TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 1019

MEDICA - ODONTOLOGICA

TREATMENT OF ACUTE OTITIS MEDIA

by

Paula Tähtinen

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2012

From the Department of Pediatrics, University of Turku, Turku, Finland,
and the National Graduate School of Clinical Investigation

Supervised by

Aino Ruohola, MD, PhD
Department of Pediatrics
University of Turku
Turku, Finland

Professor Olli Ruuskanen, MD, PhD
Department of Pediatrics
University of Turku
Turku, Finland

Reviewed by

Docent Harri Saxén, MD, PhD
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

Professor Anne Pitkäranta, MD, PhD
Department of Otorhinolaryngology
University of Helsinki
Helsinki, Finland

Opponent

Docent Marjo Renko, MD, PhD
Department of Pediatrics
University of Oulu
Oulu, Finland

ISBN 978-951-29-5047-8 (PRINT)
ISBN 978-951-29-5048-5 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2012

To Miikka
and our lovely children Vilma and Olivia

ABSTRACT

Paula Tähtinen
Treatment of Acute Otitis Media
Department of Pediatrics, University of Turku, Turku, Finland
and the National Graduate School of Clinical Investigation
Annales Universitatis Turkuensis, Medica-Odontologica,
Turku, Finland, 2012

Background: Acute otitis media (AOM) is the most common bacterial infection in young children, but the optimal management of AOM remains controversial. The aim of this study was to assess the efficacy of antimicrobial treatment, either immediate or delayed, for AOM and to compare parental experiences regarding the management of AOM in two countries with very different treatment guidelines.

Methods: Altogether, 322 children participated in a randomized, double-blind, placebo-controlled trial. Children 6–35 months of age with AOM received amoxicillin-clavulanate or placebo for 7 days. The primary outcome was the time to treatment failure. In the second study, the delayed antimicrobial treatment group consisted of recipients of placebo who had received rescue treatment. The immediate antimicrobial treatment group consisted of children allocated to amoxicillin-clavulanate group. Parental expectations and opinions were evaluated by questionnaires sent via public day care in Turku, Finland, and Utrecht, the Netherlands.

Results: Treatment failure occurred significantly more often in children receiving placebo as compared to antimicrobial treatment (45% vs. 19%, $P<0.001$). Delayed initiation of antimicrobial treatment did not worsen the recovery from AOM, but it was associated with worsening of the child's condition, prolongation of symptoms, and absenteeism from day care and parental absenteeism from work. According to the comparative questionnaire, antimicrobial use was more common in Finland than in the Netherlands. Finnish parents believed more often than Dutch parents that antimicrobials are necessary in the treatment of AOM.

Conclusions: Children with AOM benefit from antimicrobial treatment. Delayed initiation of antimicrobial does not worsen the overall recovery from AOM, but it might increase the symptom burden and create economic losses. Treatment practices and parental expectations seem to interact with each other. This needs to be considered when AOM treatment guidelines are updated.

Key words: Antimicrobials, acute otitis media, children, guidelines, international differences, parental experiences, treatment, wait-and-see approach

TIIVISTELMÄ

Paula Tähtinen
Äkillisen välikorvatulehduksen hoito
Lastenklinikka, Turun yliopisto, Turku ja Valtakunnallinen kliininen tutkijakoulu
Annales Universitatis Turkuensis, Medica-Odontologica, Turku, Suomi, 2012

Tausta: Äkillinen välikorvatulehdus on pienten lasten yleisin bakteeri-infektio, jonka hoidosta ollaan edelleen montaa eri mieltä. Tämän tutkimuksen tavoitteena oli selvittää antibiootin (joko välitön tai viivästetty) teho äkillisen välikorvatulehduksen hoidossa. Vertasimme myös vanhempien kokemuksia äkillisen välikorvatulehduksen hoidosta kahdessa maassa, joiden hoitosuosituksen eroavat huomattavasti.

Menetelmät: Yhteensä 322 lasta osallistui satunnaistettuun, kaksoissokkoutettuun, lumelääkekontrolloituun tutkimukseen. 6–35 kuukauden ikäiset lapset, joilla oli äkillinen välikorvatulehdus, saivat joko amoksisilliini-klavulaanihappoa tai lumelääkettä 7 vuorokauden ajan. Ensisijainen tulosmuuttuja oli hoidon epäonnistuminen. Toisessa tutkimuksessa ne lapset, joille oli hoidon epäonnistumisen vuoksi aloitettu avoin antibiootti tutkimuksen aikana, muodostivat viivästetty antibioottihoito -ryhmän. Ne lapset, jotka oli satunnaistettu saamaan amoksisilliini-klavulaanihappoa, muodostivat välitön antibioottihoito -ryhmän. Vanhempien mielipiteitä kartoittaneen kyselytutkimuksen kaavakkeet lähetettiin vanhemmille päivähoitopaikkojen avulla Suomen Turussa ja Hollannin Utrechtissa.

Tulokset: Hoito epäonnistui selvästi useammin lumelääkettä saaneilla kuin antibioottia saaneilla lapsilla (45% vs. 19%, $P<0.001$). Antibioottihoidon viivästyminen ei vaikuttanut korvatulehduksen paranemiseen, mutta viivästymiseen saattoi liittyä lapsen voinnin huononeminen, oireiden pitkittyminen, poissaolo päivähoidosta ja vanhempien poissaolo töistä. Kyselytutkimus osoitti, että antibioottien käyttö korvatulehdusten hoidossa oli yleisempää Suomessa kuin Hollannissa. Suomalaiset vanhemmat kokivat hollantilaisia useammin, että antibiootti on tarpeen välikorvatulehduksen hoidossa.

Johtopäätökset: Antibiootti on tehokas äkillisen välikorvatulehduksen hoidossa. Antibioottihoidon viivästyminen ei vaikeuta välikorvatulehduksesta paranemista, mutta se voi lisätä oireilua ja aiheuttaa kustannuksia. Hoitokäytännöt ja vanhempien odotukset ovat vuorovaikutuksessa keskenään. Tämä tulisi ottaa huomioon uusia äkillisen välikorvatulehduksen hoitosuosituksia laatiessa.

Avainsanat: Antibiootti, äkillinen välikorvatulehdus, lapset, hoitosuositus, kansainväliset erot, vanhempien kokemukset, hoito, aktiivinen seuranta

TABLE OF CONTENTS

ABBREVIATIONS

AOM	acute otitis media
CI	confidence interval
ET	Eustachian tube
H. influenzae	*Haemophilus influenzae*
MEE	middle ear effusion
M. catarrhalis	*Moraxella catarrhalis*
OM	otitis media
OME	otitis media with effusion
RTI	respiratory tract infection
PCV	pneumococcal conjugate vaccine
PCR	polymerase chain reaction
S. pneumoniae	*Streptococcous pneumoniae*

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I–III. The original publications have been reproduced with the kind permission of the copyright holders.

I Tähtinen PA, Laine MK, Huovinen P, Jalava J, Ruuskanen O, Ruohola A. A placebo-controlled trial of antimicrobial treatment for acute otitis media. *N Engl J Med 2011;364:116–26.*

II Tähtinen PA, Laine MK, Ruuskanen O, Ruohola A. Delayed versus immediate antimicrobial treatment for acute otitis media. *Pediatr Infect Dis J; in press.*

III Tähtinen PA, Boonacker CWB, Rovers MM, Schilder AGM, Huovinen P, Liuksila PR, Ruuskanen O, Ruohola A. Parental experiences and attitudes regarding the management of acute otitis media - a comparative questionnaire between Finland and the Netherlands. *Fam Pract 2009;26:488–92.*

1 INTRODUCTION

Acute otitis media (AOM) is one of the most common diseases of young children and a major indication for antimicrobial treatment in the outpatient setting (Vergison et al. 2010). It has been estimated that in Finland, with a population of 5 million, approximately 500 000 health care visits are made annually because of AOM (Niemelä et al. 1999); in the United States, with a population of 305 million, the corresponding number exceeds 11 million (Centers for Disease Control and Prevention 2008).

Despite the large number of published AOM treatment trials, the optimal management of AOM remains controversial. Several meta-analyses have concluded that antimicrobial treatment provides only modest benefit to the management of AOM as compared to symptomatic treatment (Rosenfeld et al. 1994, Del Mar et al. 1997, Rovers et al. 2006, Vouloumanou et al. 2009, Coker et al. 2010, Sanders et al. 2010, Shekelle et al. 2010). However, meta-analyses are based on numerous individual original trials, all of which have different outcomes and follow-up schedules. In addition, the original trials have been criticized for biased patient selection, lack of strict diagnostic criteria, and suboptimal spectra or dosages of antimicrobial agents (Bain 2001, Dagan and McCracken 2002, Pichichero and Casey 2008a, Pichichero and Casey 2008b). Thus, a recent update of a Cochrane Database Systematic Review concluded that further high-quality research is urgently needed to study the effect of antimicrobial treatment on the management of AOM and to identify subgroups of children who benefit the most from antimicrobial treatment (Sanders et al. 2010).

A wait-and-see approach, in which antimicrobial treatment is initiated only if the child's condition does not improve or if it worsens after an observation period, was introduced to reduce unnecessary antimicrobial use (van Buchem et al. 1985). This approach has been shown to be applicable for primary care and well accepted by parents (Little et al. 2001, Siegel et al. 2003, McCormick et al. 2005, Spiro et al. 2006, Chao et al. 2008). However, the consequences of delaying antimicrobial treatment on the recovery of the child with AOM are uncertain.

The current confusion regarding the treatment of AOM is reflected in the treatment guidelines of AOM which vary notably in the developed countries. Countries such as Finland and the Netherlands present two extremes: the Finnish guidelines recommend primarily antimicrobial treatment for AOM if the diagnosis is certain, while the Dutch guidelines suggest antimicrobial treatment only in selected cases of AOM (Appelman et al. 2006, Heikkinen et al. 2010).

The purpose of this study was to evaluate the efficacy of antimicrobial treatment for AOM. A randomized, double-blind, placebo-controlled trial was conducted in the age group with the highest incidence of AOM. In order to avoid the bias of previous trials, strict diagnostic criteria and an antimicrobial agent with optimal antimicrobial coverage

and dosage was used. The effect of delayed versus immediate antimicrobial treatment on the recovery of children with AOM was also studied. Parental experiences and opinions regarding the management of AOM were evaluated by using a comparative questionnaire between Finland and the Netherlands.

2 REVIEW OF THE LITERATURE

"The literature dealing with the bacteriology of acute otitis is so extensive that a review must be limited to only the most important studies, chiefly of recent date."

A thesis by E. A. Lahikainen, University of Turku, year 1953

2.1 Definition

Otitis media (OM) is an inflammation of the middle ear, which ultimately results in collection of middle ear effusion (MEE) (Bluestone and Klein 2007). OM can be further classified into AOM and otitis media with effusion (OME). The current terminology of OM (Table 1) is based on the report of an expert panel at the Seventh OM Research Conference in 1999. The panel convened to establish consensus on the confusing terminology surrounding OM and its complications (Bluestone et al. 2002).

Table 1. Otitis media: terminology and definitions.

TERM	DEFINITION
Otitis media (OM)	Inflammation of the middle ear. No reference to etiology or pathogenesis.
Acute otitis media (AOM)	Acute infection of the middle ear with rapid onset of signs and symptoms; middle ear effusion present
Otitis media with effusion (OME)	An inflammation of the middle ear with middle ear effusion, but no signs or symptoms of acute infection.
Middle ear effusion (MEE)	Fluid in the middle ear, regardless of etiology, pathogenesis, pathology, or duration. Can be serous, mucoid, or purulent, or a combination of these.
Otorrhea	Discharge from the ear.

In 2004, the American Academy of Pediatrics and the American Academy of Family Physicians published clinical practice guidelines on the diagnosis and management of AOM (Lieberthal et al. 2004). The guidelines included a definition of AOM which required three components:

1. History of acute onset of signs and symptoms
2. Presence of MEE
3. Signs and symptoms of middle ear inflammation

The diagnosis of AOM is certain if all three criteria are met. Acute otorrhea through a tympanostomy tube or a perforation of the tympanic membrane is also considered to be AOM. The recently updated Finnish guidelines on the management of AOM use the same diagnostic criteria for AOM as the American Academy of Pediatrics guidelines (Heikkinen et al. 2010).

2.2 Epidemiology

AOM is a disease of early childhood. It affects over 80% of all children before they reach the age of three (Teele et al. 1989). The incidence of AOM peaks in children 6–18 months of age (Teele et al. 1989, Alho et al. 1991, Chonmaitree et al. 2008). About 20–30% of children suffer from recurrent AOM, i.e., three or more episodes in 6 months or four or more episodes in 12 months (Sipilä et al. 1987, Teele et al. 1989). A young age at first presentation of AOM is significantly associated with a risk of recurrent episodes of AOM (Teele et al. 1989).

AOM is usually a complication of a viral respiratory tract infection (RTI), and the overall incidence of AOM complicating RTI is approximately 40% (Heikkinen and Ruuskanen 1995, Vesa et al. 2001, Chonmaitree et al. 2008). The association between viral RTI and AOM also explains the strong seasonal variation of AOM. Several studies from Finland have shown that the frequency of AOM episodes is lowest at around midsummer and highest in the winter, which concurs with the prevalence of viral RTI (Sipilä et al. 1988, Ruuskanen et al. 1989, Joki-Erkkilä et al. 1998, Vesa et al. 2001).

The incidence of AOM increased from the 1970s to the 1990s, probably due to the mounting popularity of day care for children (Lanphear et al. 1997, Joki-Erkkilä et al. 1998). Other explanations for the higher incidence rate in the 1990s may include changes in diagnostic practices, easier access to medical care, and increased parental awareness of AOM and its treatment. Joki-Erkkilä et al. (2000) demonstrated that the clinical picture of AOM was milder in the middle of the 1990s as compared to the late 1970s. This finding supports the hypothesis that currently children tend to visit the doctor at an earlier stage of the disease than previously. However, some recent studies imply that the consultation rates for AOM have decreased since the beginning of the 21st century (Plasschaert et al. 2006, Williamson et al. 2006). Perhaps the adoption of a wait-and-see policy, stricter diagnostic criteria, and a decreased general exposure to tobacco smoke contribute to the decreased incidence of AOM (Taylor et al. 2012).

The incidence of AOM may further decrease as the use of pneumococcal conjugate vaccine (PCV) becomes more popular. 7-valent PCV covers seven (4, 6B, 9V, 14, 18C, 19F, and 23) out of 91 *Streptococcus pneumoniae (S. pneumoniae)* serotypes. In randomized clinical trials, 7-valent PCV has reduced the number of AOM episodes from any cause by 6%–7% and AOM episodes caused by the serotypes present in the vaccine by 57% (Black et al. 2000, Eskola et al. 2001). In observational studies, the effect of 7-valent PCV on the number of AOM episodes has been even stronger and an average reduction of 19% in the incidence of AOM has

been reported (Taylor et al. 2012). The difference between the results of clinical trials and observational studies may be explained by herd immunity, although other factors independent of PCV may also be involved.

A new 10-valent PCV covers 10 *S. pneumoniae* serotypes and has a protein D carrier against nontypable *Haemophilus influenzae (H. influenzae)* (Vesikari et al. 2009). The protein D carrier increases the potential of the vaccine to prevent AOM. In a large Czech study, 11-valent PCV with a protein D carrier reduced the overall incidence of AOM by 34% (Prymula et al. 2006). The introduction of 13-valent PCV, which covers the seven serotypes in the 7-valent PCV and an additional six serotypes, has been estimated to decrease the incidence of pneumococcal AOM even further (Bryant et al. 2010, Shea et al. 2011). In addition, viral vaccines have a high potential to decrease AOM episodes by preventing viral RTI. Influenza vaccine prevents one third of all AOM episodes and up to 83% of AOM episodes associated with influenza infection (Heikkinen et al. 1991, Clements et al. 1995, Belshe et al. 1998).

2.3 Risk factors and genetics

According to a meta-analysis that pooled data from 22 observational studies, the most important environmental risk factor for AOM is day care outside the home (Uhari et al. 1996). In addition, siblings increase the risk for AOM (Uhari et al. 1996). The relative risk of OM seems to be related to the number of children in a group, which is probably due to increased exposure to RTI-causing viruses (Rovers et al. 1999). Other risk factors for AOM include parental smoking, use of a pacifier, and cow's milk allergy (Niemelä et al. 1995, Uhari et al. 1996, Jones et al. 2012). Maternal smoking increases the risk of recurrent AOM four-fold, even after the insertion of tympanostomy tubes (Hammaren-Malmi et al. 2007). Breastfeeding for at least three months may reduce the risk for AOM (Uhari et al. 1996). The role of atopic allergy as a risk factor for AOM remains uncertain.

A positive family history of AOM is a well-established risk factor for AOM (Uhari et al. 1996). Certain ethnic groups, such as Australian aboriginals and Inuits, are also more susceptible to AOM than others (Leach 1999, Morris et al. 2007, Koch et al. 2011). Part of this susceptibility may be explained by low socioeconomic status, which is an important risk factor for AOM (Paradise et al. 1997). However, some differences related to ethnicity may be due to true genetic variation. A prospective twin study suggested that there is a strong genetic predisposition to OM (Casselbrant et al. 1999), and epidemiological twin studies supported this finding (Kvaerner et al. 1997, Rovers et al. 2002). Same genes increase the risk of AOM in both sexes (Kvestad et al. 2004), although males appear to experience more episodes of AOM than females (Teele et al. 1989). Genetic variation may affect the development of AOM in many different ways: anatomic differences; physiologic function of the Eustachian tube (ET); or immune responses. Recent studies have focused on immunoglobulin markers and mucin genes and their role in a person's susceptibility to AOM. Recurrent OM appears to be associated with the HLA-A2 antigen and with the FBXO11, TLR4,

and TNF genes (Kalm et al. 1991, Rye et al. 2012). IL-6 gene polymorphism seems to contribute in recurrent OM, but the association between recurrent OM and polymorphism of cytokine genes is not yet completely understood (Revai et al. 2009).

2.4 Pathogenesis

2.4.1 Anatomy: nasopharynx, Eustachian tube, middle ear, and mastoid gas cells

The ET connects middle ear to the nasopharynx (Figure 1). The nose, the nasopharynx, the ET, the middle ear, and the mastoid gas cells compose a system of organs that play a crucial role in the development of AOM. These structures are all covered by respiratory mucosa. On the posterior wall of the nasopharynx, right above the adenoid, is the opening of the ET. The ET is surrounded by four muscles which are important for tubal function. The muscles are the tensor veli palatini, the tensor tympani, the levator veli palatini, and the salpingopharyngeus muscle (Bluestone and Klein 2007). In infants, the average length of the ET is 2 cm and the tube lies almost horizontally. In adults, the ET is about twice as long and it is angled 45˚ upwards (Ishijima et al. 2000).

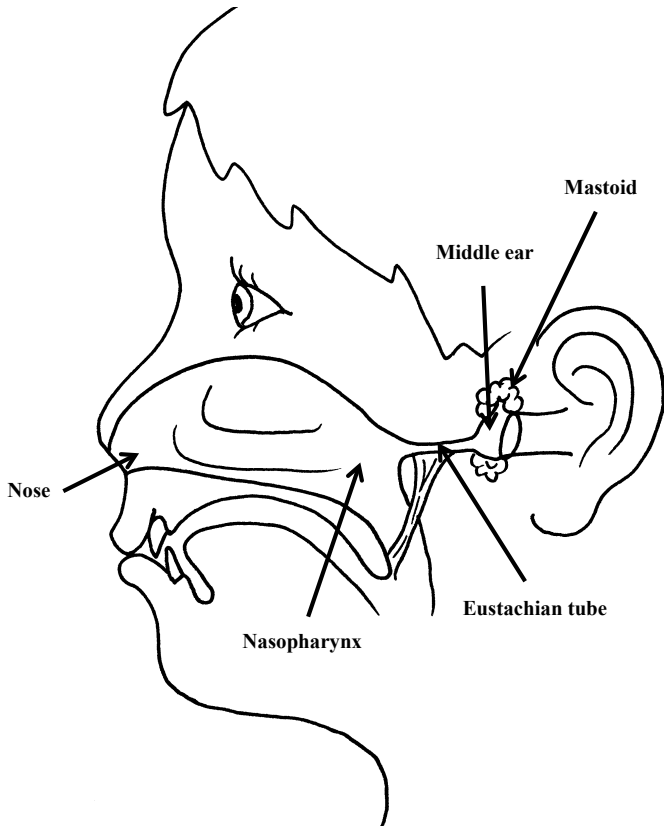

Figure 1. Anatomy of the nasopharynx, Eustachian tube, and middle ear. The child in the picture is approximately 1 year old: the Eustachian tube is short an it lies almost horizontally.
Figure by Jenni Jalkanen.

The middle ear lies within the temporal bone between the ear canal and the inner ear. Inside the middle ear are the three ossicles: the malleus, incus, and stapes. The middle ear cavity is normally a gas-filled space and its lateral wall is composed of the tympanic membrane, a flexible structure. The mastoid gas cell system is situated posterior to the middle ear cavity. The size of the mastoid gas cell system and the degree of pneumatization increases significantly from birth to school age (Bluestone and Klein 2007).

2.4.2 Physiology of Eustachian tube

Pressure regulation

The most important physiologic function of the ET is pressure regulation (ventilation) for equilibration of the gas pressure in the middle ear with surrounding atmospheric pressure (Bluestone and Klein 2007). At rest, the ET is passively closed. During swallowing, yawning, or sneezing the contraction of the tensor veli palatini muscle opens the ET and this equilibrates the pressure between the nasopharynx and the middle ear. Hearing is optimal when the gas pressure in the middle ear is the same as the air pressure in the ear canal. Children have a much poorer ability to equilibrate negative middle ear pressure by active muscular opening function than adults (Bylander et al. 1983).

Protection

The ET protects the middle ear by its functional anatomy. The tubal lumen is closed at rest, which prevents any fluid or nasopharyngeal secretions from entering the middle ear. During swallowing, the proximal end of the ET opens, but the liquid cannot enter the middle ear due to the narrow midportion (the isthmus) of the tube (Bluestone and Klein 2007). A prone position may, however, promote the reflux of secretions from the nasoprharynx to the middle ear (Winther et al. 2005). The local immunologic and mucociliary defence of the respiratory epithelium of the ET helps further to protect the middle ear from foreign microbial invaders.

Clearance

The ET removes secretions from the middle ear to the nasopharynx in two ways: by mucociliary clearance and muscular clearance (drainage). The ciliar activity in the middle ear and the ET clears secretions from the middle ear towards the opening of the ET and through ET in direction toward the nasopharynx (Stenfors et al. 1985). Similarly, the muscular pumping action of the ET drains secretions from the middle ear into the nasopharynx (Honjo et al. 1985).

Dysfunction

Several factors may interfere with the normal function of the ET. According to Bluestone and Klein (Bluestone and Klein 2007), ET dysfunction can be simply classified

as follows: “The tubal system can be either too closed or too open, or too much or too little pressure is present at either end of the ET.”

Obstruction of the ET may be caused by mucosal, inflammation-related swelling (intrinsic obstruction), or by compression by a tumor or an adenoid (extrinsic obstruction) (Bluestone and Klein 2007). Inefficient muscle function, floppy cartilage, or sudden high negative pressure may also result in functional obstruction (the ET does not open during swallowing). Children with cleft palate have functional obstruction of the ET and this predisposes to OM (Paradise and Bluestone 1974, Doyle et al. 1980). Children with craniofacial abnormalities and Down syndrome are susceptible to OM probably due to anatomical variations resulting in the ET dysfunction (Bluestone and Klein 2007).

The protective function of the ET may be lost if it is too open or too short. Some individuals have a pathologically patent ET that remains open even at rest. In small children, the ETs are shorter, more floppy, and lie more horizontal than in adults (Proctor 1967, Sadlerkimes et al. 1989, Ishijima et al. 2000). These anatomical differences promote reflux of nasopharyngeal secretions into the middle ear and explain partly why infants are more susceptible to AOM than adults. It has been suggested that, as a result of bipedalism and the development of big brain, humans are born 12 months too early (Bluestone 2008). This could explain why the immune system is immature and the ETs are too short and floppy in small children. A nonintact tympanic membrane caused by perforation or tympanostomy tube can also promote reflux to the middle ear because the normal gas cushion is disturbed and the system is too open (Bluestone and Klein 2007).

During RTI, the pressure in the middle ear is often highly negative and nasal obstruction can cause abnormal pressures in the nasopharyngeal end of the ET (Winther et al. 2002). As a result, nasopharyngeal secretions may reflux into the middle ear during swallowing and cause AOM (the Toynbee phenomenon). Gas pressures in the nasopharynx are also abnormal during scuba diving and airplane flying, and it is well known that pool diving during a RTI may result in AOM (Bluestone and Klein 2007).

Clearance is disturbed if the ciliary function is impaired. This may be caused by several factors, e.g., bacteria, bacterial toxins, and irradiation (Ohashi et al. 1989). Carson et al. (1985) reported that viral RTI results in ciliary abnormalities which may last even for 2–10 weeks after an RTI. Patients with Kartagener’s syndrome, a congenital disorder of the cilia, often have chronic MEE caused by impaired clearance (Mygind et al. 1983).

2.4.3 Viral respiratory tract infection

Henderson et al. (1982) were the first to show a strong association between viral RTI and AOM. In their 14-year longitudinal study, viral RTI tripled the risk of AOM. A large study by Ruuskanen et al. (1989) further demonstrated a correlation between viral outbreaks and concomitant increases in the incidence of AOM. Since then, several epidemiological studies have evaluated the roles of different viruses in AOM that complicates viral RTI

(Arola et al. 1990a, Uhari et al. 1995a, Vesa et al. 2001). These studies have shown that rhinoviruses and respiratory syncytial viruses are the two most common viruses associated with AOM. However, although rhinovirus was the most frequently found virus in these studies, the association between respiratory syncytial virus and AOM was stronger than between any other virus and AOM (Henderson et al. 1982, Ruuskanen et al. 1989, Arola et al. 1990a, Uhari et al. 1995a). A study that compared nasopharyngeal samples with MEE samples came to same conclusion: respiratory syncytial virus had a high ability to invade to middle ear during AOM (Heikkinen et al. 1999). Further, parainfluenza viruses and influenza viruses appeared to invade to MEE significantly more often than enteroviruses or adenoviruses. The association between viral RTI and AOM has been confirmed by several influenza vaccine trials showing that the incidence of AOM may be significantly reduced by preventing a viral RTI (Heikkinen et al. 1991, Clements et al. 1995, Belshe et al. 1998). In addition, animal studies have shown that intranasal inoculation with both viruses and bacteria simultaneously results in AOM significantly more often than inoculation of either pathogen alone (Giebink et al. 1980, Suzuki and Bakaletz 1994).

A viral RTI predisposes to AOM by disrupting the normal function of ET in several ways. After entering the nasal cavity, the virus induces inflammation in the mucosa of the nasopharyx (Matsuda et al. 1995, Fritz et al. 1999). This results in congestion of the mucosa and obstruction of the ET (Bluestone et al. 1977). Ultimately, obstruction of the ET results in a negative middle ear pressure (Bylander 1984). In addition, viruses inhibit the protective and clearance functions of the ET (Bakaletz et al. 1993, Park et al. 1993). Influenza viruses are able to suppress the leukocyte function of the host and, therefore, increase the susceptibility to secondary bacterial infection (Larson and Blades 1976, Abramson and Wheeler 1994). Data from studies with adult volunteers have suggested that viral RTI may alter the nasopharyngeal colonization rate and increase bacterial adherence to epithelial cells (Fainstein et al. 1980, Wadowsky et al. 1995). All these disruptions in the normal protective functions of the ET and nasopharynx allow pathogens to invade the middle ear cavity where the inflammatory process results in the production of MEE. Congestion of the ET further contributes to the accumulation of MEE, and ultimately a suppurative and symptomatic AOM develops.

2.4.4 Immunology

The immune system protects the body from foreign invaders, such as microbes, chemicals, and particulate foreign material. The immune tissue of the upper respiratory tract consists of the palatine tonsils, adenoid, lingual tonsils, and tubal tonsils. They form the Waldeyer's ring which acts as a gatekeeper, since pathogens that cause AOM first invade the throat or nasopharynx. The immune system also involves cells that interact with each other in order to eliminate the foreign invader (Bluestone and Klein 2007).

The immune system may be divided into innate and adaptive immunity. The innate immunity is responsible for fast and nonspecific recognition of pathogens. It does not require prior

exposure to the pathogen, nor does it develop immunologic memory. The adaptive immunity is a specific reaction to a certain antigen that results in production of antigen-specific T and B lymphocytes. The cells of the adaptive immune system remember the pathogen and create an efficient immune response in case of later re-exposition to the pathogen (Bluestone and Klein 2007).

The middle ear is covered by respiratory mucosa. If the microbe enters the middle ear, its immunologically active antigens induce ephitelial cells, dendritic cells, and mast cells to produce a local immune response. All major classes of immunoglobulins, complement components, lymphocytes, immune complexes, and various chemical inflammation mediators have been identified in the MEE of patients with acute or chronic OM (Bluestone and Klein 2007). The innate immunity is, however, considered to be the mediator of initial host response in the middle ear. It includes barrier functions, release of antimicrobial molecules, and activity of effector cells like neutrophils, macrophages, fibroblasts, natural killer cells, and eosinophils (Underwood and Bakaletz 2011).

Toll-like receptors are situated on the surface of mucosal cells. These receptors detect specific molecules at the surface of pathogens and activate several effector molecules such as cytokines, chemokines, interferons, proteases, defensins, collectins, lysozymes, and lactoferrins (Leichtle et al. 2011). Toll-like receptors activate the nuclear factor-κB via the myeloid differentiation factor-88 (MyD88) pathway. The cascade results in production of tumor necrosis factor and interleukins (Leichtle et al. 2011). In mice, deficiency or absence of genes that encode for Toll-like receptors or the signaling molecules results in failure of the organism to clear bacteria and OM will persist (Hernandez et al. 2008, Leichtle et al. 2009). Thus, the innate immunity signaling pathway via Toll-like receptors appears to be a critical step for the resolution of bacterial OM (Leichtle et al. 2011).

Defensins are antimicrobial peptides that provide rapid response protection against various bacteria and viruses. Defensins kill pathogens by forming a pore in the outer membrane. Some defensins inhibit bacterial toxins and some stimulate the inflammatory response. Especially β-defensins play an important role in killing common pathogens related to AOM (Underwood and Bakaletz 2011).

The innate and adaptive immune systems interact with each other in the middle ear. Cytokines and chemokines act as connectors between the innate and adaptive immune responses. Viral activation of epithelial cells results in cytokine and chemokine production, which activates the T and B lymphocytes of the adaptive immune system. As a result, the B lymphocytes start to produce specific immunoglobulins and T lymphocytes further stimulate the innate immune system via cytokine and chemokine production (Ogra 2000). Several studies have shown that high concentrations of immunoglobulins in the MEE facilitate clearance of bacteria and resolution of MEE (Sloyer et al. 1976, Karjalainen et al. 1991, Yamanaka and Faden 1993). Local immunity and B lymphocyte function have not developed fully in young children, a fact that may partly explain why children are at higher risk of AOM than adults (Lindberg et al. 1993).

Neutrophils kill bacteria by phagocytosis and eliminate the MEE from the middle ear. Recent studies have shown that *S. pneumoniae* and nontypable *H. influenzae* develop mechanisms that protect them from the phagocytosis (Dalia et al. 2010, Juneau et al. 2011). Bacteria may also prevent phagocytosis by forming a biofilm, an aggregate of bacteria in which they adhere to each other and form a thin layer on a surface. Biofilms have been found from the middle ear mucosa of children with chronic OM and recurrent AOM (Hall-Stoodley et al. 2006). It has been suggested that biofilms may protect the bacteria from the host's immune system and increase significantly the resistance of bacteria to antimicrobials (Underwood and Bakaletz 2011).

2.5 Microbiology

2.5.1 Colonization of the nasopharynx

The nasopharyx is colonized by bacteria within four months after birth (Faden et al. 1997). In addition to potential pathogens, a wide variety of non-pathogenic bacteria are present in the nasopharyx. These microbes interact constantly with each other. The host's innate immune response takes part in the interaction by clearing pathogens and, in this manner, by changing the composition of colonizing flora. Typically, the pathogens persist in the nasopharynx from one to five months, and several different strains of each pathogen may be present at the same time (Faden et al. 1995).

The carriage rates of the common AOM pathogens such as *S.pneumoniae, H. influenzae,* and *Moraxella catarrhalis (M. catarrhalis)* vary notably between different studies and different countries (Garcia-Rodriguez and Martinez 2002). Age, day care settings, number of siblings, viral RTI, breastfeeding, and sleeping position appear to be associated with the nasopharyngeal colonization rates, but the exact mechanisms of colonization remain uncertain. At the molecular level, bacterial adhesion to host cell carbohydrate receptors is essential for colonization. Adhesion results in a local immune response to eliminate the pathogen. The magnitude of the initial immune response is apparently associated with the colonization rate and the bacterial carriage time (Garcia-Rodriguez and Martinez 2002).

Colonization of the nasopharynx before the age of three months, especially with *M. catarrhalis,* may increase the risk of AOM (Faden et al. 1997). A high frequency of colonization appears, as such, to predispose the child to OM. The association between the nasopharyngeal flora and the middle ear pathogen was demonstrated in a study by Murphy et al. (1987). They compared paired nasopharyngeal and middle ear isolates of nontypable *H. influenzae* during AOM and found out that the strains detected from the MEE were the same as in the nasopharynx.

Non-pathogenic bacteria in the nasopharynx may antagonize colonization by pathogenic bacteria. In vitro studies suggest that α-hemolytic streptococci inhibit the colonization of *S. pneumoniae*, *H. influenzae,* and *M. catarrhalis* (Tano et al. 1999, Tano et al. 2000). This hypothesis is further supported by the finding that recolonization of the nasopharynx with α-hemolytic streptococci may protect against recurrent AOM and OME (Roos et al. 2001). Recent studies in children with AOM have shown that the common AOM pathogens predominate in the nasopharynx and that the protective, non-pathogenic bacteria are less frequent (Laufer et al. 2011, Hilty et al. 2012). Interestingly, also pathogenic bacteria appear to compete with each other. A study in children with RTI demonstrated that *S. pneumoniae* colonization inhibits colonization by *H. influenzae* (Pettigrew et al. 2008). In addition, there was an inverted association between *S. pneumoniae* and *Staphylococcus aureus* and between *H. influenzae* and *Staphylococcus aureus*.

External factors, such as antimicrobials and vaccines, can also modify the bacterial flora in the nasopharynx (Garcia-Rodriguez and Martinez 2002, Revai et al. 2006). When the pathogenic bacteria from the nasopharynx are eliminated, the burden of AOM may be reduced significantly (Vergison et al. 2010). However, several experts have warned that altering the colonization of one microbe may have consequences for other microbes in the nasopharyx (Murphy et al. 2009a, Vergison et al. 2010). In fact, the wide use of 7-valent PCV has already changed the microbiological etiology of AOM (Block et al. 2004, Casey and Pichichero 2004, Brook and Gober 2009). The worst case scenario is that this process might result in the emergence of multiresistant strains of bacteria.

2.5.2 Bacteria

In the pre-antibiotic era, *Streptococcus pyogenes* was one of the leading causes of AOM. Since the 1960s, the main bacterial pathogens causing AOM have been *S. pneumoniae*, nontypable *H. influenzae,* and *M. catarrhalis*. These three bacteria have been consistently cultured from the MEEs of patients with AOM, regardless of patient's age or country of origin (Bluestone and Klein 2007). The AOM pathogens in children are the same regardless of whether the children have an intact tympanic membrane or if they have acute otorrhea through a tympanostomy tube (Ruohola et al. 2006). Figure 2 shows the incidence of the three major pathogens in MEE samples. Data is from six studies conducted from 1980 to 2000 (Bluestone et al. 1992, Casselbrant et al. 1995, Heikkinen et al. 1999, Gehanno et al. 2001, Kilpi et al. 2001, Ruohola et al. 2006). All of these studies were conducted before the introduction of 7-valent PCV. The study by Ruohola et al. (2006) included only children with acute otorrhea through a tympanostomy tube. It used bacterial culture and polymerase chain reaction (PCR) to identify the microbes, while the other studies took MEE samples by aspiration through tympanic membrane and used culture for bacterial identification.

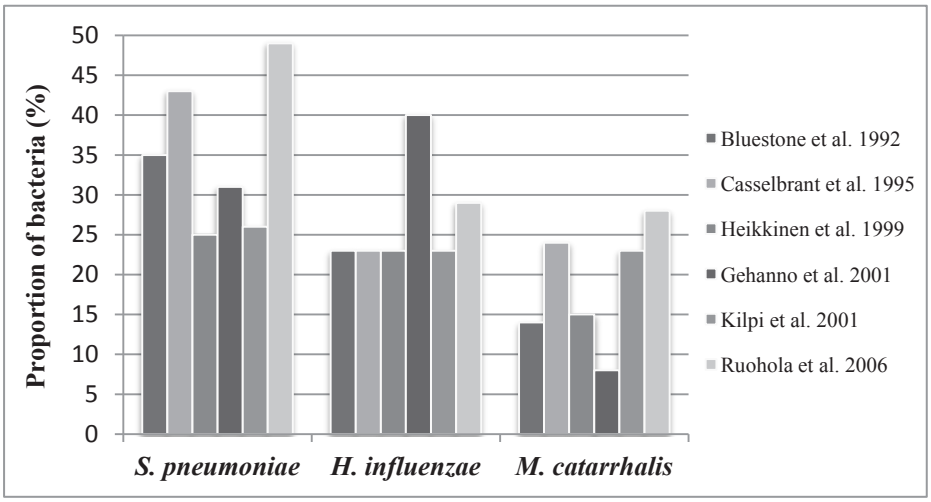

Figure 2. Bacterial etiology of acute otitis media from middle ear effusion samples. Percentages may exceed 100% because several bacteria may have been identified in middle ear simultaneously.

S. pneumoniae is one of the three most common AOM pathogens. The rate of nasopharyngeal carriage of *S. pneumoniae* is high, up to 43%, even in asymptomatic children (Syrjänen et al. 2001). The *S. pneumoniae* organism is covered by a polysaccharide capsule which protects it from immunological host defense. The polysaccharides determine the serotypes of *S. pneumoniae*. So far, 91 different serotypes have been described and they all differ by chemistry, virulence, and drug resistance (Hausdorff et al. 2000a, Hausdorff et al. 2000b, Hanage et al. 2005, Hausdorff et al. 2005).

In addition to AOM, *S. pneumoniae* causes substantial morbidity through sinusitis, pneumonia, and invasive diseases such as bacteremia and meningitis, and hence much effort has been put on prevention of pneumococcal diseases (Cartwright 2002). After the introduction of 7-valent PCV, the occurrence of invasive pneumococcal diseased has decreased dramatically. However, the effect of 7-valent PCV against AOM appears to be only modest (Black et al. 2000, Eskola et al. 2001).

H. influenzae is also a common pathogen in the nasopharynx and an important cause of AOM (Garcia-Rodriguez and Martinez 2002). It has six different capsular serotypes (a–f) and nontypable (nonencapsulated) strains. The type b strains of *H. influenzae* may cause severe invasive diseases, such as meningitis, epiglottitis, cellulitis, and pneumonia. Nowadays, these diseases can be prevented by an effective vaccine against *H. influenzae* type b. The nontypable *H. influenzae* strains may cause AOM, sinusitis, and pneumonia. AOM is often accompanied by conjunctivitis caused by the same strain of bacteria (Bodor 1982, Bodor et al. 1985, Murphy et al. 2009b). A protein D part of nontypable *H. influenzae* is included in the modern 10-valent PCV. Early studies suggest that this vaccine is cost-effective and may prevent more episodes of AOM than the 7-valent PCV alone (Prymula et al. 2006, Talbird et al. 2010).

M. catarrhalis, previously known as *Micrococcus catarrhalis, Neisseria catarrhalis, or Branhamella catarrhalis*, was regarded as a non-pathogen until the late 1970s (Murphy and Parameswaran 2009). Since then, its importance as a causative agent of AOM has been affirmed. *M. catarrhalis* accounts for approximately one fourth of AOM infections, and it is frequently found in MEE samples of children with OME. The prevalence of *M. catarrahalis* in the nasopharynx of young children is high but decreases with age. In adults, *M. catarrahalis* has an important role in chronic obstructive pulmonary disease (Murphy and Parameswaran 2009).

Other bacteria found from the MEE of patients with AOM are *Staphylococcus aureus, Diphteroids, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Proteus* (Bluestone and Klein 2007). A new species, *Alloiococcus otitidis*, has been detected in MEE of children with AOM, and its role in the pathogenesis of AOM is under investigation (Leskinen et al. 2004, Kaur et al. 2010).

The wide use of 7-valent PCV has changed the etiology of AOM. Studies in the United States using MEE samples have shown that after the introduction of 7-valent PCV, *H. influenzae* has become more prevalent and *S. pneumoniae* less prevalent as an AOM pathogen (Block et al. 2004, Casey and Pichichero 2004, Brook and Gober 2009). In addition, the proportion of non-vaccine serotypes of *S. pneumoniae* has increased while the proportion of vaccine serotypes has decreased correspondingly (Block et al. 2004, McEllistrem et al. 2005). The introduction of 10-valent and 13-valent PCV will probably decrease the amount of pneumococcal AOM even further because the non-vaccine serotypes appear to be less pathogenic than the serotypes covered by the vaccine (Shea et al. 2011). 10-valent PCV also has a protein D carrier, which allows protection against nontypable *H. influenzae.* In a randomized clinical trial, 11-valent PCV with protein D carrier reduced the number of AOM caused by nontypable *H. influenzae* by 35% (Prymula et al. 2006). So far, no epidemiological data has been presented on the overall influence of 10-valent and 13-valent PCVs on the etiology of AOM.

2.5.3 Viruses

Viruses were first detected from the middle ear in the early 1950s (Yoshie 1955). Since then, several modern molecular methods have been applied to evaluate the whole microbiological etiology of AOM. Currently, the most sensitive methods for the detection of viruses use PCR techniques.

Viruses have frequently been detected from MEE during AOM. The rate of virus detection in MEE during AOM varies from 13% to 74% (Sarkkinen et al. 1985, Chonmaitree et al. 1986, Arola et al. 1990b, Chonmaitree et al. 1992, Pitkäranta et al. 1998, Heikkinen et al. 1999, Chonmaitree and Henrickson 2000, Nokso-Koivisto et al. 2004, Ruohola et al. 2006). Table 2 summarizes the data from 6 studies on the viral etiology of AOM. The two most common viruses related to AOM are rhinovirus and respiratory syncytial virus. Human bocavirus is a recently discovered respiratory virus which has also been

identified in MEE during AOM (Ruohola et al. 2006). Another new virus, the human metapneumovirus was shown to be an AOM pathogen soon after its discovery (Suzuki et al. 2005, Ruohola et al. 2006).

Table 2. Viruses in middle ear effusion during acute otitis media.

Authors and year	No. of children/ MEE samples	Methods	Proportion of virusposi-tive MEE	Viruses in MEE
Chonmaitree et al. 1986	84 children	Culture	20%	rhinovirus, influenza virus, parainfluenza virus, enterovirus, adenovirus
Pitkäranta et al. 1998	92 children	RT-PCR	48%	rhinovirus, RSV, human coronavirus
Heikkinen et al. 1999	456 children	Culture, antigen detection	13%	RSV, influenza virus, parainfluenza virus, enterovirus, adenovirus
Chonmaitree and Henrickson 2000	65 MEE samples	Culture, RSV-EIA, RT-PCR	74%	RSV, influenza A virus, parainfluenza virus 1 and 3
Nokso-Koivisto et al. 2004	2175 MEE samples	Antigen detection, multiplex RT-PCR	38%	rhinovirus, RSV, influenza A virus, parainfluenza virus 1, 2, and 3, enterovirus, adenovirus, parechovirus
Ruohola et al. 2006	79 children	Culture, antigen detection, PCR, RT-PCR	70%	rhinovirus, RSV, influenza A virus, parainfluenza virus 3, enterovirus, nontypable picornavirus, human bocavirus, human metapneumovirus, coronavirus

MEE=middle ear effusion
PCR=polymerase chain reaction
RSV=respiratory syncytial virus
RSV-EIA=RSV antigen detection by enzyme immunoassay
RT-PCR=reverse transcription polymerase chain reaction

In conclusion, nearly all respiratory viruses have been detected from the MEE during AOM. However, the exact role of viruses in the pathogenesis of AOM is controversial, as some experts have suggested that viruses may be innocent bystanders in the middle ear rather than real pathogens (Chonmaitree et al. 2012). This debate continues. Improved assessment methods may finally give an answer to this question in the years to come.

2.5.4 Mixed infections

In most cases of AOM, bacteria and viruses are present concomitantly. In a study by Chonmaitree et al. (1992), viruses were identified in the MEE of 24% of patients with AOM and bacteria in 76% of patients. This study, together with the one by Arola et al. (1990b), suggested that a mixed viral-bacterial infection may cause prolonged symptoms of AOM. Introduction of modern molecular methods have improved the rate of detection of bacteria and viruses from MEE. In children with acute otorrhea through tympanostomy tube, bacteria were detected in 92%, viruses in 70%, and both in 66% of AOM cases (Ruohola et al. 2006).

2.6 Diagnosis

2.6.1 Symptoms

Acute symptoms, such as fever, ear pain, and respiratory symptoms are essential diagnostic criteria for AOM (Bluestone and Klein 2007). However, the specific symptoms at the time of AOM vary and often overlap with the concurrent viral RTI. Children with AOM have symptoms such as rhinitis, cough, irritability, restless sleep, and poor appetite, which are often considered to be caused by RTI (Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Kontiokari et al. 1998). The peak incidence of AOM is on the third day after the onset of RTI (Heikkinen and Ruuskanen 1994). Children with RTI and AOM cannot be distinguished from those with RTI without AOM only on the basis of symptoms (Laine et al. 2010). Furthermore, the symptoms and symptom severity do not correlate with the presence of bacterial or viral pathogens in the middle ear (McCormick et al. 2000).

Ear pain has been regarded as a specific symptom for AOM (Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Kontiokari et al. 1998). However, the absence of ear pain does not exclude AOM, since only 20–60% of children with AOM have ear pain (Arola et al. 1990a, Heikkinen and Ruuskanen 1995, Uhari et al. 1995b). The reliability of ear pain as an indicative of AOM seems to vary according to the age of the child. In younger children, the occurrence, duration, or severity of ear pain do not differentiate AOM from uncomplicated RTI, which is probably due to the poor ability of young children to express themselves verbally (Laine et al. 2010). Assessment of ear pain of children at preverbal age is done by their parents, a task that is difficult, if not impossible (Shaikh et al. 2010). Therefore, ear pain does not predict AOM in children at the otitis-

prone age, but ear pain is an indication for otoscopic examination of children with RTI (Uhari et al. 1995b, Laine et al. 2010). Other ear-related symptoms, such as ear rubbing or a sensation of having a blocked ear, were more common among children with AOM as compared to children with any acute symptoms (Niemelä et al. 1994). However, in a study including only children with RTI, ear rubbing was even more common in children without AOM than among children with AOM (Laine et al. 2010).

Fever occurs in 40–84% of children with AOM (Arola et al. 1990a, Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Uhari et al. 1995b, Kontiokari et al. 1998, Laine et al. 2010). In some studies, fever has been an indicative of AOM (Kontiokari et al. 1998) while others have found no association between the presence of fever and the presence of AOM (Niemelä et al. 1994, Heikkinen and Ruuskanen 1995, Laine et al. 2010). Fever may cause discomfort and anxiety for the child, but only seldom does fever alone raise the suspicion of the child's parents that the child may have AOM (Laine et al. 2010).

AOM is often accompanied by conjunctivitis (Laine et al. 2010). This so-called conjunctivitis-otitis syndrome was described in the 1980s, when Bodor et al. showed that nontypable *H. Influenzae* is the causative agent in almost all cases of purulent conjunctivitis associated with AOM (Bodor 1982, Bodor et al. 1985).

2.6.2 Signs on the tympanic membrane

Since the symptoms are not good predictors of AOM, otoscopic examination is crucial for the diagnosis of AOM. The presence of MEE as well as signs of middle ear inflammation are required for the diagnosis of AOM. Pneumatic otoscopy is the most important method for inspection of the tympanic membrane and for detecting MEE (Pelton 1998). The otoscopic examination, performed after careful removal of cerumen, should include an evaluation of tympanic membrane's position, color, translucency, light reflex, blood vessels, and mobility.

Otoscopic examination is, however, the most difficult part of AOM diagnosis. Cerumen removal from the ear canal of a struggling toddler is not without challenges, especially in a busy emergency room setting. For pneumatic otoscopy, the speculum should be large enough for air-tight contact, and good lightning is essential for sufficient visibility (Pelton 1998). An otomicroscope provides the best view of the tympanic membrane, but for practical reasons it is rarely used in general practice where most AOM episodes are diagnosed. Nowadays, hand-held pneumatic otoscopes with high magnification are available for easy visualization of the tympanic membrane (Macroview otoscope model 23810, Welch Allyn, Skaneateles Falls, NY, USA). It is of notice, however, that otoscopic examination is always subjective, and significant variation between the assessments of different physicians occurs (Karma et al. 1989). A diagnosis of AOM should always be based on tympanic membrane findings and, therefore, otoscopy training should play an important role in medical education.

Karma et al. (1989) carried out a large study which assessed the accuracy of different tympanic membrane findings for diagnosing AOM; tympanocentecis was the gold standard reference. They found out that the three signs predicting the presence of MEE best in children with acute symptoms were: cloudy color, bulging position, and unequivocally impaired mobility of the tympanic membrane. In a recent study by Shaikh et al. (2011), bulging of the tympanic membrane was the finding judged best to differentiate AOM from OME by AOM experts. However, the diagnostic criteria of AOM vary, and bulging of the tympanic membrane is not always required for a diagnosis of AOM (Hendley 2002). According to the American Academy of Pediatrics clinical practice guideline, the presence of MEE may be identified by any of the following: bulging of the tympanic membrane, limited or absent mobility of the tympanic membrane, or otorrhea (Lieberthal et al. 2004). A red tympanic membrane alone is not an indicative of AOM since it may be caused by crying, high fever, or even cerumen removal (Karma et al. 1989). In two studies where the diagnosis of AOM was confirmed by tympanocentesis, redness of the tympanic membrane was present in only 18% and 27% of AOM cases (Schwartz et al. 1981, Karma et al. 1989).

2.6.3 Tympanometry

Tympanometry provides objective information about the middle ear. It detects MEE by measuring acoustic admittance. Acoustic admittance describes the ease with which sound energy is transmitted from one medium to another. The movement of sound energy is most effective when the admittance levels of the two media are similar. Normally, the tympanic membrane and the three auditory ossicles help in transferring the sound energy from a high admittance medium (i.e., the air in the ear canal) to a low admittance medium (i.e., the fluid of the cochlea). In the presence of MEE this mechanism is disrupted and a higher proportion of sound energy is reflected back to the ear canal by the tympanic membrane (Brookhouser 1998).

Tympanometry sends a sound stimulus to the middle ear and measures the amount of reflected sound energy (i.e., the acoustic admittance) (Brookhouser 1998). Simultaneously, a vacuum pump changes the pressure in the ear canal from +200 daPa to -400 daPa. The admittance levels vary with the pressure in the ear canal. The results are displayed as a curve called tympanogram, where the x-axis displays the air pressure and y-axis the admittance. The height of the tympanogram illustrates the static admittance. In a healthy ear, positive and negative pressures in the ear canal move the tympanic membrane back and forward. At the highest pressures, the tympanic membrane is stretched and admittance is inhibited. As pressure decreases, admittance gradually increases. The tympanometric peak is the highest point in the tympanogram, and it indicates the point of maximum admittance. The tympanometric peak pressure demonstrates the point at which the air pressure is the same on both sides of the tympanic membrane. If the pressure is decreased even further, the tympanic membrane becomes convex and admittance is again inhibited. These changes in the admittance level can be seen in a normal tympanogram (A curve in Figure 3). MEE inhibits the movement of tympanic membrane and reduces

the admittance, which results in a straight tympanogram (B curve in Figure 3). A negative middle ear pressure results in a negative tympanometric peak pressure, and the curve is shifted to the left (C curve in Figure 3). The current classification of tympanograms (Figure 3) was introduced by Jerger (1970) and slightly modified by Orchick et al. (1978) and Zielhus et al. (1989) in the 1970s and 1980s.

Curve	**A**	**B**	**C**
Static admittance	≥0.2 mmho	<0.2 mmho	≥0.2 mmho
Peak pressure	>-100 daPa	No peak	≤-100 daPa
Middle ear status	Normal	MEE	Negative middle ear pressure with/without MEE

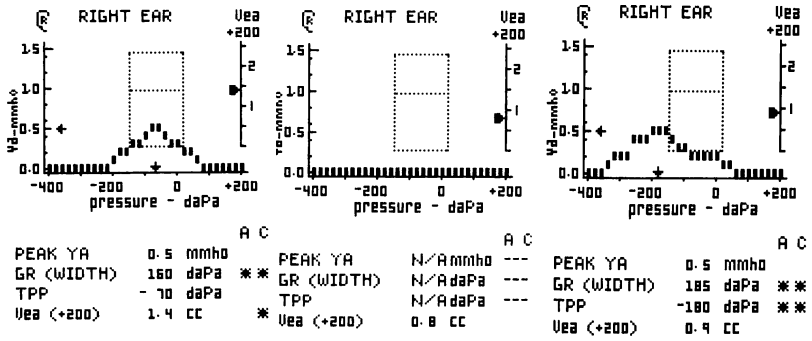

Figure 3. Classification of tympanograms.

The diagnostic value of tympanometry has been widely studied (Jerger 1970, Orchik et al. 1978, Zielhuis et al. 1989, Finitzo et al. 1992, Nozza et al. 1992, Sassen et al. 1994, Koivunen et al. 1997, Palmu et al. 1999, Saeed et al. 2004, Chianese et al. 2007). These studies have shown that tympanometry is a useful tool for detecting MEE, but its use requires some cooperation from the child. In most of the studies, a peaked tympanogram was strongly associated with healthy ear and a flat curve with MEE. Results from a large study by Smith et al. (2006) indicated that the lower the height and the greater the width of a tympanogram, the greater the probability of MEE. In conclusion, tympanometry may be used as an aid for diagnosing AOM but it cannot replace pneumatic otoscopy.

2.6.4 Acoustic reflectometry

Acoustic reflectometry determines the probability of MEE by measuring the response of the tympanic membrane to an acoustic stimulus (Kimball 1998). Acoustic reflectometry

sends a spectrum of sound energy to the ear canal and the sum of the emitted sound and the reflected sound are analyzed by an integrated microprocessor. Normally, sound waves are transmitted to the middle ear via the tympanic membrane. If the sound waves are out of phase, they are reflected back to the ear canal. The acoustic energy that is reflected back by the tympanic membrane is the reflectivity of the tympanic membrane. The instrument detects the reflectivity for each sound frequency from 1.8 to 4.4 kHz and forms a spectral gradient curve. This curve is used for calculating a spectral gradient angle. If the middle ear is filled with air, the tympanic membrane is vibrating normally and approximately half of the sound is reflected back to the ear canal. The frequency spectrum is broad, which results in a wide spectral gradient angle. If the middle ear is filled with MEE, the tympanic membrane is less mobile, and the frequency spectrum and spectral gradient angle are narrow. Thus, the angle is used to determine the probability of MEE. Spectral gradient angles are divided into 5 levels based on the probability of MEE (Kimball 1998).

Results from clinical studies have indicated that acoustic reflectometry may be helpful for confirmation of MEE (Barnett et al. 1998, Block et al. 1998, Block et al. 1999, Chianese et al. 2007, Linden et al. 2007, Teppo and Revonta 2007). The advantage of acoustic reflectometry is that it does not require a tight seal on the ear canal and it can be easily performed even if a child cooperates poorly. The measurement can also be made through a small opening in the cerumen. The reliability of acoustic reflectometry with regard to specific otoscopic diagnoses is not well studied, and hence pneumatic otoscopy examination is always needed in addition to acoustic reflectometry to differentiate AOM from OME (Lieberthal et al. 2004).

2.6.5 Tympanocentesis and myringotomy

Tympanocentesis means puncturing the tympanic membrane with a needle which is then used for aspiration of MEE (Bluestone and Klein 2007). Tympanocentesis has been used for diagnostic purposes because it provides objective evidence of the presence of MEE and enables a specific microbiologic diagnosis of AOM. Myringotomy, on the other hand, is an incision in tympanic membrane (Bluestone and Klein 2007). Myringotomy allows drainage of middle ear effusions and is primarily used for therapeutic purposes. Both procedures used to be the gold standard for the detection of MEE. However, even with proper anesthesia, tympanocentesis and myringotomy may be painful and cause anxiety for the patient. Therefore, these procedures are indicated only in selected cases of AOM: complicated AOM, seriously ill children, or patients with immunodeficiency. In these conditions, specific microbiologic diagnosis is needed and invasive procedures are therefore more justified.

2.7 Management

2.7.1 Symptomatic treatment

Symptomatic treatment is the cornerstone for the management of AOM. Whether antimicrobials are used or not, proper pain and fever management is important for the

wellbeing of the child. According to the American Academy of Pediatrics AOM treatment guidelines: "If pain is present, the clinician should recommend treatment to reduce pain." (Lieberthal et al. 2004). However, as previously pointed out, the assessment of pain is difficult in young children. Therefore, analgesics should routinely be included in the treatment of AOM, especially during the first 24 hours of an episode (Lieberthal et al. 2004). Actually, pain management should be initiated already when the parents suspect AOM.

There are only few studies evaluating the treatment of AOM-related ear pain. Bertin et al. (1996) compared ibuprofen, paracetamol (i.e., acetaminophen), and placebo for symptom relief for patients with AOM in a randomized, double-blinded, placebo-controlled trial. Ibuprofen turned out to be superior to paracetamol and placebo in the management of ear pain. Paracetamol was not significantly better than placebo. However, the dose of paracetamol was only 10 mg/kg, while in the Finnish recommendations the dose is 15–20 mg/kg (Heikkinen et al. 2010). Thus, the suboptimal dosing may partly explain why there were no significant differences in the analgetic effect between paracetamol and placebo. Overall, paracetamol and ibuprofen are well tolerated and inexpensive drugs. If administrated in adequate doses, they provide effective analgesia for mild to moderate pain (Lieberthal et al. 2004). In Finland, naproxen is also commonly used for the management of pain. Despite its anti-inflammatory nature, naproxen provides no additional effect on the resolution of MEE when combined to amoxicillin (Varsano et al. 1989). However, naproxen is well tolerated, and it has a relatively long-lasting analgesic effect. Paracetamol may be used simultaneously with ibuprofen or naproxen. All three drugs may be used as antipyretics, as well.

Anesthetic ear drops are useful especially in ear pain of sudden onset. Local application of benzocaine and lignocaine has been shown to be effective in the management of ear pain (Hoberman et al. 1997, Bolt et al. 2008). Hoberman et al. (1997) used olive oil as a placebo, while Bolt et al. (2008) used saline ear drops. In both studies, children received additional oral analgesia. Interestingly, naturopathic herbal ear drops appear to be as effective as anesthetic drops in the management of ear pain of children >6 years of age (Sarrell et al. 2001, Sarrell et al. 2003). A recent study evaluated the efficacy of homeopathic ear drops as an adjunct to standard therapy in children with AOM. Homeopathic ear drops were moderately effective in treating ear pain. However, the study was neither blinded nor placebo-controlled, and therefore the results may be biased (Taylor and Jacobs 2011).

2.7.2 Antimicrobial treatment

2.7.2.1 History

The discovery of penicillin was a significant step in the battle against bacteria, and the industrial production of penicillin in the 1940s revolutionized the treatment of several infectious diseases. Since the 1950s, antimicrobial agents have been the primary treatment for AOM. Lahikainen (1953) and Rudberg (1954) were the first to show that antimicrobial treatment significantly decreased suppurative complications and reduced the duration

of discharge after tympanocentesis. They collected MEE samples from a large number of patients with AOM and reported that the clinical recovery from AOM was clearly accelerated if the bacteria in the MEE were sensitive to the given antimicrobial agent. In a study by Lahikainen (1953), the mean duration of discharge after tympanocentesis was 9.0 days in patients treated without penicillin and 3.8 days in patients treated with penicillin. In a study by Rudberg (1954), the mean duration of discharge was 7.7 days in patients treated without antimicrobials, 5.0 in patients treated with sulphonamide, 4.2 in patients treated with penicillin and 3.9 in patients treated with suphonamide and penicillin simultaneously. The strengths of these studies are that they were conducted by a single investigator who examined the patients repeatedly throughout the follow up, and that the diagnosis was always confirmed by tympanocentesis or myringotomy, followed by microbiological analyses of MEE. However, during the past decades, numerous trials with a more sophisticated study design have been conducted to evaluate the efficacy of antimicrobial treatment for AOM.

2.7.2.2 Meta-analyses

Despite the large number of trials, the optimal management of AOM remains uncertain. Results from a meta-analysis by Rosenfeld et al. (1994) initiated the debate on whether AOM should be treated with antimicrobials or not. Since then, several meta-analyses have concluded that antimicrobial treatment provides only minor benefit to the management of AOM as compared to symptomatic treatment (Del Mar et al. 1997, Rovers et al. 2006, Vouloumanou et al. 2009, Coker et al. 2010, Sanders et al. 2010, Shekelle et al. 2010). These meta-analyses are based on numerous original studies all of which have different outcomes and follow-up schedules. It is of notice that the results of the meta-analyses may be strongly influenced by selection of the desired outcome. There are no criteria for defining what the optimal outcome of treatment of AOM should be, and the outcomes differ from study to study in relation to what the investigator decides to emphasize or consider important.

2.7.2.3 Methodological aspects

In the following review, the AOM treatment trials, which serve the basis for the meta-analyses, are evaluated separately. To adhere to the principles of evidence-based medicine, the review includes only randomized, double-blind, and placebo-controlled trials. In literature search, 11 clinical trials were found that fulfilled these three criteria (Halsted et al. 1968, Mygind et al. 1981, van Buchem et al. 1981, Thalin et al. 1985, Engelhard et al. 1989, Kaleida et al. 1991, Burke et al. 1991, Appelman et al. 1991, Damoiseaux et al. 2000, Le Saux et al. 2005, Hoberman et al. 2011). The methods of these 11 trials are summarized in Table 3.

To interpret the results of these 11 trials is difficult because of trial heterogeneity. In addition, most of the studies appear to have substantial limitations in their study design: lack of strict diagnostic criteria, inappropriate patient selection, and suboptimal

spectrum or dosage or antimicrobial agents. The following sections summarize the major methodological differences between the trials.

Diagnosis

Strict diagnostic criteria should be self-evident when evaluating the optimal treatment of AOM. Still, only 3 out of 11 trials reported to have used all three diagnostic criteria (rapid onset of symptoms, MEE, and acute inflammatory signs on the tympanic membrane) (Engelhard et al. 1989, Le Saux et al. 2005, Hoberman et al. 2011). Halsted et al. (1968) and van Buchem et al. (1981) also included children who had a red ear but no MEE in their study. These serious flaws in study design inevitably dilute the results. The diagnosis should be done by ear-nose-throat specialist or validated otoscopist to ensure that all children included in the study truly have AOM (Kaleida and Stool 1992). In the studies by Burke et al. (1991) and Damoiseaux et al. (2000), the diagnoses were done by 48 and 53 GPs, respectively. Such a large number of individual assessors most probably increase the risk of diagnostic uncertainty, despite some training beforehand.

Age range

The age range is the most visible difference between the trials. Three out of 11 trials included only children over 2 years of age (van Buchem et al. 1981, Thalin et al. 1985, Burke et al. 1991), and 4 trials had an age range up to 12–15 years (van Buchem et al. 1981, Thalin et al. 1985, Kaleida et al. 1991, Appelman et al. 1991). Only three studies included solely children at otitis-prone age, i.e., 6 months to 2 years (Engelhard et al. 1989, Damoiseaux et al. 2000, Hoberman et al. 2011). To include patients beyond the otitis prone age may significantly affect the results, as young children have a higher risk of treatment failure than older children (Rovers et al. 2006). It would also be important to study the treatment effect at age group with the highest incidence of AOM.

Exclusion criteria

In 2002, Dagan and McCracken pointed out that the results of clinical trials can be influenced, even manipulated, by patient selection (Dagan and McCracken 2002). By excluding children with severe illness or children at high risk of treatment failure, the chances of identification of any significant differences between the antimicrobial treatment group and placebo group decreases. In this review, 4 out of 11 trials (Thalin et al. 1985, Burke et al. 1991, Damoiseaux et al. 2000, Le Saux et al. 2005) had excluded children because they had required immediate antimicrobial treatment according to the study physician. In the study by Burke et al. (1991), 17% of children were excluded from the trial due to bulging eardrum or severe illness. One may severely question whether the results are reliable if no less than one fifth of otherwise eligible children were excluded only because the signs of AOM could be clearly seen. On the other hand, Appelman et al. (1991) included only children with a higher risk of treatment failure, i.e., children with recurrent AOM. They defined recurrent AOM as two or more episodes of AOM.

Table 3. Summary of the methods of 11 randomized, double-blind, and placebo-controlled trials comparing antimicrobial treatment with placebo. Diagnostic criteria, interventions, and follow-up schedule.

Authors and year	Diagnostic criteria	Diagnosis done by Diagnostic tool	Age range <2 y (%)	Intervention (dose mg/kg/vrk)	No. of children in a group	Follow-up visits
Halsted et al. 1968	1) Bulging with loss of light reflex and landmarks *or* 2) Diffusely red	Not mentioned Myringotomy	6 mo–5.5 y 75%	Ampicillin (100) Pen+sulfa (30/150) Placebo Myr for all patients	30 32 27	Days 2–4 Days 14–18
Mygind et al. 1981	1) Ear pain <24h 2) Red and inflamed TM	3 ENTs Pneumatic otoscopy	1–10 y Not mentioned	Pen (55) Placebo	72 77	Days 2–3 Day 7 1 mo 3 mo
van Buchem et al. 1981	1) Diffuse redness or bulging	12 GPs → diagnosis confirmed by 3 ENTs Not mentioned	2–12 y 27%	Amox (≈20–60) Amox+myr (≈20–60) Myr Placebo	47 48 36 40	Day 2 (no otoscopic examination) Day 7 GP Day 14 GP 1 mo ENT 2 mo ENT 1 y ENT and 2 y ENT
Thalin et al. 1985	1) Purulent AOM	Trained ENTs Otomicroscope	2–15 y 0%	Pen (50) Placebo	159 158	Days 3–4 Days 8–10 Day 30
Engelhard et al. 1989	1) Recent irritability or fever 2) MEE 3) Bulging	One ENT Otomicroscope	3–12 mo 100%	Amox-cla (≈30–60) Amox-cla+myr (≈30–60) Placebo+myr	36 34 35	Days 3–6 Days 9–11
Kaleida et al. 1991[1] *Nonsevere illness*	1) MEE *and* 2) Specified symptoms (fever, otalgia, irritability) *or* 3) Otoscopic signs (erythema/ opacification + fullness/bulging + impaired mobility)	Validated otoscopists Pneumatic otoscopy	7 mo–12 y 50%	Amox (40) Placebo	263 273	<2 years of age: Days 3–4 ≥2 years of age: Telephone calls All: 2 weeks 6 weeks→every mo

Authors and year	Diagnostic criteria	Diagnosis done by Diagnostic tool	Age range <2 y (%)	Intervention (dose mg/kg/vrk)	No. of children in a group	Follow-up visits
Kaleida et al. 1991[1] *Severe illness*	1) MEE *and* 2) Specified symptoms (fever, otalgia, irritability) *or* 3) Otoscopic signs (erythema/ opacification + fullness/bulging + impaired mobility)	Validated otoscopists Pneumatic otoscopy	7 mo–12 y 50%	Amox (40) Amox+myr (40) Placebo+myr (only children ≥2 years of age were included in the placebo+myr group)	252 198 86	<2 years of age: Days 3–4 ≥2 years of age: Telephone calls All: 2 weeks 6 weeks→every mo
Burke et al. 1991	1) Acute ear pain 2) One abnormal TM	48 trained GPs Not mentioned	3–10 y 0%	Amox (≈10–30) Placebo	114 118	Day 2 at home Days 5–7 at home Day 8 GP 1 mo (only tympanometry) 3 mo (only tympanometry)
Appelman et al. 1991 *Recurrent AOM*[2]	1) Acute onset of symptoms 2) Otoscopic signs of middle ear infection (redness or bulging of the TM)	Several GPs → diagnosis confirmed by ENTs Not mentioned	6 mo–12 y 22%	Amox-cla (≈15–28/4–9) Placebo	70 56	Visits: Day 4 (no otoscpic examination) Day 14 (no otoscopic examination) 1 mo (otolaryngologist not blinded)
Damoiseaux et al. 2000	1) Acute onset of symptoms 2) Red or bulging TM *or* 3) Acute otorrhea	53 trained GPs Not mentioned	6 mo–2 y 100%	Amox (40) Placebo	117 123	Day 4 Day 11 Week 6
Le Saux et al. 2005	1) Acute onset of symptoms (<4 days) of RTI + ear pain or fever 2) MEE 3) Redness or bulging of the TM	Several clinicians Not mentioned	6 mo–6 y 37%	Amox (60) Placebo	258 254	Telephone calls on days 1,2,3, and once between days 10–14 → clinical assessment if not improving or worse 1 mo (only tympanometry 3 mo (only tympanometry)
Hoberman et al. 2011	1) Acute onset of symptoms (<48h) 2) MEE 3) Moderate/marked bulging, or slight bulging with ear pain / marked erythema	Validated otoscopists Pneumatic otoscopy and digital images	6 mo–2 y 100%	Amox-cla (90/6) Placebo	144 147	Day 4–5 Day 10–12 Day 21–25

[1] Same trial. Before randomization, the patients were divided into two groups (nonsevere or severe) based on the severity of their illness. Illness was classified as severe if the child's temperature had been ≥39 °C within 24 hours or if the child had an ear pain score of ≥12

[2] Recurrent AOM= ≥2 AOM episodes

amox=amoxicillin, amox-cla=amoxicillin-clavulanate, AOM=acute otitis media, ENT=ear-nose-throat specialist, GP= general practioner, MEE=middle ear effusion, mo=month, myr=myringotomy, pen=penicillin, RTI=respiratory tract infection, TM=tympanic membrane, y=year

Antimicrobial agent

Two studies used penicillin as an antimicrobial agent (Mygind et al. 1981, Thalin et al. 1985). Penicillin is not effective against *H. influenzae* or *M. catarrhalis*. Thus, the treatment result may not be optimal in these studies. In addition, two studies (van Buchem et al. 1981, Engelhard et al. 1989) used an amoxicillin dose less than 40mg/kg/day for some children, and two studies (Burke et al. 1991, Appelman et al. 1991) for all children.

2.7.2.4 Results from the randomized, double-blind, placebo-controlled trials

Treatment failure as a primary outcome

The results of 10 of the trials included in this review are summarized in Figure 4. A study by van Buchem et al. (1981) is not included in the Figure 4 because it did not report treatment failure as an outcome. For the present review, treatment failure was chosen as a primary outcome because it demonstrates the overall recovery of the child and is clinically meaningful. The definition of treatment failure varied between the trials. In general, treatment failure was a non-resolution or worsening of symptom or signs during treatment period, which ultimately resulted in withdrawal from a trial or for the initiation of open antimicrobial treatment. In studies with several treatment groups (Halsted et al. 1968, Engelhard et al. 1989, Kaleida et al. 1991) the groups that were considered to be the most comparable were included in Figure 4 (i.e., myringotomy vs. myringotomy+antimicrobials instead of myringotomy vs. antimicrobials alone). As Figure 4 demonstrates, antimicrobial treatment appeared to be superior to placebo in all 10 trials: in 7 trials the difference between the two groups was statistically significant. Engelhard et al. (1989) observed as high as 53% difference in treatment failure rates between the antimicrobial treatment group and the placebo group.

Resolution of symptoms

Most of the trials used symptom resolution, especially ear pain and fever, as the primary outcome. The resolution of ear pain and fever is a significant outcome for the child, as these symptoms often cause discomfort and absence from day care. However, choosing a single symptom as an outcome measure is problematic because symptoms are not specific to AOM, the spectrum of symptoms differs between patients, and even within the same patient from day to day. Hoberman et al. (2011) used a symptom score, the AOM-SOS score, which include seven symptoms and assessment of the severity of these symptoms. The use of symptom scores is also questionable because it appears unlikely to detect any clinically meaningful differences between the treatment groups if the symptom scores do not even differentiate between children with or without AOM (Laine et al. 2010). Furthermore, an uneven distribution of the use of analgesic or antipyretic agents among the groups might flaw the results substantially. All trials allowed the use of analgesic or antipyretic agents, but only four reported them as an outcome measure (Mygind et al. 1981, Burke et al. 1991, Damoiseaux et al. 2000, Hoberman et al. 2011).

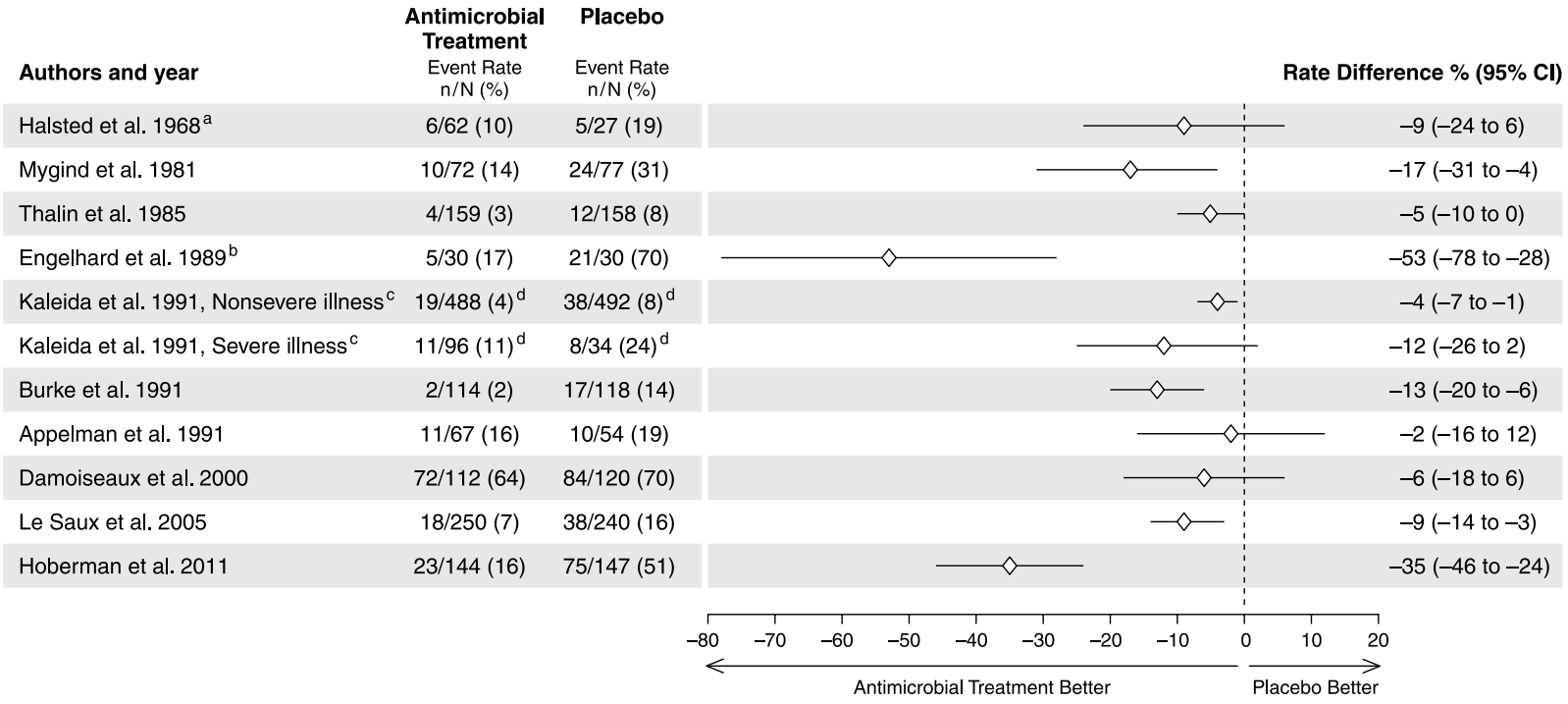

Figure 4. Treatment failure rates in acute otitis media treatment trials.

[a] Antimicrobial treatment group includes ampicillin and penicillin+sulfa groups
[b] Groups in comparison are amoxicillin-clavulanate+myringotomy and placebo+myringotomy
[c] Illness was classified as severe if the child's temperature had been ≥39 ^{0}C within 24 hours or if the child had an ear pain score of ≥12. Groups in comparison are amoxicillin+myringotomy and placebo+myringotomy
[d] Number of AOM episodes

Resolution of middle ear effusion

When evaluating the effect of antimicrobial treatment on the resolution of AOM, it is also important to examine the treatment effect on the site of the infection itself, namely middle ear. However, none of the trials in this review evaluated the resolution of MEE regularly. The studies that used the resolution of MEE as a long-term outcome reported no difference between antimicrobial treatment group and placebo group at 1–3 months after study entry (van Buchem et al. 1981, Burke et al. 1991, Damoiseaux et al. 2000, Le Saux et al. 2005).

2.7.3 Which antimicrobial agent and for how long?

Theoretically, the antimicrobial agent used for the treatment of AOM should be effective against the three most common causative agents: *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*. The choice of the optimal antimicrobial agent is based not only on its efficacy and antimicrobial effects, but also on side effects, costs, and local bacterial resistance rates. Patient-related aspects such as taste, texture, and dosing schedule need also to be taken into account, as these will affect the selection of proper antimicrobial agent.

Antimicrobial agents differ in their ability to penetrate into the middle ear by diffusion. The most important factor affecting the efficacy of an antimicrobial agent is the duration of the time that the concentration of the drug exceeds the minimal inhibitory concentration for the pathogen (Craig and Andes 1996). Craig and Andes (1996) estimated that a bacteriologic cure rate of 80–85% of AOM is achieved when the serum concentrations of antimicrobial agents exceed the minimal inhibitory concentration for approximately half of the dosing interval.

All antimicrobial agents used for the treatment of AOM provide a serum concentration high enough to eradicate penicillin-susceptible strains of *S. pneumoniae* (Craig and Andes 1996). However, for most of the penicillin-intermediate strains and penicillin-resistant strains of *S. pneumoniae*, amoxicillin appears to be the only oral antimicrobial agent that provides adequate concentrations. Ceftriaxone (intramuscularly or intravenously) is superior to amoxicillin with regard to efficacy against penicillin-resistant strains of *S. pneumoniae* (Craig and Andes 1996). Doubling the dosage of amoxicillin to from 40 to 80 mg/kg/day increases the drug concentrations in MEE (Seikel et al. 1997) and results in good clinical efficacy also against resistant strains of *S. pneumoniae* (Piglansky et al. 2003). Therefore, high-dose amoxicillin (70–90 mg/kg/day) is recommended as first-line treatment in countries where the resistance rates of *S. pneumoniae* are high (Lieberthal et al. 2004). However, the literature is lacking randomized, double-blind studies evaluating the clinical efficacy of high-dose amoxicillin in the treatment of AOM as compared to low-dose amoxicillin. Administrating amoxicillin three times a day instead of twice a day helps in maintaining the drug concentration above the minimal inhibitory

concentration between the doses, resulting in better efficacy against resistant strains of *S. pneumoniae* (McKinnon and Davis 2004).

In Western Finland, appoximately 20% of *H. influenzae* and virtually all *M. catarrhalis* strains produce β-lactamase, which makes them resistant to amoxicillin (Meurman 2012). The β-lactamase enzyme makes the drug ineffective by breaking open the β-lactam ring. Clavulanate potassium inhibits β-lactamase enzyme. Thus, adding clavulanate potassium to amoxicillin makes it more effective against β-lactamase producing strains of *H. influenzae* and *M. catarrhalis* (Bluestone and Klein 2007). In an open-label multicenter trial, high-dose amoxicillin-clavulanate was effective in the management of AOM (Dagan et al. 2001). However, in order to restrict the development of bacterial resistance, narrow-spectrum antimicrobial agents should be preferred. Thus, the use of high-dose amoxicillin-clavulanate is recommended only for children with severe illness and for children who fail to respond to the initial antimicrobial agent (Lieberthal et al. 2004). Some strains of *H. influenzae* are resistant to amoxicillin and amoxicillin-clavulanate even though they are not able to produce β-lactamase (Doern et al. 1997).

Currently, amoxicillin is used as first-line management for AOM in most countries. In Nordic countries, phenoxymethyl penicillin is also recommended as first-line management for AOM. These recommendations are largely based on meta-analyses that evaluated the comparative effectiveness of different antimicrobial agents for AOM and found no statistically significant differences between the agents in the pooled analyses (Rosenfeld et al. 1994, Coker et al. 2010). Resistant strains of *S. pneumoniae* appear to be more common among children previously treated with antimicrobial agents (del Castillo et al. 1998, Leibovitz et al. 1998). Thus, children who fail to respond to the initial antimicrobial treatment should be treated with high-dose amoxicillin, cefaclor, cefuroxime, or ceftriaxone (Lieberthal et al. 2004, Heikkinen et al. 2010). As resistant strains of *S. pneumoniae* are rapidly increasing, macrolides can no longer be recommended for the management of AOM, with the exception of children allergic to penicillin. In addition, sulfa-trimethoprim is recommended only for children allergic to penicillin (Lieberthal et al. 2004, Heikkinen et al. 2010).

The optimal duration of antimicrobial treatment is controversial. Since the 1990s, the Finnish treatment guidelines have recommended a 5-day course for the management of AOM (Puhakka et al. 1999, Heikkinen et al. 2010). In contrast, American Academy of Pediatrics treatment guidelines still recommend a 10-day course for younger children and children with severe disease (Lieberthal et al. 2004). According to a recent Cochrane Database Systematic Review, long-course antimicrobial treatment (>7 days) provides only minor benefit as compared to short-course antimicrobial treatment (Kozyrskyj et al. 2010). However, the studies included in the Cochrane review have been criticized for serious limitations of their study design, and more research is needed to establish the optimal duration of antimicrobial treatment (Paradise 1997).

2.7.4 Wait-and-see approach

The wait-and-see approach, also called as watchful waiting or a safety-net prescription, was introduced in an effort to avoid unnecessary antimicrobial treatment. In the wait-and-see approach, delayed antimicrobial treatment is initiated only for children whose condition does not improve within a couple of days.

Four open, randomized trials have compared the wait-and-see approach to immediate antimicrobial treatment in the management of AOM (Little et al. 2001, McCormick et al. 2005, Spiro et al. 2006, Neumark et al. 2007). Little et al. (2001) recruited 315 children, 6 months to 10 years of age, with AOM. The children were randomized to receive either immediate or delayed antimicrobial treatment. In the delayed antimicrobial treatment group, parents were asked to wait for 72 hours and to initiate antimicrobial treatment only if their child's condition had not improved. In this study, 24% of the children in the delayed antimicrobial treatment group ultimately used antimicrobial treatment. Immediate antimicrobial treatment significantly reduced the duration of illness and analgesics use, but this benefit emerged mainly after 24 hours, when symptoms were already resolving. In a survey that was carried out at 3 months and 1 year after study entry, Little et al. (2006) found no differences in the long-term outcomes.

In a study by McCormick et al. (2005), 233 children, 6 months to 12 years of age, with nonsevere AOM were randomized to receive either immediate antimicrobial treatment or symptom-relieving medication only (i.e., the wait-and-see group). Overall, 34% of the children in the wait-and-see group received delayed antimicrobial treatment. In the group with immediate antimicrobial treatment the failure rate was lower and symptom duration shorter than in the wait-and-see group, but the rate of adverse event was higher.

Spiro et al. (2006) examined 283 children aged 6 months to 12 years of age with AOM. The children were randomized to an immediate antimicrobial treatment group or a wait-and-see prescription group, in which the parents were advised "not to fill the antibiotic prescription unless your child either is not better or is worse 48 hours after today's visit". Delayed antimicrobial treatment was given to 38% of the children in the wait-and-see prescription group. This study found no statistically significant difference between the groups regarding the frequency of subsequent fever, ear pain, or unscheduled visits for medical care.

Neumark et al. (2007) compared immediate antimicrobial treatment with an active wait-and-see approach. They included 179 children, 2 to 16 years of age, with uncomplicated AOM. The parents were advised to revisit their health care center if the child's condition did not improve within 3 days after randomization. Altogether, 18% of children in wait-and-see group revisited because of treatment failure, but only 5% received delayed antimicrobial treatment. Children in the immediate antimicrobial treatment group had less ear pain and used less analgesics. Immediate antimicrobial treatment did not affect the overall recovery time or complication rate in this study.

In conclusion, the wait-and-see approach appears to be an effective way to reduce the use of antimicrobials (Little et al. 2001, McCormick et al. 2005, Spiro et al. 2006, Neumark et al. 2007, Grossman et al. 2010). However, three out of four randomized trials observed that immediate antimicrobial treatment was associated with a higher clinical success rate (Little et al. 2001, McCormick et al. 2005, Neumark et al. 2007). Despite the statistically significant difference favoring immediate antimicrobial treatment, all authors state that the benefit was only modest and it should be balanced against side effects, the risk of multidrug-resistant bacteria, and parents' faith in the importance of antimicrobial treatment. As wait-and-see approach is also well accepted by the parents (Little et al. 2001, Siegel et al. 2003, McCormick et al. 2005, Chao et al. 2008), it has been concluded that together with adequate symptomatic medication, the wait-and-see approach appears to be an acceptable alternative for the management of AOM.

2.7.5 Tympanocentesis and myringotomy

Until the late 1980s, tympanocentesis and myringotomy were routine treatment procedures to cure AOM, despite the lack of scientific evidence of efficacy. The use of these procedures decreased dramatically after four randomized trials had shown that the addition of myringotomy to antimicrobial treatment did not improve recovery from AOM (Lorentzen 1977, van Buchem et al. 1981, Engelhard et al. 1989, Kaleida et al. 1991). Nowadays, myringotomy is used only rarely for the treatment of AOM; a need for a specific microbiologic diagnosis is a primary indication for this procedure. Sometimes myringotomy is performed to prevent a threatening complication of AOM. Antimicrobial treatment is virtually always added to the therapy, and in most cases, tympanostomy tubes are inserted simultaneously.

2.7.6 Other treatment options

Several treatment options have been evaluated for the management of AOM. So far, no treatment has been proven superior to antimicrobial treatment. A Cochrane Database Systematic Review pooled data from 15 randomized controlled trials evaluating decongestant or antihistamine treatment for the management of AOM (Coleman and Moore 2008). The combined decongestant-antihistamine treatment showed some benefit with regard to resolution of AOM, but the clinical significance was minimal. The authors concluded that the data do not support the use of either decongestants nor of antihistamines for the management of AOM (Coleman and Moore 2008). In fact, antihistamines may even prolong the duration of MEE and should be avoided during AOM (Chonmaitree et al. 2003).

Only a few randomized, double-blind, placebo-controlled studies have evaluated the effect of oral probiotics and nasal sprays in the prophylaxis and the management of AOM. The results have been controversial. Probiotics have reduced the incidence of AOM in some studies (Roos et al. 2001, Rautava et al. 2009), while others found no

significant effect (Hatakka et al. 2001, Hatakka et al. 2007, Taipale et al. 2011). A recent review concludes that the inconsistent results may be due to the use of different strains of probiotics (Niittynen et al. 2012). The effect of probiotics in the management of AOM may also vary among different study populations. Therefore, further studies are needed to evaluate the overall effect of probiotics in the management of AOM and to identify the effective probiotic strains.

The use of homeopathic and herbal medicine is surprisingly high among children with AOM. In an Italian study, about one-half of children (1–7 years of age) used homeopathic and herbal medicine to treat AOM; the main reason being parental fear of adverse events of conventional medicine (Marchisio et al. 2011). Despite the wide use of homeopathic treatment, there is no evidence of its efficacy in the management of AOM. The literature is lacking high-quality studies comparing homeopathic treatment with placebo in the management of AOM. Jacobs et al. (2001) conducted a preliminary, randomized, placebo-controlled trial on the homeopathic treatment of AOM. In a sample-size of 75 children there were fewer treatment failures in the homeopathic treatment group than in the placebo group, but the difference was not statistically significant. The authors concluded that a larger study with almost 500 children is needed for sufficient power for identification of significant effects. A recent study from India observed that homeopathic treatment might be effective in the management of AOM, but the study was neither blinded nor placebo-controlled (Sinha et al. 2012).

Through the ages, several home remedies have been used for the management of AOM. Probably the most known are oil, garlic, and external application of heat or cold to the aching ear. No randomized, controlled trials that directly address the efficacy of these procedures on the management of AOM have been published. Therefore, they cannot be recommended as a treatment option for AOM.

2.7.7 Factors affecting management

2.7.7.5 Treatment guidelines in western countries

AOM treatment guidelines are surprisingly different among the western countries, even though they are all based on the results of the same AOM treatment trials. Treatment guidelines are created through consensus by an expert panel, and obviously the results of the trials are always interpreted through a filter of influenced personal and cultural values.

In most countries, immediate antimicrobial treatment is provided for all children less than 6 months of age (Prellner et al. 2000, Bain et al. 2003, Lieberthal et al. 2004, Appelman et al. 2006, Forgie et al. 2009, Heikkinen et al. 2010, Marchisio et al. 2010). This is understandable because, at this age group, the immune system is not fully developed and the risk of severe infections is higher than with older children. In addition, many countries recommend antimicrobial treatment for children less than 2 years of age (Prellner et al. 2000, Lieberthal et al. 2004, Heikkinen et al. 2010). These recommendations are largely based on a meta-

analysis with individual patient data showing that the risk of treatment failure is higher among children less than 2 years of age with bilateral AOM (Rovers et al. 2006). The principles of the AOM treatment guidelines in 7 different countries are presented in Table 4.

Table 4. AOM treatment guidelines in selected countries.

Country and year	< 6 months	6 months–2 years	> 2 years	First choice antimicrobial agent and dosing	Duration of antimicrobial treatment
Sweden 2000	Antimicrobial treatment	Antimicrobial treatment	***Children with perforation, general distress, or recurrent AOM*:** Antimicrobial treatment ***Others:*** Wait-and-see approach	Phenoxymethyl penicillin 50 mg/kg/day	5 days
Scotland 2003	"Children diagnosed with AOM ***should not routinely*** be prescribed antibiotics as the initial treatment." "Delayed antibiotic treatment is ***an alternative approach*** which can be applied in general practice."			Amoxicillin or amoxicillin-clavulanate	5 days
The United States 2004	Antimicrobial treatment	***Certain diagnosis:*** Antimicrobial treatment ***Uncertain diagnosis and severe illness[1]:*** Antimicrobial treatment ***Uncertain diagnosis and nonsevere illness[2]:*** Wait-and-see approach	***Certain diagnosis and severe illness[1]:*** Antimicrobial treatment ***Certain diagnosis and nonsevere illness[2]:*** Wait-and-see approach ***Uncertain diagnosis:*** Wait-and-see approach	Amoxicillin 80–90 mg/kg/day	<6 years: 10 days ≥6 years: 5–7 days
The Netherlands 2006	Antimicrobial treatment	***Most children:*** Wait-and-see approach ***Children with bilateral AOM or otorrhea:*** Antimicrobial treatment may be considered ***Children at risk for complications:*** Antimicrobial treatment	***Most children:*** Wait-and-see approach ***Children at risk for complications:*** Antimicrobial treatment	Amoxicillin 30 mg/kg/day	7 days
Canada 2009	Antimicrobial treatment	***Severe illness[1]:*** Antimicrobial treatment ***Nonsevere illness[2]:*** Wait-and-see approach	***Severe illness[1]:*** Antimicrobial treatment ***Nonsevere illness[2]:*** Wait-and-see approach	Amoxicillin 75–90 mg/kg/day	5 days
Finland 2010	***Certain diagnosis:*** Antimicrobial treatment	***Certain diagnosis:*** Antimicrobial treatment	***Certain diagnosis:*** Antimicrobial treatment	Amoxicillin 40 mg/kg/day Phenoxymethyl penicillin 66 mg/kg/day	5 days
Italy 2010	Antimicrobial treatment	***Bilateral AOM:*** Antimicrobial treatment ***Unilateral AOM and severe illness[1]:*** Antimicrobial treatment ***Unilateral AOM and nonsevere illness[2]:*** Wait-and-see approach	***Bilateral AOM and severe illness[1]:*** Antimircobial treatment ***Others:*** Wait-and-see approach	Amoxicillin 50 mg/kg/day	10 days ≥2 years: 5 days is also possible

[1] Severe illness: moderate to severe ear pain and/or fever ≥39 °C
[2] Children who do not fill the criteria for severe illness

2.7.7.6 Parental attitudes and prescription practices

In addition to treatment guidelines, parental expectations and awareness of bacterial resistance appear to influence treatment strategies. Several studies have shown that patient pressure has a significant influence on antimicrobial prescribing, even when the physician considers antimicrobial treatment unnecessary (Britten and Ukoumunne 1997, Macfarlane et al. 1997). Arason et al. (2002) compared parental expectations and antimicrobial use in four different geographic areas in Iceland. They found that parents in the area where antimicrobial use was the lowest were less likely to accept antimicrobial treatment for AOM than parents in the other areas.

Physicians also differ in their tendency to prescribe antimicrobial treatment for AOM. A large Finnish study showed that the patients' likelihood to receive antimicrobial prescription from a "high prescriber" was nearly six times higher than from a "low prescriber" (Leistevuo et al. 2005). Interestingly, "high prescribers" made the diagnosis of AOM significantly more often and a diagnosis of unspecified upper RTI more seldom than the "low prescribers". A Dutch study found out that the two most important predictors for the prescribing of antimicrobial treatment in RTI were the physician's attitude toward prescribing antimicrobials for treating sore throat and years of experience in practice (Kuyvenhoven et al. 1993).

2.7.7.7 Prognostic factors

Age and bilateral disease

Several attempts have been made to identify patients who derive the greatest benefit from antimicrobial treatment. Burke et al. (1991) found no association between the risk of treatment failure and age, gender, social class, season of the year, AOM history, bilateral or unilateral disease, symptoms at entry, or tympanic membrane findings at entry. However, young age (<2 years) has been associated with poorer outcomes in some AOM treatment studies (Kaleida et al. 1991, Appelman et al. 1991, Le Saux et al. 2005). In addition, bilateral AOM at entry appears to increase the risk of treatment failure (Kaleida et al. 1991, Hoberman et al. 2011). The strongest evidence so far regarding the prognostic factors comes from a meta-analysis with individual patient data by Rovers et al. (2006). The meta-analysis summarized data from six individual studies, and found out that children younger than 2 years of age with bilateral AOM and children with otorrhea benefit the most from antimicrobial treatment.

Severity of illness

It could be expected that children with more severe illness benefit more from antimicrobial treatment. There, in fact, is some evidence that children with less severe illness are more likely to recover without antimicrobial treatment than children with more severe illness. Kaleida et al. (1991) divided children into two groups based on the severity of symptoms at study entry. AOM was classified as severe if the child's temperature had been ≥39 °C

within 24 hours or if the child had an ear pain score of ≥12. The rate of treatment failure was highest among those children who had severe illness at study entry and who received placebo (24%). In a study by Hoberman et al. (2011), the clinical failure rates at day 10–12 were higher among children with high (>8) AOM-SOS symptom scores at entry, as compared to children with low (≤8) AOM-SOS scores. In addition, children with marked bulging of tympanic membrane had more clinical failures, as compared to children with slight or moderate bulging. On the other hand, van Buchem and colleagues (1981) reported that red or bulging tympanic membrane at study did not influence the course of AOM.

The major problem is that the definition of severe illness remains controversial, and many different severity scores have been presented (Bluestone et al. 2002). Some authors emphasize the role of symptoms while others concentrate on otoscopic signs. Symptoms may be caused by the concurrent viral RTI and they may not correlate with the severity of otoscopic signs. In fact, viral infections have been shown to cause high and prolonged fever even without concurrent bacterial infection (Putto et al. 1986). Therefore, it could be questioned whether the AOM treatment decisions should be based solely on symptom severity.

2.8 Consequences

2.8.1 Complications

The complications of AOM can be divided to intratemporal and intracranial complications. In the beginning of the 20th century, mastoiditis was a common intratemporal complication of AOM. In 1954, Rudberg reported an incidence of mastoiditis of no less than 17% in patients not treated with antimicrobials (Rudberg 1954). After the introduction of antimicrobial treatment, suppurative complications of AOM have decreased dramatically in the developed countries. However, the situation is completely different in the developing countries. It has been estimated that 51,000 young children die every year because of AOM complications. In addition, chronic suppurative OM is a major cause of hearing loss in developing countries (Vergison et al. 2010). Current AOM treatment trials do not have a sample-size large enough to detect differences in rare complications such as mastoiditis, but there is a tendency that severe bacteriological complications develop more often for children in the placebo group (van Buchem et al. 1985, Halsted et al. 1968, Damoiseaux et al. 2000, Hoberman et al. 2011).

Van Zuijlen et al. (2001) evaluated the incidence of mastoiditis in several western countries from 1991 to 1998. They found out that acute mastoiditis is more common in the Netherlands than in the other countries where antimicrobial prescription rates for AOM are higher. However, in Norway and Denmark the incidence of mastoiditis was as high as in the Netherlands, even though antimicrobial treatment was used twice as

often in Norway and Denmark as in the Netherlands. Nevertheless, the overall incidence rate of mastoiditis was low in all countries. In Sweden, the incidence of mastoiditis did not increase after the introduction of new AOM treatment guidelines which recommend a wait-and-see approach for children more than 2 years of age (Groth et al. 2011). In a retrospective cohort study from the United Kingdom, the risk of mastoiditis was 3.8 per 10,000 episodes of AOM treated without antimicrobials (Thompson et al. 2009). Antimicrobials halved the risk of mastoidits, but the overall incidence of mastoiditis was low (1.2 per 10,000 child years).

Hearing loss is the most frequent complication of AOM. The presence of MEE results in conductive hearing loss, 25 dB on average (Fria et al. 1985). Visible air-fluid level or bubbles behind the TM are associated with less severe hearing impairment. AOM may also result in sensorineural hearing loss which is often accompanied with another middle ear disease. Other intratemporal complications of AOM are petrositis, labyrinthitis, facial paralysis, external otitis, atelectasis of the middle ear, cholesteatoma, cholesterol cranluloma, tympanosclerosis, adhesive OM, and chronic suppurative OM. Perforation of the tympanic membrane may also be considered to be a complication of AOM (Bluestone et al. 2002).

Intracranial complications of AOM include meningitis, extradural abscess, subdural empyema, encephalitis, brain abscess, and lateral sinus thrombosis. These are all rare complications of AOM. In a study by van Buchem et al. (1985), 4860 children with AOM were initially treated with analgesics and nose drops alone. Two children developed mastoiditis, but none bacterial meningitis. Kilpi et al. (1991) evaluated children with bacterial meningitis and found that preceding antimicrobial treatment for AOM did not decrease the number of positive blood cultures.

The introduction of 7-valent PCV has significantly decreased the incidence of invasive pneumococcal diseases, including the complications of AOM. The decline has been largest in children below the age of 2 years (Whitney et al. 2003). The new 13-valent PCV may reduce the number of invasive pneumococcal diseases even further, but only the future will show the overall impact of PCV on the complication rate of AOM worldwide (Rubin et al. 2010, Shea et al. 2011).

2.8.2 Costs

AOM causes direct and indirect costs for the family as well as for the society. According to study from Finland, the average cost of an episode of AOM is $228 which yields a total annual cost of $138 million in Finland (Niemelä et al. 1999). In the United States, the annual cost of AOM has been estimated to be $2 billion (Shekelle et al. 2010).

Coco (2007) performed a cost-effectiveness analysis of different treatment options for AOM. It turned out that delayed prescription (i.e., the parents received a safety-net prescription) was the least costly option and immediate initiation of antimicrobial

treatment with a 7–10 day course was the most effective option for the management of AOM. The reduced costs of delayed prescription resulted from fewer health care consultations and reduced antimicrobial use.

PCV is highly cost-effective in the prevention of invasive pneumococcal disease, but it appears to have only minor impact on the economic burden of AOM (Rubin et al. 2010). A combination of PCV and nontypable *H. influenzae* vaccine would have the potential to prevent millions of AOM episodes in the future (O'Brien et al. 2009). However, as Boonacker et al. (2011) pointed out, the interpretation of the results of vaccine cost-effectiveness studies is difficult because the direct and indirect costs of AOM vary notably between studies. Overall, the economic consequences of different treatment and prevention options are difficult to estimate because the short term and long term consequences of AOM are not completely known.

3 AIMS

The aim of this study was to assess the efficacy of antimicrobial treatment, either immediate or delayed, for the recovery patients with AOM when strict diagnostic criteria and active treatment with an optimal antimicrobial coverage were used. The influence of different AOM treatment guidelines on parental experiences and opinions regarding the management of AOM was also evaluated.

The specific objectives were:

I To study the efficacy of antimicrobial treatment for AOM.

II To study if delayed, as compared to immediate, initiation of antimicrobial treatment worsens the recovery from AOM.

III To compare parental experiences and opinions regarding the management of AOM in Finland and in the Netherlands.

4 PATIENTS, MATERIALS, AND METHODS

The thesis consists of three original studies. For studies I and II, data were derived from a randomized, double-blind, placebo-controlled trial of the efficacy of antimicrobial treatment for AOM. Study III was a comparative survey that was carried out in collaboration between Finland and the Netherlands. The detailed methods are described in the original publications.

4.1 Patients and diagnostic criteria

Studies I and II

Children 6–35 months of age with acute symptoms were eligible for diagnostic screening. Three overall criteria were required for the diagnosis of AOM (Figure 5). First, MEE had to be detected by means of pneumatic otoscopic examination that showed at least two of the following tympanic-membrane findings: bulging position, decreased or absent mobility, abnormal color or opacity not due to scarring, or air-fluid interfaces. Second, at least one of the following acute inflammatory signs on the tympanic membrane had to be present: distinct erythematous patches or streaks or increased vascularity over a full, bulging, or yellow tympanic membrane. Third, the child had to have acute symptoms, such as fever, ear pain, or respiratory symptoms. Exclusion criteria were: ongoing antimicrobial treatment; spontaneous perforation of tympanic membrane; systemic or nasal steroid therapy within the 3 preceding days; antihistamine therapy within the 3 preceding days; oseltamivir therapy within the 3 preceding days; allergy to penicillin or amoxicillin; tympanostomy tube present in tympanic membrane; severe infection requiring systemic antimicrobial treatment; documented Epstein-Barr virus infection within the 7 preceding days; Down syndrome or other condition affecting middle ear diseases (e.g., cleft palate); known immunodeficiency; severe vomiting or another symptom to violate per oral dosage; poor parental co-operation due to language or other reasons; and use of any investigational drugs during the 4 preceding weeks. A parent of each child provided written informed consent. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland.

Study III

The study population consisted of children attending day care in the Turku region in Finland or the Utrecht region in the Netherlands. The parents filled out the questionnaires. To make the Finnish data comparable with the Dutch data, only children aged less than 4 years were included in the analyses, since Dutch children start primary school at the age of 4.

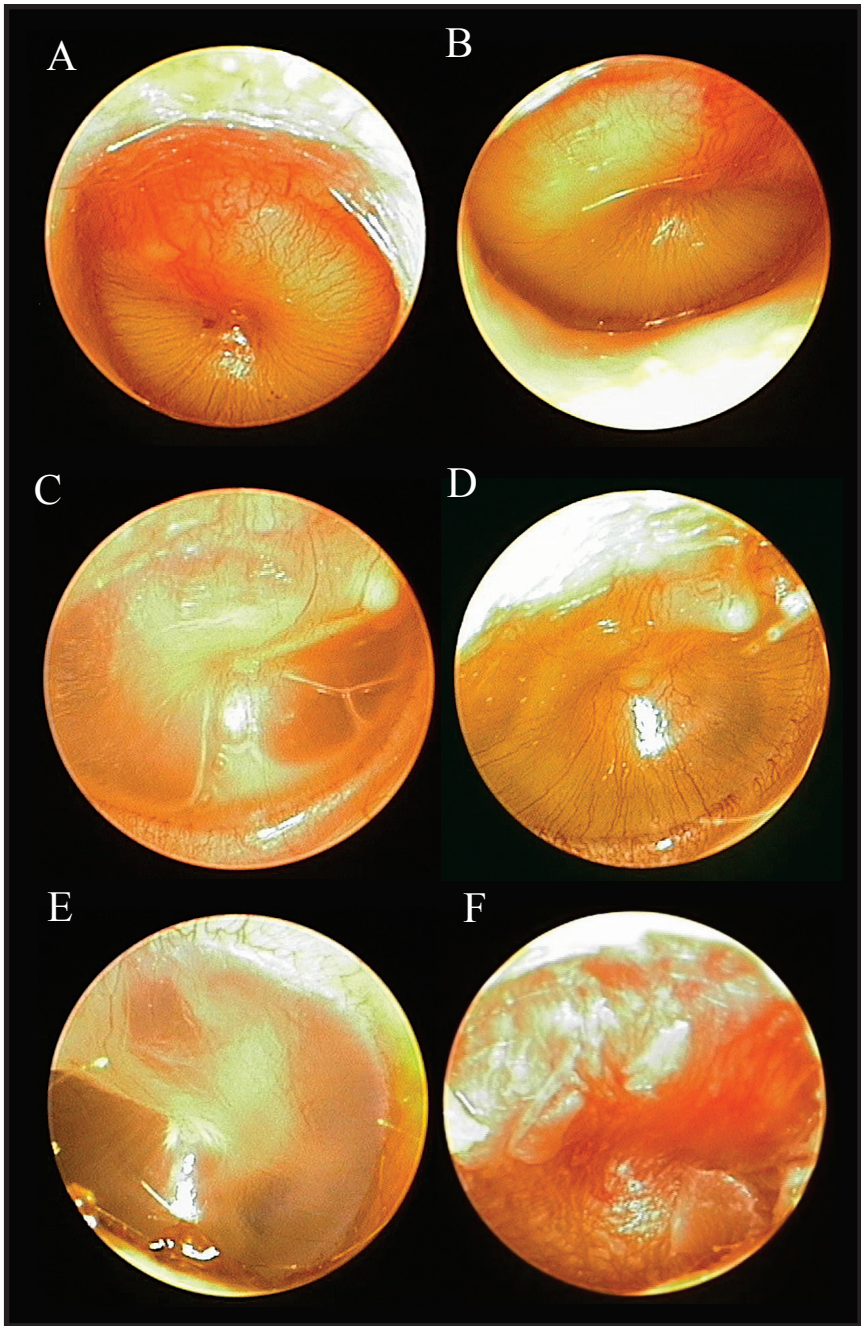

Figure 5. Examples of different otoscopic findings at the enrollment visit.
A and B: Middle ear effusion and inflammatory signs on the tympanic membrane (increased vascularity over bulging tympanic membrane). Included in the study.
C and D: Middle ear effusion but no inflammatory signs on the tympanic membrane. Not included in the study.
E: No middle ear effusion. Not included in the study.
F: Perforation of the tympanic membrane (typical cobblestone pattern). Not included in the study.

4.2 Study design

Studies I and II

The study was conducted within primary care. The patients were recruited between March 2006 and December 2008 (excluding June and July each year). At the enrollment visit (day 1), the patient's symptoms, medical history, and demographic and clinical characteristics were recorded, and a clinical examination was performed that included thorough otoscopic and tympanometric examinations.

Eligible patients were randomly assigned to receive amoxicillin-clavulanate (40/5.7 mg/kg/day, divided into two daily doses) or placebo for 7 days. The placebo was similar to the active treatment in appearance and taste. Parents were given a diary to record symptoms, doses of study drugs and any concomitant medications, absenteeism of the child from day care and of the parent from work, and adverse events. Fever was defined as a body temperature of 38 °C or higher. We highly encouraged the use of analgesic and antipyretic agents.

All children had the first scheduled follow-up visit 48–72 hours after study entry. At all visits, the study physician first asked the parents for their assessment of their child's overall condition, which was recorded as healthy, better, no improvement, or worse. Then a clinical examination, including pneumatic otoscopy examination, was performed. If the child's overall condition had not improved satisfactorily or had worsened, the physician could switch from the study drug to rescue treatment. Rescue treatment was initiated after individual consideration together with the parents. Thus, all children with treatment failure did not automatically receive rescue treatment.

For the children who continued with the study drug, the end-of-treatment visit was one day after the last dose of study drug (i.e., on day 8). For the children who received rescue treatment, the second scheduled visit was one day after the last dose of rescue treatment. This day was dependent on the initiation day of antimicrobial treatment and could be any of the days 8–16. After this visit, all children had a visit on study day 16 (± 3 days).

Additional visits were arranged whenever needed, seven days a week from 8 a.m. to 8 p.m., and the parents were encouraged to contact the study clinic if they felt their child's overall condition was not improving or was worsening. Therefore, for some children rescue treatment was initiated later than 72 hours after study entry. These children had managed well during the first 72 hours, but their overall condition had subsequently worsened.

In Study II, the delayed antimicrobial treatment group consisted of children who were primarily allocated to the placebo group, but who received rescue treatment. Although the initial study drug was discontinued, the allocation of each participant was kept blinded until the completion of the trial. Rescue treatment was an open-label antimicrobial

treatment, amoxicillin-clavulanate (40/5.7 mg/kg/day, divided into two daily doses), for 7 days. The immediate antimicrobial treatment group consisted of children who were randomized to immediately receive amoxicillin-clavulanate.

Study III

The study was carried out in collaboration with the Department of Otorhinolaryngology of the University Medical Center Utrecht, the Netherlands. In Finland, the data was collected in May and June 2006. The questionnaires were sent to all 86 public day-care centers and 345 family day-care sites in the Turku region (cities of Turku, Kaarina, Raisio, and Lieto). The day-care staff handed the questionnaires to the parents, collected them and sent them back to the study center. In the Netherlands, the data was collected in April and May 2007. The questionnaires were sent to 12 randomly chosen day-care centers. The day-care staff handed the questionnaire to the parents, but the parents were asked to send the questionnaire directly back to the study center. In the questionnaire, the parents were asked about the family background, the child's history of AOM, antimicrobial treatment and use of analgesics in previous AOM episodes, and their knowledge and attitude towards antimicrobial treatment, analgesics use and antimicrobial resistance. The questionnaires were comparable in both countries.

4.3 Outcomes

Study I

The primary outcome was the time to treatment failure, which was a composite outcome consisting of six independent components:

1. No improvement in overall condition by the time of the first scheduled visit (day 3). Unless the parents thought that their child's overall condition was improving, the case was categorized as treatment failure.
2. A worsening of the child's overall condition at any time.
3. No improvement in otoscopic signs by the end-of-treatment visit on day 8.
4. Perforation of the tympanic membrane at any time.
5. Severe infection necessitating systemic open-label antimicrobial treatment at any time.
6. Any other reason for stopping the study drug (e.g., adverse event or nonadherence to the study drug) at any time.

The time to treatment failure was the study day on which the study physician confirmed any one of the components for the first time. The secondary outcomes are summarized in Table 5.

Study II

The primary outcome was improvement during antimicrobial treatment. It was assessed one day after the last dose of antimicrobial treatment (study drug or rescue treatment). The assessment day was dependent on the initiation day of antimicrobial treatment and could be any of the days 8–16. The patient's clinical condition was defined as improved if both the child's overall condition, as assessed by parents, and otoscopic signs, as assessed by the study physician, had improved during antimicrobial treatment. The clinical condition was considered not to have improved, if the child's overall condition and/or otoscopic signs had not improved at all or had worsened during antimicrobial treatment. The secondary outcomes are summarized in Table 5.

Table 5. Summary of the outcomes and data collection methods

	Study I	**Study II**
Treatment	Antimicrobial treatment or placebo	Immediate or delayed antimicrobial treatment
Primary outcome	1) Time to treatment failure	1) Improvement during antimicrobial treatment
Assessment time for primary outcome	Days 1–8	From the initiation of antimicrobial treatment to one day after antimicrobial treatment
Secondary outcomes	2) Time to initiation of rescue treatment 3) Development of contralateral AOM 4) Analgesic or antipyretic agents' use 5) Absenteeism of the child from day care and of the parent from work 6) Treatment result at the end-of-treatment visit 7) Time to resolution of individual symptoms 8) Adverse events	2) Severe infections 3) Time to be completely asymptomatic 4) Time to completely normal otoscopic examination 5) Time to resolution of individual symptoms 6) Absenteeism of the child from day care and of the parent from work 7) Analgesic or antipyretic agents' use 8) Adverse events
Assessment time for secondary outcomes	Day 1 to end-of-treatment visit	Days 1–16

Secondary outcomes were assessed between study days 1–16. Time to be completely asymptomatic was defined as the first day after which all symptoms were absent. Time to completely normal otoscopic examination was defined as the day when the study physician observed that all otoscopic signs of OM were resolved including complete resolution of middle ear fluid. Individual symptoms were defined as resolved when they had not been present and marked in the symptom diary for two consecutive days. Data on the resolution of each symptom, absenteeism of the child from day care and of the

parent from work, and the use of analgesic or antipyretic agents, were based on diary entries.

Study III

The outcomes were parental experiences and opinions on the treatment of AOM. The individual questions are presented in Table 6 in the Results section.

4.4 Statistical analysis

Study I

The treatment failure rate was assumed to be 25% in the placebo group. To detect an absolute reduction of 15 % in the rate of treatment failure in the amoxicillin-clavulanate group as compared with the placebo group at a significance level of 5% and with a power of 90%, the minimum required number of children in each group was 130. The rate for withdrawals from the study was assumed to be 20%. Thus, at least 320 children were to be enrolled in the study.

The Kaplan-Meier method was used to analyze time-to-event data with the use of the log-rank test. Hazard ratios and confidence intervals (CIs) were calculated on the basis of a Cox regression model. Categorical outcomes were compared with the use of the χ^2-test and means were compared with Student's t-test. Absolute percentage-point differences in rates and 95% CI were calculated.

Study II

For categorical outcomes, absolute percentage-point differences in rates and 95% CIs were calculated. The main outcome was compared by the χ^2-test. Categorical outcomes were further analyzed by a logistic regression model which included the baseline characteristics in which the absolute difference between the two groups was clinically meaningful, namely exceeded 10% (i.e., day-care attendance, parental smoking, bilateral AOM, and moderate/marked bulging of the tympanic membrane). The unadjusted and adjusted odds ratios and 95% CIs were calculated. Time-to-event data were analyzed using the Kaplan-Meier method with the log-rank test.

Study III

Questionnaire data were summarized with descriptive statistics, i.e., frequencies per question. To compare the Finnish and Dutch results, percentage differences with 95% CIs were calculated. Statistical analysis was performed using SPSS statistical software (version 14.0).

5 RESULTS

5.1 Characteristics of study population

Altogether, 322 children with AOM participated in the randomized, double-blind, placebo-controlled trial (Figure 6). Baseline characteristics of the study populations are presented in the original publications.

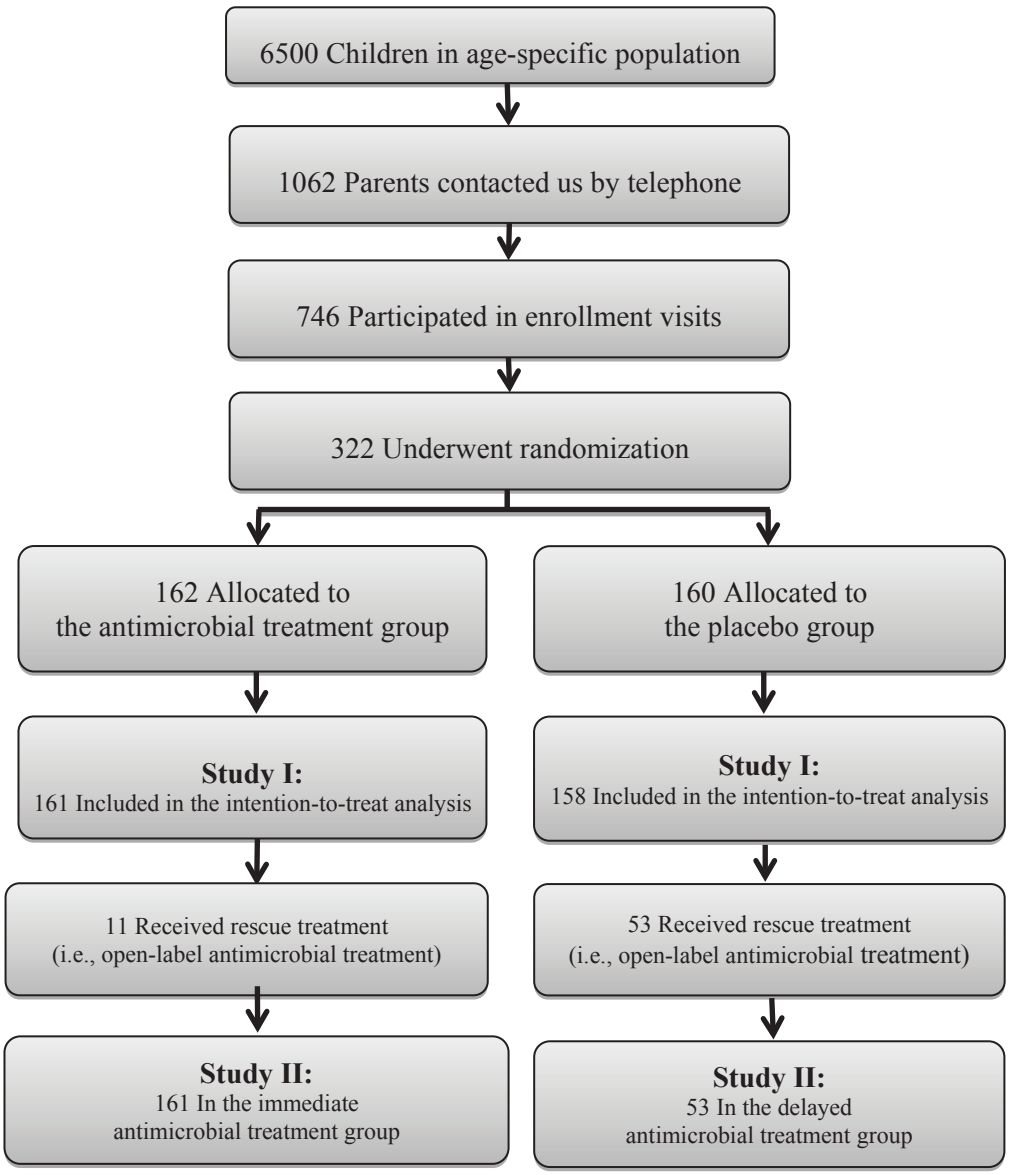

Figure 6. Flow chart of Study I and Study II.

5.2 Efficacy of antimicrobial treatment for acute otitis media

Primary outcome

Treatment failure occurred in 30 of the 161 (19%) children in the amoxicillin-clavulanate group and in 71 of the 158 (45%) of children in the placebo group (P<0.001). The groups separated in this respect already at the first scheduled visit (day 3) and the difference increased further during the entire follow-up (Figure 7A). Amoxicillin-clavulanate reduced the risk of treatment failure by 62% (hazard ratio, 0.38; 95% CI, 0.25 to 0.59; P<0.001). Each one of the six components of the treatment failure occurred less often in the amoxicillin-clavulanate group than in placebo group.

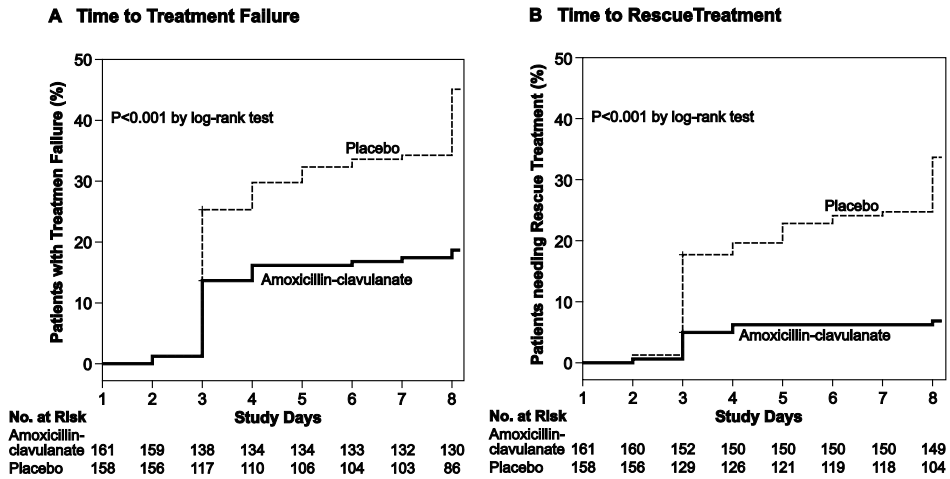

Figure 7. Cumulative incidence curves for time to treatment failure and time to rescue treatment. The figure has been published in Original Publication I.

Secondary outcomes

Rescue treatment was initiated for 11 of the 30 (37%) and 53 of the 71 (75%) treatment failure cases in amoxicillin-clavulanate and placebo groups, respectively (P<0.001). The need for rescue treatment was 81% lower in the amoxicillin-clavulanate group as compared to the placebo group (hazard ratio, 0.19; 95% CI, 0.10 to 0.36; P<0.001, Figure 7B). Contralateral AOM developed in 13 of the 159 (8%) children in the amoxicillin-clavulanate group and in 29 of the 156 (19 %) children in the placebo group (P=0.007). Altogether, 133 of the 161 (84%) children in the amoxicillin-clavulanate group and 134 of the 158 (86%) children in the placebo group received analgesic or antipyretic agents. Absenteeism from day care was reported for 107 of the 672 (16%) follow-up days and for 144 of the 568 (25%) follow-up days among day-care attendees in amoxicillin-clavulanate and placebo groups, respectively (P<0.001). Parents of day-care attendees in the amoxicillin-clavulanate treatment group missed significantly less workdays than

parents of day-care attendees in the placebo group (81/672 [12%] vs. 101/568 [18%] of follow-up days; P=0.005).

At the end of treatment visit, there was a significantly better treatment result with respect to both the overall condition and otoscopic signs in the amoxicillin-clavulanate group than in the placebo group. Overall condition had not improved or had worsened in 11 of the 161 (7%) children in the amoxicillin-clavulanate group, as compared to 47 of the 158 (30%) children in the placebo group (P<0.001). Otoscopic signs had not improved or had worsened in 8 of the 161 (5%) children in the amoxicillin-clavulanate group, as compared to 60 of the 158 (38%) children in the placebo group (p<0.001). Amoxicillin-clavulanate accelerated the resolution of fever (median time to resolution 6 and 60 hours, respectively, P<0.001 by log-rank test), poor appetite (36 vs. 72 hours, P=0.01), decreased activity (24 vs. 48 hours, P=0.02), and irritability (36 vs. 60 hours, P=0.05) but not ear pain as reported by the parents (24 vs. 36 hours, P=0.46), ear pain as reported by the children (18 vs. 36 hours, P=0.40), ear rubbing (48 vs. 48 hours, P=0.85), restless sleep (36 vs. 48, P=0.07), or excessive crying (48 vs. 60 hours, P=0.14). Adverse events were significantly more common in the amoxicillin-clavulanate group than in the placebo group (53% [85/161] vs. 36% [57/158], P=0.003).

5.3 Delayed versus immediate antimicrobial treatment for acute otitis media

Primary outcome

Improvement during antimicrobial treatment was observed in 48 of the 53 children (91%) in the delayed antimicrobial treatment group and in 155 of the 161 children (96%) in the immediate antimicrobial treatment group (P=0.10). The unadjusted odds ratio for improvement during antimicrobial treatment was 0.37 (95% CI, 0.11 to 1.27; P=0.12) for the delayed antimicrobial treatment group, as compared to immediate antimicrobial treatment group. Adjustment for baseline characteristics (day-care attendance, parental smoking, bilateral AOM, and moderate/marked bulging of the tympanic membrane), did not significantly change the odds ratio of any of the outcomes.

Secondary outcomes

In the delayed antimicrobial treatment group, one child developed blood-culture verified pneumococcal bacteremia and one child radiographically confirmed pneumonia during the wait-and-see period. The Kaplan-Meier curves showed a tendency for children in the delayed antimicrobial treatment group to become asymptomatic later, but the difference between the two groups was not statistically significant (P=0.18 by log-rank test, Figure 8A). Delayed initiation of antimicrobial treatment was associated with prolonged time to completely normal otoscopic examination (P=0.04 by log-rank test) (Figure 8B). Delayed, as compared to immediate, initiation of antimicrobial treatment

was associated with prolonged resolution of fever (median time to resolution 48 and 6 hours, respectively, P<0.001 by log-rank test), ear pain as reported by the parents (60 vs. 24 hours, P=0.04), poor appetite (84 vs. 36 hours, P=0.02), and decreased activity (60 vs. 24 hours, P=0.002). In addition, ear pain as reported by the children appeared to last longer in the delayed as compared to immediate antimicrobial treatment group (60 vs. 18 hours, P=0.15), but the difference did not reach statistical significance, since only very few children could express themselves verbally. There were no statistically significant differences in the duration of ear rubbing (36 vs. 48 hours, P=0.43), irritability (60 vs. 36 hours, P=0.62), restless sleep (60 vs. 36 hours, P=0.09), or excessive crying (60 vs. 48 hours, P=0.45) between the groups. All the differences between the groups appeared to be due to the wait-and-see period because when comparing the resolution of symptoms during antimicrobial treatment (i.e., from the initiation day of antimicrobial treatment to one day after the last dose of antimicrobial treatment), there were no statistically significant differences between the groups (data not shown).

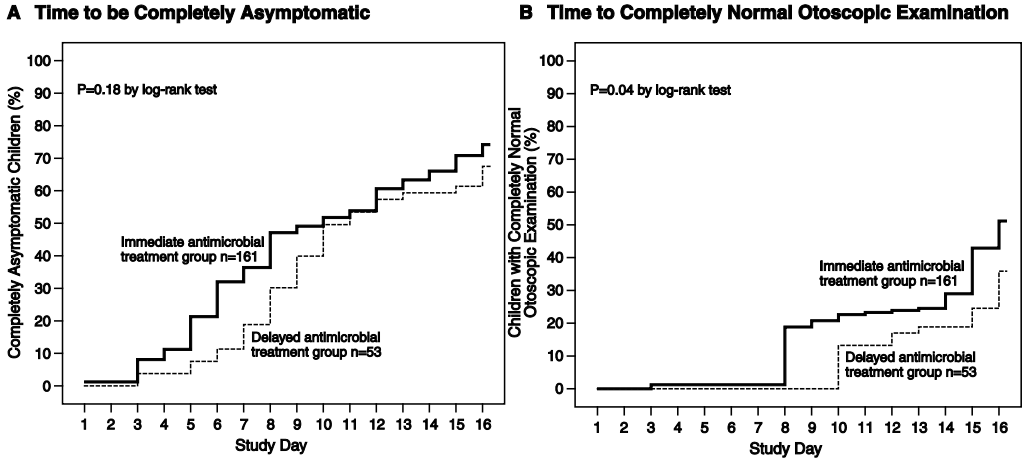

Figure 8. Cumulative incidence curves for the time to be completely asymptomatic and the time to completely normal otoscopic examination.

Among day-care attendees, the mean number of days of absenteeism from day care was 3.3 and 1.8 (P<0.001) and parental absenteeism from work was 2.1 and 1.2 (P=0.03) in the delayed and immediate antimicrobial treatment groups, respectively. In the delayed antimicrobial treatment group, 49 of the 53 (94%) children and in the immediate antimicrobial treatment group, 141 of the 161 (89%) children had received analgesic or antipyretic agents between study days 1–16 (rate difference, 6%; 95% CI, -4% to 15%). The mean number of days of usage of was higher in the delayed antimicrobial treatment group than in the immediate antimicrobial treatment group (mean number of days 5.2 vs. 4.0, P=0.01). Adverse events between study days 1–16 were equally common in the delayed, as compared to immediate, antimicrobial treatment group.

5.4 Parental experiences and opinions regarding the management of acute otitis media

Altogether 1151 families participated in this study. Results regarding antimicrobial treatment and analgesic use during AOM are summarized in Table 6. More children in Finland than in the Netherlands reported to have had at least one episode of AOM during lifetime (83% [568/686] vs. 49% [230/465]; rate difference, 34%; 95% CI, 28% to 39%). In Finland and in the Netherlands, 37% and 34% of the parents had discussed antimicrobial resistance to antibiotics with a doctor (rate difference, 3%; 95% CI, -2% to 9%). According to the parents, antimicrobial resistance had caused problems in the treatment of AOM in 20% of children in Finland and in only 2% in the Netherlands (rate difference, 18%; 95% CI, 15% to -22%). Overall, 88% of the parents in Finland and 65% in the Netherlands were worried that bacteria could become resistant to antibiotics (rate difference, 23%; 95% CI, 18% to -28%).

Table 6. Antimicrobial treatment and analgesic use during acute otitis media.

Question	**Finland** ”Yes”	**The Netherlands** ”Yes”	**Rate difference (95% CI)**
Has the doctor ever prescribed antibiotics to treat the ear infection of your child?	99% (558/563)	78% (178/227)	21% (15% to 26%)
Has the doctor ever treated the ear infection of your child without antibiotics but with wait-and-see approach?	13% (70/551)	59% (132/225)	-46% (-53% to -39%)
Do you think that antibiotics are necessary in the treatment the ear infection of your child?	85% (383/450)	55% (238/431)	30% (24% to 36%)
Has a doctor or a nurse ever recommended using painkillers in connection to the ear infection of your child?	77% (415/552)	69% (157/226)	8% (1 to 15%)
Have you ever given painkillers to your child in connection to the ear infection?	80% (441/552)	86% (195/227)	-6% (-12% to 0%)
When do you think that it is important to give your child painkillers?[1]			
- When I, as a parent, suspect that my child has an ear infection	21% (143/681)	12% (56/462)	9% (5% to 13%)
- After the doctor has diagnosed an ear infection	24% (162/681)	16% (74/462)	8% (3% to 12%)
-When the ear infection is treated with antibiotics	10% (69/681)	5% (22/462)	5% (2% to 8%)
- When the ear infection is treated without antibiotics	21% (144/681)	15% (70/462)	6% (1% to 10%)
- Only when my child seems to be in pain	84% (571/681)	91% (421/462)	-7% (-11% to -4%)

[1] The total number of “Yes” can be >100% because several answers can be given.

6 DISCUSSION

6.1 Efficacy of antimicrobial treatment for acute otitis media

The basis for this study was the controversy regarding the optimal management of AOM. Although this subject has been widely studied, there is still no consensus on whether AOM should be treated with antimicrobials or not. New, high quality evidence has been much needed to optimize the treatment of this common infection, which affects nearly every child during the first years of life. To avoid the methodological limitations of previous trials, we paid special attention to exclusion criteria, diagnosis, selection of outcomes, and the follow-up schedule (Bain 2001, Dagan and McCracken 2002, Pichichero and Casey 2008a, Pichichero and Casey 2008b).

Our results clearly show that amoxicillin-clavunalate is superior to placebo for the management of AOM. The results of the primary outcome measure, the time to treatment failure, demonstrated that the beneficial effect of antimicrobial treatment was apparent already two days after the initiation of treatment. The difference between the antimicrobial treatment group and the placebo group increased throughout the study drug period, resulting in a 26% higher treatment failure rate in the placebo group at the end-of-treatment visit. The difference in the treatment failure rates between the two groups was higher in our study than in most previous double-blind, placebo-controlled studies (Halsted et al. 1968, Mygind et al. 1981, Thalin et al. 1985, Kaleida et al. 1991, Burke et al. 1991, Appelman et al. 1991, Damoiseaux et al. 2000, Le Saux et al. 2005). Only Engelhard et al. (1989) and Hoberman et al. (2011) have reported higher differences in the failure rates between the treatment groups. In a study by Hoberman et al. (2011), 16% of the children in the antimicrobial treatment group and 51% of children in the placebo group had clinical failure. A possible explanation for the high treatment failure rates in our study and in the one by Hoberman et al. (2011) is the strict diagnostic criteria that were used in both studies. By including only patients with true AOM, we were able to evaluate the real effect of antimicrobial treatment.

Based on our results, the number needed to be treated was four for one child to benefit from antimicrobial treatment. This number is much lower than those of the meta-analyses that concluded that 7–17 children must be treated for one child to have relief of symptoms (Rosenfeld et al. 1994, Del Mar et al. 1997, Rovers et al. 2006, Vouloumanou et al. 2009, Coker et al. 2010, Sanders et al. 2010, Shekelle et al. 2010). The large numbers needed to be treated in the meta-analyses are largely based on outcome selection. Many of the meta-analyses have resolution of individual symptoms, such as ear pain or fever, as a main outcome (Del Mar et al. 1997, Rovers et al. 2006, Sanders et al. 2010). As symptoms are often caused by concurrent viral URI and spontaneous resolution of symptoms is common, the differences between the antimicrobial treatment group and placebo group may not demonstrate the most optimal effect of antimicrobial treatment. In addition,

some meta-analyses used resolution of MEE by day 30 as an outcome (Rosenfeld et al. 1995, Del Mar et al. 1997, Sanders et al. 2010). Resolution of MEE is not the most optimal outcome either because it completely ignores the symptoms. Furthermore, a point prevalence of MEE at day 30 does not take into account the fluctuating nature of AOM because relapses and reinfections may have occurred after the primary episode of AOM.

In addition to treatment failure, the secondary outcomes of our study constantly showed that antimicrobial treatment is superior to placebo. Rescue treatment was needed significantly more often in the placebo group, as compared to the antimicrobial treatment group. The decision to institute rescue treatment was based on individual consideration with the parents; in most cases, the overall condition of the child had worsened or the child still had severe symptoms two days after study entry. Therefore, the initiation of rescue treatment reflects true failure to respond to initial treatment. Our rescue treatment rates in the antimicrobial treatment group are in line with previous studies but, once again, the rates in the placebo group are much higher than in any of the previous trials (Halsted et al. 1968, Thalin et al. 1985, Kaleida et al. 1991, Burke et al. 1991, Appelman et al. 1991, Damoiseaux et al. 2000, Le Saux et al. 2005). In our study, contralateral AOM developed in 19% of children in the placebo group, as compared to 8% in the antimicrobial treatment group. Thalin et al. (1985), also noted that contralateral AOM developed significantly more often for children in the placebo group, but the overall numbers of contralateral AOM were about half from ours.

Only outcomes that favored the use of placebo were adverse events. In our study, adverse events were significantly more common than in any previous studies (Halsted et al. 1968, Mygind et al. 1981, van Buchem et al. 1981, Thalin et al. 1985, Engelhard et al. 1989, Kaleida et al. 1991, Burke et al. 1991, Appelman et al. 1991, Damoiseaux et al. 2000, Le Saux et al. 2005, Hoberman et al. 2011). Part of this can be explained by the choice of antimicrobial agent: amoxicillin-clavulanate does cause more gastroenterological adverse events than amoxicillin alone. All adverse events resolved spontaneously and did not result in discontinuation of the study drug in any case.

Interpretation of the results of symptom resolution is rather difficult because the symptoms are often considered to be caused by a concurrent RTI. In our study, the resolution of fever, poor appetite, decreased activity, and irritability was significantly faster in the antimicrobial treatment group than in the placebo group. On the other hand, there were no differences in the time to resolution of ear pain, ear rubbing, restless sleep, or excessive crying. The earliest treatment effect was seen in the resolution of fever; the difference between the groups was obvious already six hours after the initiation of study drug. This finding suggests that fever during AOM may be caused by the bacterial infection, although fever has not been shown to be indicative of AOM in most studies (Heikkinen and Ruuskanen 1995, Niemelä et al. 1994, Laine et al. 2010).

It should be noted, though, that half of the children in the placebo group did not experience treatment failure and that two thirds recovered well without antimicrobial treatment. Similarly, a meta-analysis by Rosenfeld et al. (1994) demonstrated that 81% of children in the placebo group were free from symptoms and signs of AOM within 7–14 days after study entry. Thus, it appears that not all children with AOM need antimicrobial treatment. In the future, it would be important to identify those patients who derive the greatest benefit from antimicrobial treatment. This would reduce the unnecessary use of antimicrobials and might limit the development of resistant bacteria.

A major strength of this study is the study design. The study was randomized, double-blind, and placebo-controlled, which is the gold standard for all treatment studies. We evaluated the optimal treatment of AOM at the age group with the highest incidence AOM, i.e., in children under three years of age. Unlike previous studies, we did not exclude children by symptom severity. We included children with high fever and severe ear pain; only severe infection necessitating antimicrobial treatment was a reason for exclusion from the study. On the other hand, we also included children with mild symptoms. Most importantly, we used strict diagnostic criteria, and did not include children who had MEE but no signs of acute inflammation. Furthermore, we used an adequate dose of an antimicrobial agent, amoxicillin-clavulanate, which had sufficient antimicrobial coverage. Our follow-up was more intense than in any previous treatment trials, and we performed careful otoscopic examination at every study visit. All these methodological strengths allowed us to obtain reliable data and to minimize bias.

The selection of best outcomes of AOM is, and probably will always be, debatable. Previous trials have been criticized for selecting an outcome that is not clinically relevant. Our primary outcome, the time to treatment failure, was clinically meaningful. It was a composite outcome that included both acute symptoms and otoscopic signs. Both of these elements have relevance to patient. Symptoms cause acute suffering, disturbed sleep, and absenteeism from day care and work. Otoscopic signs, on the other hand, are associated with ear pain, MEE causes reduced hearing, and persistent MEE will require insertion of tympanostomy tubes. The adverse events were also incorporated into the assessment of the child's overall condition. Therefore, our primary outcome is an adequate and sufficient measure of the net effect of treatment and the results thus demonstrate the true efficacy of antimicrobial treatment. At the same time with our study, Hoberman et al. (2011), conducted a study with a surprisingly similar study design. Their primary outcomes were the time to resolution of symptoms and the symptom burden over time, in which they reported statistically significant differences between the antimicrobial treatment group and the placebo group. In the discussion, the authors however concluded that overall clinical response may constitute the more telling measure of outcome. The fact that our treatment failure rates are very comparable with the clinical failure rates of Hoberman et al. (2011), suggests that our outcome choice has been optimal and that our results are as close to the true clinical situation as is possible at this time point.

Our study also has limitations. The evaluation of otoscopic signs is always subjective and prone to interobserver bias. To miminize bias, only qualified otoscopists were employed for this study. The overall number of otoscopists was only five, three of whom made more than 90% of the diagnoses. The interobserver agreement was excellent: the κ-value ranged from 0.80 to 0.92. Furthermore, the otoscopic findings were documented by digital pneumatic video otoscopy, which makes interpretation of the findings more objective. At trial end, an ear-nose-throat specialist assessed the videos and images obtained from 150 children; the degree of agreement with the study assessments of AOM was 95%.

Another limitation of the present study is the age range. Since most of the children were under two years of age, the results cannot be generalized to older age groups. On the other hand, this is the age group with the highest incidence of AOM. Thus, the age of most children who need treatment for AOM coincides with the age of the patients in this study.

It is also of note that our study was carried out before PCV was incorporated into the Finnish national vaccine program. Therefore, the bacterial etiology of our study may differ from the etiology of completely vaccinated population. However, widespread vaccination with PCV does not appear to change the fact that AOM still is primarily a bacterial disease where antimicrobial treatment is effective (Block et al. 2004, Casey and Pichichero 2004, Brook and Gober 2009).

We used amoxicillin-clavulanate as an active treatment to obtain optimal antimicrobial coverage for study purposes. However, the wide use of amoxicillin-clavulanate as a first-line management for AOM does not seem justifiable because it might increase the bacterial resistance and cause more adverse events than amoxicillin alone. Amoxicillin is effective against *S. pneumoniae,* which is considered to be the most important pathogen causing AOM. In complicated cases of AOM, the use of amoxicillin-clavulanate is, however, indicated as it covers all major bacterial pathogens of AOM.

Although our results show that antimicrobial treatment is effective for the management of AOM, it should not lead to increased use of antimicrobial treatment. A certain diagnosis is a key factor in the treatment of AOM. If diagnosis of AOM is not certain, antimicrobial treatment should not even be considered. On the other hand, a child with a certain diagnosis of AOM does benefit from antimicrobial treatment. Guidelines should be made as simple as possible in order to obtain physicians to follow them. A simplified treatment guideline for AOM could be as follows: "Children with bulging tympanic membrane should be treated with antimicrobials. Children without bulging tympanic membrane may be managed with wait-and-see approach if the symptoms are mild and the parents are willing to continue without antimicrobial treatment."

In conclusion, our results provide new evidence on the treatment of AOM. For children 6–35 months of age, with strictly diagnosed AOM, antimicrobial treatment is clearly beneficial. Antimicrobial treatment reduces the risk of treatment failure by improving the patient's overall condition and the otoscopic signs of the disease.

6.2 Delayed versus immediate antimicrobial treatment for acute otitis media

The wait-and-see approach with the option of delayed antimicrobial treatment is recommended by several treatment guidelines. In this approach, antimicrobial treatment is initiated within 48–72 hours after diagnosis if the child's condition does not improve or if it worsens. However, the consequences of delayed initiation of antimicrobial treatment are not fully known. We thus conducted a subanalysis of our randomized, double-blind, placebo-controlled trial to study if delayed, as compared to immediate, initiation of antimicrobial treatment worsens the recovery from AOM. To our knowledge, we were the first to analyze the delayed antimicrobial treatment group as a separate group, instead of comparing the whole wait-and-see group with the whole immediate antimicrobial treatment group. Therefore, we were able to evaluate the consequences of delaying antimicrobial treatment for AOM.

Previous studies comparing delayed versus immediate initiation of antimicrobial treatment have mainly concentrated on measuring parental satisfaction and reduction in antimicrobial use (Little et al. 2001, McCormick et al. 2005, Spiro et al. 2006). Since these outcomes have been well documented, we chose to measure the treatment effect on the patient-level. AOM is a common infection and it causes profound disturbances in the family's daily routines. Thus, also patient-related aspects need to be taken into account when choosing between immediate antimicrobial treatment and the wait-and-see approach.

Our results indicate that delayed, as compared to immediate, initiation of antimicrobial treatment does not worsen the recovery from AOM. The overall condition and otoscopic signs of more than 90% of the children in the delayed and immediate antimicrobial treatment groups improved during antimicrobial treatment. This result supports our previous finding that antimicrobial treatment is effective in the treatment of AOM. It appears that, although the overall condition of the children in the delayed antimicrobial treatment group might have worsened during the observation period, most children recovered well once antimicrobial treatment was instituted. This is important information for practicing physicians who have been advised by the guidelines to use the wait-and-see approach.

An important finding of our study was that the observation period may entail costs for the child, family, and society. In our study, delayed initiation of antimicrobial treatment appeared to predispose the child to the development of severe infections. In the delayed antimicrobial treatment group, one child developed pneumococcal bacteremia and one child developed radiographically confirmed pneumonia. Of course, we do not know if these infections could have been avoided by initiating antimicrobial treatment immediately. It is of notice, however, that many previous placebo-controlled studies have documented serious infections among children not receiving antimicrobials (Halsted et al. 1968, van Buchem et al. 1985, Damoiseaux

et al. 2000, Hoberman et al. 2011). Severe infections are rare, but very often require hospitalization, which, again, entails significant economic consequences.

Delayed initiation of antimicrobial treatment was associated with prolonged resolution of symptoms. In particular, the consequences of prolonged fever should not and cannot be dismissed. Prolongation of fever by two days is significant for the family, since fever is often considered to be a sign of more severe disease, thus causing parental anxiety. The longer duration of fever and ear pain in the delayed antimicrobial treatment group may also explain why children in the delayed antimicrobial treatment group used more analgesic and antipyretic agents than children in the immediate antimicrobial treatment group. Our results are in agreement with previous trials. In a study by Little et al. (2001) children in the delayed antimicrobial treatment group reported more pain at day 3 in comparison to immediate antimicrobial treatment group. McCormick et al. (2005) and Neumark et al. (2007) reported that immediate antimicrobial treatment was associated with improved symptom control, while Spiro et al. (2006) found no difference between the immediate antimicrobial treatment group and the wait-and-see group regarding the frequency of subsequent fever or ear pain. However, both these symptoms were associated with the initiation of delayed antimicrobial treatment. Taken together, our results challenge the view that antimicrobial treatment of strictly diagnosed AOM should be withheld to follow if symptoms resolve spontaneously without antimicrobials.

Since AOM is an acute infection, the main benefit of antimicrobial treatment could be expected at an early stage of the disease. We observed a tendency that children in the delayed antimicrobial treatment group became completely asymptomatic later than children in the immediate antimicrobial treatment group. However, regardless of treatment timing, most children were completely asymptomatic by day 16. Therefore, the costs of delayed antimicrobial treatment appear to be generated from the very first days after the diagnosis of AOM, i.e., before the initiation of antimicrobial treatment. The fact that resolution of MEE occurred later in the delayed than in the immediate antimicrobial treatment group is important especially for children with recurrent AOM who are usually diagnosed with a new episode of AOM before they even are cured from previous infection. If recovery of these children could be accelerated by immediate initiation of antimicrobial treatment, resolution of MEE could be confirmed between the episodes. This would change their diagnosis from chronic OME to recurrent AOM, and thus, improve prognosis and reduce tympanostomy tube insertions.

A study by Mepropol et al. (2008) questions if the American Academy of Pediatrics AOM treatment guidelines are consistent in all age groups. The authors found out that while guideline implementation in children less than 2 years of age reduces antimicrobial use, the price is high in the form of cost of sick days and parental absenteeism from work. Our results support this finding. In the present study, parents of children in the delayed antimicrobial treatment group missed an average of one workday more than parents of children in the immediate antimicrobial treatment group. This difference may seem small, but when multiplied with the 9 million annual episodes of AOM diagnosed

in the United States, it could be discussed that the difference in the absenteeism might actually be the most important economic consequence related to the delayed initiation of antimicrobial treatment (Soni 2008). According to the Occupational Employment Statistics for 2010, the mean wage for an 8-hour workday in the US is $170 (Bureau of Labor Statistics 2010). Thus, the costs of one-day absence from work would exceed 1.5 billion dollars in the United States every year. Our results, together with the Cochrane authors, emphasise the need to identify those children whose antimicrobial treatment should not be delayed (Sanders et al. 2010)

A reduction in antimicrobial use is an important goal, but it should not be achieved in the expense of the child and family. From the family's point of view, a reduction in the number of days of fever and a reduction in missed work days are much more important goals than a reduction in bacterial resistance in the community. Therefore, it seems understandable that most AOM episodes are still treated with antimicrobials, regardless of recommendations. On the other hand, delaying antimicrobial treatment did not worsen the recovery of the child. If the child's overall condition is good and parents feel comfortable with the wait-and-see approach, this approach, together with adequate symptomatic medication, appears to be an acceptable alternative for the management of AOM. However, as previously pointed out, the delay in the initiation of antimicrobial treatment should be adjusted according to the duration and severity of prior symptoms (Little 2006). Our results highlight the importance of individualizing treatment decisions case-by-case with consideration of the child's symptoms, history of AOM, and the overall situation of the family.

This study has obvious limitations. First, it constitutes a subanalysis of a randomized, placebo-controlled trial of antimicrobial treatment for AOM. Therefore, our results can indicate only associations, not causal effects. The sample size may also have been underpowered to detect differences between the two groups. However, this subanalysis was pre-planned and as a result, both study groups received equal antimicrobial treatment with optimal antimicrobial coverage. Second, this subanalysis was not blinded for the delayed antimicrobial treatment agent, since the rescue treatment was open-labelled. This may have resulted in assessment bias by parents and study personnel. On the other hand, parents and study personnel did not know which group the child had been allocated to, when initiation of delayed antimicrobial treatment was considered, and the allocation of each participant was kept blinded until completion of the whole trial. Third, the delayed antimicrobial treatment group was composed of children who failed to respond to study drug (i.e., placebo). Thus, at the time of the initiation of delayed antimicrobial treatment, the delayed antimicrobial treatment group may have been composed of children more ill, while the immediate antimicrobial treatment group contained children with a range of disease severity. However, symptoms of children in the delayed and immediate antimicrobial treatment groups were similar at baseline, and the impact of any confounding factors was studied by multivariable analyses.

The importance of this study lies in the fact that the results are directly applicable for clinical decision making because our study mimics the daily practice. The clinician has to choose between immediate antimicrobial treatment and the wait-and-see option without knowing how the overall condition of the child will develop. Eventually, some children will receive delayed antimicrobial treatment. Therefore, our delayed antimicrobial treatment group appears to be an appropriate representation of children who would receive delayed antimicrobial treatment also in daily practice. However, our study design was not optimal for the comparison of delayed versus immediate antimicrobial treatment. Thus, further high-quality studies are needed to evaluate the consequences of delayed initiation of antimicrobial treatment for AOM. An optimal study design would be a randomized, double-blind study in which the other group would receive antimicrobial treatment for the first 7 days and placebo for the next 3 days and the other group would receive placebo for the first 3 days and antimicrobial treatment for the next 7 days. A double-blind study design would minimize the assessment bias and provide as objective data as possible to give an answer to this important question.

6.3 Parental experiences and opinions regarding the management of acute otitis media

The comparative questionnaire on the parents' experiences and opinions regarding the management of AOM between Finland and the Netherlands showed that treatment guidelines, clinical practise and parental attitudes interact with each other. There were significant differences in parental opinions between the countries: more Finnish parents than Dutch parents were of the opinion that antimicrobials are necessary in the treatment of AOM. The faith in antimicrobial treatment of the Finnish parents may be a consequence of their own experiences. Since only a minority of AOM cases in Finland are treated by wait-and-see approach, the parents may not be aware of other options than antibiotics. The expectations of the parents may further strengthen the doctors' practice to prescribe antibiotics. Previous studies have shown that the perceptions of the physicians regarding the patients' expectations appear to be the strongest driver for the antimicrobial prescription (Britten and Ukoumunne 1997). It is also possible that Finnish parents' conception of the necessity of antimicrobials to treat AOM is based on their awareness of the Finnish guidelines that prefer antimicrobial treatment. The Dutch guidelines of AOM treatment are considerably different: wait-and-see approach is largely recommended and thus, most Dutch parents have experiences of this treatment option. In consequence, Dutch parents thought less often of antimicrobials as a necessary component to treat AOM.

The proportion of children reported to have at least one episode of AOM was higher in Finland than in the Netherlands (83% vs. 49%). It is possible, that Finnish parents may seek medical care more easily, while Dutch parents follow their child's symptoms at home. Another explanation for the difference between the countries may be that Finnish physicians have a lower threshold to diagnose AOM than their Dutch colleagues to justify

antimicrobial prescription. This kind of attitude was reported in a study by Leistevuo et al. (2005), where ”high prescribers” made the diagnosis of AOM significantly more often than ”low prescribers”.

According to the parents' perceptions, antimicrobial resistance had caused more problems in the treatment of AOM in Finland than in the Netherlands (20% vs. 2%). This result might be related to higher antimicrobial resistance rates in Finland, which are a consequence of higher antimicrobial use in Finland than in the Netherlands. In Finland, 88% of parents were worried that bacteria may become resistant to antimicrobials. The high percentage suggests that parents are aware of antimicrobial resistance and that they might be willing to take actions to decrease resistance rates. Therefore, parental education might be a good way to decrease unnecessary antimicrobial use and emergence of bacterial resistance. This view is supported by the results from a study by Arason et al. (2002) who found out that antimicrobial consumption can be influenced by increasing the awareness of parents of antimicrobial use. In our study, the fact that Finnish parents were more worried about antimicrobial resistance did not lead to reduced amount of antimicrobial prescriptions in Finland. The reason for this is probably in the Finnish treatment guidelines which recommend treating AOM primarily with antimicrobials in all cases (Heikkinen et al. 2010). However, AOM is not the only reason for antimicrobial use for outpatients. Antimicrobials are often prescribed for other infections, such as viral RTI, bronchitis, and rhinosinusitis, although guidelines by no means recommend antimicrobial treatment at the early stages of these infections. To reduce unnecessary antimicrobial use, the optimal management of cough and rhinosinusitis are also important subjects for parental education.

We were very worried about the finding that only 21% of the Finnish and 12% of the Dutch parents reported that they believe that it is important to give analgesics when they suspect AOM. Most parents would give their child analgesics only when their child seems to be in pain. This may increase unnecessary pain experiences because the assessment of ear pain is difficult in young children. Furthermore, only 10% of the Finnish and 5% of the Dutch parents reported concomitant use of analgesics and antimicrobials. This is in disagreement with treatment guidelines, which expressly recommend regular use of analgesics in all cases of AOM (Appelman et al. 2006, Heikkinen et al. 2010). Pulkki et al. (2006) reported similar results: in their large study of the 3059 patients only 10% got a prescription or recommendation to use analgesics. These results indicate that the health personnel should be encouraged to discuss analgesic use with their patients. In addition, the importance of analgesic as soon as AOM is suspected needs to be emphasized more clearly to parents.

This study has some limitatios. First, recall bias is a limitation common to all questionnaire studies. However, recall bias is a universal phenomenon. Thus, it hardly explains differences when two countries are compared. Second, the questionnaire was translated from Finnish to English and then to Dutch, and differences might have been introduced during this chain of translations. Third, since the questionnaire was limited

only to children attending day-care, the results cannot be generalized to every child under four years old.

The major strength of this study is the comparison between two western countries whose AOM guidelines differ notably. In both countries, the study was carried out at the same time of the year: at the end of the annual AOM season. The end of AOM season was chosen so that parents could remember their experiences of the treatment of AOM well.

Our results demonstrate that there is an interaction between guidelines, clinical practise and parental opinions regarding the treatment of AOM. Therefore, if treatment practices are to be changed, it is not enough to modify the guidelines for professionals. Parents should also be educated and informed about the available treatment options for AOM.

7 SUMMARY AND CONCLUSIONS

The purpose of this study was to find an answer to the question: "Should AOM be treated with antimicrobials or not?" This study provides the answer: "Yes, in most cases." Children with a definitive diagnosis of AOM benefit from antimicrobial treatment as compared to placebo. The treatment failure rate was 26% higher in the placebo group than in the amoxicillin-clavulanate group. The difference between the groups was apparent already on day 3 and increased throughout the study drug period which lasted for 7 days. Adverse events occurred significantly more often in the amoxicillin-clavulanate group, but all other outcomes favored amoxicillin-clavulanate.

Based on our results, the wait-and-see approach appears to be an acceptable treatment option for children with AOM under certain circumstances. Delayed initiation of antimicrobial treatment did not worsen the recovery from AOM, but the observation period before the initiation of delayed antimicrobial treatment might be associated with worsening of child's condition, prolongation of symptoms, and economic losses. Our results highlight the importance of individualized treatment decisions. When considering which treatment option is in the best interest of the child, practicing physicians should take into account the severity of symptoms and otoscopic signs, history of OM, and the overall situation of the family. Furthermore, if antimicrobial treatment is not initiated immediately, the child should have easy access to follow-up care.

The results of our comparative questionnaire study indicate that parents are familiar with the AOM treatment practices in their home country. In addition, treatment practices and parental expectations seem to interact. This is important information for experts who are responsible for updating AOM treatment guidelines. If we aim to change AOM treatment practices, guidelines as well as parental expectations need to be modified.

In conclusion, this study provides new evidence regarding the effect of antimicrobial treatment for the management of AOM. The results suggest that antimicrobials are superior to placebo and delaying antimicrobial treatment does not worsen the recovery from AOM. Parental education should be considered when introducing new treatment guidelines.

ACKNOWLEDGEMENTS

This study was carried out at the Department of Pediatrics in the University of Turku during the years 2006–2012. I am grateful to Professor Olli Simell, MD, Professor Jussi Mertsola, MD, and the former head of the Department of Pediatrics, Docent Marja-Riitta Ståhlberg, MD, for providing excellent facilities for research and creating an inspiring atmosphere at the department of Pediatrics. I am also grateful to the National Graduate School of Clinical Investigation for financial and educational support.

Above all, I wish to express my deepest gratitude to my supervisor, Aino Ruohola, MD, for trusting in me from the very beginning. You were always there when I needed you, and your support helped me through the laborious years of data collection. Thank you for innumerable, hilarious hours spent on your couch when we were planning data analysis and improving manuscripts. I admire your knowledge, creativity, and your enthusiasm towards research: you are an exemplary model for me. You have thought me to think independently and to trust in my own ideas. Those skills are of value in research as well as in every-day life. Thank you for teaching me that, in addition to many other things. I could not have a better supervisor than you.

I also thank my other supervisor, Professor Olli Ruuskanen, MD, for the support and guidance. It has been a true privilege to learn from such a great teacher and innovative researcher. I am proud of being a part of your legendary study group. I also thank you for providing excellent research facilities in the Pediatric Research Unit of the Turku University Hospital Foundation.

The reviewers of this thesis, Professor Anne Pitkäranta, MD, and Docent Harri Saxén, MD, are gratefully acknowledged for their careful and prompt review. Your valuable comments and constructive criticism improved the quality of this work significantly. I would also like to thank Professor Jussi Mertsola, MD, and Docent Minna Aromaa, MD, for encouraging comments and support as part of my follow-up committee. My colleagues in the "Pediatric infectious diseases study group" are warmly acknowledged for all the support during these years.

I most sincerely thank all my co-authors for the collaboration. Miia Laine, MD, is especially acknowledged for her strong contribution to data collection. Thank you, Miia, for your friendship and for sharing all those ups and downs with me during the clinical phase of the Baby Elephant Study. You help has been invaluable. I would also like to thank Professor Pentti Huovinen, MD, Docent Jari Jalava, PhD, and Pirjo-Riitta Liuksila, PhD, for expertise and support. Professor Maroeska Rovers, PhD, Chantal Boonacker, PhD, and Professor Anne Schildrer, MD, are highly acknowledged for their valuable collaboration in the parental questionnaire study.

I am grateful to Raakel Luoto, MD, and Elina Lahti, MD, for help with the data collection. Thank you for taking such a good care of our study patients while I was

at home taking care of my own “Baby Elephant”. Research nurses Kaisa Erkkilä, RN, Sari Rajamäki, RN, and Maarit Rosenblad, RN, as well as Kati Thorn, MD, are acknowledged for their enjoyable collaboration and skilful assistance at the study clinic. I also want to thank Kaisa and Kati for our ongoing friendship and joyful conversations at the study clinic. Laura Lindholm, MSc, is acknowledged for her help with the bacteriological analyses, and Kati Vuorenoja, MSc, for processing the bacteriological analyses forward. I want to thank Maria Ruohola, Ulla Torkko, and Anne Nurmi for data handling, Tero Vahlberg, MSc, for assistance in statistical analyses, and Timo Kattelus, graphic designer, for creating high-quality figures for this thesis at a nearly impossible timetable. Tuomo Puhakka, MD, is warmly acknowledged for assessing digital images and videos of tympanic membrane findings of our study patients. Tiina Purhonen, MA, is acknowledged for revising the language of the original articles and Robert Paul, MD, for revising the language of the thesis.

I warmly thank the staff at the emergency department of the health center of the City of Turku for cordially welcoming us to work with them during the data collection. The personnel of the health centers and well-baby clinics are acknowledged for their contribution to patient recruitment and the personnel of the day care units for their help with handing the questionnaires to the parents. I also thank the staff of ward 415 for their collaboration. I am extremely grateful to all the families who participated in our study. Your excellent collaboration and your commitment to the study gave me strength during those busy years of data collection.

I sincerely thank all my colleagues in the Pediatric Research Unit of the Turku University Hospital Foundation for sharing all these long days with me. I direct my special thanks to Anna Pärtty, MD, Heli Silvennoinen, MD, Mira Huhtala, MD, and Johanna Olli, MNSc, for stimulating conversations and mental support during the final crush of finishing my thesis. Johanna Uitti, MD, Kjell Helenius, MD, and Santtu Heinonen, MD, are acknowledged for cheerful moments at international congresses and at home. Thank you for making the research work fun.

I am truly fortunate to have wonderful friends around me. Thank you all for your friendship and our enjoyable moments together. Linda Pääjärvi, Jaana Schneider, Minna Saarikoski-Johansson, Julia Heinonen, Lara Lehtoranta, Laura Kauppinen and Aija Jalonen are especially acknowledged for sharing the joys and sorrows of being a mother and a working woman. Jenni Jalkanen, Elina Penttilä, Johanna Loukamaa, Nina Nevalainen, and Emmi Rees: thank you for being there despite the geographic distance.

I thank my parents-in-law, Marja-Terttu and Markku Tähtinen, for helping our family in so many ways and for taking care of our children whenever needed. My sister-in-law, Milla Tähtinen, her husband Markus Heinonen, and their lovely children Mikael and Max are acknowledged for sharing so many good times together. Thank you all for taking me into your wonderful family.

I am grateful to my mother Marja Ruokonen for all the love and support throughout my life. You have always put the wellbeing of my sister and me ahead of your own. Thank you so much for taking care of Vilma and Olivia; they were in your loving hands while I was at work. I know that my late father Urpo Ruokonen would have been proud of me right now. My dear sister Hanna Peri and my brother-in-law Janne Peri are also acknowledged for their endless support and for unforgettable moments together.

Finally, to my beloved husband Miikka. No words exist to express my gratitude for all the love, patience, and support you have given me during these years. Thank you for taking care of our precious children and the household when I have been working. Without you, this thesis would not have been possible. You have always believed in me and stood beside me even when days were rainy. I am fortunate and deeply grateful to have a husband like you. Thank you for all our past days together and for the days to come. I also thank our precious children, Vilma and Olivia, for always reminding me of what is truly important in life. Your smiles and kisses make me forget all the worries and make me feel so fortunate. To quote the words of my supervisor, Aino: "Dear children, if you can be proud of this work one day, it was worth all our efforts."

The following foundations and institutes are acknowledged for their financial support: the National Graduate School of Clinical Investigation; the Foundation for Paediatric Research; the Research Funds from Specified Government Transfers; the Maud Kuistila Memorial Foundation; the Emil Aaltonen Foundation; the Finnish Cultural Foundation; the Finnish-Norwegian Medical Foundation; the Finnish Medical Society Duodecim; Turku University Foundation; and the Medical Faculty of the University of Turku.

Turku, May 2012

Paula Tähtinen

REFERENCES

Abramson JS, Wheeler JG. Virus-induced neutrophil dysfunction: role in the pathogenesis of bacterial infections. Pediatr Infect Dis J 1994;13:643-52.

Alho OP, Koivu M, Sorri M, Rantakallio P. The occurrence of acute otitis media in infants. A life-table analysis. Int J Pediatr Otorhinolaryngol 1991;21:7-14.

Appelman C, van Balen F, van de Lisdonk E, van Weert HE. NGH practice guideline ´Acute otitis media`. Huisarts wet 1990;33:242-5. Updated December 2006. Available at: http://www.artsennet.nl/Richtlijnen/Richtlijn/Otitis-media-acuta-bij-kinderen-2.htm, in dutch. [Accessed 14 May 2012]

Appelman CL, Claessen JQ, Touw-Otten FW, Hordijk GJ, de Melker RA. Co-amoxiclav in recurrent acute otitis media: placebo controlled study. BMJ 1991;303:1450-2.

Arason VA, Sigurdsson JA, Kristinsson KG, Gudmundsson S. Tympanostomy tube placements, sociodemographic factors and parental expectations for management of acute otitis media in Iceland. Pediatr Infect Dis J 2002;21:1110-5.

Arola M, Ruuskanen O, Ziegler T, et al. Clinical role of respiratory virus infection in acute otitis media. Pediatrics 1990a;86:848-55.

Arola M, Ziegler T, Ruuskanen O. Respiratory virus infection as a cause of prolonged symptoms in acute otitis media. J Pediatr 1990b;116:697-701.

Bain J. Treatment of acute otitis media: are children entered into clinical trials representative? Br J Gen Pract 2001;51:132-3.

Bain J, Townsley P, Boyle K, et al. Diagnosis and management of childhood otitis media in primary care: guideline no 66. Edinburgh: Scottish Intercollegiate Guidelines Network 2003. Available at: http://www.sign.ac.uk/guidelines/fulltext/66/index.html. [Accessed 14 May 2012]

Bakaletz LO, Daniels RL, Lim DJ. Modeling adenovirus type 1 induced otitis media in the chinchilla: effect on ciliary activity and fluid transport function of Eustachian tube mucosal epithelium. J Infect Dis 1993;168:865-72.

Barnett ED, Klein JO, Hawkins KA, Cabral HJ, Kenna M, Healy G. Comparison of spectral gradient acoustic reflectometry and other diagnostic techniques for detection of middle ear effusion in children with middle ear disease. Pediatr Infect Dis J 1998;17:556-9.

Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. N Engl J Med 1998;338:1405-12.

Bertin L, Pons G, d'Athis P, et al. A randomized, double-blind, multicentre controlled trial of ibuprofen versus acetaminophen and placebo for symptoms of acute otitis media in children. Fundam Clin Pharmacol 1996;10:387-92.

Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Pediatr Infect Dis J 2000;19:187-95.

Block SL, Mandel E, McLinn S, et al. Spectral gradient acoustic reflectometry for the detection of middle ear effusion by pediatricians and parents. Pediatr Infect Dis J 1998;17:560-4.

Block SL, Pichichero ME, McLinn S, Aronovitz G, Kimball SB. Spectral gradient acoustic reflectometry: detection of middle ear effusion in suppurative acute otitis media. Pediatr Infect Dis J 1999;18:741-4.

Block SL, Hedrick J, Harrison CJ, et al. Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. Pediatr Infect Dis J 2004;23:829-33.

Bluestone CD, Cantekin EI, Beery QC. Effect of inflammation on ventilatory function of eustachian tube. Laryngoscope 1977;87:493-507.

Bluestone CD, Stephenson JS, Martin LM. 10-year review of otitis media pathogens. Pediatr Infect Dis J 1992;11:S7-S11.

Bluestone CD, Gates GA, Klein JO, et al. Definitions, terminology, and classification of otitis media. Ann Otol Rhinol Laryngol 2002;111 Suppl 188:8-18.

Bluestone CD, Klein JO. Otitis media in infants and children. 4th edition. Hamilton: BC Decker Inc 2007.

Bluestone CD. Impact of evolution on the eustachian tube. Laryngoscope 2008;118:522-7.

Bodor F. Conjunctivitis-otitis syndrome. Pediatrics 1982;69:695-8.

Bodor FF, Marchant CD, Shurin PA, Barenkamp SJ. Bacterial etiology of conjunctivitis otitis media syndrome. Pediatrics 1985;76:26-8.

Bolt P, Barnett P, Babl FE, Sharwood LN. Topical lignocaine for pain relief in acute otitis media: results of a double-blind placebo-controlled randomised trial. Arch Dis Child 2008;93:40-4.

Boonacker CW, Broos PH, Sanders EA, Schilder AG, Rovers MM. Cost effectiveness of pneumococcal conjugate vaccination against acute otitis media in children: a review. Pharmacoeconomics 2011;29:199-211

Britten N, Ukoumunne O. The influence of patients' hopes of receiving a prescription on doctors' perceptions and the decision to prescribe: a questionnaire survey. BMJ 1997;315:1506-10.

Brook I, Gober AE. Bacteriology of spontaneously draining acute otitis media in children before and after the introduction of pneumococcal vaccination. Pediatr Infect Dis J 2009;28:640-2.

Brookhouser PE. Use of tympanometry in office practice for diagnosis of otitis media. Pediatr Infect Dis J 1998;17:544-51.

Bryant KA, Block SL, Baker SA, Gruber WC, Scott DA; PCV13 Infant Study Grp. Safety and immunogenicity of a 13-valent pneumococcal conjugate vaccine. Pediatrics 2010;125:866-75.

Bureau of Labor Statistics. Occupational employment statistics. May 2010. Available at: http://www.bls.gov/oes/current/oes_nat.htm. [Accessed 14 May 2012]

Burke P, Bain J, Robinson D, Dunleavey J. Acute red ear in children: controlled trial of non-antibiotic treatment in general practice. BMJ 1991;303:558-62.

Bylander A, Tjernstöm O, Ivarsson A. Pressure opening and closing functions of the eustachian-tube in children and adults with normal ears. Acta Otolaryngol 1983;95:55-62.

Bylander A. Upper respiratory tract infection and eustachian tube function in children. Acta Otolaryngol 1984;97:343-9.

Carson JL, Collier AM, Hu SS. Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. N Engl J Med 1985;312:463-8.

Cartwright K. Pneumococcal disease in western Europe: burden of disease, antibiotic resistance and management. Eur J Pediatr 2002;161:188-95.

Casey JR, Pichichero ME. Changes in frequency and pathogens causing acute otitis media in 1995-2003. Pediatr Infect Dis J 2004;23:824-8.

Casselbrant ML, Mandel EM, Kurs-Lasky M, Rockette HE, Bluestone CD. Otitis-media in a population of black American and white American infants, 0-2 years of age. Int J Pediatr Otorhinolaryngol 1995;33:1-16.

Casselbrant ML, Mandel EM, Fall PA, et al. The heritability of otitis media - a twin and triplet study. JAMA 1999;282:2125-30.

Centers for Disease Control and Prevention. NAMCS, National Ambulatory Medical Care Survey. 2008. Available at: http://www.cdc.gov/nchs/data/ahcd/namcs_summary/namcssum2008.pdf. [Accessed 14 May 2012]

Chao JH, Kunkov S, Reyes LB, Lichten S, Crain EF. Comparison of two approaches to observation therapy for acute otitis media in the emergency department. Pediatrics 2008;121:e1352-6.

Chianese J, Hoberman A, Paradise JL, et al. Spectral gradient acoustic reflectometry compared with tympanometry in diagnosing middle ear effusion in children aged 6 to 24 months. Arch Pediatr Adolesc Med 2007;161:884-8.

Chonmaitree T, Howie VM, Truant AL. Presence of respiratory viruses in middle ear fluids and nasal wash specimens from children with acute otitis media. Pediatrics 1986;77:698-702.

Chonmaitree T, Owen MJ, Patel JA, Hedgpeth D, Horlick D, Howie VM. Effect of viral respiratory tract infection on outcome of acute otitis media. J Pediatr 1992;120:856-62.

Chonmaitree T, Henrickson KJ. Detection of respiratory viruses in the middle ear fluids of children with acute otitis media by multiplex reverse transcription polymerase chain reaction assay. Pediatr Infect Dis J 2000;19:258-60.

Chonmaitree T, Saeed K, Uchida T, et al. A randomized, placebo-controlled trial of the effect of antihistamine or corticosteroid treatment in acute otitis media. J Pediatr 2003;143:377-85.

Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. Clin Infect Dis 2008;46:815-23.

Chonmaitree T, Ruohola A, Hendley JO. Presence of viral nucleic acids in the middle ear: acute otitis media pathogen or bystander? Pediatr Infect Dis J. 2012;31:325-30.

Clements DA, Langdon L, Bland C, Walter E. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. Arch Pediatr Adolesc Med 1995;149:1113-7.

Coco AS. Cost-effectiveness analysis of treatment options for acute otitis media. Ann Fam Med 2007;5:29-38.

Coker TR, Chan LS, Newberry SJ, et al. Diagnosis, microbial epidemiology, and antibiotic treatment of acute otitis media in children: a systematic review. JAMA 2010;304:2161-9.

Coleman C, Moore M. Decongestants and antihistamines for acute otitis media in children. Cochrane Database Syst Rev 2008:CD001727.

Craig WA, Andes D. Pharmacokinetics and pharmacodynamics of antibiotics in otitis media. Pediatric Infect Dis J 1996;15:255-9.

Dagan R, Hoberman A, Johnson C, et al. Bacteriologic and clinical efficacy of high dose amoxicillin/clavulanate in children with acute otitis media. Pediatr Infect Dis J 2001;20:829-37.

Dagan R, McCracken GH. Flaws in design and conduct of clinical trials in acute otitis media. Pediatr Infect Dis J 2002;21:894-902.

Dalia AB, Standish AJ, Weiser JN. Three surface exoglycosidases from *Streptococcus pneumoniae*, NanA, BgaA, and StrH, promote resistance to opsonophagocytic killing by human neutrophils. Infect Immun 2010;78:2108-16.

Damoiseaux RA, van Balen FA, Hoes AW, Verheij TJ, de Melker RA. Primary care based randomised, double blind trial of amoxicillin versus placebo for acute otitis media in children aged under 2 years. BMJ 2000;320:350-4.

del Castillo F, Baquero-Artigao F, Garcia-Perea A. Influence of recent antibiotic therapy on antimicrobial resistance of *Streptococcus pneumoniae* in children with acute otitis media in Spain. Pediatr Infect Dis J 1998;17:94-7.

Del Mar C, Glasziou P, Hayem M. Are antibiotics indicated as initial treatment for children with acute otitis media? A meta-analysis. BMJ 1997;314:1526-9.

Doern GV, Brueggemann AB, Pierce G, Holley HP Jr, Rauch A. Antibiotic resistance among clinical isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 and detection of beta-lactamase-positive strains resistant to amoxicillin-clavulanate: results of a national multicenter surveillance study. Antimicrob Agents Chemother 1997;41:292-7.

Doyle WJ, Cantekin EI, Bluestone CD. Eustachian tube function in cleft palate children. Ann Otol Rhinol Laryngol Suppl 1980;89:34-40.

Engelhard D, Cohen D, Strauss N, Sacks TG, Jorczak-Sarni L, Shapiro M. Randomized study of myringotomy, amoxycillin/clavulanate, or both for acute otitis-media in infants. Lancet 1989;2:141-3.

Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med 2001;344:403-9.

Faden H, Duffy L, Williams A, Krystofik DA, Wolf J. Epidemiology of nasopharyngeal colonization with nontypable *Haemophilus influenzae* in the first 2 years of life. J Infect Dis 1995;172:132-5.

Faden H, Duffy L, Wasielewski R, Wolf J, Krystofik D, Tung Y. Relationship between nasopharyngeal colonization and the development of otitis media in children. J Infect Dis 1997;175:1440-5.

Fainstein V, Musher DM, Cate TR. Bacterial adherence to pharyngeal cells during viral infection. J Infect Dis 1980;141:172-6.

Finitzo T, Friel-Patti S, Chinn K, Brown O. Tympanometry and otoscopy prior to myringotomy: issues in diagnosis of otitis media. Int J Pediatr Otorhinolaryngol 1992;24:101-10.

Forgie S, Zhanel G, Robinson J. Management of acute otitis media - a summary. Paediatr Child Health 2009;14:457-60.

Fria TJ, Cantekin EI, Eichler JA. Hearing acuity of children with otitis media with effusion. Arch Otolaryngol 1985;111:10-6.

Fritz RS, Hayden FG, Calfee DP, et al. Nasal cytokine and chemokine responses in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. J Infect Dis 1999;180:586-93.

Garcia-Rodriguez JA, Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J Antimicrob Chemother 2002;50:59-73.

Gehanno P, Panajotopoulos A, Barry B, et al. Microbiology of otitis media in the Paris, France, area from 1987 to 1997. Pediatr Infect Dis J 2001;20:570-3.

Giebink GS, Berzins IK, Marker SC, Schiffman G. Experimental otitis-media after nasal inoculation of *Streptococcus pneumoniae* and influenza A virus in chinchillas. Infect Immun 1980;30:445-50.

Grossman Z, Silverman BG, Porter B, Miron D. Implementing the delayed antibiotic therapy approach significantly reduced antibiotics consumption in Israeli children with first documented acute otitis media. Pediatr Infect Dis J 2010;29:595-9.

Groth A, Enoksson F, Hermansson A, Hultcrantz M, Stalfors J, Stenfeldt K. Acute mastoiditis in children in Sweden 1993-2007 -no increase after new guidelines. Int J Pediatr Otorhinolaryngol 2011;75:1496-501.

Hall-Stoodley L, Hu FZ, Gieseke A, et al. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA 2006;296:202-11.

Halsted C, Lepow ML, Balassanian N, Emmerich J, Wolinsky E. Otitis media. Clinical observations, microbiology, and evaluation of therapy. Am J Dis Child 1968;115:542-51.

Hammaren-Malmi S, Saxén H, Tarkkanen J, Mattila PS. Passive smoking after tympanostomy and risk of recurrent acute otitis media. Int J Pediatr Otorhinolaryngol 2007;71:1305-10.

Hanage WP, Kaijalainen TH, Syrjänen RK, et al. Invasiveness of serotypes and clones of *Streptococcus pneumoniae* among children in Finland. Infect Immun 2005;73:431-5.

Hatakka K, Savilahti E, Pönkä A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ 2001;322:1327-9.

Hatakka K, Blomgren K, Pohjavuori S, et al. Treatment of acute otitis media with probiotics in otitis-prone children - a double-blind, placebo-controlled randomised study. Clin Nutr 2007;26:314-21.

Hausdorff WP, Bryant J, Kloek C, Paradiso PR, Siber GR. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. Clin Infect Dis 2000a;30:122-40.

Hausdorff WP, Bryant J, Paradiso PR, Siber GR. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. Clin Infect Dis 2000b;30:100-21.

Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. Lancet Infect Dis 2005;5:83-93.

Heikkinen T, Ruuskanen O, Waris M, Ziegler T, Arola M, Halonen P. Influenza vaccination in the prevention of acute otitis-media in children. Am J Dis Child 1991;145:445-8.

Heikkinen T, Ruuskanen O. Temporal development of acute otitis media during upper respiratory tract infection. Pediatr Infect Dis J 1994;13:659-61.

Heikkinen T, Ruuskanen O. Signs and symptoms predicting acute otitis media. Arch Pediatr Adolesc Med 1995;149:26-9.

Heikkinen T, Thint M, Chonmaitree T. Prevalence of various respiratory viruses in the middle ear during acute otitis media. N Engl J Med 1999;340:260-4.

Heikkinen T, Huovinen P, Jero J, et al. Välikorvatulehdus. Käypä hoito -suositus. Duodecim 2010;126:573-4. Available at: www.kaypahoito.fi, in Finnish. [Accessed 14 May 2012]

Henderson FW, Collier AM, Sanyal MA, et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. N Engl J Med 1982;306:1377-83.

Hendley JO. Clinical practice. Otitis media. N Engl J Med 2002;347:1169-74.

Hernandez M, Leichtle A, Pak K, et al. Myeloid differentiation primary response gene 88 is required for the resolution of otitis media. J Infect Dis 2008;198:1862-9.

Hilty M, Qi W, Brugger SD, et al. Nasopharyngeal microbiota in infants with acute otitis media. J Infect Dis 2012;205:1048-55.

Hoberman A, Paradise JL, Reynolds EA, Urkin J. Efficacy of Auralgan for treating ear pain in children with acute otitis media. Arch Pediatr Adolesc Med 1997;151:675-8.

Hoberman A, Paradise JL, Rockette HE, et al. Treatment of acute otitis media in children under 2 years of age. N Engl J Med 2011;364:105-15.

Honjo I, Hayashi M, Ito S, Takahashi H. Pumping and clearance function of the eustachian tube. Am J Otolaryngol 1985;6:241-4.

Ishijima K, Sando I, Balaban C, Suzuki C, Takasaki K. Length of the eustachian tube and its postnatal development: computer-aided three-dimensional reconstruction and measurement study. Ann Otol Rhinol Laryngol 2000;109:542-8.

Jacobs J, Springer DA, Crothers D. Homeopathic treatment of acute otitis media in children: a preliminary randomized placebo-controlled trial. Pediatr Infect Dis J 2001;20:177-83.

Jerger J. Clinical experience with impedance audiometry. Arch Otolaryngol 1970;92:311-24

Joki-Erkkilä VP, Laippala P, Pukander J. Increase in paediatric acute otitis media diagnosed by primary care in two Finnish municipalities - 1994-5 versus 1978-9. Epidemiol Infect 1998;121:529-34.

Joki-Erkkilä VP, Pukander J, Laippala P. Alteration of clinical picture and treatment of pediatric acute otitis media over the past two decades. Int J Pediatr Otorhinolaryngol 2000;55:197-201.

Jones LL, Hassanien A, Cook DG, Britton J, Leonardi-Bee J. Parental smoking and the risk of middle ear disease in children: a systematic review and meta-analysis. Arch Pediatr Adolesc Med 2012;166:18-27.

Juneau RA, Pang B, Weimer KE, Armbruster CE, Swords WE. Nontypeable *Haemophilus influenzae* initiates formation of neutrophil extracellular traps. Infect Immun 2011;79:431-8.

Kaleida PH, Casselbrant ML, Rockette HE, et al. Amoxicillin or myringotomy or both for acute otitis-media: results of a randomized clinical trial. Pediatrics 1991;87:466-74.

Kaleida PH, Stool SE. Assessment of otoscopists accuracy regarding the diagnosis of middle-ear effusion in children. Otoscopic validation. Am J Dis Child. 1992;146:433-5.

Kalm O, Johnson U, Prellner K, Ninn K. HLA frequency in patients with recurrent acute otitis-media. Arch Otolaryngol Head Neck Surg 1991;117:1296-9.

Karjalainen H, Koskela M, Luotonen J, Herva E, Sipilä P. Occurrences of antibodies against *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Branhamella catarrhalis* in middle ear effusion and serum during the course of acute otitis media. Acta Otolaryngol 1991;111:112-9.

Karma PH, Penttilä MA, Sipilä MM, Kataja MJ. Otoscopic diagnosis of middle ear effusion in acute and non-acute otitis media. 1. the value of different otoscopic findings. Int J Pediatr Otorhinolaryngol 1989;17:37-49.

Kaur R, Adlowitz DG, Casey JR, Zeng M, Pichichero ME. Simultaneous assay for four bacterial species including *Alloiococcus otitidis* using multiplex-PCR in children with culture negative acute otitis media. Pediatr Infect Dis J 2010;29:741-5.

Kilpi T, Anttila M, Kallio MJ, Peltola H. Severity of childhood bacterial meningitis and duration of illness before diagnosis. Lancet 1991;338:406-9.

Kilpi T, Herva E, Kaijalainen T, Syrjänen R, Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. Pediatr Infect Dis J 2001;20:654-62.

Kimball S. Acoustic reflectometry: spectral gradient analysis for improved detection of middle ear effusion in children. Pediatr Infect Dis J 1998;17:552-5.

Koch A, Homøe P, Pipper C, Hjuler T, Melbye M. Chronic suppurative otitis media in a birth cohort of children in Greenland: population-based study of incidence and risk factors. Pediatr Infect Dis J 2011;30:25-9.

Koivunen P, Alho OP, Uhari M, Niemelä M, Luotonen J. Minitympanometry in detecting middle ear fluid. J Pediatr 1997;131:419-22.

Kontiokari T, Koivunen P, Niemelä M, Pokka T, Uhari M. Symptoms of acute otitis media. Pediatr Infect Dis J 1998;17:676-9.

Kozyrskyj A, Klassen TP, Moffatt M, Harvey K. Short-course antibiotics for acute otitis media. Cochrane Database Syst Rev 2010;8:CD001095.

Kuyvenhoven M, de Melker R, van der Velden K. Prescription of antibiotics and prescribers characteristics. A study into prescription of antibiotics in upper respiratory tract infections in general practice. Fam Pract 1993;10:366-70.

Kvaerner KJ, Tambs K, Harris JR, Magnus P. Distribution and heritability of recurrent ear infections. Ann Otol Rhinol Laryngol 1997;106:624-32.

Kvestad E, Kvaerner KJ, Røysamb E, Tambs K, Harris JR, Magnus P. Otitis media: genetic factors and sex differences. Twin Research 2004;7:239-44.

Lahikainen EA. Clinico-bacteriologic studies on acute otitis media: aspiration of tympanum as diagnostic and therapeutic method. Acta Otolaryngol 1953;107 (Suppl):1-82.

Laine MK, Tähtinen PA, Ruuskanen O, Huovinen P, Ruohola A. Symptoms or symptom-based scores cannot predict acute otitis media at otitis-prone age. Pediatrics 2010;125:e1154-61.

Lanphear BP, Byrd RS, Auinger P, Hall CB. Increasing prevalence of recurrent otitis media among children in the United States. Pediatrics 1997;99:e1.

Larson HE, Blades R. Impairment of human polymorphonuclear leukocyte function by influenza virus. Lancet 1976;1:283

Laufer AS, Metlay JP, Gent JF, Fennie KP, Kong Y, Pettigrew MM. Microbial communities of the upper respiratory tract and otitis media in children. Mbio 2011;2:e00245.

Le Saux N, Gaboury I, Baird M, et al. A randomized, double-blind, placebo-controlled noninferiority trial of amoxicillin for clinically diagnosed acute otitis media in children 6 months to 5 years of age. Can Med Assoc J 2005;172:335-41.

Leach AJ. Otitis media in Australian Aboriginal children: an overview. Int J Pediatr Otorhinolaryngol 1999;49 Suppl 1:S173-8.

Leibovitz E, Raiz S, Piglansky L, et al. Resistance pattern of middle ear fluid isolates in acute otitis media recently treated with antibiotics. Pediatr Infect Dis J 1998;17:463-9.

Leichtle A, Hernandez M, Pak K, et al. TLR4-mediated induction of TLR2 signaling is critical in the pathogenesis and resolution of otitis media. Innate Immun 2009;15:205-15.

Leichtle A, Lai Y, Wollenberg B, Wasserman SI, Ryan AF. Innate signaling in otitis media: pathogenesis and recovery. Curr Allergy Asthma Rep 2011;11:78-84.

Leistevuo J, Huikko S, Rautakorpi U, et al. Prescription rates and diagnostic patterns are stable: A comparison of high-, medium- and low-prescribing primary care physicians treating community-acquired respiratory tract infections. Scand J Infect Dis 2005;37:465-70.

Leskinen K, Hendolin P, Virolainen-Julkunen A, Ylikoski J, Jero J. *Alloiococcus otitidis* in acute otitis media. Int J Pediatr Otorhinolaryngol 2004;68:51-6.

Lieberthal AS, Ganiats TG, Cox EO, et al. American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. Pediatrics 2004;113:1451-65.

Lindberg K, Freidj A, Rynnel-Dagöö B, Hammarström L. Anti pneumococcal antibody activity in nasopharyngeal secretions in healthy adults and children. Acta Otolaryngol 1993;113:673-8.

Lindén H, Teppo H, Revonta M. Spectral gradient acoustic reflectometry in the diagnosis of middle-ear fluid in children. Eur Arch Otorhinolaryngol 2007;264:477-81.

Little P, Gould C, Williamson I, Moore M, Warner G, Dunleavy J. Pragmatic randomised controlled trial of two prescribing strategies for childhood acute otitis media. BMJ 2001;322:336-42.

Little P. Delayed prescribing - a sensible approach to the management of acute otitis media. JAMA 2006;296:1290-1.

Lorentzen P, Haugsten P. Treatment of acute suppurative otitis-media. J Laryngol Otol 1977;91:331-40.

Macfarlane J, Holmes W, Macfarlane R, Britten N. Influence of patients' expectations on antibiotic management of acute lower respiratory tract illness in general practice: questionnaire study. BMJ 1997;315:1211-4.

Marchisio P, Bianchini S, Galeone C, et al. Use of complementary and alternative medicine in children with recurrent acute otitis media in Italy. Int J Immunopathol Pharmacol 2011;24:441-9.

Marchisio P, Bellussi L, Di Mauro G, et al. Acute otitis media: From diagnosis to prevention. Summary of the Italian guideline. Int J Pediatr Otorhinolaryngol 2010;74:1209-16.

Matsuda K, Tsutsumi H, Okamoto Y, Chiba C. Development of interleukin 6 and tumor necrosis factor alpha activity in nasopharyngeal secretions of infants and children during infection with respiratory syncytial virus. Clin Diagn Lab Immunol 1995;2:322-4.

McCormick DP, Lim-Melia E, Saeed K, Baldwin CD, Chonmaitree T. Otitis media: can clinical findings predict bacterial or viral etiology? Pediatr Infect Dis J 2000;19:256-8.

McCormick DP, Chonmaitree T, Pittman C, et al. Nonsevere acute otitis media: a clinical trial comparing outcomes of watchful waiting versus immediate antibiotic treatment. Pediatrics 2005;115:1455-65.

McEllistrem MC, Adams JM, Patel K, et al. Acute otitis media due to penicillin-nonsusceptible *Streptococcus pneumoniae* before and after the introduction of the pneumococcal conjugate vaccine. Clin Infect Dis 2005;40:1738-44.

McKinnon PS, Davis SL. Pharmacokinetic and pharmacodynamic issues in the treatment of bacterial infectious diseases. Eur J Clin Microbiol 2004;23:271-88.

Meropol SB, Glick HA, Asch DA. Age inconsistency in the American Academy of Pediatrics guidelines for acute otitis media. Pediatrics 2008;121:657-68.

Meurman O. Antibioottiresistenssitilanne Varsinais-Suomessa 2011. Antimicrobial resistance rates in Western Finland year 2011. 2012. Available at:

http://www.tykslab.fi/fi/6543. [Accessed 14 May 2012]

Morris PS, Leach AJ, Halpin S, et al. An overview of acute otitis media in Australian Aboriginal children living in remote communities. Vaccine 2007;25:2389-93.

Murphy TF, Bernstein JM, Dryja DM, Campagnari AA, Apicella MA. Outer membrane protein and lipooligosaccharide analysis of paired nasopharyngeal and middle ear isolates in otitis media due to nontypable *Haemophilus influenzae* - pathogenetic and epidemiologic observations. J Infect Dis 1987;156:723-31.

Murphy TF, Bakaletz LO, Smeesters PR. Microbial interactions in the respiratory tract. Pediatr Infect Dis J 2009a;28:S121-6.

Murphy TF, Faden H, Bakaletz LO, et al. Nontypeable *Haemophilus influenzae* as a pathogen in children. Pediatr Infect Dis J 2009b;28:43-8.

Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. Clin Infect Dis 2009;49:124-31.

Mygind N, Meistrup-Larsen KI, Thomsen J, Thomsen VF, Josefsson K, Sørensen H. Penicillin in acute otitis media: a double-blind placebo-controlled trial. Clin Otolaryngol 1981;6:5-13.

Mygind N, Pedersen M, Nielsen MH. Primary and secondary ciliary dyskinesia. Acta Otolaryngol 1983;95:688-94.

Neumark T, Mölstad S, Rosén C, et al. Evaluation of phenoxymethylpenicillin treatment of acute otitis media in children aged 2-16. Scand J Prim Health Care 2007;25:166-71.

Niemelä M, Uhari M, Jounio-Ervasti K, Luotonen J, Alho OP, Vierimaa E. Lack of specific symptomatology in children with acute otitis-media. Pediatr Infect Dis J 1994;13:765-8.

Niemelä M, Uhari M, Möttönen M. A pacifier increases the risk of recurrent acute otitis media in children in day care centers. Pediatrics 1995;96:884-8.

Niemelä M, Uhari M, Möttönen M, Pokka T. Costs arising from otitis media. Acta Paediatrica 1999;88:553-6.

Niittynen L, Pitkäranta A, Korpela R. Probiotics and otitis media in children. Int J Pediatr Otorhinolaryngol 2012;76:465-70.

Nokso-Koivisto J, Räty R, Blomqvist S, et al. Presence of specific viruses in the middle ear fluids and respiratory secretions of young children with acute otitis media. J Med Virol 2004;72:241-8.

Nozza RJ, Bluestone CD, Kardatzke D, Bachman R. Towards the validation of aural acoustic immittance measures for diagnosis of middle-ear effusion in children. Ear Hear 1992;13:442-53.

O'Brien MA, Prosser LA, Paradise JL, et al. New vaccines against otitis media: projected benefits and cost-effectiveness. Pediatrics 2009;123:1452-63.

Ogra PL. Mucosal immune response in the ear, nose and throat. Pediatr Infect Dis J 2000;19:S4-8.

Ohashi Y, Nakai Y, Ikeoka H, et al. Mucociliary disease of the middle ear during experimental otitis media with effusion induced by bacterial endotoxin. Ann Otol Rhinol Laryngol 1989;98:479-84.

Orchik DJ, Dunn JW, McNutt L. Tympanometry as a predictor of middle ear effusion. Arch Otolaryngol 1978;104:4-6.

Palmu A, Puhakka H, Rahko T, Takala AK. Diagnostic value of tympanometry in infants in clinical practice. Int J Pediatr Otorhinolaryngol 1999;49:207-13.

Paradise JL, Bluestone CD. Early treatment of universal otitis media of infants with cleft palate. Pediatrics 1974;53:48-54.

Paradise JL. Short-course antimicrobial treatment for acute otitis media: not best for infants and young children. JAMA 1997;278:1640-2.

Paradise JL, Rockette HE, Colborn DK, et al. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. Pediatrics 1997;99:318-33.

Park K, Coticchia J, Bakaletz LO, Lim DJ. Effect of influenza A virus on ciliary activity and dye transport function in the chinchilla eustachian tube. Ann Otol Rhinol Laryngol 1993;102:551-8.

Pelton SI. Otoscopy for the diagnosis of otitis media. Pediatr Infect Dis J 1998;17:540-3.

Pettigrew MM, Gent JF, Revai K, Patel JA, Chonmaitree T. Microbial interactions during upper respiratory tract infections. Emerg Infect Dis 2008;14:1584-91.

Pichichero ME, Casey JR. Diagnostic inaccuracy and subject exclusions render placebo and observational studies of acute otitis media inconclusive. Pediatr Infect Dis J 2008a;27:958-62.

Pichichero ME, Casey JR. Comparison of study designs for acute otitis media trials. Int J Pediatr Otorhinolaryngol 2008b;72:737-50.

Piglansky L, Leibovitz E, Raiz S, et al. Bacteriologic and clinical efficacy of high dose amoxicillin for therapy of acute media in children. Pediatr Infect Dis J 2003;22:405-12.

Pitkäranta A, Virolainen A, Jero J, Arruda E, Hayden FG. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. Pediatrics 1998;102:291-5.

Plasschaert AI, Rovers MM, Schilder AG, Verheij TJ, Hak E. Trends in doctor consultations, antibiotic prescription, and specialist referrals for otitis media in children: 1995-2003. Pediatrics 2006;117:1879-86.

Prellner K, Andersson K, Augustsson I, et al. Treatment for acute inflammation of the middle ear. Consensus statement. 2000. Available at: www.mfr.se. [Accessed 14 May 2012]

Proctor B. Embryology and anatomy of eustachian tube. Arch Otolaryngol 1967;86:503

Prymula R, Peeters P, Chrobok V, et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*: a randomised double-blind efficacy study. Lancet 2006;367:740-8.

Puhakka H, Hagman E, Heikkinen T, et al. Evidence-based guidelines for treatment of acute otitis media in children. Duodecim 1999;115:2155-61.

Pulkki J, Huikko S, Rautakorpi UM, et al. Management of pain in acute otitis media in Finnish primary care. Scand J Infect Dis 2006;38:265-7.

Putto A, Ruuskanen O, Meurman O. Fever in respiratory virus infections. Am J Dis Child 1986;140:1159-63.

Rautava S, Salminen S, Isolauri E. Specific probiotics in reducing the risk of acute infections in infancy - a randomised, double-blind, placebo-controlled study. Br J Nutr 2009;101:1722-6.

Revai K, McCormick DP, Patel J, Grady JJ, Saeed K, Chonmaitree T. Effect of pneumococcal conjugate vaccine on nasopharyngeal bacterial colonization during acute otitis media. Pediatrics 2006;117:1823-9.

Revai K, Patel JA, Grady JJ, Nair S, Matalon R, Chonmaitree T. Association between cytokine gene polymorphisms and risk for upper respiratory tract infection and acute otitis media. Clin Infect Dis 2009;49:257-61.

Roos K, Håkansson EG, Holm S. Effect of recolonisation with ”interfering” alpha streptococci on recurrences of acute and secretory otitis media in children: randomised placebo controlled trial. BMJ 2001;322:210-2.

Rosenfeld RM, Vertrees JE, Carr J, et al. Clinical efficacy of antimicrobial drugs for acute otitis-media - metaanalysis of 5400 children from 33 randomized trials. J Pediatr 1994;124:355-67.

Rovers MM, Zielhuis GA, Ingels K, van der Wilt GJ. Day-care and otitis media in young children: a critical overview. Eur J Pediatr 1999;158:1-6.

Rovers M, Haggard M, Gannon M, Koeppen-Schomerus G, Plomin R. Heritability of symptom domains in otitis media: A longitudinal study of 1,373 twin pairs. Am J Epidemiol 2002;155:958-64.

Rovers MM, Glasziou P, Appelman CL, et al. Antibiotics for acute otitis media: A meta-analysis with individual patient data. Lancet 2006;368:1429-35.

Rubin JL, McGarry LJ, Strutton DR, et al. Public health and economic impact of the 13-valent pneumococcal conjugate vaccine (PCV13) in the United States. Vaccine 2010;28:7634-43.

Rudberg RD. Acute otitis media: comparative therapeutic results of sulphonamide and penicillin administered in various forms. Acta Otolaryngol 1954;113 (Suppl):1-79.

Ruohola A, Meurman O, Nikkari S, et al. Microbiology of acute otitis media in children with tympanostomy tubes: prevalences of bacteria and viruses. Clin Infect Dis 2006;43:1417-22.

Ruuskanen O, Arola M, Putto-Laurila A, et al. Acute otitis media and respiratory virus infections. Pediatr Infect Dis J 1989;8:94-9.

Rye MS, Blackwell JM, Jamieson SE. Genetic susceptibility to otitis media. Laryngoscope 2012;122:665-75.

Sadlerkimes D, Siegel MI, Todhunter JS. Age-related morphologic differences in the components of the eustachian tube/middle ear system. Ann Otol Rhinol Laryngol 1989;98:854-8.

Saeed K, Coglianese CL, McCormick DP, Chonmaitree T. Otoscopic and tympanometric findings in acute otitis media yielding dry tap at tympanocentesis. Pediatr Infect Dis J 2004;23:1030-4.

Sanders SL, Glasziou PP, Del Mar CB, Rovers MM. Antibiotics for acute otitis media in children 2010. Cochrane Database Syst Rev 2010;1:CD000219.

Sarkkinen H, Ruuskanen O, Meurman O, Puhakka H, Virolainen E, Eskola J. Identification of respiratory virus antigens in middle ear fluids of children with acute otitis media. J Infect Dis 1985;151:444-8.

Sarrell EM, Mandelberg A, Cohen HA. Efficacy of naturopathic extracts in the management of ear pain associated with acute otitis media. Arch Pediatr Adolesc Med 2001;155:796-9.

Sarrell EM, Cohen HA, Kahan E. Naturopathic treatment for ear pain in children. Pediatrics 2003;111:e574-9.

Sassen ML, van Aarem A, Grote JJ. Validity of tympanometry in the diagnosis of middle ear effusion. Clin Otolaryngol Allied Sci 1994;19:185-9.

Schwartz RH, Stool SE, Rodriguez WJ, Grundfast KM. Acute otitis media: toward a more precise definition. Clin Pediatr 1981;20:549-54.

Seikel K, Shelton S, McCracken GH. Middle ear fluid concentrations of amoxicillin after large dosages in children with acute otitis media. Pediatr Infect Dis J 1997;16:710-1.

Shaikh N, Kearney DH, Colborn DK, et al. How do parents of preverbal children with acute otitis media determine how much ear pain their child is having? J Pain 2010;11:1291-4.

Shaikh N, Hoberman A, Kaleida PH, et al. Otoscopic signs of otitis media. Pediatr Infect Dis J 2011;30:822-6.

Shea KM, Weycker D, Stevenson AE, Strutton DR, Pelton SI. Modeling the decline in pneumococcal acute otitis media following the introduction of pneumococcal conjugate vaccines in the US. Vaccine 2011;29:8042-8.

Shekelle PG, Takata GS, Newberry SJ, et al., editors. Management of acute otitis media: Update. Rockville, MD: Agency for Healthcare Research and Quality 2010. Available at: http://www.ncbi.nlm.nih.gov/books/NBK56132/. [Accessed 14 May 2012]

Siegel RM, Kiely M, Bien JP, et al. Treatment of otitis media with observation and a safety-net antibiotic prescription. Pediatrics 2003;112:527-31.

Sinha MN, Siddiqui VA, Nayak C, et al. Randomized controlled pilot study to compare homeopathy and conventional therapy in acute otitis media. Homeopathy 2012;101:5-12.

Sipilä M, Pukander J, Karma P. Incidence of acute otitis-media up to the age of 11/2 years in urban infants. Acta Otolaryngol 1987;104:138-45.

Sipilä M, Karma P, Pukander J, Timonen M, Kataja M. The Bayesian approach to the evaluation of risk factors in acute and recurrent acute otitis media. Acta Otolaryngol 1988;106:94-101.

Sloyer JL, Howie VM, Ploussard JH, Schiffman G, Johnston RB. Immune response to acute otitis media: association between middle ear fluid antibody and clearing of clinical infection. J Clin Microbiol 1976;4:306-8.

Smith CG, Paradise JL, Sabo DL, et al. Tympanometric findings and the probability of middle-ear effusion in 3686 infants and young children. Pediatrics 2006;118:1-13.

Soni A. Ear Infections (otitis media) in children (0-17): use and expenditures, 2006. Statistical Brief No. 228. Agency for Healthcare Research and Quality 2008. Available at: http://www.meps.ahrq.gov/mepsweb/data_files/publications/st228/stat228.pdf. [Accessed 14 May 2012]

Spiro DM, Tay KY, Arnold DH, Dziura JD, Baker MD, Shapiro ED. Wait-and-see prescription for the treatment of acute otitis media: a randomized controlled trial. JAMA 2006;296:1235-41.

Stenfors LE, Albiin N, Bloom GD, Hellström S, Widemar L. Mast cells and middle ear effusion. Am J Otolaryngol 1985;6:217-9.

Suzuki K, Bakaletz LO. Synergistic effect of adenovirus type 1 and nontypable *Haemophilus influenzae* in a chinchilla model of experimental otitis media. Infect Immun 1994;62:1710-8.

Suzuki A, Watanabe O, Okamoto M, et al. Detection of human metapneumovirus from children with acute otitis media. Pediatr Infect Dis J 2005;24:655-7.

Syrjänen RK, Kilpi TM, Kaijalainen TH, Herva EE, Takala AK. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Finnish children younger than 2 years old. J Infect Dis 2001;184:451-9.

Taipale T, Pienihäkkinen K, Isolauri E, et al. *Bifidobacterium animalis* subsp lactis BB-12 in reducing the risk of infections in infancy. Br J Nutr 2011;105:409-16.

Talbird SE, Taylor TN, Knoll S, Frostad CR, Garcia Marti S. Outcomes and costs associated with PHiD-CV, a new protein D conjugate pneumococcal vaccine, in four countries. Vaccine 2010;28:G23-9.

Tano K, Olofsson C, Grahn-Håkansson E, Holm SE. In vitro inhibition of *S. pneumoniae*, nontypable *H. influenzae* and *M. catharralis* by alpha-hemolytic streptococci from healthy children. Int J Pediatr Otorhinolaryngol 1999;47:49-56.

Tano K, Grahn-Håkansson E, Holm SE, Hellström S. Inhibition of OM pathogens by alpha-hemolytic streptococci from healthy children, children with SOM and children with rAOM. Int J Pediatr Otorhinolaryngol 2000;56:185-90.

Taylor JA, Jacobs J. Homeopathic ear drops as an adjunct to standard therapy in children with acute otitis media. Homeopathy 2011;100:109-15.

Taylor S, Marchisio P, Vergison A, Harriague J, Hausdorff WP, Haggard M. Impact of pneumococcal conjugate vaccination on otitis media: A systematic review. Clin Infect Dis 2012;54:1765-73.

Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first 7 years of life in children in greater Boston: a prospective, cohort study. J Infect Dis 1989;160:83-94.

Teppo H, Revonta M. Comparison of old, professional and consumer model acoustic reflectometers in the detection of middle-ear fluid in children with recurrent acute otitis media or glue ear. Int J Pediatr Otorhinolaryngol 2007;71:1865-72.

Thalin A, Densert O, Larsson A, Lyden E, Ripa T. Is penicillin necessary in the treatment of acute otitis media? Proceedings of the International Conference on Acute and Secretory Otitis Media, Part 1, Jerusalem, Israel. November 1985;441-6.

Thompson PL, Gilbert RE, Long PF, Saxena S, Sharland M, Wong ICK. Effect of antibiotics for otitis media on mastoiditis in children: a retrospective cohort study using the United Kingdom general practice research database. Pediatrics 2009;123:424-30.

Uhari M, Hietala J, Tuokko H. Risk of acute otitis media in relation to the viral etiology of infections in children. Clin Infect Dis 1995a;20:521-4.

Uhari M, Niemelä M, Hietala J. Prediction of acute otitis media with symptoms and signs. Acta Paediatr 1995b;84:90-2.

Uhari M, Mäntysaari K, Niemelä M. A meta-analytic review of the risk factors for acute otitis media. Clin Infect Dis 1996;22:1079-83.

Underwood M, Bakaletz L. Innate immunity and the role of defensins in otitis media. Curr Allergy Asthma Rep 2011;11:499-507.

van Buchem FL, Dunk JH, van't Hof MA. Therapy of acute otitis media: myringotomy, antibiotics, or neither? A double-blind study in children. Lancet 1981;2:883-7.

van Buchem FL, Peeters MF, van't Hof MA. Acute otitis media: a new treatment strategy. Br Med J 1985;290:1033-7.

Van Zuijlen DA, Schilder AG, Van Balen FA, Hoes AW. National differences in incidence of acute mastoiditis: relationship to prescribing patterns of antibiotics for acute otitis media? Pediatr Infect Dis J 2001;20:140-4.

Varsano IB, Volovitz BM, Grossman JE. Effect of naproxen, a prostaglandin inhibitor, on acute otitis media and persistence of middle ear effusion in children. Ann Otol Rhinol Laryngol 1989;98:389-92.

Vergison A, Dagan R, Arguedas A, et al. Otitis media and its consequences: beyond the earache. Lancet Infect Dis 2010;10:195-203.

Vesa S, Kleemola M, Blomqvist S, Takala A, Kilpi T, Hovi T. Epidemiology of documented viral respiratory infections and acute otitis media in a cohort of children followed from two to twenty-four months of age. Pediatr Infect Dis J 2001;20:574-81.

Vesikari T, Wysocki J, Chevallier B, et al. Immunogenicity of the 10-valent pneumococcal non-typeable haemophilus influenzae protein D conjugate vaccine (PHiD-CV) compared to the licensed 7vCRM vaccine. Pediatr Infect Dis J 2009;28:S66-76.

Vouloumanou EK, Karageorgopoulos DE, Kazantzi MS, Kapaskelis AM, Falagas ME. Antibiotics versus placebo or watchful waiting for acute otitis media: a meta-analysis of randomized controlled trials. J Antimicrob Chemother 2009;64:16-24.

Wadowsky RM, Mietzner SM, Skoner DP, Doyle WJ, Fireman P. Effect of experimental influenza A virus infection on isolation of *Streptococcus pneumoniae* and other aerobic bacteria from the oropharynges of allergic and nonallergic adult subjects. Infect Immun 1995;63:1153-7.

Whitney CG, Farley MM, Hadler J, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med 2003;348:1737-46.

Williamson I, Benge S, Mullee M, Little P. Consultations for middle ear disease, antibiotic prescribing and risk factors for reattendance: a case-linked cohort study. Br J Gen Pract 2006;56:170-5.

Winther B, Hayden FG, Arruda E, Dutkowski R, Ward P, Hendley JO. Viral respiratory infection in schoolchildren: effects on middle ear pressure. Pediatrics 2002;109:826-32.

Winther B, Gwaltney JM Jr, Phillips CD, Hendley JO. Radiopaque contrast dye in nasopharynx reaches the middle ear during swallowing and/or yawning. Acta Otolaryngol 2005;125:625-8.

Yamanaka N, Faden H. Local antibody response to P6 of nontypable *Haemophilus influenzae* in otitis-prone and normal children. Acta Otolaryngol 1993;113:524-9.

Yoshie C. On the isolation of influenza virus from mid-ear discharge of influenza otitis media. Jpn J Med Sci Biol 1955;8:373-7.

Zielhuis GA, Rach GH, van den Broek P. Screening for otitis media with effusion in preschool children. Lancet 1989;1:311-4.