Acute Otitis Media. Aspects of diagnosis and prophylaxis

Foglé-Hansson, Margaretha

2006

Department of Otorhinolaryngology
Clinical Sciences, Lund
Lund University, Sweden

ACUTE OTITIS MEDIA

Aspects of diagnosis and prophylaxis

Margaretha Foglé-Hansson

LUNDS
UNIVERSITET
Medicinska fakulteten
The Faculty of Medicine, Lund University

Lund 2006
Skövde and Lund University 2006

Printed in Sweden by Media-Tryck, Lund
ISSN 1652-8220
ISBN 91-85559-54-7

To Roland, Annika and Marika

ABSTRACT

Acute otitis media. Aspects of diagnosis and prophylaxis.

Acute otitis media (AOM) is one of the most common childhood diseases, and the most common cause of antibiotic treatment in small children. Most children will experience one or two episodes of AOM during preschool age, but some will suffer from repeated attacks and become otitis-prone. Complications with severe, sometimes fatal, infections such as acute mastoiditis and meningitis are now rare, but do still exist.

The major pathogens found in AOM are *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*, and *Streptococcus pyogenes.* During recent decades, all these common upper airway bacteria have developed various degrees of resistance to antibiotics. Several studies have related this problem to a large consumption of antibiotics, especially in small children. It would be of great benefit to reduce the antibiotic consumption in these small children without increasing the incidence of serious complications.

In the first two studies, intermittent antibiotic prophylaxis at upper respiratory tract infections (URTI) was tested as a method of reducing the number of AOM episodes in young, otitis-prone children. Long time prophylaxis with antibiotics has been shown to be effective, but greatly increases antibiotic consumption. The prophylactic model studied here was treatment with a short-term penicillin V course of 5 days at episodes of URTI. This seemed effective in the slightly older patients in the first study, but ineffective in the younger, truly otitis-prone children in the second study. The children in this study were treated with large quantities of pcV during the study period of one year, but there was no change in the upper airway bacteria regarding either susceptibility to β-lactams in the *S. pneumoniae* or β-lactamase production in the *H. influenzae*.

Most complications in AOM are caused by Gram-positive bacteria; most AOM caused by Gram-negative bacteria will heal without treatment. If we could differentiate the potentially dangerous bacteria from the more harmless ones at the time of diagnosis, we might be able to chose treatment more judiciously and thus lower the number of antibiotic treatments without raising the number of complications. An experimental study showed that we were able to predict whether the type of bacteria responsible for the infection was Gram-positive (*S. pneumoniae* and *S. pyogenes*) or Gram-negative (*H. influenzae* and *M. catarrhalis*). The same was possible in humans studied at the ENT department in Skövde during optimal conditions.

In summary; short-term prophylaxis with penicillin V at URTI in children did not reduce the number of episodes of AOM in the younger, truly otitis-prone children. The antibiotic susceptibility for penicillin in the *S. pneumoniae* during these repeated courses of pcV did not change; neither did the *H. influenzae* produce more β-lactamase.

It was possible to predict whether a Gram-positive or Gram-negative bacterium was the causative pathogen in an experimental and a human setting, further emphasising the need for better diagnosis in AOM.

CONTENTS

ABBREVIATIONS **1**

ORIGINAL PAPERS **2**

INTRODUCTION **3**

1.1 Definition of OM *3*
1.2 Diagnosis of AOM *4*
2. EPIDEMIOLOGY 6
3. COMPLICATIONS 6
4.MICROBIOLOGY 7
4.1.Viruses *7*
4.2. Bacteria *7*
4.3 Antibiotic resistance to β-lactams *9*
5. TREATMENT OF AOM 10
5.1 Antibiotics *10*
5.2 Surgical treatment of AOM *13*
6. PREVENTION 13

AIMS **15**

MATERIAL AND METHODS **16**

1.1 Study populations *16*
1.2 Study design *17*
1.3 Bacterial culturing and susceptibility testing *19*
1.4 Prophylactic antibiotic drugs *20*
2.1 Animals and surgical procedure *20*
2.2 Otomicroscopy *20*
2.3 Photo documentation *20*
2.4 Inocula *20*
2.5 ELISA *21*
2.6 Statistical methods *21*

RESULTS **22**

Paper I. Can a short-term prophylactic course of PcV during URTI reduce the incidence of AOM in rAOM children? *22*
2. Paper II. Can the same prophylactic treatment policy in younger rAOM infants reduce the number of AOM episodes? *22*
3. Paper III. Did multiple courses of PcV have an effect on the bacteria in the nasopharyngeal space of the infants included in study II? *23*
4 Paper IV. Was it possible to determine the bacterial agent of an AOM in an experimental setting with the use of an otomicroscope? *24*
5 Paper V. Was it possible to predict the different types of AOM-inducing pathogens in the middle cavity in a human material? *25*

DISCUSSION **26**

Is PcV treatment during URTI an effective strategy against rAOM? *26*

Do frequent PcV courses affect the bacterial flora of the nasopharynx in small children? *27*

Can a better diagnostic procedure optimize the treatment of antibiotics in an episode of AOM? *27*

CONCLUSIONS **29**

SVENSK SAMMANFATTNING **31**

ACKNOWLEDGEMENTS **33**

REFERENCES **35**

ABBREVIATIONS

AOM	acute otitis media
rAOM	recurrent acute otitis media
ELISA	enzyme-linked immunosorbent assay
ENT	ear, nose, and throat
Hib	*Haemophilus influenzae* type b
H. influenzae	*Haemophilus influenzae*
IL	interleukin
M. catarrhalis	*Moraxella catarrhalis*
MIC	minimum inhibitory concentration
NTHi	nontypeable *Haemophilus influenzae*
PBP	penicillin-binding protein
PCR	polymerase chain reaction
PcV	penicillin V
RSV	respiratory syncytial virus
SOM	secretory otitis media
S. pneumoniae	*Streptococcus pneumoniae*
S. pyogenes	*Streptococcus pyogenes*
TM	tympanic membrane
URTI	upper respiratory tract infection

ORIGINAL PAPERS

This thesis is based on the following publications and manuscripts, which will be referred to in the text by their respective Roman numerals:

I. Prellner, K, Foglé-Hansson, M. Jörgensen F, Kalm O, Kamme C. Prevention of Recurrent Acute Otitis Media in Otitis-prone Children by Intermittent Prophylaxis with Penicillin. Acta Otolaryngol (Stockh)1994;114:182-187.

II. Foglé-Hansson M, White P, Hermansson A, Prellner K. Short-term penicillin-V prophylaxis did not prevent acute otitis media in infants. Int J Pediatr Otorhinolaryngol 59 (2001) 119-123.

III. Foglé-Hansson M, White P, Hermansson A. Pathogens in acute otitis media – impact of intermittent penicillin V prophylaxis on infant nasopharyngeal flora. Int J Pediatr Otorhinolaryngol (2003) 67; 511-516.

IV. Foglé-Hansson M, White P, Hermansson A, Melhus Å. Otomicroscopic findings and systemic interleukin-6 levels in relation to etiologic agent during experimental acute otitis media. APMIS 114; 2006: 285-291.

V. Foglé-Hansson M, White P, Hermansson A. Prediction of upper respiratory tract bacteria in acute otitis media in children. Submitted.

INTRODUCTION

Acute otitis media (AOM) is primarily a childhood disease, although it can also occur among adults. Episodes in adults are often severe, and it is recommended that adult patients should always be treated with antibiotics (Sugita). In children, however, AOM is often a self-limiting disease which heals without any treatment in the majority of cases. Almost all children experience at least one episode of AOM before school age, but on average will only suffer one or two episodes in total. However, approximately 5% of children will experience recurrent episodes of AOM (rAOM), often defined as at least 3 episodes of AOM over a 6-month period or 4 episodes over a year (Ingvarsson). These "otitis-prone" children constitute a problematic group, both for their families and for the health care system (Alho).
The treatment of AOM has been under discussion for several reasons. AOM is not only the most common reason for antibacterial treatment in children, but it is also difficult to diagnose and there is a need for a better diagnostic procedure.

1.1 Definition of OM

The definition of AOM is still under debate. Pathophysiologically, AOM is an inflammation in the mucous membrane of the middle ear with the development of a purulent effusion which becomes trapped in the middle ear. Cytokines are known to play an important part in this process; the earliest to emerge during experimental AOM is IL-6 (Melhus 2000), which seems to be totally independent of the infecting agent.
There are different clinical definitions of AOM in different parts of the world, and definitions may also have changed over time. In Evidence Based OM (1999), Bluestone states that "AOM is the rapid onset of signs and symptoms, such as otalgia and fever, and of acute infection in the middle ear" (Bluestone). A Swedish textbook states that "AOM is a disease with a rather rapid course. The symptoms seen are earache and hearing impairment. When examining the patient you find a red, thickened, often bulging tympanic membrane (TM), which sometimes ruptures and causes otorrhea" (Anniko). The presence of purulent middle ear effusion together with signs of acute infection is also the definition given by Karma (Karma). The purulent middle ear effusion makes the tympanic membrane bulge and become more or less immobile. Acute discharge from the ear by spontaneous perforation or through a tympanostomy tube is also defined as an AOM (Karma 1987, Rosenfelt & Bluestone). In

several countries the definition is wider, for example in Holland, where the definition of AOM is an infection of the middle ear with an acute onset and a duration of less than three weeks, characterized by an abnormal eardrum, and sometimes accompanied by earache, fever, perforation of the eardrum, or general illness (guidelines of the Dutch College of General Practitioners). Under this definition, a purulent effusion is not necessary for the diagnosis of AOM.

The natural course of an untreated episode of AOM is not fully known. Comparisons with the pre-antibiotic era are not reliable since both the bacterial spectrum and living conditions have changed. The *Streptococcus pyogenes* (*S. pyogenes*) were the predominant bacteria in the middle ear effusion during the pre-antibiotic era. Modern studies of "watchful waiting", which use a selected material, do not give an adequate picture of what would happen if all AOM were left without treatment. Furthermore, in all such studies that have been conducted, interventions were of course made as soon as the patients showed signs of complications. It has, however, been shown that a majority of cases will heal without intervention, although most studies have found the period of pain to be longer in the untreated cases (Del Mar, van Buchem).

Secretory otitis media (SOM), a non-purulent effusion trapped in the middle ear space, is often seen after an episode of AOM, but might also appear without a known preceding episode of AOM. In all probability, the etiology is multifactorial. SOM in conjunction with upper respiratory tract infections (URTI) of viral origin might be misinterpreted as an episode of AOM.

1.2 Diagnosis of AOM

In most parts of the world, patients with AOM are mainly seen by General Practitioners (GPs) and paediatricians, and only to a small extent by Ear, Nose, and Throat (ENT) specialists, who are usually consulted only in cases of complications and surgical treatment.

The gold standard of a diagnosis of AOM is a full view of the TM and growth of bacteria from the middle ear effusion (Rosenfelt, Karma 1987, Pichichero). In current practice, tympanocentesis is seldom performed, since it is considered an unethical procedure in a child who displays neither severe symptoms nor a threat of complications. There is no easy way of performing a painless tympanocentesis on a child without general anaesthesia; however, it can be performed in adults under local anaesthesia.

Blomgren has reported on the skills of diagnosing AOM in general practitioners, paediatricians, and otorhinolaryngologists using pneumatic otoscopy and tympanometry (Blomgren 2003), and showed that pure otoscopy is uncertain in diagnosing an AOM. A tympanometry curve may add further information, but there are still differences in diagnostic skills between GPs, paediatricians, and ENT surgeons (Blomgren 2003).

AOM is hardest to diagnose in very young children, since they have relatively narrow ear-canals and are rarely cooperative. Signs and symptoms such as URTI, fever, and malaise are insufficient for a diagnosis of AOM (Rothman). Rothman reported in 2003 that a TM which is cloudy, bulging, and distinctively immobile may strongly suggest an AOM (Rothman). Earache is another symptom highly indicative of an AOM (Palmu), but has the disadvantage that a young child may not be able to verbally convey its presence. Many countries now have written guidelines on AOM; and in some of these guidelines, for example those of the Dutch (The Dutch Collegue of General Practitioners), the criteria for a diagnosis of AOM are very wide.

In everyday practice, AOM is usually clinically verified by otoscopy alone. Several authors have pointed out the necessity of using a pneumatic otoscope; the mobility of the tympanic membrane provides essential information about the status of the middle ear (Bluestone, Rothman, Rosenfelt 1994, Blomgren). In addition to the pneumatic otoscope, tympanometry might also be used. While tympanometry can reveal the presence of effusion in the middle ear, it cannot distinguish the type of effusion as purulent or non-purulent. Otomicroscopy is a diagnostic tool mainly used by ENT specialists; however, more frequent use in primary care would be desirable. Its great advantages are the magnification of the TM and the ability to use both hands during investigation, which can enable a diagnostician to clear obscuring material from the ear canal in order to see the TM properly.

AOM is probably very often confused with an SOM in conjunction with a URTI (Garbutt), particularly if the pneumatic otoscope is not used to test the mobility of the TM. A red TM with a normal mobility may also be caused by the child shouting and be a normal status of the TM.

2. Epidemiology

AOM is a common disease, with 50% of all children experiencing their first AOM before the age of one year (Ruuskanen 1994, Alho 1991, Sipilä). The incidence peaks at around 12 months of age and then declines (Pukander, Teele 1989, Alho). The incidence also varies throughout the year, with a lower number of infections during the summer months. The incidence of AOM is higher today compared to 30 years ago (Joki-Erkkilä 2000), but most episodes have milder symptoms and complications are fewer. Here again, the criteria for AOM might affect the figures. The milder symptoms might partly be explained by a shift in the causes of AOM from *S. pyogenes* and pneumococci towards NTHi.

Several risk factors for AOM have been reported during the last two decades. Verified negative factors include male gender, absence of breast feeding, and young age (Teele 1989, Duncan, Alho), as well as early day care attendance (Teele 1991) and parental smoking (Stenström). A genetic predisposition to AOM has also been verified (Casselbrant 1999, Kvearner).

3. Complications

The complications to AOM are often divided into two categories; extracranial and intracranial. The extracranial complications are labyrintitis, mastoiditis, and facial palsy. Labyrintitis is an infection spread from the middle ear cavity to the inner ear, with severe dizziness and perhaps hearing loss as a result. Mastoiditis is the spread from the middle ear to the mastoid cavity with an infection into the mastoid bone. Facial palsy depends on the facial nerve being affected by the purulent effusion under pressure in the middle ear. All these extracranial complications might, if left untreated, give rise to intracranial, life threatening complications such as meningitis, sinus thrombosis, and intracranial abscesses.

All suspected complications are treated with high-dose intravenous antibiotics and surgical intervention.

During the pre-antibiotic era, severe complications of AOM were relatively frequent (Lahikainen, Rudberg), and surgical interventions a common procedure. Such complications are still a reality, but are no longer as frequent. Surgical treatment is still an option when complications threaten, but only after antibiotic treatment. In the 1950s, the *S. pyogenes* were the major cause of AOM (Lahikainen 1953, Lundgren 1949). Lahikainen showed that patients

with AOM caused by *S. pyogenes* sought medical advice earlier than those with AOM caused by *H. influenzae*. Untreated patients had AOM symptoms for 12 days, while patients with *H. influenza* AOM had symptoms for only 6 days (Lahikainen). The *S. pyogenes* infections had a mean discharge time of 12.4 days without any penicillin and 3.8 days with treatment (Lahikainen).

Meningitis and mastoiditis still occur as complications to AOM today (<4/100000) (van Zuijlen). The most common pathogen in such cases is *S. pneumoniae* with a normal susceptibility to penicillin (Luntz, Spratley, Leskinen). Although *S. pyogenes* are currently only responsible for 5% of all AOM in children, they are still the second most common bacteria found in mastoiditis (Block). Recently, reports have been published regarding increasing rates of mastoiditis (Leskinen, Gehanno, van Zuijlen) as a possible consequence of restricted antibiotic use during AOM.

4.Microbiology

4.1.Viruses

There are several groups of viruses considered as pathogens in AOM, including respiratory syncytial virus (RSV), influenza A and B, and adenovirus (Heikkinen 2000). Viral infections are often seen before bacterial AOM, and PCR studies have shown a high frequency of viral infections in conjunction with AOM (Pitkaranta). Giebink has shown that there is a synergistic effect between virus and bacteria in experimental AOM; 67% of chinchillas inoculated with *S pneumoniae* and influenza A developed AOM, compared to only 4% of the animals inoculated only with virus and 21% of the animals inoculated only with bacteria (Giebink).

4.2. Bacteria

The major causes of AOM are upper respiratory tract (URT) bacteria such as *Streptococcus pneumoniae (S. pneumoniae), Streptococcus pyogenes (S. pyogenes), Haemophilus influenzae (H. influenzae),* nontypeable *Haemophilus influenzae* (NTHi), and *Moraxella catarrhalis (M. catarrhalis*) (Jacobs). Gram-positive *S. pyogenes* and *S. pneumoniae* are more virulent bacteria than the Gram-negative NTHi and *M. catarrhalis*. It has been shown that around 30%

of healthy children below the age of 3 years harbour bacteria in the nasopharyngeal space. The number of positive nasopharyngeal cultures increases during AOM to more than 60% (Faden 1990).

Nasopharyngeal cultures in AOM are not always positive for otitis pathogens; 25-30% show no growth or only skin pathogens such as *Staphylococcus epidermidis* (Del Beccaro). In our bacterial study, 14% of cultures were sterile (Foglé-Hansson 2003). A high percentage of "sterile" cultures may, however, be the result of the time spent in transit to the laboratory, or the presence of other bacteria not cultured for.

Biofilm formation, where the bacteria accumulate but are protected against antibiotic treatment and will not yield a positive result when cultured, have been shown as a mechanism in *H. influenzae* (Post 04). This phenomenon might explain some of the negative cultures in treatment-resistant cases.

4.2.1 S. pneumoniae

The major bacterial pathogen during episodes of AOM, with a reported frequency of around 50% of the total amount of bacteria, is *S. pneumoniae,* an encapsulated Gram-positive diplococcus with approximately 90 serotypes. In Scandinavia, the most common types found in AOM are serotypes 3, 6, 9, 14, 18, 19, and 23 (Orange & Grey). *S. pneumoniae* is not only the pathogen most frequently associated with complications in AOM, but also that most frequently associated with the most severe and life-threatening complications, such as mastoiditis and meningitis (Spratley, Luntz, Leskinen). Fifty-eight percent of complications in AOM are caused by S. pneumoniae (Niv).
The recovery rate from pneumococcal AOM without therapy is approximately 20% (Klein, Howie, Rosenfeld).

4.2.2. S. pyogenes (group A streptococcus)

This bacterium is a highly virulent Gram-positive coccus that is often a cause of tonsillitis, AOM, and other infections, and is also feared as an invasive bacterium. Niv reports that 23% of AOM complications such as acute mastoiditis are caused by *S. pyogenes* (Niv 04), although only 5% of all AOM episodes are caused by this pathogen (Block, Segal). The recovery rate of untreated AOM caused by *S. pyogenes* is lower than in the *S. pneumoniae* (Rudberg).

4.2.3. H. influenzae

The *H. influenzae* can be divided into two categories; either encapsulated (types a – f) or nontypeable. Nontypeable *Haemophilus influenzae* (NTHi) is a small Gram-negative cocco-bacillus. Of the 40% of all AOM episodes induced by an *H. influenzae*, 95% are caused by NTHi. Infections with NTHi are often seen in young children with rAOM (Kilpi), but are also found in older children. The bacteria are often found in nasopharyngeal cultures from healthy children (Faden). Among the capsulated *H. influenzae*, type b is the most virulent; however, it is uncommon today, as most children are vaccinated against Hib in order to avoid invasive diseases such as meningitis, pneumonia, and epiglottitis. Acute mastoiditis in AOM is caused by NTHi in 12% of cases (Niv). The recovery rate from untreated AOM caused by *H. influenzae* is around 50% (Klein 1993, Howie). There has been speculation over a heightened risk for development of SOM after NTHi AOM as well as a greater risk for rAOM (Kilpi). In experimental studies, extensive changes in the middle ear mucosa with goblet cell depletion have been seen in conjunction with NTHi AOM (Westman 2002).

4.2.4. M. catarrhalis

This bacterium, previously known as *Branhamella catarrhalis*, was recognized as an otitis media pathogen in the 1980s (Kovatch). This Gram-negative bacterium lacks a capsule and has a low virulence. *M. catarrhalis* has been shown experimentally to cause fewer inflammatory reactions in the infected middle ear than the *H. influenzae* (Westman 1999). Today, it is present in approximately 25% of AOM episodes. It is also common in the nasopharyngeal space of healthy children (Faden 1991, Verduin). It has been suggested that the presence of *M. catarrhalis* may hinder the emergence of reduced susceptibility to penicillins in *S. pneumoniae* in children with rAOM (Joki-Erkkäla 2002). There is a spontaneous recovery from *M. catarrhalis* AOM in 80% of cases without any antibiotic treatment. Complications in infections caused by this bacterium are limited.

4.3 Antibiotic resistance to β-lactams

β-lactamases are enzymes produced by several bacteria. They hydrolyze the β-lactam ring in the penicillins, thus making the bacteria resistant to penicillin (Manninen). Another type of resistance pattern is the chromosomally-induced alteration of the penicillin-binding proteins (PBP) in the bacterial wall which makes the bacteria resistant to β-lactam antibiotics

(Clairoux). In Sweden today, 12% of all *H. influenzae* and almost all *M. catarrhalis* are β-lactamase producing (STRAMA 2005).

In the 1960s, the first reports appeared of a decreased susceptibility to penicillin in the *S. pneumoniae* (Hansman). The pneumococci had earlier been fully susceptible to β-lactam antibiotics, which interact with the PBP in the bacterial cell wall.

By the early 1990s, these problems had manifested in several countries. Reports from Iceland were showing an alarming rise in the isolation frequency of pneumococci with a decreased susceptibility to penicillin among the *S. pneumoniae* (Arason). The same tendency was noted in Sweden, where the isolation frequency peaked at 10% in 1996. Several measures have been undertaken to address this problem both in Iceland and in Sweden, and today the level is 5% in southern Sweden and around 3% in the rest of Sweden (STRAMA 2005). However, the isolation frequencies are dependent on the number of isolations studied.
Many countries in Europe and many states in the USA report high levels of decreased susceptibility to penicillin (around 50%), but the northern part of Europe shows declining figures (STRAMA 2005).

5. Treatment of AOM

5.1 Antibiotics

The first antibiotics that were used to treat AOM were members of the sulpha group. While they are still in use to some extent, they have fallen out of favour due to severe adverse drug reactions and their tendency to promote resistance problems. However, adverse reactions are very seldom seen in children, and so in some cases these substances are still used in children.

In Sweden, trimethoprim-sulphamethoxazole is recommended as an alternative when there is a therapy failure in patients with penicillin allergy treated with macrolides.

In 1949, Lundgren reported on penicillin treatment of AOM (Lundgren). Reports on antibiotic use in AOM also came from the United States where several reports were published during the following years (Rutherford, Fry, Medical Research Council). Most countries have a tradition of antibiotic treatment of AOM, but in some countries, such as Holland, the use of antibiotics is and has been quite strict (van Buchem).

The penicillins (penicillin V for oral administration and penicillin G for intravenous administration) rapidly became a favourable and effective treatment, easy to administer and access (Lundgren, Rudberg). Oral administration was possible by the beginning of the 1950s.

Penicillin V and G both belong to the β-lactam group. They are bactericidal, have a low cost, and are non-toxic. They have a narrow spectrum, being effective against Gram-positive upper respiratory tract bacteria. PcV is rather poorly absorbed from the gastrointestinal tract and has a very poor effect on Gram-negative bacteria. Depending on context, this narrow spectrum can be either an advantage or a disadvantage. The low cost of the penicillins is their other main advantage; however, they also have the disadvantage that their oral forms are unpleasant-tasting. In 1971, a Swedish study showed that PcV in a dosage of 50 mg/day/kg bodyweight administered twice a day was fully capable of eradicating 90% of the pathogens causing AOM (Kamme 1971) at that time. During the 1960s and 1970s, penicillin was administered three times a day, but findings in the 1980s (Rundcrantz) revealed that twice-daily administration resulted in better patient compliance while still being as effective as the earlier regimen of three times a day. Today, however, after 20 years of this administration policy, there are again discussions of whether thrice-daily administration would be a better choice, since its longer time over MIC could help combat the decreased susceptibility in the upper respiratory pathogens.

PcV is still the first-line antibiotic for bacterial URTI in Sweden (Consensus 2000). In 2004, Ander reported that out of 400 prescriptions for AOM, 70% were for PcV (Ander).

Development of semisynthetic penicillins such as ampicillin and amoxicillin was achieved by the end of the 1950s, and these drugs replaced penicillin G and V in most countries, becoming the first-line antibiotics in the USA for treating AOM. They are better absorbed from the gastrointestinal tract than the penicillins, and also have a broader spectrum. They are active against both *S. pneumoniae* and *H. influenzae.* They give higher antibiotic concentration in the middle ear effusion than PcV, and in high doses may even eradicate *S. pneumoniae* with a decreased susceptibility to β-lactam antibiotics (Lister, Canafax). The oral mixture tastes much better than PcV, and is thus easier to get children to accept. A further step was the binding of clavulanic acid to amoxicillin to neutralize the β-lactams produced by the *H. influenzae* and *M. catarrhalis*.

The macrolides were introduced during the 1970s, and became very popular in the treatment of AOM, sometimes together with the sulfisoxazoles. Although macrolides have a limited effect against the *H. influenzae*, they are recommended in cases of penicillin allergy in Sweden and in many other countries. The macrolides have a good effect on *M. catarrhalis*-induced infections; however, concentrations of macrolides are very low in the middle ear effusion.

The cephalosporins are semisynthetic broad-spectrum antibiotics which were introduced during the 1970s and rapidly became popular. Intravenously administered cephalosporins are the drug of choice to address complications of AOM because they are effective against all the common AOM pathogens. Peroral cephalosporins have a poor pharmacokinetic profile and cannot be compared to intravenous cephalosporins.

During recent decades, problems have risen with antibiotic resistance in several bacteria (Arason, Bronswear, Cars). Discussions have been initiated globally concerning the wide use of antibiotics (Rosenfelt, Gonzales).

The advantage of antibiotic treatment is the elimination of the bacteria which caused the infection. However, studies of AOM treatment have shown that 17 patients need to be treated in order to mitigate earache in just one of them (Del Mar).

The spontaneous healing of episodes of AOM is less common in infants than in older children, because of their inability to produce antibodies (Damoiseaux).

The disadvantage of antibiotic treatment is the resistance problem, which now exists all over the world (Arason, Bronswear, Cars).

In Holland, primary AOM treatment recommendations include analgesia and perhaps nose drops, and in patients under 2 years a second visit after 24 hours. If the AOM deteriorates, antibiotics in the form of ampicillin are recommended. Amoxicillin is also the recommended treatment in Finland, while in Norway the recommendation is for no antibiotics during the first two days, and PcV if the condition persists (Puhakka, Norwegian, Dutch guidelines). In Sweden, PcV treatment is recommended in children younger than 2 years and in adults; children aged between 2 and 16 years can be left without antibiotics in uncomplicated AOM if

their condition justifies it and they are otherwise healthy, that is, with a normal temperature and no pain. In the USA, amoxicillin is recommended, but again the patient might be left without antibiotics in benign cases (Lieberthal).

5.2 Surgical treatment of AOM

At the threat of complications, antibiotics are used together with a tympanocentesis (Karma 1987). This procedure clears the purulent discharge from the middle ear, thus reducing further damage to the inner ear. It also enables bacterial culture of the aspirate, which is the only way to conclusively prove the existence of a middle ear infection and select the right antibiotic treatment. A tympanostomy often only stays open for a day or two, and sometimes there is a need for repetitive tympanostomies. There are reports of placing tympanostomy tubes through the TM during acute infection; the tubes then function as drainage to keep the middle ear clean from infectious material (Spratley). This is not, however, the general treatment in Sweden today.

If acute mastoiditis deteriorates, surgery, performed as a mastoidectomy, is mandatory (Nadol).

6. Prevention

RAOM in a child places a psychological, economic, and medical burden on both the whole family and society in general. Several ways of attacking the problem have been tried during the last 50 years.

Virus isolation from the middle ear effusion in AOM is verified in approximately 20-40% of AOM cases (Heikkinen 2003, Canafax). Recent PCR studies have shown that the viral engagement together with bacteria during AOM is much higher than virological culturing (Pitkaranta, Heikkinen 2003); vaccination against viruses may therefore be able to prevent AOM (Heikkinen 2003).

In the 1960s, adenoidectomy was often used as a prevention against AOM. The indication for this procedure was altered in the 1980s to become more of a prevention against snoring and

impaired nasal breathing in young children. Today the pendulum has swung back the other way, and adenoidectomy is again being discussed as prevention against rAOM. In 2004, Koivunen reported on the lack of influence of adenoidectomy in children with rAOM below the age of 2 years (Koivunen). The nasopharyngeal space is again considered as a reservoir for bacteria, activated during respiratory illness. Most children below the age of 3 harbour bacteria even when in full health (Faden 1991), but when they become infected, the number of pathogens increases (Faden 1990).

Tympanostomy tubes, with or without adenoidectomy (Casselbrant 1992), have been used for decades in treatment of rAOM (Gonzalez, Gebhart, Bluestone). The effect of tubes has been reported to be an approximately 50% reduction of new episodes of AOM (Gonzales, Gebhart). Long-term side effects such as tympanosclerosis without any hearing disabilities have been reported in 59% of cases (Tos), and open perforations in 4% of cases after a 21-month follow-up (Casselbrant 1992). Most tube treatments are not for rAOM but for SOM.

Antimicrobial prophylaxis was started in the late 1950s by Ensign, who studied sulphamethoxypyridazine as a long-term prophylactic among a group of Indian children with draining ears (Ensign). Today we know that long-term treatment is effective against rAOM (Persico, Gaskins, Casselbrant 1992), but the impact on bacterial resistance, like the negative side effects of the medication, is a negative factor.

Regarding vaccination, today we have pneumococcal vaccines of 23 serotypes, non-conjugated and conjugated 7-valent vaccine Prevenar®. The 23-valent vaccine (Pneumovax®), containing plain capsular polysaccharides of *S. pneumoniae*, is not immunogenic in small children. A protein conjugate vaccine against *S. pneumoniae* has been introduced (Prevenar®) and is widely used today as a protection against invasive pneumococcal disease such as meningitis and pneumonia. Large studies have shown that it only reduced the total number of all AOM by 6% (Eskola, Black). There was, however, a reduction of 34% in the episodes caused by pneumococcus and 57% in the episodes caused by the strains induced in the vaccine (Eskola). New protein-conjugated pneumococcus vaccines are under development, and there are reports of a 11-valent vaccine, conjugated to a recombinant non-lipidated form of protein D, which has been tested and shows interesting

results (Prymula) as well as 9- and 11-valent pneumococcal vaccines and vaccines against RSV and para-influenzae virus (Straetemans, Heikkinen 2003). The possibility of reducing the number of URTI and thereby the number of AOM by vaccination against the most common viruses (influenza A) inducing URTI has been studied by several groups (Heikkinen, 1991, Hoberman 2003); Heikkinen has reported a 30% reduction of episodes of AOM (Heikkinen 1991).

Breast-feeding has been shown in several reports to be a good prophylactic agent against rAOM (Duncan). The effect is probably a transmission of immunoglobulins from the mother, and it persists as long as breast-feeding is continued.

Humoral intervention includes gammaglobulins in infusion and injection. These have been tried by Kalm, Jörgensen, and Shurin without any effect (Kalm, Jörgensen, Shurin). Giebink reported on a human bacterial polysaccharide immune globulin effective in preventing AOM in chinchillas (Giebink) but there have been no successful results reported in humans.

Chewing-gum with xylitol has been proven to decrease the number of episodes of AOM (Uhari), but its disadvantage is that it must be chewed 5-6 times a day, which is a problem for most patients.

AIMS

The aim of this thesis was to explore possibilities to limit the use of antibiotics in treatment and prevention of AOM without risking complications.

The specific aims were:

- to investigate whether PcV treatment during URTI is an effective strategy against rAOM;
- to examine the effect of frequent PcV courses on the bacterial flora of the nasopharynx in small children; and
- to investigate whether a clinical diagnose of causative agent in AOM might be possible.

MATERIAL AND METHODS

1.1 Study populations

Paper I

Paper I included 76 children (25 girls and 51 boys) below the age of 18 months, all of whom had experienced at least three episodes of AOM prior to inclusion (see Table 1).

Table 1. Patients' characteristics; study I.

Patients	PcV	Placebo
Number	35	41
Males	25 (71%)	26 (63%)
Median age at inclusion in months (range)	12.2 (5-18)	11.6 (5-18)
Median age at first AOM in months (range)	5.2 (1-11)	6.2 (1-12)
Mean no. Of AOM before inclusion (range)	5.4 (3-15)	4.8 (3-12)

Paper II

Paper II included 70 children (29 girls and 41 boys), all of whom had had their first episode of AOM before the age of 6 months (see Table 2).

Table 2. Patients' characteristics; study II.

Patients	PcV	Placebo
Number	30	40
Males	19 (63%)	20 (50%)
Median age at inclusion in months (range)	5.0 (2-8)	5.0 (2-8)
Median age at first AOM in months (range)	4.5 (1-6)	4.0 (1-6)
No. of AOM before inclusion	0	0

Paper III

In paper III, the nasopharyngeal cultures obtained in study II were investigated further. A total of 703 cultures were studied regarding decreased susceptibility to penicillin in the *S.*

pneumoniae, β-lactamase production in the *H. influenzae*, and the presence of *H. influenzae* and *M. catarrhalis* in the nasopharyngeal space.

Paper IV

Thirty-six male Sprague-Dawley rats, each weighing approximately 250-300 grams, were challenged with *S. pneumoniae*, NTHi*, M. catarrhalis*, and *S. pyogenes*. Twenty-four were examined daily using otomicroscopy and photography, and the remaining twelve were used as controls and for IL-6 detection.

Paper V

Paper V included 82 patients, both children and adults, who had presented at the ENT clinic in Skövde with an AOM in need of acute treatment due to severe symptoms such as a high temperature or severe pain, or the failure of previous antibiotic treatment. The sample comprised 44 males and 38 females, 72 of whom were below the age of 7, eight of whom were aged 7-16, and two of whom were adults. The median age of the whole group was 29 months (6-539).

1.2 Study design

Paper I

The children were placed at random in one of two groups: a prophylactic medication group of PcV in the otitis dose of 50 mg/kg bodyweight b.i.d., and a placebo group. When symptoms of URTI occurred (high temperature, running nose, cough), the child was given the study medication at home. The children were examined at the ENT clinic three days after initiation of the study medication, or earlier if signs of deterioration were noted. If an AOM was found at the examination, the parents were given a prescription of PcV, while if the ears showed a normal tympanic membrane or signs of SOM, the study medication was continued for a total of ten days. Every month the children were examined by an ENT specialist. At all visits, samples for bacterial cultures from the nasopharyngeal space were collected. The total observation time for each patient was 4.5 months.

Paper II

The children were randomized into two groups at the beginning of the study. When URTI developed, the children in the treatment group were given PcV, while children in the control group were given placebo. After three days, or earlier if signs of acute illness appeared, they were examined by one of the authors. If an AOM had developed then PcV was prescribed, while if the child did not show any signs of AOM then the 5-day course of study medication was continued.

Every other month a control visit was performed at the clinic.

Additionally, if patients developed acute symptoms, they could visit the clinic and be treated according to the findings.

At every visit, a nasopharyngeal sample was taken for bacterial culturing. The total observation time for each patient was 11 months.

Paper III

The children in the second paper made 860 visits to the clinics in Lund and Skövde and provided a total of 703 cultures.

Several aspects of these cultures were studied, the most important being decreased susceptibility to penicillin in the *S. pneumoniae* during repeated PcV treatment.

H. influenzae were investigated regarding β-lactamase production and chromosomal resistance, and the frequency of isolation of *H. influenzae* and *M. catarrhalis* from the nasopharynx was also studied.

The results of the cultures were divided by treatment group (PcV or placebo) and by the three different types of visits;

1. "prophylactic visits", where the child had been given the test medication and controlled;
2. "acute visits", with no previous treatment at home, generally because the parents were convinced that their child was suffering from a new AOM and wanted a prompt examination;
3. "control visits", in which the child came for examination in a normal state of health.

Paper IV

A total of 36 male Sprague-Dawley rats were inoculated with bacteria, either *S. pneumoniae, S. pyogenes*, NTHi, or *M. catarrhalis*. Six animals in each group were anesthetized, inspected, and documented by photography daily for five consecutive days. Their TMs were inspected

using otomicroscopy and a test protocol was made of the findings. A photo was also taken of every case. Three animals in each group were investigated solely regarding IL-6 levels in sera, which were taken daily through a tail vein. On the fourth day the TMs were inspected, and the animals were sacrificed and samples for culturing taken from their middle ears.

Paper V

The patients were treated according to clinical practice for an acute severe episode of AOM, with an acute tympanocentesis, bacterial culturing from the middle ear and/or the nasopharyngeal space, and later antibiotic treatment. The patients were examined by otomicroscopy and the TMs were photographed at the outpatient clinic. Rectal temperature was also measured.

The investigator made a preliminary prediction of the causative agent of the present AOM. A tympanocentesis was then performed under either local or general anaesthesia and a specimen was retrieved from the middle ear effusion for culturing. A sample was also taken from the nasopharyngeal space and the TM was photographed. The findings from the examination as well as the prediction of a specific bacterium were documented in the operating room papers, the referral paper to the microbiological laboratory, and a special examination document. A control visit was made three months later.

1.3 Bacterial culturing and susceptibility testing

Culturing was performed under standard conditions at the microbiological laboratory at the University Hospital in Lund and the Capio Diagnostik in Skövde. The nasopharyngeal and middle ear aspirates were placed in a transport medium and taken to the laboratory. The sample was placed in broth and shaken for 30 seconds. With a sterile bent glass rod, 0.1 ml of the broth was inoculated on blood agar and hematin agar plates overnight. The blood agar plate was incubated anaerobically in 37^{o} C and the hematin agar plate in 6% CO_2 overnight. The isolates were identified and tested for susceptibility to antibiotics according to the laboratory routine and the recommendation of SRGA. Penicillin resistance was tested in the *S. pneumoniae* by the disk diffusion test using 1-µg oxacillin disks. *S. pneumoniae* with an MIC value of ≥0.125 mg/l were classified as having a reduced susceptibility to penicillin and those with an MIC >2.0 mg/l as being resistant. Detection of chromosomal penicillin resistance in *H. influenzae* was performed with a disc diffusion test, with β-lactamase-stable cephalosporin

(loracabef) as an indicator. Nitrocefin was used to detect β-lactamase in both *H. influenzae* and *M. catarrhalis*.

1.4 Prophylactic antibiotic drugs

In the studies with prophylactic antibiotics in papers I and II, PcV (Kåvepenin®, Astra 50 mg/ml) was used in 50 mg/kg bodyweight/day administered twice daily.

2.1 Animals and surgical procedure

Inoculation was performed during general anaesthesia with either intravenous methohexital or intraperitoneal chloralhydrate. The bulla of the right ear of the rat was exposed through a submental, midline incision and blunt dissection through the tissue. The inoculum (0.05 ml of either *S. pyogenes, S. pneumoniae*, NTHi, or *M. catarrhalis*) was injected through the bulla wall with a fine needle. Proper deposition of the inoculum was verified using otomicroscopy. All experiments were approved by the Animal Ethics Committee at Lund University.

2.2 Otomicroscopy

During all studies, both experimental and human, inspection of the tympanic membranes was made by otomicroscopy. The findings were recorded both in experimental protocols and by photography in papers IV and V.

2.3 Photo documentation

Photo documentation was performed using a Ricoh camera and a special TTS-lens (through the lens) connected to an endoscope. The camera being analogue, slides were produced of the film. The endoscope was a Hopkins endoscope; 3 mm wide, 60 mm long, and 0^{o}. A special flash was used, a Storz flash generator 600 BA.

2.4 Inocula

The inocula used in the animal study (paper IV) were *S. pneumoniae* strain L1 (type 3*),* NTHi strain 3655 (nontypeable, biotype II), *M. catarrhalis* strain 483, and *S. pyogenes* strain 5955. The concentrations used were 10^{5} cfu/ml for the streptococcal strains, 10^{7} cfu/ml for the NTHi

(Melhus 1994), and 10^8 cfu/ml for the *M. catarrhalis* (Westman 1999), and were chosen after dose-finding experiments.

2.5 ELISA

Serum levels of IL-6 were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Endogen, Inc., Woburn, MA, USA, detection limit: 16 pg/ml). The assay was performed according to the manufacturer's instructions.

2.6 Statistical methods

Student's t-test was used to determine the significance of group differences in mean values for quantitative variables (e.g. age and number of AOM episodes). The Chi-squared test was used to evaluate group differences in the distribution of qualitative characteristics (e.g. the proportion of infections progressing to AOM episodes). The Wilcoxon rank-sum test was used to determine differences between groups. Cohen's kappa-test was used to validate the difference between the groups in the prediction situation. This method shows the agreement between the test and the outcome measured against the agreement that can be expected on the basis of chance.

RESULTS

Paper I. Can a short-term prophylactic course of PcV during URTI reduce the incidence of AOM in rAOM children?

There were 72 episodes of URTI in the penicillin V group, and 78 in the placebo group. Of these, 17 episodes in the penicillin group and 39 in the placebo group developed into AOM. This is a significant difference ($p< 0.001$). The children treated prophylactically with PcV showed a significantly lower isolation frequency of *S. pneumoniae* in their nasopharyngeal cultures, compared to those who were given placebo ($p<0.001$), while there was no difference in growth of *H. influenzae* and *M. catarrhalis*. Among the children seeking acute medical advice without initiating self-medication, there were 98 visits with 67 episodes of AOM.

Table 3. Results; paper I.

Patients	PcV n=35	Placebo n=41
Follow-up months/child (range)	4.8 (2-6)	4.5 (2-6)
Mean no. of URTI during prophylaxis (child)	72 (2.0)	78 (1.9)
Mean no. of AOM during prophylaxis (child)	17 (0.5)	39 (1.0)

Conclusion: PcV prophylaxis during URTI reduced the total number of registered AOM in these children.

A true episode of AOM was found in 68% of visits (67 out of 98) by children seeking medical advice due to the parents' being convinced that the child was suffering from an AOM.

2. Paper II. Can the same prophylactic treatment policy in younger rAOM infants reduce the number of AOM episodes?

Of the 90 children included, 70 completed the study; 30 in the penicillin V group and 40 in the placebo group. Twenty children left the study due to medical complications such as diarrhoea and asthma, and three did not like the medicine. There were no differences between the two groups regarding age at inclusion, age at first episode of AOM, study period, or number of infections (see Table 4).

The results in the two groups did not differ statistically. There were 254 visits without previous "home medication", since the parents were convinced that the child was suffering from severe AOM. These visits mainly occurred in Lund. In 61% (n=154) of these visits the children proved to have a true episode of AOM.

Table 4. Results; paper II.

Patients	PcV n=30	Placebo n=40
Follow-up months/child (range)	11.2 (6-13)	11.2 (6-13)
Mean no. of URTI during prophylaxis (child)	134 (4.4)	179 (4.4)
Mean no. of AOM during prophylaxis (child)	42 (1.4)	58 (1.5)

Conclusion: PcV prophylaxis during URTI in this study did not reduce the total number of registered AOM.

A true episode of AOM was found in 61% of children seeking medical advice due to the parents' being convinced that the child was suffering from an AOM.

3. Paper III. Did multiple courses of PcV have an effect on the bacteria in the nasopharyngeal space of the infants included in study II?

The 70 children followed in Lund and Skövde in study II yielded a total of 703 nasopharyngeal cultures. The cultures were classified into three groups according to when they were taken; during prophylactic treatment, during acute illness, or during control visits. The results show a significant decrease in the presence of *S. pneumoniae* in the nasopharyngeal space after treatment with PcV compared to placebo ($p<0.001$). There was no change over the study period in the frequency of pneumococci with a decreased susceptibility to penicillin. There was no increase in the incidence of either β-lactamase production or chromosomally altered PBP in the *H. influenzae*, even though these patients were given up to seven courses of PcV during the study, in contrast to the one course which would be given to the average child in Sweden of the same age over the same time.

In the PcV group, 72 of the 282 cultures (30%) were positive for *H. influenzae*; the corresponding figure in the placebo group was 123 out of 421 (29%). Regarding *M. catarrhalis*, 156 of 282 cultures (55%) were positive in the PcV group, and 233 of 421 (55%) in the placebo group; this was not a significant difference.

Table 5. Total number of *S. pneumoniae* positive cultures by treatment group and type of visit

Type of visit	PcV (n=30)	Placebo (n=40)	p	Total
"Prophylactic"	41	104	0.001	145
"Control"	48	78	n.s.	126
"Acute"	29	45	n.s.	74
Total	118	227		345

Conclusion: Multiple courses of PcV neither induced more strains of *S. pneumoniae* with a decreased susceptibility to penicillin nor increased the amount of β-lactamase producing *H. influenzae*. The number of *H. influenzae* and *M.catarrhalis* positive cultures were unchanged in the nasopharynx..

4 Paper IV. Was it possible to determine the bacterial agent of an AOM in an experimental setting with the use of an otomicroscope?

All the rats inoculated with bacteria developed AOM, defined as an opaque or purulent-looking effusion in the middle ear. The rats inoculated with the Gram-positive bacteria *S. pyogenes* and *S. pneumoniae* developed an AOM with a purulent-looking effusion in the middle ear, thick membranes, dilated vessels, and some perforations. The Gram-negative bacteria, NTHi and *M. catarrhalis*, induced an milder-looking AOM. The tympanic membranes in the Gram-negative group were thinner and sometimes retracted, the middle ear showed a more opaque effusion, and there were no perforations. The findings during the study were noted and documented by photography. After the study, randomly sorted slides with different bacteria were validated (AH, PW). The investigators were able to differentiate Gram-positive from Gram-negative bacteria in an experimental acute otitis media both with the otomicroscope and with the slides.

The values of interleukin-6 showed an answer for the *S. pneumoniae* on day 1-3, but not for the *S. pyogenes*, and no values for the Gram-negative bacteria on days 2 and 3, except *H. influenzae* on day 2, indicating that this cytokine is of limited value in differentiating various bacteria from each other in the rat model.

At the end of the study, cultures were taken from all the middle ears. All cultures from the *S. pneumoniae*, *S. pyogenes,* and NTHi groups were positive. The *M. catarrhalis* group only showed one positive culture out of six.

Conclusion: There seems to be a possibility of separating the Gram-positive from the Gram-negative bacteria in an experimental AOM purely by examination of the TM in an otomicroscope.

These results indicate further study to see if the findings are also valid in humans.

5 Paper V. Was it possible to predict the different types of AOM-inducing pathogens in the middle cavity in a human material?

Of the 82 patients included in this study, it was possible to validate the causative bacteria in 63. Among the excluded patients, nine showed no growth in their cultures from the nasopharyngeal and middle ear space, and the remaining ten had cultures showing both *S. pneumoniae* and *H. influenzae/M. catarrhalis*. Of the remaining 63 patients, accurate predictions were made for 47, with a κ-value of 0.47 (p=0.001). If we calculate the results and include the patients with both *S. pneumoniae* and *H. influenzae* in their nasopharyngeal space and predict that the result would be a pneumococcus-like picture on the TM the κ-value then rises to 0.56 (p=0.001).

More than 50% of the middle ear cultures showing *S. pneumoniae* came from patients with temperature over 38.5^{o}; this was also the case in 18% of the *H. influenzae* and *M. catarrhalis*-induced episodes of AOM (p=0.01).

Table 6. Prediction of causative agents and findings in cultures from middle ear effusion.

n=63	**Cultures**		n
Prediction	*S. pneumoniae*	*H. influenzae/M. catarrhalis*	
S. pneumoniae	18	6	24
H. influenzae/M. catarrhalis	10	29	39
Total	28	35	63

Conclusion: In this study it seemed possible to differentiate Gram-positive from Gram-negative bacteria in humans suffering from AOM by examining the TM with an otomicroscope and testing the mobility of the TM.

DISCUSSION

Is PcV treatment during URTI an effective strategy against rAOM?

In our first study (paper I) there was a statistical effect of intermittent prophylactic PcV treatment during URTI in preventing new episodes of AOM. No other studies of intermittent prophylaxis have been performed, but similar results were reported by Persico, Casselbrant, and Gaskins regarding long-term antibiotic prophylaxis. In the following study (paper II) we were not able to repeat these findings; no protective effect was noted. There were two major differences between the studies; the age of the children included and the inclusion criteria. In the first study, the mean age at inclusion was 12 months and the observation time was 4.5 months, while in the second study the children were much younger — the mean age at inclusion was only 5 months — and the observation time was 11 months. The children included in the first study were found to experience only a few episodes of AOM; 35 out of 76 (46%) only experienced at most one episode of AOM, which would not make them "otitis-prone". In contrast, in the second study, only 14% had an incidence of AOM this low. It is possible, therefore, that we did not manage to include "otitis-prone" children in study I but managed to do so in study II. The children in study I were also older and on the verge of leaving the worst period of recurrent AOM episodes.

It should also be noted that in the first study, the children were included on the grounds of a medical history obtained from a parent of at least three episodes of AOM before the age of 18 months, while in the second study, children were included only if they had experienced an episode of AOM as verified by an ENT surgeon before the age of 6 months.

Discrepancies have been found between diagnoses of AOM made by ENT surgeons and by general practitioners (Blomgren 2003). In a sample of 50 children, general practitioners diagnosed 20% more instances of AOM. Clinical signs alone are a poor determinant for AOM; this was apparent in our studies, where only 61% of children seeking acute advice because the parent were convinced that the child was suffering from a new episode of AOM were actually diagnosed with AOM when examined by an ENT surgeon. These findings are similar to the results of Ingvarsson, who reported an AOM frequency of 46% in children seeking advice because of otalgia (Ingvarsson) and 59% tympanocentesis positive AOM in Finnish children seeking acute advice due to ear pain (Karma 1989). An interesting observation was the greater number of acute visits to the ENT clinic in Lund than in Skövde during the second study (185/254, 73%). A possible explanation for this is that it is easy to

access the ENT clinic in Lund at all times, while in Skövde the clinic's opening hours more closely resemble office hours.

Of the 70 children in the second study, 24 had two or three episodes of AOM, 21 had more than four, and 15 had more than seven. With short-term prophylaxis, very otitis-prone children might get another infection as soon as the treatment period is over.

The results in study II are probably more representative of a group of rAOM infants than those in the first study.

Do frequent PcV courses affect the bacterial flora of the nasopharynx in small children?

In this study, repeated treatment with PcV neither affected susceptibility to penicillin in the *S. pneumoniae* nor induced more β-lactamase production in the *H. influenzae*. These results differ from those of several studies where promotion of a decreased susceptibility to penicillin in the *S. pneumoniae* has been observed when using broader β-lactam antibiotics (Cohen, Arason, Melander, Ghaffar, Dabernat). In Iceland, the promotion of pneumococci with a reduced susceptibility to penicillin seemed to depend on high antibiotic use, particularly of β-lactam antibiotics and trimethoprim-sulpha (Arason). In 1988, Eliasson reported increasing amounts of β-lactamase producing bacteria among children treated with PcV, ampicillin, and cefaclor, in contrast to our findings regarding PcV treatment. Molstad also reported in 1999 on a decreased use of broad-spectrum antibiotics and the reduction of pneumococci with a decreased susceptibility to PcV.

Can a better diagnostic procedure optimize the treatment of antibiotics in an episode of AOM?

Earlier studies in animals have shown that there are different otomicroscopical findings dependent on bacteriological species (Hermansson, Melhus 1994, Westman 1999, Cayé-Thomasen). We were able to differentiate the Gram-positive (*S. pneumoniae* and *S. pyogenes*) from the Gram-negative (NTHi and *M. catarrhalis*) episodes of AOM both in humans and in an experimental setting in rats.

Our results mirror the findings of Palmu, who reported on a way of selecting the *S. pneumoniae* in an AOM by correlating the more severe findings on the TM with a high temperature of the patient. He also chose to correlate the findings on the TM with eye symptoms in the *H. influenzae* infections (Palmu). These findings resemble ours, where 50% of the patients with *S. pneumoniae* AOM had a temperature higher than 38.5^{o}, while only 18% of those with *H. influenzae* and *M. catarrhalis* AOM had a temperature this high (p=0.01). Hotomi used a score system to show that *S. pneumoniae* infections had the highest symptom scores (Hotomi).

Several authors have stressed the need for a more effective and correct diagnosis of AOM when treatment should be more select. It is important to minimize the number of unnecessary antibiotic treatments (Palmu, Blomgren 2004, Del Mar, Dagan, Bluestone, Rosenfelt) without increasing the number of complications.

In conclusion, we found that we were able to differentiate the Gram-positive *S. pneumoniae* from the Gram-negative NTHi and *M. catarrhalis* both in animals and in humans by clinical examination alone (otomicroscopy and testing of the mobility of the TM). These results could help reduce antibiotic intake, since they offer the possibility of differentiating infections caused by the more hazardous Gram-positive bacteria from those caused by the more benign Gram-negative bacteria. In treating only the potentially harmful Gram-positive bacteria we might be able to achieve a more restricted use of antibiotics without risking further complications in the patients.

CONCLUSIONS

Prophylactic short-term PcV had no effect in infants between 2 and 20 months of age in preventing AOM during attacks of URTI.

There was a tendency toward a positive effect of short-term prophylactic PcV in older children (5-22.5 months).

Frequent PcV courses did not alter the nasopharyngeal flora with respect to either decreased susceptibility to penicillin in the *S. pneumoniae* or increased β-lactamase production in the *H. influenzae*. The PcV treatment did not change the frequency of carriage of *H. influenzae* and *M. catarrhalis* in the nasopharyngeal space.

In an experimental study in rats, we were able to differentiate Gram-positive from Gram-negative pathogens using otomicroscopy. IL-6 was of limited diagnostic value in separating different bacteriological agents during experimental AOM.

In humans, we were able to differentiate the *S. pneumoniae* from the NTHi and the *M. catarrhalis* during episodes of AOM by clinical investigation including otomicroscopy and testing of the mobility of the TM.

In conclusion, we have found that prophylactic antibiotic treatment in URTI in children does not prevent episodes of AOM, frequent PcV courses do not induce either decreased susceptibility to penicillin in the *S. pneumoniae* or increased β-laktamase production in the *H. influenzae* and we have also shown that it is possible to differentiate Gram-positive bacteria from Gram-negative bacteria during both experimental and human episodes of AOM by clinical examination of the TM.

SVENSK SAMMANFATTNING

Akut mellanöreinflammation (akut otitis media, AOM) är en av barnaålderns vanligaste sjukdomare och en av dem som oftast föranleder antibiotikabehandling. De flesta barn får bara någon enstaka AOM, men cirka 5% av dem som insjuknar drabbas sedan av upprepade infektioner (s.k. otit-benägna barn) och blir till ett problem både för patienten, dennes familj och samhället. Den frekventa antibiotikaanvändningen vid dessa tillstånd har också lett till en resistensutveckling mot antibiotika hos de bakterier som orsakar AOM. Syftet med denna avhandling var att undersöka om förebyggande antibiotikabehandling vid symptom på övre luftvägsinfektion (ÖLI) hindrade utvecklingen av AOM hos otitbenägna barn och hur detta påverkade de aktuella bakterierna ur resistenssynpunkt. Dessutom avsåg man, att undersöka om man enbart genom mikroskopisk inspektion av trumhinnan kunna fastställa vilken bakterie som orsakat den aktuella infektionen.

I studie I inkluderades barn under 18 månaders ålder, som haft minst 3 AOM. Vid tecken till ÖLI (snuva, temperaturstegring, hosta) gavs försöksmedicin i form av penicillin V (PcV) eller placebo. Inom 3 dygn undersöktes barnen med öronmikroskopi för att bekräfta eller utesluta AOM. Bakterieodling från nässvalget togs också vid undersökningstillfället. Patienterna följdes under 4.5 månader. I gruppen som fått PcV noterades en minskad frekvens AOM jämfört med placebogruppen. Odlingarna från de patienter som erhållit försöksmedicin i minst 2 dagar visade att *S. pneumoniae* (pneumococcer) förekom i en lägre frekvens hos dem som fått PcV jämfört med de som fått placebo.

Den andra studien omfattade barn som före 6 månaders ålder haft en AOM konstaterad av öronläkare. I övrigt var uppläggningen identisk med den i studie I frånsett att patienterna följdes under 12 månader. De förändrade urvalskriterierna för patienter motiverades av att frekvensen AOM hos de i studie I inkluderade barnen varit lägre än förväntad. Resultaten visade att båda grupperna fick lika hög frekvens AOM, någon förebyggande effekt av PcV kunde inte noteras. Däremot hade 48/70 inkluderade barn haft mer än 3 AOM-episoder under studietiden, vilket talar för att urvalskriterierna fångat upp otit-benägna barn.

Det tredje arbetet är en bakteriologisk studie baserad på prov tagna från patienterna i studie II. Sammanlagt omfattade materialet 703 odlingar. Patienterna, som deltog i studie II, hade under studieåret konsumerat 7 respektive 5 kurer antibiotika i PcV- respektive placebogruppen.

Trots detta kunde man inte hos någon av de isolerade stammarna av *S. pneumoniae* eller *H. influenzae* i något avseende konstatera en ökad resistens.

I den fjärde studien framkallades AOM experimentellt hos olika grupper av råttor med de vid otit vanligast förekommande bakterierna. Trumhinnans utseende under försöksperioden dokumenterades och hos en del av försöksdjuren bestämdes också halten av det vid inflammatoriska processer bildade cytokinet IL-6. Resultatet visade att man genom trumhinnans utseende kunde avgöra vilka djur som infekterats med komplikationsbenägna bakterier (*S. pneumoniae* och *S. pyogenes*) från de där de ofarligare bakterierna (*H. influenzae* och *M. catarrhalis*) var etiologiskt agens. Däremot kunde inte halten av cytokinet IL-6 i blod korreleras till vilken bakterie som orsakat den aktuella AOM:en.

I det avslutande femte arbetet undersöktes 82 patienter med AOM. Härvid gjordes öronmikroskopi samt bakterieodling från mellanörat och nässvalget. Utifrån trumhinnans utseende angav undersökaren troligt etiologiskt agens och detta jämfördes sedan med resultatet av bakterieodlingarna. Av 63 bedömbara patienter kunder man hos 47 ange om infektionen orsakats av komplikationsbenägna bakterier eller sådana med mindre benägenhet för detta.

Sammanfattningsvis har avhandlingen visat att förebyggande antibiotikabehandling vid ÖLI hos otitbenägna barn minskar frekvensen AOM hos äldre barn men ej hos yngre. Upprepade behandlingar med PcV orsakar inte någon typ av resistensutveckling hos de vid AOM aktuella bakterierna. Det finns dessutom en klar möjlighet att enbart utifrån trumhinnebilden, både vid djurexperimentell och human otit, avgöra om komplikationsbenägna bakterier (*S. pneumoniae/S. pyogenes*) eller ofarligare sådana (*H. influenzae/M. catarrhalis*) är etiologiskt agens. Det senare kan resultera i att man i den kliniska situationen ibland kan avvakta med antibiotikabehandling även vid AOM.

ACKNOWLEDGEMENTS

After 15 years of examining, treating, and studying many children, both healthy and ill, new-born and older, this work has come to an end. Many people have helped and supported me throughout these years, and I would like to make particular mention of a few:

Hans Rundcrantz, former head of the ENT department in Skövde, who introduced me to the clinical field of ear, nose, and throat diseases. He also made me take the first step into this work in 1990, when our collaboration with the ENT clinic at Lund's University Hospital began. He has always supported me and guided me through the research world among friends and colleagues around the world.

Ann Hermansson, my tutor and friend, who has always been enthusiastic and innovative, helping me through the ups and downs of my work while carrying on her own extensive research and clinical work. She has also most unselfishly opened her home for me during several visits to Lund.

Åsa Melhus, my co-tutor and friend, who with her vast knowledge of bacteriology has read the manuscripts and initiated fruitful discussions as well as providing me with all the bacterial strains needed for the work.

Peter White, my co-tutor, friend, and room-mate in Lund, who with his logical way of thinking and writing and his never ending sense for details helped me during the entire work. He has also been a superb discussion partner during long working hours as well as an excellent host when I had the privilege of being a guest in his home.

Mats Bende, present head of the ENT clinic in Skövde, with his never ending enthusiasm for research. He has never denied me a period of stay in Lund, always supported my work, and looked forward to new papers to read.

Karin Prellner, professor at the ENT clinic in Lund, who enthusiastically introduced me to this research field and always believed in my work.

All my doctor colleagues at the ENT department in Skövde, who have taken care of our daily work while I have had the privilege of taking care of my "own" patients or visiting Lund.

All colleagues at the ENT department in Lund, for their kindness and encouraging words while looking for Peter White's chocolate in our office.

All other medical staff at the ENT department in Skövde, the staff at the Central Operation Theatre, and Peter and Cathrin at the photo department who helped me with the documentation.

Karin Brundell-Frej, who has been a great help with the statistics.
Barbro Kahl-Knutsson and her colleagues, for handling the bacterial cultures during the studies.
Mikael Thörnlund at Storz, for his total devotion to our technical equipment. Several times it failed to work but he always managed in some fantastic way to help.
The former secretaries in Lund, Ulla Wide and Birgitta Nilsson, who always handled different secretarial emergencies with a smile.
Professors Rune Andersson and Hans Hedelin at the FoU department at the Central Hospital in Skövde, for believing in my work and providing financial support.
My husband Roland, and our daughters Annika and Marika. All of them never complaining about a wife and mother on the run to the hospital in the middle of the night to take photos or going away to Lund for a week. Without you, none of this would have been possible!

Financial support was provided by the Swedish Medical Research Council (K1999-73X-006857-16C), Malmö Health Services District Foundations, the Department of Research and Development at the Central Hospital of Skövde, Sweden, and Ronald McDonald Children's Foundation.

REFERENCES

Alho OP, Koivu M, Sorri M, Rantakallio P. The occurrence of acute otitis media in infants. A life-table analysis. Int J Pediatr Otorhinolaryngol. Feb 1991;21(1):7-14.

Alho OP, Koivu M, Sorri M. What is an "otitis-prone" child? Int J Pediatr Otorhinolaryngol, 1991; 21:201-209.

Ander Lundborg A, Eggertsen R. Akut otitis media behandlas inte enligt rekommendationer. Läkartidningen. 2004;101;41:3142-3146.

Anniko M. Swedish textbook in Ear,- Nose, and Throat-diseases, Head and Neck Surgery. Liber AB, Stockholm 2001

Arason VA, Kristinsson KG, Sigurdsson JA, Stefansdottir G, Molstad S, Gudmundsson S. Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. Bmj. Aug 17 1996;313(7054):387-391.

Black S, Shinefield H, Fireman B, Lewis E, Ray P et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. Pediatr Infect Dis J. 2000;Mar;19(3)1:187-195.

Block SL, Hendrick JA, Kratzer J, Nemeth MA, Tack KJ. Five-day twice daily cefdinir therapy for acute otitis media: microbiologic and clinical efficacy. Pediatr Infect Dis J. 2000;Dec;19(12 suppl):S153-158.

Blomgren K, Pitkaranta A. Is it possible to diagnose acute otitis media accurately in primary health care? Fam Pract. Oct 2003;20(5):524-527.

Blomgren K, Pohjavuori S, Poussa T, Hatakka K, Korpela R, Pitkaranta A. Effect of accurate diagnostic criteria on incidence of acute otitis media in otitis-prone children. Scand J Infect Dis. 2004;36(1):6-9.

Blomgren K, Pitkaranta A. Current challenges in diagnosis of acute otitis media Int J Pediatr Otorhinolaryngol 2005;69:295-299.

Bluestone CD. Evidence-based otitis media. 1999(B.C. Decker Inc.):86.

Bronzwaer SL, Cars O, Buchholz U, et al. A European study on the relationship between antimicrobial use and antimicrobial resistance. Emerg Infect Dis. Mar 2002;8(3):278-282.

Canafax DM YZ, et al. Amoxicillin middle ear fluid penetration and pharmacokinetics in children with acute otitis media. Pediatr Infect Dis J. 1998;Febr,17(2):149-156.

Cars O. Steering an appropriate course: principles to guide antibiotic choice, Respir. Med. 95 (Suppl A) 2001:S20-S25.

Casselbrant ML, Mandel EM, Fall PA, Rockette HE, Kurs-Lasky MMS, Bluestone CD, Ferrell RE. The heritability of otitis media. Jama. 1999;282, no 22(Dec 8):2125-2130.

Casselbrant ML, Kaleida PH, Rockette HE, et al. Efficacy of antimicrobial prophylaxis and of tympanostomy tube insertion for prevention of recurrent acute otitis media: results of a randomized clinical trial. Pediatr Infect Dis J. Apr 1992;11(4):278-286.

Cayé-Thomasen P, Hermansson A, Tos M, Prellner K. Changes in globlet cell density in rat middle ear mucosa in acute otitis media. Am J Otol. 1995;16:75-82.

Clairoux N, Picard M, Brochu A, Rousseau N, Gourde P, Beauchamp D, Parr TR Jr, Bergeron MG, Malouin F. Molecular basis of the non-beta-lactamase-mediated resistance to beta-lactam antibiotics in strains of *Haemophilus influenzae* isolated in Canada. Antimicrob Agents Chemother. 1992;36(7):1504-1513.

Cohen R Bingen E, Varon E, de la Rocque F, Brahimi N, Levy C, Boucherat M, Langue J, Geslin P. Change in nasopharyngeal carriage of Streptococcus pneumoniae resulting from antibiotic therapy for acute otitis media in children. Pediatr Infect Dis J. 1997;Jun;16(6):555-560.

Dabernat H Geslin P, Megraud FF, Bégué P, Boulesteix J, Dubreuil C, de la Roque F, Trinh A, Scheimberg A. Effects of cefixime or co-amoxiclav treatment on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae in* children with acute otitis media. J Antimicrob Chemother. 1998;41:253-258.

Dagan R, Klugman KP, William AC, Baquero F. Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy. J Antimicrob Chemother 2001, 47:129-140.

Damoiseaux RA, Sanders LA, van Balen FA, Rijkers G. Sibling history of recurrent acute otitis media correlates with low IgG2 anti-pneumococcal polysaccaride antiboddy levels. Pediatr Infect Dis J 2000 Feb;19(2):176-7.

Del Beccaro MA, Mendelman MP, et al. Bacteriology of acute otitis media: a new perspective. J Pediatr. 1992;120(1):81-84.

Del Mar C, Glasziou P, Hayem M. Are antibiotics indicated as initial treatment for children with acute otitis media? A meta-analysis BMJ 1997 May 24;314(7093):1526-9.

Duncan B, Ey J, Holberg CJ, Wright AL, Martinez FD, Taussig LM. Exclusive breast-feeding for at least 4 months protects against otitis media. Pediatrics. 1993;91(5):867-872.

The Dutch Collegue of general practitioners. Guidelines for acute otitis media. 1992.

Eliasson I, Holst E, Molstad S, Kamme C. Emergence and persistence of beta-laktamase-producing bacteria in the upper respiratory tract in children treated with beta-lactam antibiotics. Am J Med. 1990 May 14;88(5A):51S-55S.

Ensign PR, Urbanich EM, Moran M. Prophylaxis for otitis media in an Indian population. Am J Public Health. Feb 1960;50(2):195-199.

Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Engl J Med. Feb 8 2001;344(6):403-409.

Faden H, Waz MJ, Bernstein JM, Brodsky L, Stanievich J, Ogra PL. Nasopharyngeal flora in the first three years of life in normal and otitis-prone children. Ann Otol Rhinol Laryngol. Aug 1991;100(8):612-615.

Faden H, Stanievich J, Brodsky L, Bernstein J, Ogra PL. Changes in nasopharyngeal flora during otitis media of childhood. Pediatr Infect Dis J 1990;9:623-626.

Fogle-Hansson M, White P, Hermansson A, Prellner K. Pathogens in acute otitis media-impact of intermittent penicillin V prophylaxis on infant nasopharyngeal flora. Int J Pediatr Otorhinolaryngol. 2003;67:511-516.

Fry J F. Antibiotics in acute tonsillitis and acute otitis media. BMJ. 1958;Oct 11:883-886.

Fulghum RS, Brinn JE, Smith AM, Daniel III HJ, Loesche PJ. Experimental otitis media in gerbils and chinchillas with *Streptococcus pneumoniae, Haemophilus influenzae* and other aerobic and anaerobic bacteria. Infect Immun. 1982;36(2):802-810.

Garbutt J, Jeffe DB, Shackelford P. Diagnosis and treatment of acute otitis media: an assessment. Pediatrics. Jul 2003;112(1 Pt 1):143-149.

Gaskins JD, Holt RJ, Kyong CU, Weart CW, Ward J. Chemoprophylaxis of recurrent otitis media using trimethoprim/sulfamethoxazole. Drug Intell Clin Pharm 1982 May;16(5):387-90.

Ghaffar F Muniz LS, Katz K, Reynolds J, Smith JL, Davis P, Freidland IR, McCracken Jr H. Effects of Amoxicillin/clavunate or Azithromycin on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* in children with acute otitis media. Clin Infect Dis. 2000;31:875-880.

Gebhart DE. Tympanostomy tubes in the otitis prone child. Laryngoscope 1981 Jun; 91(6):849-66.

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, Nov 30(2):445-450.

Gonzales R, Malone DC, Maselli JH, Sande MA. Excessive antibiotic use for acute respiratory infections in the United States. Clin Infect Dis. Sep 15 2001;33(6):757-762.

Gonzalez C, Arnold JE, Woody EA, et al. Prevention of recurrent acute otitis media: chemoprophylaxis versus tympanostomy tubes. Laryngoscope. Dec 1986;96(12):1330-1334.

Hansman D, Bullen M. A resistant pneumococcus. Lancet ii:1997, July 29:264-65.

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 1992 Sep;146(9):1018-9.

Heikkinen T. The role of respiratory viruses in otitis media. Vaccine. Dec 8 2000;19 Suppl 1:S51-55.

Heikkinen T, Chonmaitree T. Importance of respiratory viruses in acute otitis media. Clin Microbiol Rev. Apr 2003;16(2):230-241.

Hermansson A, Emgård P, Prellner K, Hellström S. A rat model for pneumococcal otitis media. Am J Otolaryngol. 1988;9:97-101.

Hoberman A GD, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled study. JAMA. 2003;290:1608-1616.

Hotomi M, Yamanaka N, Shimada J, Ikeda Y, Faden H. Factors associated with clinical outcomes in acute otitis media. Ann Otol Rhinol Laryngol. 2004;113:846-852.

Howie VM, Pluossard JH. Efficacy of fixed combination antibiotics versus separate components in otitis media. Effectiveness of erythromycin estrolate, triple sulphonamide, ampicillin, erythromycin estrolate- triple sulphonamide, and placebo in 280 patients with acute otitis media under two and one-half years of age. Clin Pediatr (Phila). 1972;11(4):205-214.

Ingvarsson L, Lundgren K. Penicillin treatment of acute otitis media in children. A study of the duration of treatment. Acta Otolaryngol. Sep-Oct 1982;94(3-4):283-287.

Jacobs MR et al. Prevalence of antimicrobial-resistant pathogens in middle ear fluid: multinational study of 917 children with acute otitis media. Antimicrob Agents Chemother. 1998;42(3):589-595.

Joki-Erkkila VP, Aittoniemi J, Vuento R, Puhakka H. Beta-Lactamase-producing *Moraxella catarrhalis* may prevent the emergence of penicillin-resistant *Streptococcus pneumoniae* in children with recurrent acute otitis media. Int J Pediatr Otorhinolaryngol. 2002;63(3):219-222.

Joki-Erkkila 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(3):197-201.

Jorgensen F, Andersson B, Hanson LA, Nylen O, Svanborg Eden C. Gamma-globulin treatment of recurrent acute otitis media in children. Pediatr Infect Dis J. Jun 1990;9(6):389-394.

Kalm O, Prellner K, Christensen P. The effect of intravenous immunoglobulin treatment in recurrent acute otitis media. Int J Pediatr Otorhinolaryngol. Sep 1986;11(3):237-246.

Kamme C, Lundgren K. Frequency of typable and non-typeable Haemophilus influenzae strains in children with acute otitis media and results of penicillin V treatment. Scand J Infect Dis 1971; 3:225-228.

Karma PH, Palva T, Kouvalainen K, et al. Finnish approach to the treatment of acute otitis media. Report of the Finnish Consensus Conference. Ann Otol Rhinol Laryngol Suppl. Mar-Apr 1987;129:1-19.

Karma PH, Penttila MA. Sipila MM, Kataja MJ. Otoscopic diagnosis of middle ear effusion in acute and non-acute otitis media I. The value of different otoscopic findings. Int J Otorhinolaryngol 1989 Febr; 17(1):37-49.

Kilpi T, Herva E, Kaijalainen T, Syrjanen 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. Jul 2001;20(7):654-662.

Klein JO. Microbiologic efficacy of antibacterial drugs for acute otitis media. Pediatr Infect Dis J. 1993;12:973-975.

Koivunen P, Uhari M, Luotonen J, Kristo A, Raski R, Pokka T, Alho OP. Adenoidectomy versus chemoprophylaxis and placebo for recurrent otitis media in children aged under 2 years: randomised controlled trial. Bmj. 2004;febr 28;328(7438):487.

Kovatch AL, Wald E, Michaels RH. Beta-lactamase-producing *Branhamella catarrhalis* causing otitis media in children. J Pediatr. 1983;102(2):261-264.

Kvaerner KJ, Tambs K, Harris JR, Magnus P. Distribution and heritability of recurrent ear infections. Ann Otol Rhinol Laryngol. 1997;Aug;106(8):624-632.

Lahikainen. Clinico-bacteriologic studies on acute otitis media. Thesis. 1953.

Leskinen K, Jero J. Complications of acute otitis media in children in southern Finland. Int J Pediatr Otorhinolaryngol. Mar 2004;68(3):317-324.

Lieberthal AS GT. Diagnosis and Management of Acute Otitis Media. Pediatrics. 2004;113:1451-1465.

Lister Pong A, Chartrand SA, Sanders CC. Rationale behind high-dose amoxicillin therapy for acute otitis media due to penicillin-non-susceptible pneumoncocci: support from in vitro pharmacodynamic studies. Antimicrob Agents Chemother. 1997;41(9):1926-1932.

Lundgren N. Penicillinterapi vid akuta otiter. Nordisk medicin. 1949;42:1401-1409.

Luntz M, Brodsky A, Nusem S, et al. Acute mastoiditis- the antibiotic era: a multicenter study. Int J Pediatr Otorhinolaryngol. Jan 2001;57(1):1-9.

Manninen R, Huovinen P, Nissinen A. Increasing antimicrobial resistance in, *Streptococcus pneumoniae Haemophilus influenzae* and *Moraxella catarrhalis* in Finland. J Antimicrob Chemother. Sep 1997;40(3):387-392.

Medical Research Council´s working-party for research in general practice. Acute otitis media in general practice. Lancet, 1957,Sept 14:510-514.

Melander E, Ekdahl K, Jonsson G, Molstad S. Frequency of penicillin-resistant pneumococci in children is correlated to community utilization of antibiotics. Pediatr Infect Dis J. 2000;Dec;19(12):1172-1177.

Melhus A, Hermansson A, Forsgren A, Prellner K. A resolved pneumococcal infection protects against nontypeable Haemophilus influenzae: an evaluation of different routes of whole cell immunization in protection against experimental acute otitis media. Int J Pediatr Otorhinolaryngol. 1997;Mar 6;39(2):119-131.

Melhus A, Hermansson A, Prellner K. Nontypeable and encapsulated *Haemophilus influenzae* yield different clinical courses of experimental otitis media. Acta Otolaryngol. 1994;114:289-294.

Melhus A, Ryan AF. Expression of cytokine genes during pneumococcal and nontypeable *Haemophilus influenzae* acute otitis media in the rat. Infect Immun. 2000 Jul;68(7):4024-31.

Molstad S, Cars O. Major change in the use of antibiotics following a national programme: Swedish Strategic Programme for the Rational Use of Antimicrobial Agents and Surveillance of Resistance (STRAMA) Scand J Infect Dis 1999;31(2):191-5.

Nadol JB Schuhknecht HF. Surgery of the Ear and Temporal Bone. Raven Press N.Y. 1993. 1993:148-149.

Nasrin D, Collignon PJ, Roberts L, Wilson EJ, Pilotto LS, Douglas RM. Effect of beta lactam antibiotic use in children on pneumococcal resistance to penicillin: prospective cohort study. Bmj. 2002; Jan 5;324(7328):28-30.

Niv A, Nash M, Slovik Y, Fliss DM, Kaplan D, Leibovitz E et al. Acute mastoiditis in infancy: The Soroka experience: 1900-2000. Int J Pediatr Otorhinolaryngol. 2004;Nov;68(11):1435-1439.

Norwegian guidelines in treatment of acute otitis media.

Orange M GB. Pneumococcal serotypes causing disease in children in Alabama. Pediatr Infect Dis J. 1993;12(3):244-246.

Palmu AA JJ, Kaijalainen T, Leinonen M, Karma P, Kilpi TM. Association of clinical signs and symptoms with pneumococcal acute otitis media by serotype-implications for vaccine effect. Clin Infect Dis. 2005;Jan 1;40(1):52-57.

Persico M, Podoshin L, Fradis M, Gruschka D, Golan V et al. Recurrent acute otitis media – prophylactic penicillin treatment: a prospective study. Part I. Int J Pediatr Otorhinolaryngol. 1985;10:37-46.

Pichichero ME, Pichichero CL. Persistent acute otitis media: Persistent acute otitis media: I. Causative pathogens. Pediatr Infect Dis J. Mar 1995;14(3):178-183.

Pitkaranta A, Virolainen A, Jero J, Arruda E, Heyden FG. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. Pediatrics. 1998;Aug;102(2 Pt1):291-295.

Post JC, Stoodley P, Hall-Stoodley L, Ehrlich GD. The role of biofilms in otolaryngological infections. Curr Opin Otolaryngol Head Neck Surg. 2004;Jun;12(3):185-190.

Prellner K. Consensus for treatment of acute otitis media in Sweden. Landstingsförbundet, Medicinska Forskningsrådet och Socialstyrelsen. 2000.

Prymula R, Peeters P, Chrabok V, Kriz P, Novakova E, Kaliskova E, Kohl I et al. Pneumococcal capsular polysaccarides conjugate to protein D for prevention of acute otitis media caused by both *S. pneumoniae* and non-typable *H. influenzae*: a randomized double-blind efficacy study. Lancet 2006;367:740-48.

Puhakka P. Guidelines in acute otitis media in Finland. Duodecim 1999;115:17-23.

Pukander J, Karma P, Sipilä M. Occurence and recurrence of acute otitis media among children. Acta Otolaryngol (Stockh). 1982;94:479-486.

Rosenfelt RM, Vertrees JE, Carr J, Cipolle RJ, Uden DL, Giebink SG, Canafax DM. Clinical efficacy of antimicrobial drugs for acute otitis media: metaanalysis of 5400 children from thirty-three randomised trials. J Pediatr. 1994;124(3):355-367.

Rothman R, Owens T, Simel DL. Does this child have acute otitis media? Jama. 2003 Sep 24;290(12):1633-1640.

Rudberg R. Acute otitis media, comparative therapeutic results of sulphonamide and penicillin administered in various forms. Acta Otolaryngol. 1954;Suppl 113:7-79.

Rundcrantz H, Sundför A. Förenklad dosering av penicillin. Läkartidningen. 1974;71(1-2):71-72.

Rutherford MH. Evaluation of antibiotic therapy of acute otitis media. Eye, Ear,Nose and Throat Monthly. 1953;XXXII, Oct:579-581.

Ruuskanen O, Heikkinen T. Otitis media: etiology and diagnosis. Pediatr Infect Dis J. 1994 Jan;13(1 Suppl):S23-6.

Segal N, Givon-Lavi N, Leibovitz E, Yagupsky P, Leiberman A, Dagan R. Acute otitis media caused by *Streptococcus pyogenes* in children. Clin Infect Dis. 2005;Jul 1;41(1):35-41.

Shurin PA, Wegman DL, Ambrosino D, Tholl J, Overman M, Bauer T, Siber GR. Prevention of pneumococcal otitis media in chinchillas with human bacterial polysaccharide immune globulin. J Clin Microbiol. 1988;Apr;26(4):755-9.

Sipilä M, Pukander J, Karma P. Incidence of Acute Otitis Media up to the Age of 11/2 Years in Urban Infants. Acta Otolaryngol(Stockh) 1987;104:138-145.

Spratley J, Silveira H, Alvarez I, Pais-Clemente M. Acute mastoiditis in children: review of the current status. Int J Pediatr Otorhinolaryngol. 2000 Nov 30;56(1):33-40.

Stenström R, Bernard PAM, Ben-Simhon H. Exposure to environmental tobacco smoke as a risk factor for recurrent acute otitis media in children under the age of five years. Int J Pediatr Otorhinolaryngol. 1993;27(2):127-136.

Straetemans M, Sanders EAM, Veenhoven RH, Schilder AGM, Damoiseaux RAMJ, Zielhuis GA. Pneumococcal vaccines for preventing acute otitis media. The Cochrane Database of Systemic Reviews Collaboration. 2004; Issue 1. Art no.:CD001480. DOI:10.1002/14651858.CD001480.pub2.

STRAMA Swedish Strategic Programme for the Rational Use of Antimicrobial Agents and Surveillance of Resistance, The Swedish Institute for Infectious Disease Control (SMI) 2005.

Sugita R, Fujimaki Y, Deguchi K. Bacteriological features and chemotherapy of adult acute purulent otitis media. J Laryngol Otol. Jul 1985;99(7):629-635.

Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. J Infect Dis 1989 Jul;160(1):83-94.

Teele DW. Strategies to control recurrent acute otitis media in infants and children. Pediatr Ann. Nov 1991;20(11):609-610, 612-614, 616.

Tos M, Stangerup SE. Hearing loss in tympanosclerosis caused by grommets. Arch Otolaryngol Head Neck Surg. 1989;Aug;115(8):931-5.

Uhari M, Tapiainen T, Kontiokari T. Xylitol in preventing acute otitis media. Vaccine. 2000;Dec 8;19 Suppl 1:S144-147.

van Buchem FL, Peeters MF, van 't Hof MA. Acute otitis media: a new treatment strategy. Br Med J (Clin Res Ed). Apr 6 1985;290(6474):1033-1037.

Van Zuijlen, Diederick A, Schilder A, van Balen FAM, 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(2):140-144.

Verduin CM Cees H, Fleer A, van Dijk H, van Belkum A. *Moraxella catarrhalis*: From emerging to established pathogen. Clin Microbiol Rev. 2002;15(1):125-144.

Westman E, Melhus A, Hellström S, Hermansson A. *Moraxella catarrhalis*-induced purulent otitis media in the rat middle ear. Structure, protection, and serum antibodies. APMIS. 1999;107:737-746.

Westman E, Melhus A. The treatment of *Haemophilus influenzae* acute otitis media with amoxicillin protects against reinfection but not against structural changes. J Antimicrobial Chemother. 2002 Jan;49(1):141-7.