* was isolated in only one case.  

During an investigation of a community outbreak of pneumonia due to *Mycoplasma*, 59% of children with otitis media were shown (by isolation of the organism from the pharynx or by antibody responses) to have had an infection with *Mycoplasma pneumoniae*. Thus, it is likely that mycoplasma infection causes disease in all parts of the respiratory tract, including the middle ear. Patients with respiratory disease due to *Mycoplasma pneumoniae* may have accompanying otitis media, but the organism appears to play a limited role in the overall picture of AOM in children.

**CHLAMYDIA**

*Chlamydia trachomatis* is the etiologic agent of a mild but prolonged pneumonitis in infants. Many infants with pneumonia due to *C. trachomatis* have otitis media. Tipple and colleagues isolated the organism from ear aspirates of three of 11 infants with chlamydial pneumonia. Chang and colleagues recovered *C. trachomatis* from the middle-ear effusions of two of 12 children with AOM and one of 14 children with persistent middle-ear effusion. One of the children with acute infection was 10 months old, but the report did not provide ages for the other two children. *C. trachomatis* was not isolated from middle-ear fluids obtained at the time of tympanostomy tube placement in 68 children aged 9 months to 8 years. Thus, *C. trachomatis* is associated with acute respiratory infections, including otitis media, in young infants (younger than 6 months).  

*C. pneumoniae* has been associated with acute respiratory diseases, including pharyngitis, bronchitis, and pneumonia, but infrequently with otitis media. *C. pneumoniae* has been isolated from one child with AOM (together with *Streptococcus pneumoniae*), and serology or PCR of throat swabs suggested an association with otitis media (not further defined) in 11 Swedish children. The organism has been isolated from some patients with persistent middle-ear effusion: none of 75 Seattle children and 5 of 53 Danish children. The mean age of the Danish children with identification of *C. pneumoniae* (by PCR) in persistent middle-ear effusion was older than that of children in whom the organism could not be detected, suggesting a possible role in otitis media with effusion in school-aged children but not in infants or preschoolers.

**UNCOMMON MICROORGANISMS**

*Corynebacterium diphtheriae*

Diphtheritic otitis media may accompany diphtheritic croup and nasopharyngitis. Although
many cases cannot be differentiated from other forms of purulent otitis, diphtheritic membranes may form and be recognized in the middle ear. Complications are frequent, including destruction of the tympanic membrane and ossicles and invasive infection of contiguous structures, leading to necrosis of the mastoid process, temporal bone, and labyrinth.135,136 Thirteen cases of otitis media due to *Corynebacterium diphtheriae* were among 3,916 cases reported to the Centers for Disease Control and Prevention of the US Public Health Service for the years 1959 to 1970. Five cases of diphtheritic otitis occurred among 1,433 cases of diphtheria seen at the Los Angeles County Hospital during the 10-year period beginning June 1941.137

**Mycobacterium tuberculosis**

At the turn of the century, tuberculous otitis was an occasional cause of severe middle-ear disease, particularly in the very young. Turner and Fraser reported a series of cases at the Royal Infirmary in Edinburgh for the period 1907 to 1914; 51 (2.8%) were due to tuberculosis, and 84% of these cases occurred in the first year of life.138 Today, the disease is seen in underdeveloped areas of the world, but occasional cases occur in the United States. Bovine tuberculosis was responsible for 29 cases of chronic otorrhea in children seen in Kampala between 1969 and 1972139; 11 cases of tuberculous otitis media were reported in Capetown children between 1967 and 1971140; three patients with tuberculous otitis media were seen at the Children’s Memorial Hospital in Oklahoma City141; and two children had concurrent tuberculous arthritis and otitis.142 Tuberculous infection should be considered when chronic otorrhea occurs in recent immigrants from areas with high rates of infection and in patients who are immunocompromised. Skolnik and colleagues have reviewed the literature of tuberculosis of the middle ear.143

When otitis media occurs as the only apparent focus of tuberculous infection, the disease is usually due to ingestion of infected cow’s milk. The infection may also occur in patients with active pulmonary disease; the middle ear is infected from the upper respiratory tract.

Tuberculous otitis media is characterized by a painless, watery otorrhea through single or multiple perforations of the tympanic membrane; enlarged periauricular lymph nodes; a high incidence of facial paralysis; and early hearing loss. Mastoiditis is a frequent complication.144

The diagnosis of tuberculous otitis media is based on demonstration of acid-fast bacilli within a granuloma in biopsy materials, with or without the culture of *Mycobacterium tuberculosis* from the biopsy material, aural drainage, or aspirate of middle-ear fluid. Chemotherapy shortens the course and severity of the disease, but persistent hearing loss is frequent.

**Non-tuberculous Mycobacteria**

Case reports of persistent otorrhea or otitis media and mastoiditis due to *Mycobacterium fortuitum*,145 *Mycobacterium avium-intracellulare*,146 *Mycobacterium chelonae*,147 and *Mycobacterium chelonae abscessus*148 indicate that these organisms may cause acute or chronic middle-ear disease. Chronic otorrhea due to non-tuberculous mycobacterial infections may occur in the child with tympanostomy tubes.147,148

**Clostridium tetani**

Orogenous tetanus usually occurs as a sequela of chronic otitis media. *Clostridium tetani* multiplies in the purulent drainage in the external ear canal and may gain access to the middle ear.149 The organism may also be present in the oropharynx, and it is possible that infection in the middle ear occurs through the eustachian tube. *Clostridium tetani* was isolated from swabs of middle-ear fluid of eight children admitted to Children’s Hospital in Bangkok with otitis media, otorrhea, trismus, and other signs of tetanus.150
Parasitic Infections

The only parasite that has been associated with otitis media is *Ascaris lumbricoides*. Roundworms may be vomited through the mouth or nostrils, enter the eustachian tube, and produce an inflammatory reaction in the middle ear. The worm perforates the tympanic membrane and emerges through the external canal. A case report described infection in an 18-month-old child who was brought to a Bombay clinic with a worm emerging from the ear. A 7.5-cm roundworm, *A. lumbricoides*, was removed from the canal and middle ear.151

Patients with HIV infection may have invasion of the sinuses and middle ear with opportunistic pathogens. Dunand and colleagues reported five cases of sinusitis due to *M. sporidium*, *Cryptosporidium*, and *Acanthamoeba* species, including one adult patient with recurrent AOM who had cryptosporidial oocysts identified in the middle-ear fluid.152

Fungi

Although otitis externa is often associated with fungi, otitis media is rarely associated with these organisms. Cases in which fungi have been implicated in AOM include patients with prior systemic disease due to the organism or complicated by granulation tissue. The case of a 12-year-old boy with a mass extending into the middle ear from the nasal cavity and infiltrated with *Blastomyces dermatitidis* was reported by Istorico and colleagues.153 Mycotic infections of the mastoid and middle ear, including *Candida* species and *Aspergillus* species, were identified in patients with chronic suppurative otitis media and with human immunodeficiency syndrome.154–156

MICROBIAL PRODUCTS

Biofilms

Microorganisms can attach to and grow on exposed surfaces to form biofilms. Biofilms are ubiquitous and have been associated with a number of infectious diseases, including intravascular catheter infections, native valve endocarditis, periodontitis, otitis media and chronic infection in patients with cystic fibrosis.157 *Staphylococcus epidermidis* and other coagulase-negative staphylococci (CoNS) can adhere to and grow on surfaces of synthetic polymers used in the manufacture of intravascular catheters. The strains of CoNS produce a mucoid substance (a slime or glycocalix or biofilm) that stimulates adherence of microcolonies to various surfaces in the environment and on epithelium.

The biofilm envelopes the bacteria and protects the organisms from the activity of antibacterial drugs and from host defenses including humoral and phagocytic cell functions. Biofilms have been identified also in the lungs of cystic fibrosis (CF) patients infected with *Pseudomonas aeruginosa*. Of interest is the therapeutic benefit of macrolide antibiotics in CF patients: the macrolides have minimal *Pseudomonas* activity but appear to disrupt the biofilm and reduce the synthesis of the matrix polysaccharide.158

Ehrlich and colleagues have studied mucosal biofilm formation in the middle ear.159 Chinchillas were infected with viable *H. influenzae* injected in the middle ear by a trans-bulla approach. The animals were treated with ampicillin 72 hours following infection in a dosage sufficient to sterilize the middle ear infection. Scanning electron microscopy and confocal laser scanning microscopy identified biofilm formation as early as 24 hours after infection, with persistence for 21 days in all animals that had developed middle-ear effusions. The mucosal biofilm in the otitis media model is comparable to biofilms identified in intravascular catheters infected with CoNS. The investigators suggest that the mucosal-biofilm concept may shed light on the pathogenesis of otitis media with effusion and lead to effective therapies for decreasing the duration of middle-ear effusion after episodes of AOM.
**Endotoxin**

Endotoxin has been detected in middle-ear effusions that contain nontypeable *H. influenzae*. Endotoxins are lipopolysaccharide complexes on the surface of gram-negative bacteria that have many biologic effects, including production of fever and inflammation. Physiologic activities persist after death of the organism. DeMaria and colleagues detected endotoxin in 80% of 89 middle-ear fluids obtained at tympanostomy tube placement. Not only was endotoxin present in all except one fluid from which *H. influenzae* was cultured, but it was also present (although in lesser concentrations) in fluids that had negative results at culture and fluids that cultured *Streptococcus pneumoniae* (which does not contain endotoxin). The source of endotoxin for the middle-ear fluids that did not contain endotoxin-producing microorganisms is unknown. Endotoxin may play a role in the pathogenesis of inflammation in the middle ear; purified endotoxin from killed *H. influenzae* induced production of middle-ear fluid.

**Interferon**

Local production of interferon in the middle ear was suggested by findings of higher concentrations in specimens of middle-ear fluid compared with levels in serum. The presence of interferon suggests a current or antecedent viral infection; Salonen and colleagues recovered interferon in middle-ear fluids positive for RSV antigen and failed to detect interferon in antigen-negative specimens. In contrast, Howie and colleagues demonstrated the presence of interferon in middle-ear fluids containing pathogenic bacteria in the absence of detectable viruses, suggesting bacterial induction of interferon. The role of interferon in middle-ear infection remains to be explained.

**Neuraminidase**

Neuraminidase identified at neutral pH was found in middle-ear fluids from patients with AOM and otitis media with effusion. LaMarco and colleagues noted that almost all fluids that grew *Streptococcus pneumoniae* had neuraminidase activity, whereas only about one-third of fluids that grew other bacteria, or no bacteria, had evidence of the enzyme. The plasma of patients lacked neuraminidase activity, indicating that the enzyme in the middle-ear fluid originated in the middle ear and was not a transudate from blood. Because mammalian neuraminidases have optimal activity near pH 4, the authors concluded that microorganisms were the source of the neutral pH neuraminidase. The enzyme may be an important factor in the pathogenesis of disease caused by *Streptococcus pneumoniae*.

**BACTERIOLOGY OF RELAPSE AND RECURRENCE**

*Relapse* is defined as reappearance of signs and symptoms after initial response during therapy or within 4 days after therapy ends; the organism responsible for the original infection has not been successfully eradicated. *Recurrence* is defined as reappearance of signs and symptoms 5 to 14 days after therapy ends; a new organism or event is responsible for the renewed signs of acute otitis media. These definitions of relapse and recurrence were provided in guidelines for anti-infective drugs for acute otitis media in 1992 by the Food and Drug Administration and the Infectious Diseases Society of America.

Studies of relapse and recurrence suggest varied microbiology for antibiotic failures. Tymanocenteses provided information about the initial isolate and the isolate recovered after initiation of drug; some of the isolates of relapse and recurrence were either the same or a different organism but were susceptible to the initial drug; some were the same or a different organism and were resistant to the initial drug; and many of the aspirates were sterile. Pichichero and Pichichero noted a shift in prevalence of resistant bacterial strains, including penicillin-resistant *Streptococcus pneumoniae* and β-lactamase–producing *H. influenzae* and *Moraxella catarrhalis*.
BACTERIOLOGIC STUDIES OF CHRONIC OTITIS MEDIA WITH EFFUSION

In the past, chronic and persistent middle-ear effusions have been assumed to be sterile because several reports described unsuccessful attempts to culture bacteria from them. Senturia and coworkers, however, were able to identify bacteria by means of smears and cultures from 42% of children with otitis media with effusion. Since then, other workers have reported similar results. The studies were performed by investigators in Columbus, Ohio, Boston, Minneapolis, Galveston, Finland, and Pittsburgh (see Figure 1) and included children who had persistent effusion for at least 2 months and had material obtained for culture at the time of myringotomy or tympanostomy tube placement. From 21 to 50% of the children had bacteria in the middle-ear fluid; *Streptococcus pneumoniae*, *H. influenzae*, *Moraxella catarrhalis*, or group A streptococcus was isolated from 10 to 22% of the fluids (Table 5). A higher incidence of respiratory pathogens was noted in children aged 3 years or younger in the Boston study. In addition to the classic bacteria, other microorganisms isolated from aspirates of middle-ear fluid in patients with otitis media with effusion include *Chlamydia pneumoniae*, *Mycobacterium chelonae*, and a unique, slowly growing gram-positive coccus as yet unidenti-

---

**Table 5. BACTERIOLOGIC RESULTS OF 179 CHRONIC MIDDLE-EAR EFFUSIONS IN CHILDREN**

<table>
<thead>
<tr>
<th>Type of Organism</th>
<th>Serous (48 ears)</th>
<th>Mucoid (112 ears)</th>
<th>Purulent (19 ears)</th>
<th>Total (179 ears)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>4</td>
<td>4</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>8*</td>
<td>11</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em></td>
<td>2</td>
<td>4</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>4</td>
<td>3</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>15</td>
<td>12</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Others†</td>
<td>31</td>
<td>22</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>Percentage of ears with organism²</td>
<td>52</td>
<td>43</td>
<td>68</td>
<td>83</td>
</tr>
</tbody>
</table>

*Includes one effusion with *H. influenzae* type b; no ampicillin-resistant organisms were identified.

†This number is smaller than the sum of each percentage above because some ears contained more than one type of organism.

Adapted from Riding KH, et al. 190

---

In summary, the middle-ear fluid of asymptomatic children may harbor bacterial pathogens similar to those identified in AOM, including *Streptococcus pneumoniae* and *H. influenzae*. The significance of this finding is uncertain at present. There were only minimal differences in the rates of isolation of bacteria from serous, mucoid, or purulent fluids (see Table 5). Bacterial pathogens associated with AOM are eradicated from the middle-ear fluids by appropriate antimicrobial agents within 3 to 4 days. The results of studies of children with persistent middle-ear effusion suggest that the organisms gain entry again to the middle-ear fluid and may be isolated from those fluids but apparently do not cause significant clinical signs (apart from the persistence of the effusion) in many children. The bacteria may be present without provoking an inflammatory response or they may produce a low-grade or subclinical infection, or the effusion may represent an immune response to the prolonged presence of the bacteria. Specific antibody to the bacteria isolated is present in the middle-ear fluid of children with persistent effusion. This finding suggests that the bacteria are not passive in chronic middle-ear effusion but elicit an
immunologic response and may be involved in the production and persistence of fluid.

**OTITIS MEDIA IN THE NEWBORN INFANT**

Bacteriologic data are available from aspiration of middle-ear fluids of 169 neonates with otitis media studied in the 1970s. A recent study of AOM in infants younger than two years of age found that, even in the first month of life, *Streptococcus pneumoniae* and *H. influenzae* are the bacteria isolated most frequently in the very young, as is the case in older infants and children. A recent study of AOM in infants younger than two years of age found that, even in the first month of life, the pneumococcus was responsible for approximately half the isolates. However, organisms associated with local and systemic infection in the newborn infant—group B *Streptococcus*, *Staphylococcus aureus*, and gram-negative enteric bacilli—may be responsible for acute otitis media in the neonate. When term infants who have had no problems with delivery or nursery experience develop otitis media 2 weeks or more after hospital discharge, the bacterial pathogens are most likely to be *Streptococcus pneumoniae* and *H. influenzae*.

**BACTERIOLOGIC STUDIES OF ACUTE OTORRHEA IN CHILDREN WITH TYPANOSTOMY TUBES**

Acute otorrhea through a patent tympanostomy tube occurs in about 50% of ears with tubes. In part, the bacteriology of the drainage from the middle ear is comparable to that of acute otitis media. Acute infection in the child with tympanostomy tubes is due to the same pathogens, but there is no opportunity for a suppurative abscess to develop; instead, the infection is in the form of a mucositis with infected secretions. Some children with tympanostomy tubes may have the middle-ear space invaded by organisms present in the external canal. Mandel and colleagues obtained cultures of otorrhea by swabbing the external canal close to the tympanostomy tube. Pathogens typical of acute otitis media (*Streptococcus pneumoniae, H. influenzae*, and *Moraxella catarrhalis*) were found in 42% of episodes of acute otorrhea in children with tympanostomy tubes; organisms that colonize the external canal (*P. aeruginosa* or *Staphylococcus aureus*) were found in 44% of episodes of otorrhea.

**BACTERIOLOGIC STUDIES OF THE EXTERNAL EAR CANAL**

The microbial flora of the external canal is similar to the flora of skin elsewhere on the body. In various microbiologic studies, there is a predominance of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and diphtheroids and, to a lesser extent, anaerobic bacteria such as *P. acnes* and anaerobic cocci. Pathogens responsible for infection of the middle ear—*Streptococcus pneumoniae, H. influenzae*, and *Moraxella catarrhalis*—are uncommonly found in cultures of the external auditory canal when the tympanic membrane is intact. Isolation of *Staphylococcus epidermidis*, *Staphylococcus aureus*, diphtheroids, or certain anaerobic bacteria from cultures of middle-ear fluids may represent contamination of the fluid by organisms present in the external canal. Adequate cleansing of the external canal is necessary before tympanocentesis is performed for the purpose of microbiologic diagnosis.

Diseases of the external canal are not considered in this monograph. The interested reader is referred to a monograph by Senturia and colleagues.

**REFERENCES**


Beswick AJ, Lawley B, Fraise AP, et al. Detection of Alloiococcus otitis in mixed bacterial populations from...
124 OTITIS MEDIA IN INFANTS AND CHILDREN


caused by both *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*: a randomized double-blind efficacy study. Lancet 2006;367:740–748.
