Management of chronic mucosal otitis media in children

Behandeling van chronische otitis media bij kinderen

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(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit van Utrecht

op gezag van de rector magnificus, prof.dr. J.C. Stoof,

ingevolge het besluit van het colege van promoties

in het openbaar te verdedigen op

dinsdag 9 maart 2010 des middags te 2.30 uur

door

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geboren op 9 april 1981 te Warnsveld

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The research described in this thesis was financially supported by the Health Care Insurance Board (CVZ).

Publication of this thesis was kindly supported by Adacon Management BV, Artu-Biologicals Europe BV, Atos Medical BV, Daleco Pharma BV, Decos Audiology, Dos Medical, Electro Medical Instruments BV, Entermed BV, Garant Optiek BV, GlaxoSmithKline BV, Nationale Hoorstichting/ Sponsor Bingo Loterij, Oticon Medical BV, Roche Nederland BV, Schering Plough BV, Schoonenberg Hoorcomfort, Stallergenes BV, Veenhuis Medical Audio BV

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Chapter 1

General Introduction

GENERAL INTRODUCTION

Chronic active mucosal otitis media (COM) is one of the most common childhood chronic infectious diseases worldwide, affecting children both in developing and in industrialized countries.[1, 2] COM is defined as a chronic inflammation of the middle ear mucosa in which the tympanic membrane is not intact because of a perforation or tympanostomy tube, and otorrhea is present.[3-5] Consensus regarding the definition of chronic is lacking. The duration of symptoms varies from more than 2 weeks[6] to 3 months.[7-11] In this thesis, we use a definition of persistent symptoms of otorrhea for more than 3 months.

COM causes considerable morbidity and is a major global cause of hearing impairment in children. Moreover, it may lead to serious extracranial and intracranial complications, such as mastoiditis and meningitis.[12-20] This calls for proactive management of the condition by the ENT surgeon. Evidence regarding the most effective approach for chronically discharging ears, however, is incomplete. Few methodologically sound randomized controlled trials have been performed; comparison of its results is hampered by differences in inclusion criteria, interventions, outcome measures, and follow-up. [8, 21-27] While in most Western countries topical and systemic antibiotics are the mainstay of treatment in children with COM, in the Netherlands there is a strong preference for surgical treatment.

Trimethoprim/sulfamethoxazole (TMP-SMX) has been used for many years in the management of upper respiratory tract infections in children. It was found to be effective as a prophylaxis in recurrent acute otitis media.[28, 29] It is an inexpensive antimicrobial drug and well tolerated by children. At the Wilhelmina Children's Hospital of the University Medical Center Utrecht we have managed children with COM empirically with prolonged courses of TMP-SMX for many years. The results of a retrospective analysis of the outcome in 48 of these children aged 1 to 12 years was promising: 52% of children were cured, 25% had improved and 23% did not respond. Such a retrospective approach, however, provides limited evidence since confounding by indication and effects of natural course and placebo cannot be precluded.

We therefore initiated a randomized placebo-controlled trial to establish the clinical effectiveness of TMP-SMX in children aged 1 to 12 years with a documented history of continuous otorrhea for at least 12 weeks.

Even more important than medical or surgical management of COM, is to establish measures to prevent the condition. This requires knowledge of individual and environmental factors that put children at risk for developing COM. Such data are scarce. Similarly, the role of the innate and adaptive immune defence system in the development of COM in children has not been clarified.[30]

Repeated or prolonged antibiotic treatment is a major factor for selection of resistance in the microbial flora of humans. Therefore, it is important to study not only the potential

beneficial effects of trimethoprim-sulfamethoxazole on clinical parameters, but also its effects on carriage and antibiotic susceptibility of the microbial flora.[31-33]

OBJECTIVE

The main objective of this thesis is to study the (cost-)effectiveness of a 6-12 week course of TMP-SMX in children with chronic active mucosal otitis media.

Apart from the clinical effects on the middle ear and the child's well-being, the effects on the microbial flora of the middle ear, nasopharynx and gastro-intestinal tract will be studied. To identify children at risk for developing COM, we will study potential risk factors for COM including innate immunity genetic polymorphisms.

OUTLINE OF THE THESIS

In **Chapter 2**, we present an overview of the current evidence regarding the aetiology, pathogenesis and management of COM in children.

Chapter 3 focuses on the intrinsic and extrinsic characteristics of children with COM. In **Chapter 3.1** a case-control study is presented comparing potential risk factors in children with COM with those in a control group of children from the general population and in children managed with tympanostomy tubes for persistent middle ear effusion. In **Chapter 3.2** the role of polymorphisms in the pathogen recognition receptor genes TLR2, TLR4, CD14 and MyD88 in COM, is studied in a case-control design. The genetic Toll-like R, CD14 and Myd88 profile in children with COM is compared with these profiles in population controls.

In **Chapter 4.1** we present the clinical results of our randomized placebo controlled trial on the effectiveness of oral TMP-SMX in children with COM. The cost-effectiveness of this intervention is presented in **Chapter 4.2**.

Chapter 5 focuses on the effects of prolonged treatment with TMP-SMX on the microbial flora of the children with COM. In **Chapter 5.1** the optimal approach for sampling the nasopharyngeal flora is presented. In **Chapter 5.2** we study the effects of TMP-SMX on carriage of potential pathogens in the nasopharynx and development of antimicrobial resistance in these bacteria. Since the intestinal flora is known to be the main reservoir for the emergence and spread of antimicrobial resistance, we report on the effects of prolonged oral use of TMP-SMX on resistance and integron prevalence in the intestinal flora of the children with COM participating in our trial and in population controls in **Chapter 5.3**.

In **Chapter 6** the results are discussed in the light of clinical practice, and recommendations are made for future research. Summaries in English and Dutch complete this thesis.

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Chapter 2

Chronic suppurative otitis media: A review

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International Journal of Pediatric Otorhinolaryngology. 2006 Jan;70(1):1-12.

ABSTRACT

Objectives: Chronic suppurative otitis media (CSOM) remains one of the most common childhood chronic infectious diseases worldwide. Although microbial, immunological, and genetically determined factors, as well as Eustachian tube characteristics, are supposed to be involved in the pathogenesis of CSOM, many aspects of the pathogenesis of CSOM still need to be clarified. Optimal treatment strategy has not been established yet. The objective of this review is to present and evaluate the current state of knowledge of CSOM.

Design: Systematic narrative review.

Methods: A PubMed search (1966–January 2005) was performed for studies on epidemiology, pathogenesis, clinical management, and complications of CSOM. All included articles were categorized according to level of evidence.

Results: Five hundred and fifty papers were identified, of which 79 were found to be relevant for this review. The definition of CSOM was found to vary. CSOM is a multifactorial disease. Regarding management of CSOM, there is no consensus as to what the optimal management strategy should entail. No convincing evidence is available for most medical and surgical therapies. Topical quinolones have proven effective, but need further monitoring regarding adverse effects.

Conclusions and recommendations: Important goals in research of CSOM should be achieving consensus about the definition of CSOM and gaining more in-depth knowledge of the pathogenesis of CSOM, especially the role of innate and adaptive immunity. There is also a need for further well-designed studies on the effectiveness of various management strategies for CSOM.

INTRODUCTION

Chronic suppurative otitis media (CSOM) remains one of the most common childhood chronic infectious diseases worldwide, affecting diverse racial and cultural groups both in developing and industrialized countries. It involves considerable morbidity and can cause extra- and intracranial complications.[1-5]

There are still many questions about the pathogenesis of CSOM and consequently about what the optimal management – medical and/or surgical – should entail.

In this article, the current state of knowledge in epidemiology, pathogenesis, complications, and management of CSOM is reviewed systematically from a clinical perspective, the ultimate aim being to provide the clinician a tool in the management of this condition. In this connection, future research goals will be identified.

METHODS

Search strategy and selection criteria

A PubMed search was done for articles dating from 1966 to January 2005 with the MESH heading "Otitis media, Suppurative" in combination with text- and keywords complications, drug therapy, epidemiology, etiology, genetics, history, immunology, microbiology, pathology, physiopathology, surgery, and therapy. All studies in English on chronic suppurative otitis media in children and adults, containing these text- and keywords were considered for inclusion. After critical assessment of the abstracts identified by the initial search, the full content of all potentially relevant papers was reviewed for final selection and data extraction.

The following articles were excluded: those dealing with topics other than CSOM, e.g. cholesteatoma; those focussing on technical aspects of surgery in CSOM; those with information included in more recent updates on CSOM.

Subsequently, a review of identified report bibliographies and a manual search of standard textbooks on ENT surgery was undertaken.

RESULTS

The search resulted in 550 citations, which after applying the criteria for in- and exclusion left 79 articles for inclusion; 42 additional references were also used. The articles were independently categorized by two authors (M.V. and M.M.R.) in levels of evidence (Table 2.1).[6]

Table 2.1 References categorized in level of evidence

Level of evidence	Study design	References (PubMed search)	References (additional)
1a	Systematic review of RCTs*	2	-
1b	Individual RCT* (good quality)	-	-
2a	Systematic review of cohort studies	-	-
2b	Individual cohort study, incl. cohort of cases; RCT* of low quality, e.g. <80 % follow-up, allocation bias, low power	18	8
3a	Systematic review of case-control studies	-	-
3b	Individual case-control study	3	3
3c	Surveys	15	1
4	Case-series, poor-quality cohort and case-control studies	22	9
5	Expert opinions	18	12
Other	e.g. in vitro studies, animal models, book chapters, etc	1	9
Total		79	42

*RCT: Randomised Controlled Trial

Definition

The textbook definition of CSOM is chronic inflammation of the middle ear and mastoid mucosa in which the tympanic membrane is not intact (perforation or tympanostomy tube) and discharge (otorrhea) is present.[7-9] There is, however, no consensus about the duration of the symptoms. The World Health Organisation (WHO)[10] defines CSOM as "otorrhea through a perforated tympanic membrane present for at least 2 weeks", while others define "chronic" as symptoms persisting for more than 6 weeks.[1,11-14] Since it is accepted that CSOM is preceded by acute otitis media (AOM) treated either incomplete or unsuccessfully[11,15,16], these variations in definition of duration of symptoms suggest that the transition from otorrhea as a sign of AOM to that of CSOM is not clearly established.

CSOM should be distinguished from tympanostomy tube otorrhea (TTO), which is the most common complication of tympanostomy tube placement[17,18], but as with AOM and CSOM, the transition from TTO to CSOM is not well defined. At the same time, CSOM should be distinguished from chronic OME (otitis media with effusion), in which no perforation or active infection is present, as well as from a chronic perforation of the

tympanic membrane (TM), in the absence of middle-ear infection.[19] If a cholesteatoma is present, the term chronic suppurative otitis media with cholesteatoma is used.

Epidemiology

The divergent definitions of CSOM and the inclusion of patients with cholesteatoma in reported prevalences of CSOM, preclude an accurate estimate of the true prevalence and incidence of CSOM. CSOM most often occurs in the first 5 years of life[20], and it is most common in developing countries, in special populations such as children with craniofacial anomalies[21], and in certain racial groups[19]. Highest prevalences of CSOM in children are reported among the Inuits of Alaska, Canada and Greenland, American Indians, and Australian Aborigines, and range from 7% to 46%.[19,22-25] Intermediate prevalences are reported in the South Pacific Islands, Africa, Korea, India, and Saudi Arabia, ranging from 1% to 6%.[19,26-28] The lowest prevalences are found in highly developed industrial countries such as the UK and the US: <1%.[19,29]

Risk factors

Fliss et al. have identified a history of acute and recurrent otitis media, parental history of chronic otitis media, and crowded conditions (i.e. large families with several siblings, large day care centres) as significant risk factors for CSOM.[30] They could not establish an association between CSOM and allergy, recurrent upper respiratory infections, breast-feeding, sex, parental age, or passive smoking. From a clinical perspective, however, some of these risk factors for AOM are likely to play a role in CSOM.[31-33] No quantitative data on risk factors for CSOM, such as odds ratios or prognostic models that can predict which children will develop CSOM, are available.

Pathogenesis

The pathogenesis of CSOM is multifactorial: environmental versus genetically determined factors as well as anatomical and functional characteristics of the Eustachian tube are involved. The following paragraphs describe the main causative factors for CSOM in greater detail.

Eustachian tube function

The Eustachian tube has three important functions with respect to the middle ear: ventilation, protection, and clearance. Both endogenous and exogenous factors can impair these functions and therefore cause OM (otitis media).[5,19,34,35] When a perforation of the tympanic membrane is present, either spontaneously or due to a tympanostomy tube, the middle ear "gas cushion" is lost, resulting in reflux of nasopharyngeal secretions through the Eustachian tube and consequent contamination of the middle ear with potential respiratory pathogens.[11,19,35] Infants and young children are especially at

risk for such reflux because their Eustachian tubes are short, horizontal, and "floppy".[19,35] Similarly, Down syndrome and craniofacial anomalies such as cleft palate affect both the anatomy and function of the Eustachian tube and so predispose to CSOM.[21] Yuceturk et al. studied Eustachian tube function (automatic Toynbee test, tympanometry, Valsalva's manouvre) in 60 ears with CSOM and 146 control ears, finding Eustachian tube dysfunction in 72% (95% CI, 61–83) versus 35% (95% CI, 27–43), respectively, ($p < 0.05$).[34]

Reduced ciliary function of the middle ear and Eustachian tube mucosa has been associated with impairment of clearance of middle-ear secretions and may, therefore, facilitate the progression from AOM/OME into CSOM.[36,37]

Gastroesophageal reflux may also contribute to Eustachian tube dysfunction and subsequent middle-ear infection.[38,39]

Microbiology

In CSOM, bacteria can reach the middle ear either from the nasopharynx through the Eustachian tube or from the external ear canal through a non-intact tympanic membrane.[11,19,35] The aerobic micro-organisms most frequently isolated in CSOM are *Pseudomonas aeruginosa* (in 18–67% of ears), *Staphylococcus aureus* (14–33%), Gram-negative organisms, such as *Proteus* spp., *Klebsiella* spp., and *Escherichia* spp. (4–43%), and *Haemophilus influenzae* (1–11%).[13,15,40-49] The most frequently isolated anaerobic organisms are *Bacteroides* spp. (1–91%) and *Fusobacterium* spp. (4–15%) (Table 2.2).[42-47] In CSOM, the middle-ear environment is thought to be more tolerant to unusual organisms like *P. aeruginosa*, *S. aureus*, and anaerobes; therefore, it is still uncertain whether these bacteria are true pathogens in CSOM or might reflect secondary invaders or contamination from the external auditory canal.[2,7] The large variability in recovery rates of aerobic and anaerobic bacteria may be related to differences in timing of sampling during the course of the disease, prior use of antibiotics, and differences in sampling- and processing techniques, e.g. sterilizing the auditory canal before sampling, transport media, or delays in inoculation.[2,7,11] Fungi are also thought to play a role in CSOM, especially *Aspergillus* spp. and *Candida* spp..[50,51] In some populations, especially those inhabiting hot, humid regions where fungi may well flourish, fungi are isolated in 50% of cases with CSOM.[50,51]

Recently, concern has risen about secondary fungal overgrowth as a complication of treatment with quinolone eardrops.[52]

Recently, bacterial biofilms have gained attention as a source of chronic infections. A biofilm is a population of bacterial cells growing on a surface, enclosed in an exopolysaccharide matrix; being difficult to eradicate, they could be the source of persistent infections.[53,54] Biofilms may attach to damaged tissue, such as exposed osteitic bone and ulcerated middle-ear mucosa, or to otologic implants such as tympanostomy tubes, and are therefore thought to cause persistent infection in CSOM.[11,54-56]

Table 2.2 Most frequently isolated aerobic and anaerobic micro-organisms in chronic suppurative otitis media (percentage per ear)[a]

Author [ref]	Aerobic micro-organisms				Anaerobic micro-organisms	
	Pseudomonas aeruginosa	*Staphylococcus aureus*	Gram-neg. rods[b]	*Haemophilus influenzae*	*Bacteroides* spp.	*Fusobacterium* spp.
Kenna et al.[15]	67	20	4-8[c]	4-6	n/a	n/a
Fliss et al.[48]	62	14	7-15	10-11	n/a	n/a
Arguedas et al.[13]	26	14	14-22	1	n/a	n/a
Indudharan et al.[40]	36	31	10-15	8	n/a	n/a
Brook et al.[46]	20	22	20-43	9	24-61	15
Brook et al.[44]	22	15	15-35	9	17-71	8-11
Miró et al.[41]	18	25	5-10	2[d]	n/a	n/a
Jonsson et al.[42]	30	30	21[e]	2	23	4
Erkan et al.[43] [f]	37	23	21-40	n/a	11-34	5
Papastavros et al.[45]	55	29	6-9	n/a	5-10	n/a
De Uzeda et al.[47]	18	33	31-42[g]	9	24-91	7-11
Ibekwe et al.[49]	25	23	4-7[g]	7	1	n/a

[a] Minimum and maximum (min – max) data are given when more than one gram negative rod or species was identified

[b] Escherichia spp, Klebsiella spp, Proteus spp

[c] Data on Klebsiella species not provided

[d] Only total number of Haemophilus spp is provided

[e] Data on Klebsiella spp and Escherichia spp not provided

[f] Percentage per case

[g] Data on Escherichia spp not provided

Immunology and genetics

In general, immunoglobulins IgG and IgA are most important in the defence against mucosal infections like CSOM. Secretory IgA (SIgA) is synthesized locally by plasma cells in the mucosa of the middle-ear cavity and may be important in preventing bacteria from attaching to and colonizing the middle-ear mucosa. Children with CSOM may lack SIgA.[57] IgG-class immunoglobulins facilitate phagocytosis directly or via complement activation. IgG and IgG-subclass concentrations depend on age.[58] Children with recurrent upper respiratory infections may have low specific IgG-subclass (mostly IgG2) antibody levels (10–20% of cases).[59,60] For CSOM no such data are available. An essential condition for immunoglobulins to act is their adherence to the bacterial wall, i.e. coating.[58] In CSOM, intense SIgA and IgG coating of bacteria is common, but when *P. aeruginosa* is the causative agent of the infection, no bacterial coating is seen. This may well explain why infections caused by *P. aeruginosa* are so difficult to eradicate.[57,61]

Although many have reported on inflammatory mediators in AOM and OME, evidence about the specific role of such factors in CSOM, like local cytokine production, is not yet available.

Genetic determinants of CSOM are still unknown. Against the background of twin studies in OM, showing a higher concordance rate in OM for monozygotic twins than for dizygotic twins, a genetic component is also likely for CSOM.[62,63]

Low serum levels of mannose-binding lectin (MBL) and polymorphisms of Fc gamma receptor have been associated with recurrent upper respiratory infections and otitis media in childhood.[64-66] Further research into these and other modifier genes is necessary to confirm their role in CSOM.

CSOM in systemic conditions

CSOM may occur as a part of a systemic condition, e.g. M. Wegener, tuberculosis, and Histiocytosis X[21,67,68], where the mastoid and middle ear may be the localization of this specific inflammation.

Complications and sequelae of CSOM

The most common sequela of CSOM is hearing loss, either conductive or sensorineural; this may affect a young child's language development and school progress. Chronic infection of the middle ear, causing oedema of the middle-ear lining and discharge, tympanic membrane perforation, and possibly ossicular chain disruption, results in a conductive hearing loss ranging from 20 to 60 dB.[7,69,70]

There is some evidence that CSOM causes sensorineural hearing loss. Animal studies have shown that inflammatory mediators, penetrating into the inner ear through the round window membrane, can cause the loss of hair cells in the cochlea.[71,71] A recent study in humans has shown loss of outer and inner hair cells in the basal turn of the cochlea in patients with CSOM.[73]

Four studies have been identified reporting on sensorineural hearing loss in CSOM.

In one retrospective study of 218 patients (average age 35 years) with unilateral CSOM the bone conduction threshold was 9–14 dB lower in diseased ears than in healthy ears.[74] In another retrospective study of 121 patients (average age 37 years) with unilateral CSOM, the bone conduction threshold was 10–12 dB in the diseased ear compared to 3–4 dB in the healthy ear.[75]

On the other hand, in two prospective studies, one of 286 patients with unilateral CSOM (average age 50 years) and the other of 87 children with bilateral CSOM (average age 5.5 years), no effect of the infection on bone conduction thresholds was found.[69,76] No evidence was found suggesting that children are more susceptible to develop sensorineural hearing loss in CSOM than adults.[69,74-76]

CSOM can result in serious extracranial and intracranial complications. The reported overall extra- and intracranial complication rate in CSOM varies from 0.7% to 3.2%; extracranial complications alone from 0.5% to 1.4% and intracranial complications from 0.3% to 2.0%.[3,79] The incidence of complications appeared higher in pediatric patients than in adults with CSOM. With CSOM being more frequent in children, however, no adequate comparison between complication rates in children and adults can be made.

The most frequent extracranial complications are facial paralysis, subperiosteal abscess, mastoiditis, and labyrinthitis, with reported incidences of 13–58%, 40–68%, 14–74%, 7–34% of all extracranial complications, respectively.[3,4,77-80]

The most common intracranial complications of CSOM are meningitis, cerebral abscess, lateral sinus thrombosis, extradural abscess, otic hydrocephalus, and encephalitis, with reported incidences of 21–72%, 18–42%, 2–26%, 7–16%, 5–11%, and 2% of all intracranial complications, respectively.[3,4,77-80]

Clinical management

Topical treatment

In developing countries, antiseptic drops, e.g. aluminium acetate, boric acid, iodine powder, and povidone-iodine are commonly used for CSOM because of their low cost and availability.[81-83] Eardrops containing antimicrobial agents either with or without an anti-inflammatory component have been promoted as an effective therapy for CSOM since the 1950s.[84-86] Since the 1990s, fluoroquinolone drops have become available.[33,41,87-92]

The effectiveness of ototopical drops was evaluated in a Cochrane Review.[82] CSOM was defined according to WHO criteria, i.e. otorrhea through a perforated tympanic membrane present for at least 2 weeks. Overall success percentages (dry ear) of ototopical drops varied from 40% to 100%. It was concluded that treatment with antibiotic or antiseptic eardrops accompanied by aural toilet was more effective in resolving otorrhea than no treatment (OR 0.37, 95% CI, 0.24–0.57) or aural toilet alone (OR 0.31, 95% CI, 0.23–0.43). Topical antibiotics were not more effective than topical antiseptics (OR 1.34, 95% CI, 0.64–2.81), and topical treatment with antibiotic or antiseptic eardrops was more effective than systemic antibiotics (OR 0.46, 95% CI, 0.30–0.69). The benefit of combinations of topical antibiotics and steroids as compared to topical antibiotics alone has not been evaluated formally. Combined treatment with topical and systemic antibiotics was not more effective than treatment with topical antibiotics alone in terms of otorrhea resolution (OR 1.71, 95% CI, 0.88–3.34). Topical quinolones appeared to be more effective than non-quinolone eardrops (OR 0.26, 95% CI, 0.16–0.41).

Another systematic review by Abes et al.[93] also showed that quinolone eardrops were more effective than non-quinolones. They found a 2.67 times higher cure rate with topical 0.3% ofloxacin otic solution, than with other topical or systemic antibiotics (95% CI,

2.04–3.50). In a third non-systematic review, however, the authors stated that topical quinolones were not superior to topical aminoglycosides.[94]

The overall quality of the studies included in these three reviews[82,89,93-97], was generally considered low. The definition of CSOM, duration of discharge (3 weeks–40 years), age range of the patients (21 months–79 years) and follow-up varied considerably. No consistent relation between duration of CSOM or age of the patients and outcome was found. In all studies, outcome was defined as resolution of otorrhea; no data regarding the quality of the tympanic membrane or hearing were given.

The risk of ototoxicity by ototopical preparations has been the subject of discussion for many years.[84,98] Potential ototoxicity of antibiotics, solvents, and antiseptics has been demonstrated in animal studies, but information regarding these adverse effects in humans is scarce.[85] In the studies reviewed by Acuin et al. negligible rates of ototoxicity were found.[82]

Secondary fungal overgrowth causing otitis externa has been reported as a side effect of treatment with topical quinolones. With the growing enthusiasm for the use of these eardrops, the incidence of this complication is found to increase.[52]

Systemic treatment

Systemic antibiotics are advised both as initial therapy for CSOM[21,51,96] and as secondary when topical therapy fails[1,7,11,15,99-102]. In Table 2.3, the results of the available studies on systemic treatment of CSOM are summarized; the success rate of systemic antibiotics appears to be quite high, approximately 70%. Overall, the methodological quality of these studies was low. Because of heterogeneity of study populations and study design the data could not be pooled, nor could subgroups of patients with better or poorer outcome be identified.

An expert panel called together by the American Academy of Otolaryngology—Head and Neck Surgery concluded recently that systemic therapy should only be considered in patients with CSOM showing signs of complicated or invasive infections or signs of systemic disease.[84] Consensus is lacking as to which antibiotic to use systemically as well as about the duration of treatment in CSOM[11,103,104]; both broad-spectrum antibiotics, as well as culture-directed therapy, have been advocated as initial oral therapy for CSOM.[11,103]

Surgical treatment

Tympanomastoidectomy has been advocated as the surgical treatment of choice in CSOM since the 1970s.[7,16,105] However, no prospective, randomised, controlled trials justifying this guidance have been published.[82] Only three retrospective studies on surgical treatment for CSOM are available. Vartianen et al. reported the outcome of 221 ears with CSOM in children and adults managed with either a one-stage tympanomastoidectomy (84%) or a mastoidectomy, with planned second-stage tympanoplasty (15%).[105] The

Table 2.3 Systemic treatment for chronic suppurative otitis media

Author	N	Average age (range)	Type of study	Antibiotic type	Duration of treatment	Initial/secondary treatment	Route of administration (oral, IV, IM)	Follow-up	Succes rate (%)
Khanna et al.[51]	110	n/a	RCT*	culture directed AB vs co-trimoxazole	14 days max	Initial	oral	2 weeks	85 vs 75
Fliss et al.[1]	48	4 years (1-12 years)	RCT*	mezclocillin vs ceftazidime	10-14 days	Secondary	IV	12 days	100 both
Kenna et al.[15]	36	(1-17 years)	Case report	various types of AB	3-35 days	Secondary	IV	4-20 months	89
Dagan et. al.[101]	37	(6 months-16 years)	Cohort of cases	ceftazidime	21 days max	Secondary	IV/IM	12 months	76
Esposito et al.[100]	52	8.4 years (6-12 years)	Case report	ceftazidime	7-10 days	Secondary	IM	40 days max	67
Somekh et al.[99]	30	4.2 years (1-12 years)	RCT*	ceftazidime vs aztreonam	14 days max	secondary	IV	90 days	73 vs 53
Legent et al.[96]	75	42.6 years	RCT*	ciprofloxacin vs amoxycillin/clavulanic acid	9 days	Initial	oral	10 days	58 vs 37

*RCT: Randomized Controlled Trial

overall success rate, defined as dry ear plus an intact, mobile eardrum, was 73% (95% CI, 67–79). No differences were found between results in children and adults. Another report by the same authors, limited to children with CSOM, showed an equally successful outcome of (tympano)mastoidectomy, namely 74% (95% CI, 59–89).[16] Balyan et al. analyzed the surgical outcome in 323 patients (age range 4–68 years) with CSOM managed by: (I) tympanoplasty and mastoidectomy (discharging ears); (II) tympanoplasty alone (discharging ears); or (III) tympanoplasty alone (dry ears).[106] Graft success rates in groups I–III were 91% (95% CI, 83–98), 86% (95% CI, 73–99), and 90% (95% CI, 85–93), respectively. Mean residual air-bone gaps were 17, 20, and 19 dB, respectively. The success percentage of surgery appeared to be higher in children below 16 years of age. The effect of duration of symptoms on outcome was not studied. The authors concluded that results of tympanoplasty combined with mastoidectomy are no better than tympanoplasty alone in patients with CSOM.

Studies comparing surgical versus medical therapies for CSOM are not available.

Regarding the timing of mastoid surgery and tympanoplasty for CSOM in children, opinions vary. Procter[9] recommends that mastoid surgery should be delayed until puberty when possible, while Bluestone et al.[7] and Vartiainen[16,105] consider mastoid surgery indicated in all cases of CSOM that do not respond to conservative treatment, regardless of age.

Regarding tympanoplasty alone in children with CSOM, some authors recommend this operation only in children older than 10–12 years of age because of a higher incidence of surgical failures in younger children.[107,108] Other authors state that age does not affect the outcome of tympanoplasty in children with CSOM.[16,109,110]

In cases of therapy-resistant CSOM radical mastoidectomy may be considered, with or without mastoid obliteration.[111-114] In one retrospective case study of 16 patients (average age 44 years) who had undergone a radical revision mastoidectomy, 80% obtained a dry ear (95% CI, 60–99).[111] In another case-control study of 30 patients with CSOM who had been managed by revision mastoidectomy with mastoid obliteration using a temporoparietal fascial flap, 96% (95% CI, 89–100) had a dry ear at 12 months follow-up versus 10% (95% CI, 1–21) of 30 conservatively treated patients with CSOM.[114]

Hearing revalidation

Bone-anchored hearing aids (BAHA) became available in the 1980s. They are considered a good alternative to conventional bone-conduction hearing aids in patients with chronic draining ears.[115-118] A retrospective study of 69 patients with CSOM previously using a conventional hearing aid showed a reduction of discharge in 84% of patients following fitting with a BAHA; 58% of patients were more satisfied with their BAHA than with their previous aid.[116] Audiologically, patients with CSOM do not perform better with BAHAs than with air conducting aids.[115,118]

Up till the 1980s CSOM was considered a contraindication for cochlear implantation because of the risk of spread of the infection to the intracranial space.[119] However, two recent studies which included 19 patients with CSOM, one with an average follow-up period of 18 months and the other 4 years, showed that cochlear implantation could be safely achieved in these patients.[120,121]

DISCUSSION

A large number of studies have been published about CSOM, but the great variability of these studies precluded pooling of the results in a meta-analysis or a systematic review. Therefore, this review is narrative. As such, it might be prone to bias in the selection of articles, interpretation of results, and recommendations.

To minimize such bias, we performed an elaborate PubMed search using a MESH heading, which ensured us of finding all articles about suppurative otitis media. We included all articles that provided us actual and relevant information about CSOM; all other subjects than CSOM were excluded. This study shows that most articles on CSOM are expert opinions or case-series, using very variable definitions of CSOM, small study populations, and short follow-up duration, hence overall yielding poor evidence.

We, therefore, believe that future research in CSOM should be directed to:

1. Achieving consensus regarding the definition of the disease, including its duration. The WHO definition of CSOM as "otorrhea present for at least 2 weeks", is not of much help to the clinician who is generally faced with patients with a much longer duration of symptoms. The various stages of this disease, especially the transitional phase from AOM to CSOM, need to be defined more clearly.
2. Identifying the risk factors and pathogenesis of CSOM. It is assumed that factors involved in AOM also have an important role in CSOM, but good evidence for this assumption is lacking. Optimal management of the disease and advice to the parents should be based on the knowledge of the risk factors and pathogenesis of CSOM. In particular, the role of innate and adaptive immunity, e.g. modifier genes, as well as the role of bacterial biofilms needs further study, as these can be targets for new therapies.
3. Developing prognostic models including factors that can predict which children will develop CSOM.
4. Since so few well-designed studies of current medical and surgical therapies are available, management of CSOM is still controversial. Topical quinolones are a promising option in the management of CSOM. Prospective and well-controlled studies are needed to establish the role of both medical and surgical therapies, including the optimal duration of treatment, for various stages of CSOM.

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Chapter 3

Epidemiology of chronic mucosal otitis media in children

Chapter 3.1

Predictors of chronic suppurative otitis media in children

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Archives Otolaryngology Head and Neck Surgery. 2006;132:1115-8.

ABSTRACT

Objective: To determine which factors predict development of chronic suppurative otitis media (CSOM) in children.

Design: Case-control study, with univariate and multivariate logistic regression analysis applied to determine which factors independently predict CSOM.

Subjects: Prognostic factors for CSOM were identified in (1) 100 children with CSOM and 161 controls aged 1 to 12 years and (2) 83 children who developed CSOM in the presence of a tympanostomy tube and 136 children with tympanostomy tubes who did not develop CSOM.

Results: Independent predictors for CSOM were previous tympanostomy tube insertion (odds ratio [OR], 121.4 [95% confidence interval {CI}, 38.9-379.3]); having had more than 3 upper respiratory tract infections in the past 6 months (OR, 12.2 [95% CI, 3.5-42.3]); having parents with a low education level (OR, 14.1 [95% CI, 2.9-68.6]); and having older siblings (OR, 4.4 [95% CI, 1.6-12.6]). Independent predictors for CSOM after tympanostomy tube insertion were having experienced more than 3 episodes of otitis media in the past year (OR, 4.9 [95% CI, 2.2-11.0]; attending day care (OR, 3.6 [95% CI, 1.7-7.8]); and having older siblings (OR, 2.6 [95% CI, 1.2-5.5]).

Conclusions: Treatment with tympanostomy tubes is the most important prognostic factor for CSOM in children. In children who are being treated with tympanostomy tubes for persistent middle ear effusion, the most important prognostic factor for CSOM is a history of recurrent episodes of acute otitis media. This information should be taken into consideration and discussed with parents when considering insertion of tympanostomy tubes in children.

INTRODUCTION

Chronic suppurative otitis media (CSOM) is defined as a chronic inflammation of the middle ear and mastoid mucosa in which the tympanic membrane is not intact and discharge is present.[1-3] This condition can occur in the presence of a tympanic membrane perforation or after tympanostomy tube insertion. Despite improvements in overall patient health and in their access to otolaryngologic pediatric care, management of CSOM remains a challenge.

There is no consensus regarding the most effective treatment for CSOM, and both medical and surgical therapies have substantial failure rates.[4-7] Therefore, prevention or early treatment of CSOM is important. This requires knowledge of factors that predict CSOM in children. Such data are scarce.[8] Since it is thought that CSOM begins with an episode of otitis media (OM),[9-10] it is likely that prognostic factors for OM also play a role in the development of CSOM. These include intrinsic factors such as race,[10] age,[11] previous upper respiratory tract infections (URTIs) and/or acute OM,[11-13] educational level of the parents,[14-15] and extrinsic (environmental) factors such as parental smoking,[16] day-care attendance,[11, 13, 16-17] and bottle vs breastfeeding.[17] Herein, we report the results of our study of prognostic factors for OM in children with CSOM, in healthy controls and in children treated with tympanostomy tubes for persistent middle ear effusion.

METHODS

Patients and controls

The patient group of our case-control study consisted of children aged 1 to 12 years with a documented history of at least 3 months of continuous otorrhea through either a tympanic membrane perforation or a tympanostomy tube. They were referred to the department of Pediatric Otorhinolaryngology of the University Medical Center Utrecht between February 2003 and June 2005 to participate in a study on the effectiveness of prolonged treatment with sulfamethoxazole-trimethoprim. Exclusion criteria for this study were (1) cholesteatoma, (2) known immune deficiency other than for IgA or IgG subclass deficiency, (3) Down syndrome, (4) craniofacial anomalies, (5) cystic fibrosis, (6) primary ciliary dyskinesia, (7) allergy to sulfamethoxazole-trimethoprim, and (8) continuous use of antibiotics for more than 6 weeks in the past 6 months.

Prognostic factors in the patient group were compared with those in 2 control groups. The first control group consisted of "healthy" children recruited at 2 day care centers and a primary school in the vicinity of Utrecht. Between November 2003 and January 2004, parents were asked to fill in a questionnaire with information on possible prognostic factors for CSOM. Of the 400 questionnaires distributed, 163 were filled out and returned to the study center.

The second control group consisted of children treated with tympanostomy tubes for persistent OM with effusion between December 1999 and July 2002. They participated in a prospective cohort study on the immune status and eustachian tube function in children with recurrent OM with effusion. Inclusion and exclusion criteria have been described in detail elsewhere.[18] Baseline information on prognostic factors for OM was collected at study entry.[18] None of the participants developed CSOM during follow-up. Distribution of prognostic factors in this control group with tympanostomy tubes without CSOM was compared with children in the patient group who developed CSOM in the presence of a tympanostomy tube.

Prognostic factors

The following potential prognostic factors for CSOM were studied in the group of children with CSOM and in the healthy controls: age (<4 vs ≥4 years), sex (male vs female), previous OM episodes (yes vs no), OM in first year of life (yes vs no), recurrent OM episodes in the year before study entry (>3 vs ≤3 episodes), parental history of OM (yes vs no), recurrent URTIs (rhinitis, cough, and/or sore throat) in the 6 months before study entry (>3 vs ≤3 episodes), previous tympanostomy tube insertion (yes vs no), siblings (>2 vs ≤2 siblings), older siblings (yes vs no), smoking in the household (yes vs no and >10 vs ≤10 cigarettes per day), educational level of the mother (low vs average or above average), birth weight (<2500 g vs ≥2500 g), gestational age (<37 weeks vs ≥37 weeks), having been breastfed (>3 vs ≤3 months), and atopy (yes vs no).

For the comparison between the children who developed CSOM in the presence of a tympanostomy tube and the controls with tympanostomy tubes without CSOM, the same factors were studied except for URTIs, previous tympanostomy tube insertion, educational level of the mother, birth weight, and atopy, since these factors were not measured in the controls. Prognostic factors associated with day care, however, were added: attending day care (yes vs no and >2 vs ≤2 days per week) and group size (>10 vs ≤10 children per group).

Statistical analysis

The association between each of the factors and the outcome measures (CSOM and CSOM in the presence of tympanostomy tubes) was determined by applying univariate logistic regression analysis. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

Predictors that were univariately associated with the outcome ($P \leq .10$) were included in multivariate logistic regression analyses. The predictive accuracy of the models was estimated by their reliability (goodness of fit) using Hosmer-Lemeshow statistics that evaluate the correspondence between a model's predicted probabilities and the observed frequencies over groups spanning the entire range of probabilities. The prog-

nostic value of the models was evaluated using the c-index. This index has a theoretical range of 0.5 (no prognostic value) to 1.0 (maximum prognostic value). The c-index is equal to the area under the receiver operating curve.[19] All analyses were performed using SPSS statistical software, version 12.0 (SPSS Inc, Chicago, Ill).

RESULTS

Of the 100 patients with CSOM, 83 children had developed CSOM in the presence of tympanostomy tubes and 17 in the presence of a tympanic membrane perforation. One hundred sixty-three children served as healthy controls, and 136 as the control group with tympanostomy tubes without CSOM. Characteristics of these children are reported in Table 3.1.1 and Table 3.1.2.

Table 3.1.1. Univariate analysis of potential prognostic factors for CSOM in patients with CSOM and "healthy" controls.

Potential prognostic factors	Patients with CSOM (N=100) N (%)	"Healthy" controls (N=161) N (%)	OR	95%CI
<4 years of age	49 (49)	71 (44)	1.2	0.7 – 2.0
Gender (boys)	55 (55)	78 (49)	1.3	0.8 – 2.1
Previous OM episodes	68 (68)	61 (38)	3.5	2.1 – 5.9
OM in first year of life	27 (40)	35 (22)	2.3	1.2 – 4.2
>3 OM episodes in year before study entry	24 (36)	12 (8)	6.8	3.1 – 14.8
Parental history of OM	42 (43)	79 (53)	0.7	0.4 – 1.1
>3 URTI's in 6 months before study entry	58 (58)	15 (9)	13.4	6.9 – 26.1
Previous tympanostomy tube insertion	89 (89)	9 (6)	135.7	54.1 – 340.3
Siblings				
More than 2	13 (13)	10 (6)	2.3	1.0 – 5.4
Older siblings	73 (73)	64 (40)	4.1	2.4 – 7.1
Parental smoking				
Yes	29 (29)	19 (12)	3.1	1.6 – 5.8
>10 cigarettes/day	16 (16)	7 (4)	4.5	1.8 – 11.5
Low education level mother	19 (19)	9 (6)	4.0	1.7 – 9.3
Birth weight < 2500 g	15 (15)	8 (5)	3.4	1.4 – 8.3
Breast fed for >3 months	34 (34)	82 (51)	0.5	0.3 – 0.8
Atopy	39 (39)	46 (29)	1.5	0.9 – 2.6

Abbreviations: CI, confidence interval; CSOM, chronic suppurative otitis media; OM, otitis media; OR, odds ratio; URTI, upper respiratory tract infection (rhinitis, cough, and/or sore throat). *Unless otherwise noted, data are reported as number (percentage) of subjects.

Table 3.1.2. Univariate analysis of potential prognostic factors for CSOM in patients with CSOM in the presence of tympanostomy tubes and controls with tympanostomy tubes without CSOM.

Potential prognostic factors	TT* and CSOM Patients N= 83 N (%)	TT** controls N= 136 N (%)	OR	95%CI
<4 years of age	41 (49)	32 (24)	3.2	1.8 – 5.7
Gender (boys)	46 (55)	68 (50)	1.2	0.7 – 2.2
Previous OM episodes	57 (69)	55 (44)	2.8	1.6 – 5.0
OM in first year of life	24 (41)	26 (21)	2.6	1.3 – 5.1
>3 OM episodes in year before study entry	22 (39)	17 (14)	4.0	1.9 – 8.3
Parental history of OM	37 (45)	49 (39)	1.2	0.7 – 2.2
Siblings				
More than 2	12 (16)	11 (8)	1.9	0.8 – 4.6
Older siblings	59 (71)	65 (48)	2.6	1.5 – 4.7
Parental smoking				
Yes	25 (30)	46 (34)	0.8	0.5 – 1.5
>10 cigarettes/day	15 (18)	20 (15)	1.2	0.6 – 2.6
Attending day-care (for children <4 yrs)	35 (42)	21 (16)	4.0	2.1 – 7.5
>2 days per week	10 (12)	4 (3)	4.5	1.4 – 14.8
>10 children per group	24 (29)	15 (11)	3.2	1.6 – 6.6
Gestational age <37 weeks	10 (12)	12 (9)	1.4	0.6 – 3.4
Breastfed for >3 months	31 (37)	48 (36)	1.1	0.6 – 1.9

Abbreviations: CI, confidence interval; CSOM, chronic suppurative otitis media;
OM, otitis media; TT, tympanostomy tubes; OR, odds ratio. *Unless otherwise noted, data are reported as number (percentage) of subjects.

Table 3.1.3. Independent predictors for CSOM in an otherwise "healthy" population

Prognostic factors*	OR	95% CI
Previous tympanostomy tube insertion	121.4	38.9 – 379.3
>3 URTI's in previous 6 months	12.2	3.5 – 42.3
Low education level mother	14.1	2.9 – 68.6
Older siblings	4.4	1.6 – 12.6

*intercept = -4.4
Abbreviations: CI, confidence interval; OR, odds ratio; CSOM, chronic suppurative otitis media; URTI, upper respiratory tract infection.

Prognostic factors for CSOM in an otherwise healthy population

Independent predictors for CSOM were previous tympanostomy tube insertion (OR, 121.4 [95% CI, 38.9-379.3]); having had more than 3 URTIs in the past 6 months (OR, 12.2 [95% CI, 3.5-42.3]); having older siblings (OR, 4.4 [95% CI, 1.6-12.6]); and having

Table 3.1.4. Independent predictors for CSOM in children with tympanostomy tubes

Prognostic factors*	OR	95% CI
>3 OM episodes in past year	4.9	2.2 – 11.0
Day-care	3.6	1.7 – 7.8
Older siblings	2.6	1.2 – 5.5

*intercept = -2.1
Abbreviations: CI, confidence interval; OM, otitis media; CSOM, chronic suppurative otitis media; OR, odds ratio.

parents with a low education level (OR, 14.1 [95% CI, 2.9-68.6]). The goodness-of-fit test indicated an acceptable fit of the multivariate model (P = .94), and the c-index was 0.98 (Table 3.1.3).

Prognostic factors for CSOM in children with tympanostomy tubes

Independent predictors for CSOM in children with tympanostomy tubes were having experienced more than 3 episodes of acute OM in the past year (OR, 4.9 [95% CI, 2.2-11.0]); attending day care (for children younger than 4 years) (OR, 3.6 [95% CI, 1.7-7.8]); and having older siblings (OR, 2.6 [95% CI, 1.2-5.5]). The goodness-of-fit test indicated an acceptable fit of the model (P = .93), and the c-index was 0.75 (Table 3.1.4).

DISCUSSION

This is the first study to our knowledge to identify independent predictive factors for CSOM. In otherwise healthy children, previous tympanostomy tube insertion, having older siblings, more than 3 URTIs in the past 6 months, and a low education level of the mother were independent predictors for CSOM. The prognostic value of the model, as indicated by the c-index, was very high, indicating that clinicians can reliably use this information to predict CSOM in children.

So far, only Fliss et al[8] have compared risk factors for CSOM in a population of 88 children with CSOM (12 with tympanostomy tubes and 76 with perforations) and in 76 healthy controls. They found that a history of acute OM, a parental history of chronic OM, and crowded conditions (larger families and large day care centers) were risk factors for CSOM. However, they did not study whether these factors were independently associated with CSOM.

Since most of our CSOM group had chronic otorrhea in the presence of tympanostomy tubes, we also studied which factors predict CSOM in children treated with tympanostomy tubes for persistent OM with effusion. Attending day care, having older siblings, but most of all having experienced more than 3 acute OM episodes in the past year were

independent predictors for CSOM in these children. Apparently, children treated with tympanostomy tubes because of persistent middle ear effusion have an increased risk of CSOM when they are also prone to recurrent acute OM episodes.[20]

Since we performed a case-control study, the proportion of patients with CSOM (a relatively rare condition) is much higher than in the general population. Therefore, the ORs provided cannot be used to calculate absolute risks for CSOM. However, the ORs do indicate which factors predict CSOM in children with and without tympanostomy tubes.

In conclusion, treatment with tympanostomy tubes is the most important prognostic factor for CSOM in children. In children who are being treated with tympanostomy tubes for persistent middle ear effusion, this is a history of recurrent episodes of acute OM. This information should be taken into consideration and discussed with parents when considering insertion of tympanostomy tubes in children.

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Chapter 3.2

CD14, MyD88 and TLR-2/-4 polymorphisms do not predispose to chronic otitis media in children

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Submitted (Box nomenclatuur)

ABSTRACT

Objective: Pathogen recognition receptors (PRRs) are specialized innate immune molecules that play a role in the first line of defense against infections. Several studies suggest that functional polymorphisms in the genes encoding these PRRs may alter the susceptibility to pathogens that play a role in otitis media. Therefore the objective of the present study was to evaluate whether functionally relevant pathogen recognition receptor polymorphisms predispose to chronic active mucosal otitis media (COM).

Design: case-cohort study.

Methods: In 90 children with COM and 375 controls TLR2 SNPs (-16934; Pro631His; Arg-753Gln), TLR4 SNPs (Thr399Ile), CD14 SNPs (-651; -260), MyD88 SNPs (1944), genotype and haplotype frequencies were compared. Genotyping was carried out with sequence specific polymerase chain reaction (SSP-PCR). Haplotypes for the SNP positions of the TLR2 gene and the CD14 gene were estimated from unphased genotype data using the Baysian statistical method. Subgroup analysis were performed for the children with COM according to age (≤3/>3 years).

Results: The frequency of the wild type and mutant SNP alleles of the TLRs, CD14 and MyD88 genes as well as the genotype frequencies and haplotype frequencies of CD14 and TLR2 did not differ between the patient and the control group. Subgroup analyses according to age did not influence the results.

Conclusions: Polymorphisms of the TLR2, TLR4, CD14 or MyD88 genes do not appear to be associated with an increased susceptibility to COM.

INTRODUCTION

Chronic active mucosal otitis media (COM) is one of the most common chronic infectious diseases, affecting children both in developing and industrialized countries. It causes considerable morbidity, may lead to extra- and intracranial complications and is a major global cause of hearing impairment in children.[1]

Evidence about factors predisposing children to COM is scarce. It is assumed that the multifactorial determinants of otitis media also play a role in predisposing to COM.[1] As such, two core elements are recognized: microbial load versus the response of the immune system. The immune system responds in two ways: by the innate immune pathway which is the first line of defence and not specific for microbes, and by the adaptive immune pathway, which develops in response to microbe antigen exposure. The innate immune response acts through pathogen recognition receptor molecules (PRRs) recognizing highly conserved pathogen associated molecular patterns (PAMPs) and thus differentiate between host and non-self molecules.[2, 3] These receptors, such as Toll-like receptors (TLRs) and CD14, are situated on surface epithelial cells and act as a first line of defense.[4, 5] Recognition of pathogens results in activation of downstream signalling molecules such as MyD88[3, 6], which eventually leads to induction of direct antimicrobial activity, phagocytosis and triggering and shaping of the adaptive immune response.[7] TLR4 is the main receptor for lipopolysaccharide (LPS) from Gram-negative bacteria such as *P. aeruginosa* (PA), whereas TLR2 interacts with a series of other bacterial ligands, including lipopeptides, peptidoglycan,and lipoteichoic acid of Gram-positive bacteria, such as Streptococcus and Staphylococcus.[8, 9] These bacteria are involved in COM.[1, 5, 7, 10, 11] One of the co-receptors of the TLR4 receptor complex, CD14, is also a high-affinity LPS-binding protein. Slight variations of the genes encoding TLR2, TLR4 and CD14 have been called single nucleotide polymorphisms (SNPs) and a set of closely linked SNPs a haplotype.[2, 3] They may lead to functional variations of these receptors occasionally altering the interaction and susceptibility to pathogens [2, 3, 9, 10-14] that also play a role in COM. It is unknown whether these functional polymorphisms also alter the clinical susceptibility to COM.

The objective of the present study was to establish whether several previously described TLR2, TLR4, CD14 and MyD88 gene polymorphisms are associated with COM. We therefore compared the prevalence of these genetic polymorphisms in children with COM with that in members of a birth cohort.

MATERIAL AND METHODS

Study population

In this case control study functional SNPs of a number of PRR genes in a group of children with chronic active mucosal otitis media were compared to frequencies of these SNPs from unselected participants from a birth cohort. Patients were children aged 1 to 12 years with a documented history of at least 3 months of continuous otorrhea through either a tympanic membrane perforation or a tympanostomy tube. They were referred to the department of Paediatric Otorhinolaryngology of the University Medical Center Utrecht between February 2003 and June 2005 to participate in a randomized controlled trial on the effectiveness of a 6 to 12 week course of sulfamethoxazol-trimethoprim. Exclusion criteria were reported in detail elsewhere.[15] Importantly, children with a known immune deficiency other than partial IgA or IgG subclass deficiency were excluded.

The control group consisted of members of a birth cohort of 1493 children born in Nijmegen, The Netherlands, between September 1982 and September 1983. In 2004, the home addresses of 1055 of the original cohort members could be traced and 406 subjects provided a blood sample.[16]

The medical ethical committee of the University Medical Center Utrecht had approved the study protocol. Written informed consent was obtained from all subjects or from their parents.

Single Nucleotide Polymorphisms in TLR, TLR4, CD14 and MyD88

Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) and stored at -20°C until analysis. Genotyping was carried out with sequence specific polymerase chain reaction (SSP-PCR) for the following polymorphisms TLR2 Pro631His, TLR2 Arg753Gln, TLR2/ -16934, TLR4 Thr399Ile, CD14/ -651, CD14 -260 and MyD88/ 1944. These polymorphisms were previously reported to result in an altered function of the pathogen recognition receptor.[2, 3, 10-12] The specific primer sequences are described in Table 3.2.1. SSP primers were manufactured by Sigma-Aldrich Chemie, Zwijndrecht, the Netherlands. The PCR conditions were as previously described.[17]

Statistical analysis

The allele frequencies were first tested for Hardy-Weinberg equilibrium to determine whether the allele frequencies found were according to the expected frequencies in a population that would be stable and in genetic equilibrium. Then, haplotypes for the SNP positions of the TLR2 gene and the CD14 gene were estimated from unphased genotype data using the Baysian statistical method in PHASE 2.1 (http://www.stat.washington.edu/stephens/software.html).[18, 19] Rate differences and 95% confidence intervals were calculated to compare the patient group and the control group for SNP polymorphisms and haplotypes. Subgroup analysis were performed for the children with COM according to age (≤3/>3 years).

Table 3.2.1. Specific primer sequences used to determine polymorphisms in TLR2, TLR4, CD14 and MyD88.

dbSNP identifier	Gene	SNP	Amino acid change	Primer sequence
rs4696480	TLR2	-16934 T/A		ATTGAAGGGCTGCATCTGGT
				ATTGAAGGGCTGCATCTGGA
				GTGTGCCCCAAAGCTCATG
rs5743704	TLR2	1892 C/A	p631h	CTGCTGGGAGCTTTCCTGG
				CTGCTGGGAGCTTTCCTGT
				AGCAAG CACTGGCCAAAGTCT
rs5743708	TLR2	2257 A/G	R753Q	AGGTCTTGGTGTTCATTATCTTCT
				GGTCTTGGTGTTCATTATCTTCC
				ATGATGTGGGCCTGGCTC
rs4986791	TLR4	8851 C/T	T399I	TCTCAAAGTGATTTTGGGACAAC
				TTCTCAAAGTGATTTTGGGACAAT
				GAGAGAGGTCCAGGAAGGTC
rs5744455	CD14	-651 C/T		AAGGGGGAATTTTTCTTTAGACC
				GAAGGGGGAATTTTTCTTTAGACT
				CTGAGGTTCGGAGAAGTTGC
rs2569190	CD14	-260 C/T		CAGAATCCTTCCTGTTACGGC
				CAGAATCCTTCCTGTTACGGT
				CTGAGGTTCGGAGAAGTTGC
rs4988457	MyD88	1944 C/G	intron	CTGGACAGTGCACAGCTAGC
				CTGGACAGTGCACAGCTAGG
				CTCTGAGGAGTATCATCTTGGGAA

RESULTS

Genetic polymorphisms were determined in 90 children with COM and in 375 unselected controls. The median age of the children with COM was 47 months and 74% had had their first episode of otorrhea before the age of 2 years. More than 90% of the participants in both groups were of Caucasian ethnicity.

All genotype frequencies of both groups were within Hardy-Weinberg Equilibrium, except for CD14/ -651 in the COM group (p=0.03) (*data not shown)*.

The allele carrier frequencies of the polymorphisms in the TLR2, TLR4, CD14 and MyD88 genes are presented in Figure 3.2.1. The frequency distribution of the wild type and mutant SNP alleles did not differ between the patients and the controls .

Table 3.2.2 shows the genotype frequencies for TLR2, TLR4, CD14 and MyD88 categorised in a dominant mutation model (at least one mutation variant of the SNP) and in

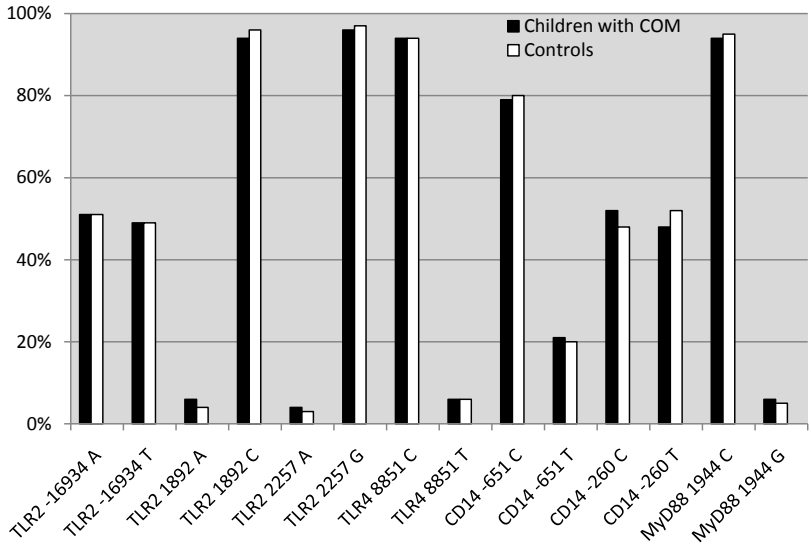

Figure 3.2.1: The frequency of wild type and mutant SNP alleles of children with COM and the healthy controls

a recessive model (homozygote mutant). There was a 10% difference in the genotype CD14/ -260 frequency in the homozygote model between children with COM and the controls (RD 10 [0; 20]). For all other genotypes, deviations were smaller than 10%.

PHASE 2.1 software constructed 4 different haplotypes from the TLR2 genotype data and 3 different haplotypes from the CD14 genotype data (Table 3.2.3). Haplotype frequencies did not differ between the patients and the controls.

Age did not influence genotype and haplotype frequencies in the patient group.

DISCUSSION

This is the first study on previously reported functional polymorphisms in the genes encoding TLR2, TLR4, CD14 and MyD88 in children with chronic active mucosal otitis media. We found no differences in genotype, allele (carrier) or haplotype frequencies between children with COM and unselected members of a birth cohort. It is therefore unlikely that these variations alter children's susceptibility to COM.

Most studies on functional polymorphisms in the TLR2, TLR4, CD14 and MyD88 genes indicating a potentially increased susceptibility to pathogens that play a role COM have been performed in vitro and in mice.[7, 9, 10, 13, 14, 20, 21] Studies in humans on the direct impact

Table 3.2.2. Genotype frequencies for TLR2, TLR4, CD14 and MyD88 SNPs categorised in dominant mutation models and recessive models

	Children with COM		Healthy controls		% difference (95%CI)
TLR2 -16934		N=90		N=375	
AA	19	(21%)	102	(27%)	-6 [-16; 4]
AT + TT	71	(79%)	273	(73%)	6 [-4; 16]
AA + AT	72	(80%)	279	(74%)	6 [-3; 15]
TT	18	(20%)	96	(26%)	-6 [-15; 3]
TLR2 1892		N=90		N=375	
CC	79	(88%)	345	(92%)	-4 [-11; 3]
CA + AA	11	(12%)	30	(8%)	4 [-3; 11]
CC + CA	90	(100%)	374	(100%)	
AA	0	(0%)	1	(0%)	
TLR2 2257		N=90		N=375	
GG	82	(91%)	356	(95%)	-4 [-10; 2]
GA + AA	8	(9%)	19	(5%)	4 [-2; 10]
GG + GA	90	(100%)	375	(100%)	
AA	0	(0%)	0	(0%)	
TLR4 8851		N=90		N=375	
CC	80	(89%)	333	(89%)	0 [-7; 7]
CT + TT	10	(11%)	42	(11%)	0 [-7; 7]
CC + CT	90	(100%)	372	(99%)	1 [0; 2]
TT	0	(0%)	3	(1%)	-1 [-2; 0]
CD14 -651		N=90		N=372	
CC	61	(68%)	242	(65%)	3 [-8; 14]
CT + TT	29	(32%)	130	(35%)	-3 [-14; 8]
CC + CT	82	(91%)	356	(96%)	-5 [-11; 1]
TT	8	(9%)	16	(4%)	5 [-1; 11]
CD14 -260		N=90		N=372	
CC	27	(30%)	75	(20%)	10 [0; 20]
CT + TT	63	(70%)	297	(80%)	-10 [-20; 0]
CC + CT	67	(74%)	284	(76%)	-2 [-12; 8]
TT	23	(26%)	88	(24%)	2 [-8; 12]
Myd88 1944		N=90		N=375	
CC	80	(89%)	334	(89%)	0 [-7; 7]
CG + GG	10	(11%)	41	(11%)	0 [-7; 7]
CC + CG	90	(100%)	375	(100%)	
GG	0	(0%)	0	(0%)	

Table 3.2.3. Haplotypes of TLR2; SNP sequence: -16934, 1892 and 2257 and haplotypes of CD14; SNP sequence: -651, -260

Haplotype	Children with COM		Healthy controls		Percentage difference [95%CI]	
TLR2	(N=90)		(N=375)			
TCG*	81	(45%)	350	(47%)	-2	[-10; 6]
TCA	8	(4%)	19	(3%)	1	[-2; 4]
ACG	80	(44%)	350	(47%)	-3	[-11; 5]
AAG	11	(6%)	31	(4%)	2	[-2; 6]
CD14	(N=90)		(N=372)			
CC#	57	(32%)	214	(28%)	4	[-4; 12]
CT	86	(48%)	384	(52%)	-4	[-12; 4]
TC	37	(21%)	145	(19%)	2	[-5; 9]

*TCG = wild type for SNP -16934, Pro631His and Arg753Gln, respectively
#CC = wild type for SNP -651 and -260, respectively

of these polymorphisms on the susceptibility to infectious diseases have been sparse and results have been inconsistent due to considerable heterogeneity and limited numbers of participants.[3, 10, 11]

Our results need to be interpreted in light of several limitations.

First, polymorphisms in innate immunity genes may have the highest impact early in life between 4 and 11 months of age, when maternally derived antibodies have waned and the adaptive immune system of the young child is not yet fully developed.[11, 12] Patients in our study developed their symptoms of COM between 1 and 12 years of age, so may have been at an age when innate immunity may be less important in the susceptibility to COM. On the other hand, 74% of the patients had their first episode of otorrhea before the age of 2 years and a subgroup analyses according to age (≤3 versus >3 years) did not show a higher frequency of the studied polymorphisms in the younger subgroup with COM. This shows that there is no increased susceptibility to COM in younger children carrying the studied polymorphisms.

Second, since our control population was a birth cohort, it is likely that some of its members have a history of COM. However, the prevalence of COM in the general population of The Netherlands as reflected by our birth cohort is less than 1 percent. It is therefore unlikely that this has influenced our results.

Third, the adult subjects available for evaluation of functional polymorphisms in the PRR genes (N=406) may not be representative for the original birth cohort of 1328 members. However, no important differences were found in baseline characteristics between participating subjects and the entire birth cohort.[16] Moreover, it is unlikely that selection according to genetic differences or susceptibility to infections occurred.

Fourth, we studied only a restricted number of previously reported functional polymorphisms of single genes and found no association with COM. In the future, whole gene analyses or tagging genes should determine whether other genetic variations are associated with COM.

In conclusion, we have found no evidence supporting the hypothesis that previously reported functional polymorphisms of the TLR2, TLR4, CD14 and MyD88 genes are associated with an increased susceptibility to chronic active otitis media.

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Chapter 4

Trial results

Chapter 4.1

Effectiveness of trimethoprim/sulfamethoxazole for children with chronic active otitis media: A randomized, placebo-controlled trial

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Pediatrics 2007 May;119(5):897-904.

ABSTRACT

Objectives: The goal was to determine the clinical effectiveness of prolonged outpatient treatment with trimethoprim/sulfamethoxazole for children with chronic active otitis media.

Methods: We performed a randomized, placebo-controlled trial with 101 children (1–12 years of age) with chronic active otitis media (defined as otorrhea for ≥12 weeks). In addition to a short course of steroid and antibiotic eardrops, children were assigned randomly to receive 6 to 12 weeks of orally administered trimethoprim/sulfamethoxazole (18 mg/kg, 2 times per day) or placebo and were monitored for 1 year.

Results: At 6 weeks, 28% of children in the trimethoprim/sulfamethoxazole group and 53% of children in the placebo group had otomicroscopic signs of otorrhea. At 12 weeks, these values were 32% and 47%, respectively. At 1 year, the numbers of children with otorrhea were similar in the 2 groups (25% and 20%, respectively). One child in the trimethoprim/sulfamethoxazole group developed a skin rash. Vomiting or diarrhea was reported for 9% of the trimethoprim/sulfamethoxazole group and 2% of the placebo group. Pure-tone hearing levels and health-related quality of life improved during the study but did not differ between the trimethoprim/sulfamethoxazole group and the placebo group. *Pseudomonas aeruginosa* was the most frequently isolated bacteria in the otorrhea samples from both groups.

Conclusions: A 6- to 12-week course of high-dose, orally administered trimethoprim/sulfamethoxazole therapy is beneficial for children with chronic active otitis media. The treatment effect is most pronounced with the shorter course and disappears if administration of the medication is discontinued.

INTRODUCTION

Chronic active (mucosal) otitis media (COM) is a common infectious disease, affecting children in both developing and industrialized countries.[1–3] It causes considerable morbidity and is a major global cause of hearing impairment in children. Moreover, it may lead to serious extracranial and intracranial complications, such as mastoiditis and meningitis.[4–8] An active approach in the management of COM is therefore important.[6] Evidence regarding the most effective medical or surgical treatment of COM is incomplete; few randomized, controlled trials have been performed, and inclusion criteria, outcome measures, and follow-up methods in those studies vary considerably.[9–16]

Trimethoprim/sulfamethoxazole has been used for many years for the treatment of upper respiratory tract infections in children. It is an inexpensive antimicrobial drug and is well tolerated by children. When used for prophylaxis for recurrent acute otitis media, it was found to be effective.[17,18] A retrospective analysis of data for children with COM treated with trimethoprim/sulfamethoxazole for a prolonged period at our hospital showed promising results. We therefore initiated a randomized, placebo-controlled trial of a 6- to 12-week course of orally administered trimethoprim/sulfamethoxazole in addition to a short course of steroid and antibiotic eardrops for children with COM who had experienced failure of conventional management with topical medications and/or short-term systemic antibiotic treatment. In this article, we report on both the clinical effectiveness and bacteriologic findings.

METHODS

Patients

We conducted a randomized, placebo-controlled trial between February 2003 and June 2006. Otorhinolaryngologists and pediatricians from across the Netherlands referred potential participants (ie, children with COM who had experienced failure of conventional management with topical medications and/or short-term systemic antibiotic treatment) to the pediatric otorhinolaryngology department of the University Medical Center Utrecht. Inclusion criteria were age of 1 to 12 years and a documented history of ≥3 months of continuous otorrhea through either a tympanic membrane perforation or a tympanostomy tube. We excluded children with (1) cholesteatoma, (2) known immunodeficiency other than for IgA or IgG subclasses, (3) Down syndrome, (4) craniofacial anomalies, (5) cystic fibrosis, (6) primary ciliary dyskinesia, (7) allergy to trimethoprim/sulfamethoxazole, or (8) continuous use of antibiotics for >6 weeks in the past 6 months. The medical ethics committee of the University Medical Center Utrecht approved the study protocol.

Randomization

Children whose parents gave informed consent were assigned randomly to receive either trimethoprim/sulfamethoxazole (18 mg/kg, administered orally, 2 times per day) or placebo for 6 to 12 weeks. A computer-generated randomization list was prepared by an independent data manager and sent to the hospital pharmacist, who then provided numbered boxes with bottles filled with a blinded suspension of either trimethoprim/sulfamethoxazole or placebo, with identical taste, bottle appearance, and fluid appearance. At entry into the trial, the investigator responsible for seeing the study participants allocated the next available number on the randomization list and the corresponding box with blinded suspension to each participant. The investigators remained blinded to the randomization until the end of the study.

When otorrhea was found to be present in either ear at the first control visit after 6 weeks, study medication administration was continued for another 6 weeks. Administration of the study medication was discontinued if both ears were found to be free of otorrhea and parents confirmed that they had seen no signs of otorrhea during the previous week. Parents were instructed to start study medication treatment again if symptoms of otorrhea recurred between the follow-up visits at 6 and 12 weeks. At inclusion and if otorrhea was present at the 6-week and 12-week follow-up visits, hydrocortisone/bacitracin/colistin eardrops were prescribed in addition to the study medication for 7 to 10 days, in both the trimethoprim/sulfamethoxazole group and the placebo group. These eardrops were chosen because they are widely used in the Netherlands and are generally considered safe.[6] From July 2004 onward, hydrocortisone/neomycin/polymyxin B eardrops were prescribed because the former combination was no longer available in the Netherlands. During the first 12 weeks, local otorhinolaryngologists and pediatricians were allowed to prescribe additional medication, except for trimethoprim/sulfamethoxazole, to the participants according to their regular practice. After the second control visit at 12 weeks, administration of the study medication was discontinued irrespective of the presence or absence of otorrhea. At that time, children were referred back to their local doctors. Treatment was unblinded for an independent doctor, who informed the parents and local doctors about the assigned treatment by letter. The letter also included a treatment recommendation in case otorrhea was still present or recurred, that is, trimethoprim/sulfamethoxazole (18 mg/kg, 2 times per day, for 6–12 weeks) for the placebo group and azithromycin (5 mg/kg, once per day, for 6–12 weeks) for the trimethoprim/sulfamethoxazole group. At that stage, local otorhinolaryngologists and pediatricians were free to follow the recommendations or to manage symptoms of otorrhea according to their regular practice.

Inclusion and Follow-up Monitoring

At inclusion, disease-specific questionnaires, including information on potential risk factors for ear disease, duration of otorrhea before study entry, and previous treatments, were completed. At inclusion and at the 3 follow-up visits at 6 weeks, 12 weeks, and 1 year, parents completed 1 generic and 2 disease-specific questionnaires on health-related quality of life, namely, the Child Health Questionnaire parental form,[19] a 6-item otitis media questionnaire,[20] and a visual analog scale measuring ear-related quality of life.[20] At these visits, the ears of the children were examined with an otomicroscope. The following features were noted: tympanostomy tube, tympanic membrane perforation, otorrhea, and middle ear effusion. If otorrhea was present, then a swab was taken from the otorrhea before suction cleaning was performed. To test for adverse reactions to the study medication, venous samples were taken for complete blood counts and hepatic (aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltranspeptidase) and renal (urea and creatinine) function tests at inclusion, at 6 weeks, and at 12 weeks. For children >3 years of age, pure-tone air conductive hearing levels were measured at frequencies of 500, 1000, 2000, and 4000 Hz.Parents kept a diary of study medication and additional medication used for their child's' ear disease, including eardrops. These data were collected at the follow-up visits. During those visits, adverse effects of the study medication were noted and the empty and full bottles of study medication were weighed, to determine compliance rates.

Microbiologic Investigation

At inclusion and at the follow-up visits, study physicians took otorrhea samples by using flexible, sterile, rayon-tipped swabs (Medical Wire & Equipment Co, Corsham, Wiltshire, United Kingdom). The samples were immediately stored in Stuart's transport medium at room temperature. Samples were transported to the microbiology laboratory and plated, within 18 hours after sampling, onto sheep blood (5%), *Haemophilus*, and MacConkey agar plates for the isolation of potential aerobic pathogens. The culture plates were incubated aerobically at 37°C (MacConkey agar) and <5% carbon dioxide (blood and *Haemophilus* agars). They were examined at 24 and 48 hours. Colonies suspected to be *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, or aerobic Gram-negative bacteria were identified with previously described methods.[21]

Study Outcomes

The primary end point was otomicroscopic signs of otorrhea in the presence of a tympanostomy tube or tympanic membrane perforation at follow-up times of 6 weeks, 12 weeks, and 1 year. Secondary outcome measures were use of medication other than the study medication for ear disease, adverse effects of the study medication, ear/nose/

throat operations, health-related quality of life, pure-tone hearing levels, and bacteriologic findings for the otorrhea samples.

Statistical Aspects

Assuming a spontaneous recovery of 25% and a treatment effect of trimethoprim/sulfamethoxazole of 50% (based on a retrospective study of children treated with trimethoprim/sulfamethoxazole for COM at our hospital) and using an α of 0.05 and a power of 0.80, we calculated that each group should consist of 50 children. Rate differences (RDs) with 95% confidence intervals (CIs) were calculated at the 3 control visits, to compare the 2 groups for otomicroscopic results, use of medication other than the study medication for ear disease, and adverse effects of the study medication. To detect possible effect modification, subgroup analyses were performed according to age (≤3 years or >3 years) and duration of otorrhea before study entry (≤6 months or >6 months), as prespecified in the trial protocol. Health-related quality-of-life instrument scores were transformed linearly onto scales of 0 to 100. The differences between the scores at the follow-up visits and at study entry were calculated and presented for each domain. Differences in domain scores between the groups at follow-up times of 0 weeks, 6 weeks, 12 weeks, and 1 year were tested with the Mann-Whitney *U* test, because these scores were not normally distributed. Box and whisker plots were used to compare the pure-tone hearing levels (air conduction at 500, 1000, 2000, and 4000 Hz) between the 2 groups. Percentage differences with 95% CIs were calculated for the bacteriologic findings. All analyses were performed on an intention-to-treat basis.

RESULTS

Study Group

Between February 2003 and November 2005, 101 children were enrolled; 50 were allocated to the trimethoprim/sulfamethoxazole group and 51 to the placebo group. The flow of the participants through the trial is presented in Figure 4.1.1. At baseline, clinical characteristics did not differ significantly between the 2 groups (Table 4.1.1). The median age of the study participants was 50 months (interquartile range: 55 months). The compliance rates for both the trimethoprim/sulfamethoxazole group and the placebo group were good (ie, >90% of the prescribed study medication was used).

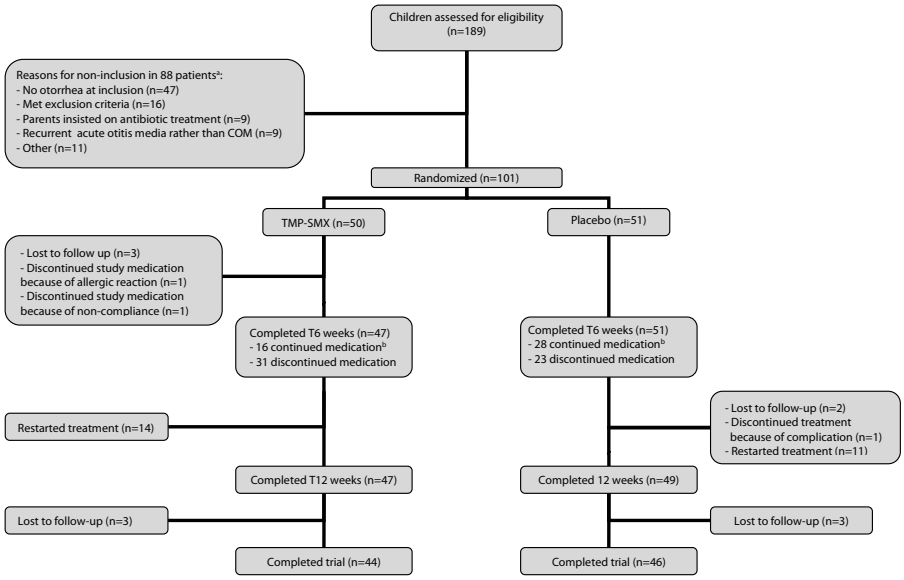

Figure 4.1.1. Flow of participants through the trial.
TMP-SMX indicates trimethoprim/sulfamethoxazole; T6, 6-week follow-up; T12, 12-week follow-up. [a]The number exceeds 87 because >1 reason could be indicated. [b]Administration of the study medication was discontinued if both ears were found to be free from otorrhea and parents confirmed that they had seen no signs of otorrhea during the previous week.

Outcomes

At the 6-week follow-up visit, otorrhea was present for 28% of children in the trimethoprim/sulfamethoxazole group and 53% in the placebo group (RD: –25%; 95% CI: –44% to –6%; number needed to treat [NNT]: 4 children) (Table 4.1.2). At 12 weeks, the RD was still –15% (95% CI: –34% to 4%; NNT: 7 children) in favor of the trimethoprim/sulfamethoxazole group. At 1 year, there was no difference between the 2 groups (RD: 5%; 95% CI: –12% to 22%). At follow-up times of 6 weeks, 12 weeks, and 1 year, more children in the trimethoprim/sulfamethoxazole group had bilateral intact tympanic membranes and aerated middle ears than did those in the placebo group.

Otomicroscopic results were also analyzed according to age and duration of otorrhea before study entry. At the 6-week follow-up visit, otorrhea was present in 8 children (29%) with >6 months of otorrhea before study entry who were treated with trimethoprim/sulfamethoxazole and 14 (67%) who were treated with placebo (RD: –38%; 95% CI: –64% to –12%; NNT: 3 children). For the children with 3 to 6 months of otorrhea before study entry, these values were 5 (26%) versus 13 (43%) (RD: –17%; 95% CI: –44% to 10%; NNT: 6 children). At the 12-week follow-up visit, these numbers were 11 (39%) versus 15 (71%)

Table 4.1.1: Characteristics of patients with COM in the TMP-SMX group and the placebo group; number (percentage)

Characteristics	TMP-SMX N=50	Placebo N=51
Male	28 (56)	26 (51)
Age in months [a]	48 (12; 144)	51 (15; 143)
Duration of otorrhea before study entry in months [a]	8 (3; 113)	5 (3; 116)
Previous treatment		
Ototopical drops	50 (100)	50 (98)
Systemic antibiotics	48 (96)	46 (90)
Surgery		
adenoidectomy and/or tonsillectomy	30 (60)	36 (71)
tympanostomy tubes	45 (90)	46 (90)
tympanoplasty and/or mastoidectomy	5 (10)	7 (14)
Number of siblings [a]	2 (0; 6)	1 (0; 3)
Family history of OM (parents, siblings)	29 (58)	28 (55)
Number of upper respiratory tract infections in 6 months before study entry [a]	6 (0; 8)	6 (0; 7)
Daycare or school in year before study entry	45 (90)	47 (92)
Parental smoking	16 (32)	13 (26)
Use of systemic antbiotics during the last 2 weeks	3 (6)	2 (4)
Unilateral versus bilateral COM [b]	25 (50) vs 25 (50)	30 (59) vs 21 (41)
Tympanostomy tubes [b]	32 (64)	29 (57)
Tympanic membrane perforation [b]	24 (48)	27 (53)

[a] median (range)
[b] at inclusion
Note: 11 children had a tympanostomy tube in one ear and a tympanic membrane perforation in the other; 6 in the TMP-SMX group and 5 in the placebo group

(RD: –32%; 95% CI: –59% to –5%; NNT: 3 children) and 4 (21%) versus 8 (29%) (RD: –8%; 95% CI: –33% to 17%; NNT: 13 children), respectively. At the follow-up time of 1 year, the treatment effect was no longer affected by the duration of otorrhea before study entry. Age did not influence the effectiveness of trimethoprim/sulfamethoxazole.

During the first 6 weeks, 38 (83%) of the children in the trimethoprim/sulfamethoxazole group and 39 (77%) of the children in the placebo group used antibiotic eardrops (RD: 6%; 95% CI: –10% to 22%). After the first 6 weeks, antibiotic eardrops were used slightly more often in the placebo group than in the trimethoprim/sulfamethoxazole group; 21 (55%) in the trimethoprim/sulfamethoxazole group and 26 (67%) in the placebo group (RD: –12%; 95% CI: –34% to 10%) used eardrops between follow-up times of 6 weeks and 12 weeks. Between follow-up times of 12 weeks and 1 year, these figures were 78% and 82% (RD: –4%; 95% CI: –22% to 14%), respectively. Systemic antibiotics other than

Table 4.1.2: Otomicroscopic results at the follow-up visits; number (%)

Otoscopy	T6 weeks			T12 weeks			T1 year		
	TMP-SMX (N=47)	Placebo (N=51)	% Difference (95%CI)	TMP-SMX (N=47)	Placebo (N=49)	% Difference (95%CI)	TMP-SMX (N=44)	Placebo (N=46)	% Difference (95%CI)
Otorrhea[a] in the presence of:	13 (28)	27 (53)	-25 (-44; -6)	15 (32)	23 (47)	-15 (-34; 4)	11 (25)	9 (20)	5 (-12; 22)
tympanostomy tube	6 (13)	11 (22)	-9 (-24; 6)	9 (19)	10 (20)	-1 (-17; 15)	5 (11)	2 (4)	7 (-4; 18)
tympanic membrane perforation	7 (15)	16 (31)	-16 (-32; 0)	6 (13)	13 (28)	-15 (-31; 1)	4 (9)	6 (13)	-4 (-4 (-17; 9)
Bilateral intact tympanic membrane and aerated middle ear	2 (4)	0 (0)	4 (-2; 10)	4 (9)	0 (0)	9 (1; 17)	7 (16)	5 (11)	5 (-9; 19)

[a] in either ear

TMP-SMX: trimethoprim-sulfamethoxazole

the study medication (mostly amoxicillin) were used by 4 children (11%) in the trimethoprim/ sulfamethoxazole group and 7 children (18%) in the placebo group between inclusion and the follow-up time of 12 weeks (RD: –7%; 95% CI: –23% to 9%). Between 12 weeks and 1 year, these figures were 23 (62%) and 18 (47%), respectively (RD: 15%; 95% CI: –7% to 37%), and trimethoprim/sulfamethoxazole and azithromycin were used most frequently. Ear/nose/throat surgery (tympanostomy tube insertion or removal, $n = 13$; adenotonsillectomy, $n = 7$; tympanomastoidectomy and/or tympanoplasty, $n = 5$) was performed for similar numbers of children in the 2 groups between follow-up times of 12 weeks and 1 year, that is, 13 (30%) in the trimethoprim/sulfamethoxazole group and 11 (24%) in the placebo group (RD: 6%; 95% CI: –12% to 24%).

Figure 4.1.2 shows box and whisker plots of the hearing levels for children >3 years of age. Pure-tone air conduction levels at 500, 1000, 2000, and 4000 Hz could be determined for 20 children in the trimethoprim/sulfamethoxazole group and 18 children in the placebo group. Although hearing levels generally improved, no differences between the groups were found.

During the study, the health-related quality-of-life scores improved substantially in both the trimethoprim/sulfamethoxazole and placebo groups (data not shown). Mean scores for the trimethoprim/sulfamethoxazole and placebo groups for the 6-item otitis media questionnaire, Child Health Questionnaire, and visual analog scale were the same at all visits.

Table 4.1.3 shows that, at follow-up times of 6 weeks, 12 weeks, and 1 year, there were no differences in the proportions of culture-positive otorrhea samples for the trimethoprim/ sulfamethoxazole group and the placebo group (6 weeks: RD: 0%; 95% CI: –14% to 14%; 12 weeks: RD: 3%; 95% CI: –20% to 26%; 1 year: RD: –8%; 95% CI: –23% to 7%). At follow-up times of 6 and 12 weeks, *Pseudomonas aeruginosa* was the most frequently isolated microorganism in both groups and, in contrast to most other organisms, was found more frequently in the otorrhea samples of the trimethoprim/ sulfamethoxazole group than in those of the placebo group, that is, 9 (56%) vs 12 (38%) at 6 weeks (RD: 18%; 95% CI: –12% to 48%) and 7 (50%) vs 9 (31%) at 12 weeks (RD: 19%; 95% CI: –12% to 50%). At the follow-up time of 1 year, no differences were found between the groups.

Adverse Effects

During the first 6 weeks, vomiting or diarrhea (potential adverse effects of the study medication) were reported for 9% of the trimethoprim/sulfamethoxazole group and 2% of the placebo group (RD: 7%; 95% CI: –2% to 16%; number needed to harm: 14 children). Between 6 and 12 weeks, no differences between the groups were found. One child in the trimethoprim/sulfamethoxazole group developed a skin rash; she was treated with cetirizine and administration of the study medication was discontinued, after which the

rash disappeared. Treatment of COM was continued with azithromycin. Complete blood count and hepatic and renal function tests at inclusion and at follow-up times of 6 weeks and 12 weeks did not show any abnormalities in either group.

Two children developed mastoiditis during the first 12 weeks of follow-up monitoring, and their randomization codes were unblinded immediately. One child was allocated to trimethoprim/sulfamethoxazole; he was treated with a mastoidectomy and amoxicillin/clavulanic acid, administered intravenously for 7 days and orally for 14 days. The other child was allocated to placebo; he was treated with intravenously administered amoxicillin/clavulanic acid for 7 days, followed by 6 weeks of trimethoprim/sulfamethoxazole. Both children recovered well with this therapy.

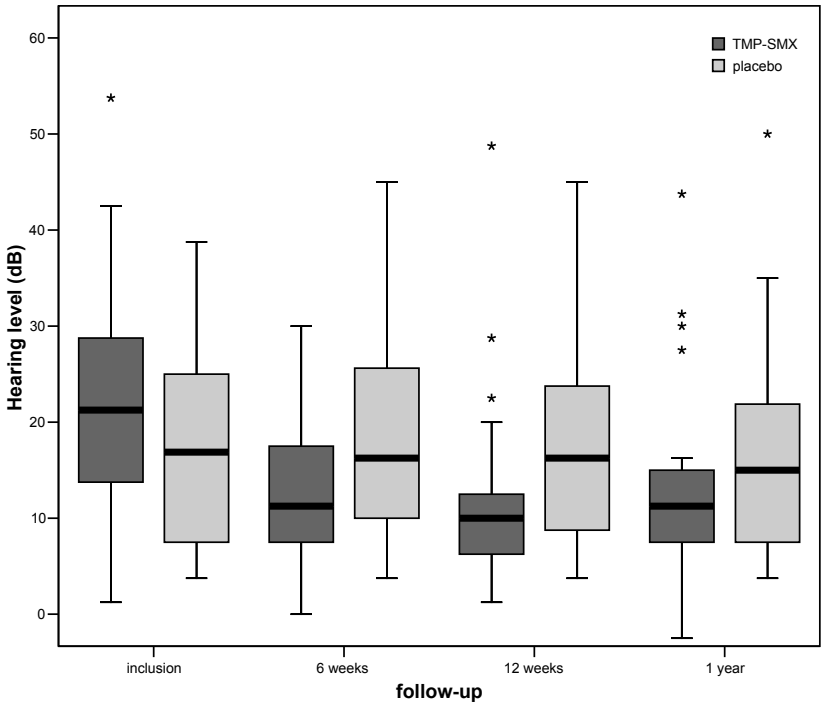

Figure 4.1.2. Box and whisker plots[22] of the average air-conduction levels at 500, 1000, 2000, and 4000 Hz at inclusion and at 6 weeks, 12 weeks, and 1 year.
Values are expressed as the median (horizontal line in each box), with the quartiles (top and bottom of the box) and range (I bar), excluding out-of-range observations ([a] ≥1.5 times the interquartile range beyond the quartile). TMP-SMX indicates trimethoprim/sulfamethoxazole.

Table 4.1.3: Bacteriological findings in the otorrhea samples of the children at inclusion and during follow-up (per sample taken)

Bacterial species	N (%)																	
	Inclusion				T6 weeks					T12 weeks								
	TMP-SMX N=74		Placebo N=70		TMP-SMX N=16		Placebo N=32		Difference (95%CI)	TMP-SMX N=14		Placebo N=29		Difference (95%CI)	TMP-SMX N=13		Placebo N=11		Difference (95%CI)
Positive culture	68	(92)	59	(84)	15	(94)	30	(94)	0 (-14;14)	12	(86)	24	(83)	3 (-20;26)	12	(92)	11	(100)	-8 (-23;7)
S.pneumoniae	10	(14)	7	(10)	0	(0)	5	(16)	-16 (-29;-3)	0	(0)	4	(14)	-14 (-27;-1)	1	(8)	1	(9)	-1 (-23;21)
Hemolytic Streptococci Group A	4	(5)	1	(1)	0	(0)	2	(6)	-6 (-14;2)	1	(7)	3	(10)	-3 (-20;14)	1	(8)	0	(0)	8 (-7;23)
H.influenzae	13	(18)	23	(33)	0	(0)	6	(29)	-29 (-45;-13)	1	(7)	9	(31)	-24 (-45;-3)	2	(15)	1	(9)	6 (-20;32)
S.aureus	15	(20)	14	(20)	2	(13)	6	(19)	-6 (-27;15)	1	(7)	6	(21)	-14 (-34;6)	2	(15)	1	(9)	6 (-20;32)
P.aeruginosa	32	(43)	22	(31)	9	(56)	12	(38)	18 (-12;48)	7	(50)	9	(31)	19 (-12;50)	6	(46)	6	(55)	-9 (-49;31)
M. catarrhalis	1	(1)	6	(9)	0	(0)	1	(3)	-3 (-9;3)	0	(0)	3	(10)	-10 (-21;1)	0	(0)	0	(0)	0 (0;0)
Other organisms	53	(72)	23	(33)	10	(63)	12	(38)	25 (-4; 54)	4	(29)	10	(34)	-5 (-34;24)	7	(54)	9	(82)	-28 (-63;7)
Total n species	128		95		21		44			14		44			19		18		

TMP-SMX: trimethoprim-sulfamethoxazole

DISCUSSION

This is the first placebo-controlled, randomized trial of systemic antibiotic treatment for patients with COM. It showed that a 6- to 12-week high-dose course of trimethoprim/sulfamethoxazole in addition to a short course of steroid and antibiotic eardrops had a cure rate of 68% at the follow-up time of 12 weeks and was clinically more effective than placebo for children with COM who had experienced failure of conventional management with topical medications or short-term systemic antibiotic therapy. This treatment effect was most pronounced during the first 6 weeks. Children with a history of otorrhea for >6 months benefited more from trimethoprim/sulfamethoxazole therapy than did those with a shorter history of otorrhea. Age did not influence the effectiveness of trimethoprim/sulfamethoxazole. Pure-tone hearing levels and health-related quality of life improved during the study but did not differ between the trimethoprim/sulfamethoxazole group and the placebo group.

The effect of treatment with trimethoprim/sulfamethoxazole for COM was studied in one previous trial, in which a 2-week course of trimethoprim/sulfamethoxazole was compared with a course of antibiotics based on culture results.[9] After a maximal follow-up period of only 14 days, otorrhea resolved for 75% of the trimethoprim/sulfamethoxazole group and 85% of the patients treated with culture-directed antibiotics. Other studies using various systemic antibiotics for COM found similar success rates of ~70%.[10–14,16] Inclusion and outcome criteria, route of drug administration, and follow-up periods in those studies varied considerably, and no study was placebo controlled.[2]

Our results need to be interpreted in light of several limitations. First, the children in our study had persistent symptoms of COM despite conventional management with topical medications, short-term systemic antibiotic therapy, and/or previous ear surgery. Because the majority of children with otorrhea seen by generalist physicians respond well to conventional management, our results should be applied to patients with similarly complicated COM.

Second, because the Netherlands is known for its restrictive policy regarding systemic antibiotic treatment for otitis media, it is possible that before study entry the participants had received fewer courses of systemic antibiotics and more courses of topical antibiotics than would be expected in other countries. A meta-analysis by Macfadyen et al,[16] however, showed that topical antibiotics, such as those used by our participants before study entry, were more effective than short courses of systemic antibiotic therapy in resolving otorrhea. Therefore, we think that our results can be extrapolated to countries where short-term systemic antibiotic therapy is used more frequently for the management of COM.

Third, children in our study all received suction cleaning and topical treatment, in addition to the study medication, when otorrhea was present. Antibiotic eardrops

were used slightly more frequently in the placebo group than in the trimethoprim/sulfamethoxazole group between follow-up times of 6 and 12 weeks. This might have influenced the high cure rate in the placebo group at 12 weeks, which might have resulted in an underestimation of the real treatment effect of trimethoprim/sulfamethoxazole. Other important factors for the small treatment effect with the longer course might have been the natural course of COM and regression to the mean.

Fourth, at the follow-up time of 12 weeks, the parents and the local otolaryngologist or pediatrician were informed about the assigned treatment, and doctors were free to manage additional symptoms of COM in both groups either according to our advice (with a 6-week course of antibiotics) or according to their own practice. Our follow-up data revealed that 10 children in the placebo group and 7 children in the trimethoprim/sulfamethoxazole group indeed received a prolonged course of antibiotics after the follow-up period of 12 weeks. This might have added to the dilution of the effect after 12 weeks.

Fifth, the choice of trimethoprim/sulfamethoxazole for COM could be questioned, because *P aeruginosa*, which is the most common organism in COM and was present in 54 (38%) of otorrhea samples at inclusion, is known to be unsusceptible to trimethoprim/ sulfamethoxazole. This is reflected by our culture results; during treatment with trimethoprim/ sulfamethoxazole, the proportion of otorrhea samples positive for *P aeruginosa* did not change, whereas that of bacteria that are not intrinsically resistant to trimethoprim/sulfamethoxazole, such as *H influenzae* and *S pneumoniae*, decreased. Because trimethoprim/sulfamethoxazole was effective during the first 6 to 12 weeks of our study, *P aeruginosa* seems to be a secondary microorganism in COM, rather than the causative microorganism.

CONCLUSIONS

A 6- to 12-week course of high-dose, oral, trimethoprim/sulfamethoxazole therapy is beneficial for children suffering from COM. The treatment effect is most pronounced with the shorter course and disappears if administration of the medication is discontinued.

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Chapter 4.2

Trimethoprim-sulfamethoxazole in children with chronic otitis media: a randomized comparison of costs and effects

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Otology & Neurotology. 2008; 29(7):961-4.

ABSTRACT

Objective: To study the cost-effectiveness of a 6- to 12-week course of high-dose oral trimethoprim-sulfamethoxazole in children with chronic active otitis media (COM).

Study design: Cost-effectiveness study including both direct and indirect costs alongside a randomized placebo-controlled trial.

Patients: One hundred one children aged 1 to 12 years with a documented history of COM for at least 3 months treated.

Intervention: Six to 12 weeks of oral trimethoprim-sulfamethoxazole 18 mg/kg twice daily versus placebo.

Main outcome measures: Incremental cost-effectiveness in terms of costs per number needed to treat (NNT) to cure 1 patient (incremental cost-effectiveness ratio [ICER]). Curation was defined as no otomicroscopic signs of otorrhea in either ear.

Results: After 6 weeks of follow-up, the difference in mean cost per patient between the trimethoprim-sulfamethoxazole and placebo groups was Euro100 (US $126). The NNT was 4 (clinical effect), and the corresponding ICER was Euro400 (US $504), that is, the average extra costs to cure 1 child from otorrhea is Euro400 (US $504). After 12 weeks of follow-up, the difference in mean costs between both groups was Euro159 (US $201), the NNT was 7, and the corresponding ICER was Euro1,113 (US $1,407).The mean costs after 1 year of follow-up were Euro1,601 (US $2,021) in the trimethoprim-sulfamethoxazole group and Euro1,164 (US $1,469) in the placebo group. Because the clinical effect of trimethoprim-sulfamethoxazole disappeared after its discontinuation, we did not calculate an ICER at 1 year of follow-up.

Conclusion: In children with active COM, direct and indirect costs of a 6- to 12-week course of high-dose oral trimethoprim-sulfamethoxazole are modest in the light of its short-term clinical benefit.

INTRODUCTION

Chronic active otitis media (COM) is one of the most common infectious diseases, affecting children both in developing and industrialized countries.[1,2] It causes considerable morbidity and is a major global cause of hearing impairment in children.[2] Moreover, it may lead to serious extracranial and intracranial complications, such as mastoiditis and meningitis.[3,4]

Direct and indirect costs related to the management of otitis media are high.[5-10] So far, however, the costs of various treatments of COM have not been studied in the light of their clinical effectiveness. This is an essential element in the discussion about optimal management of COM in different settings.

In a recent randomized placebo-controlled trial, we have shown that a 6- to 12-week course of high-dose oral trimethoprim-sulfamethoxazole is clinically effective in children with COM.[11] The objective of this study is to establish whether trimethoprim-sulfamethoxazole is also cost-effective.

METHODS

Patients

This cost-effectiveness study was performed alongside a randomized placebo-controlled trial conducted in a tertiary care university hospital in the Netherlands between February 2003 and June 2006. The study was approved by the medical ethical committee of the hospital. The design of the study has been reported previously.[11] In brief, children aged 1 to 12 years with a documented history of at least 3 months of continuous otorrhea through either a tympanic membrane perforation or a tympanostomy tube were included in the study. Children with cholesteatoma, known immune deficiency other than for immunoglobulin A or G subclasses, Down syndrome, craniofacial anomalies, cystic fibrosis, primary ciliary dyskinesia, and allergy to trimethoprim-sulfamethoxazole or those who had used antibiotics continuously for more than 6 weeks in the past 6 months were excluded from the study.

Randomization

Children for whom informed consent was obtained were randomly assigned to either oral trimethoprim-sulfamethoxazole (18 mg/kg, twice daily) or placebo, for 6 weeks. When otorrhea was found to be present in either ear at the first follow-up visit after 6 weeks, the treatment was continued for another 6 weeks. The study medication was discontinued if both ears were found to be free of otorrhea and parents confirmed that they had seen no signs of otorrhea during the previous week. Parents were instructed to

start the study medication again if symptoms of otorrhea recurred between the first follow-up visit at 6 weeks and the second at 12 weeks. In addition to the study medication, both the trimethoprim-sulfamethoxazole and placebo groups were given combined antibiotic/steroid eardrops for 7 to 10 days at inclusion and if otorrhea was present at either of the follow-up visits. Between February 2003 and June 2004, hydrocortisone/bacitracin/colistin eardrops were prescribed. From July 2004 onward, hydrocortisone/neomycin/polymyxin B eardrops were prescribed because the former combination was no longer available in the Netherlands. All study medication was discontinued after 12 weeks of follow-up irrespective of the presence or absence of otorrhea. At that time, children were referred back to their local physician. Treatment was unblinded by an independent physician, who informed the parents and local physicians about the assigned treatment by letter. The letter also included a treatment recommendation in case otorrhea was still present or would recur. Local physicians were, however, free to follow these recommendations or to manage symptoms of otorrhea according to their regular practice.

At 1 year after inclusion, children were examined once more by the study physician.

Outcome measures

The primary clinical outcome used for this economic evaluation is the number needed to treat (NNT) to cure 1 patient. Curation was defined as no otomicroscopic signs of otorrhea in either ear.

Costs

Costs were estimated at the patient level for the year 2005. A societal perspective was used, and costs were retrieved from available sources where possible. When unavailable, unit costs were estimated in a separate costing study. Parents recorded resources used, such as over-the-counter (OTC) drugs and outpatient visits in diaries. Out-of-pocket expenses such as "baby-sitters" and travel expenses were also recorded in these diaries. The study physician verified diary entries during the follow-up visits. Costs of medication, including antibiotics, were derived from the Dutch formulary[12] and increased with a pharmacist's fee.[13] Costs of OTC drugs and complementary and alternative medicines were based on average retail prices.

Indirect costs to society related to parental leave of absence were estimated using the friction-cost method.[13] The indirect cost related to parental lost of housekeeping activities was valued by using the costs related to housekeeping as paid work.

Costs per patient were calculated by multiplying the prospectively measured quantities per patient with unit cost prices. For medication, the costs of a standard dose per week were calculated. If more than 1 cost price was available, the mean price was used. The costs of physician consultations (family physician; pediatrician; or ear, nose, and

throat surgeon) and hospitalization were valued according to current Dutch guidelines for pharmacoeconomic evaluation.[13] The costs of ear, nose, and throat operations were calculated based on resource use and personnel input. Older prices were adjusted to the price level of 2005 with price index numbers.[13] The exchange rate of June 2005 was used to compare the euro with the US dollar (Euro1 = US $1.26210).

Statistical analysis

Rate differences (RDs) with 95% confidence intervals (CIs) were calculated at the 3 follow-up visits, to compare the 2 groups for otomicroscopic signs of otorrhea. The balance between costs and effects was assessed by head-to-head comparisons using incremental cost-effectiveness ratios (ICERs) calculated by multiplying the estimated differences in costs with the NNT. The ICER, therefore, reflects the costs to society of treating the number of patients that is required to clinically cure 1 patient. For all analyses, a short time horizon was used, and therefore, time preference or discount rate was not accounted for. Bootstrapping was used to represent the uncertainty of the calculated costs and effects due to sampling variation.[14]

RESULTS

Study group

In total, 101 children were enrolled in the study between February 2003 and November 2005. Fifty were randomly allocated to the trimethoprim-sulfamethoxazole group; and 51, to the placebo group. At baseline, clinical characteristics did not differ between the 2 groups. The median age of the study participants was 50 months (interquartile range [IQR], 22-76 mo), and the median duration of otorrhea before study entry was 8 months (IQR, 4-22 mo) for the trimethoprim-sulfamethoxazole group and 5 months (IQR, 4-18) for the placebo group. Before study entry, 50 (100%) of the children in the trimethoprim-sulfamethoxazole group and 50 (98%) in the placebo group had used ototopical drops; 48 (96%) and 46 (90%) children had used systemic antibiotics, respectively.

Clinical effectiveness

At 6 weeks of follow-up, 72% of the children in the trimethoprim-sulfamethoxazole group and 47% in the placebo group were clinically cured (RD, 25%; 95% CI, 6-44%; NNT, 4). At 12 weeks of follow-up, these percentages were 68% and 53% (RD, 15%; 95% CI, -4 to 34%; NNT, 7), respectively. At 1 year, there was no difference between the 2 groups (RD, -5%; 95% CI, -12 to 22%) (Table 4.2.1).

Table 4.2.1: The number of clinically cured children at 6, 12 and 52 weeks follow-up

	6 weeks				12 weeks				1 year		
	TMP-SMX N = 47	Placebo N = 51	RD % (95% CI)	NNT	TMP-SMX N = 47	Placebo N = 49	RD % (95% CI)	NNT	TMP-SMX N = 44	Placebo N = 46	RD % (95% CI)
Clinically cured children	34 (72%)	24 (47%)	25 (6 – 44)	4	32 (68%)	26 (53%)	15 (-4 – 34)	7	33 (75%)	37 (80%)	-5 (-22 – 12)

Clinically cured children: Children found to be free of otorrhea in either ear on otomicroscopy. CI indicates confidence interval; RD, rate difference; TMP-SMX, trimethoprim-sulfamethoxazole.

Table 4.2.2: Resources used and cost estimates (in €, basic year 2005)

Resources	Cost estimate, unit costs in €	Source
Adenoidectomy	135.20	Costing study
Adenoidectomy combined with tympanostomy tubes	270.95	Costing study
Adenotonsillectomy	125.01	Costing study
Attico-antrotomy	1003.00	Costing study
Mastoidectomy	704.50	Costing study
Tympanoplasty	1597.00	Costing study
Tympanostomy tubes	208.00	Costing study
Day care in relation to sugery	235.87	Guideline
Hospitalisation per day	359.04	Guideline
Consultation ENT-specialist / pediatrician	61.46	Guideline
Consultation general practitioner	20.81	Guideline
Consultation paramedical professional	38.68	Guideline
Leave of absence (per hour)	36.03	Guideline
Lost of housekeeping activities (per hour)	8.55	Guideline
Pharmacists fee (per prescription)	6.45	Guideline
Prescribed medication	Several	Dutch Formulary
OTC drugs and CAM	Several	Retail prices

Abbreviations:
Guideline: Dutch Guideline for pharmacoeconomic research [13]
OTC: Over the counter
CAM: Complementary and alternative medicines

Costs

Table 4.2.2 shows a detailed overview of the most relevant cost estimates. After 6 weeks of follow-up, the mean costs per patient in the trimethoprim-sulfamethoxazole group were Euro337 (US $425) versus Euro237 (US $299) in the placebo group. After 12 weeks of follow-up, these costs were Euro594 (US $750) and Euro435 (US $549), respectively. The mean costs per patient after 1 year of follow-up were Euro1,601 (US $2,021) in the trimethoprim-sulfamethoxazole group and Euro1,164 (US $1,469) in the placebo group.

Cost-effectiveness

After 6 weeks of follow-up, the NNT was 4 (clinical effect), and the corresponding ICER was Euro400 (US $504), that is, the average extra cost to cure 1 child from otorrhea is Euro400 (US $504). After 12 weeks, the NNT was 7, and the corresponding ICER was Euro1,113 (US $1,407).

Because the clinical effect of trimethoprim-sulfamethoxazole disappeared after its discontinuation, we did not calculate an ICER after 1 year of follow-up.

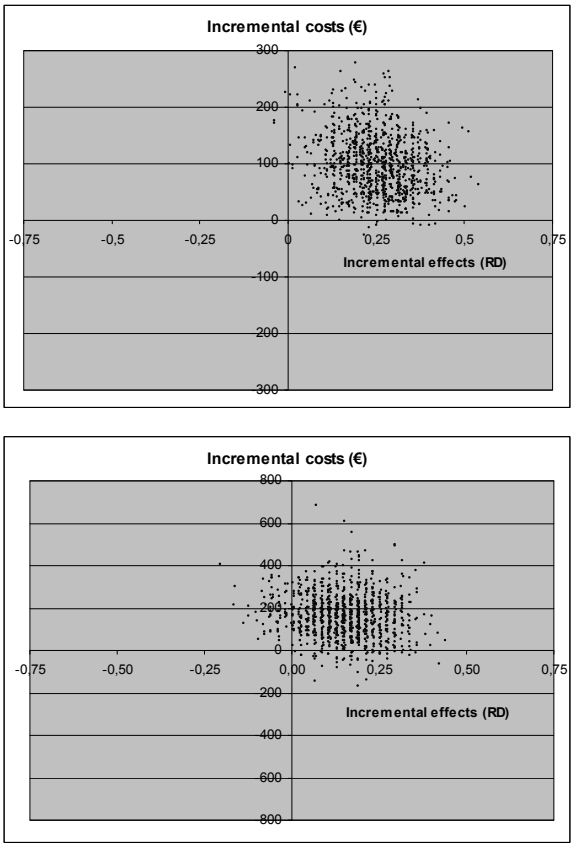

Figure 4.2.1A. Incremental costs and effects for TMP-SMX treatment as compared to placebo, after 6 weeks follow-up

Figure 4.2.1B. Incremental costs and effects for TMP-SMX treatment as compared to placebo, after 12 weeks follow-up
Legend for figure 1A and 1B:
RD = difference in proportion of clinically cured children between the TMP-SMX and the placebo group
To calculate the NNT à 1 / RD

The 1,000 bootstrap replicates of the incremental costs and effects for the trimethoprim-sulfamethoxazole treatment as compared with placebo after 6 and 12 weeks are depicted in Figure 4.2.1, A and B, respectively. After 6 weeks, 99% of the data points show advantage of trimethoprim-sulfamethoxazole over placebo at higher costs. In 1% of the bootstrapped estimates, trimethoprim-sulfamethoxazole treatment was more effective and less costly. After 12 weeks, these percentages were 89% and 5%, respectively. In 6% of the bootstrapped estimates, trimethoprim-sulfamethoxazole treatment was less effective and more costly.

DISCUSSION

This is the first randomized controlled study of the cost-effectiveness of prolonged systemic antibiotics in children with COM. It shows that trimethoprim-sulfamethoxazole offers a means to achieve clinical cure in young children with COM at modest costs.

The major strength of our study is that costs were measured prospectively, alongside a randomized controlled trial. Furthermore, by using a societal perspective, all relevant costs were taken into account. On the other hand, some limitations are worth considering.

First, we calculated the cost-effectiveness on the basis of the NNTs. This approach limited us to the use of a nongeneric and unidimensional clinical outcome (i.e., clinical curation) rather than a generic outcome such as quality-adjusted life years (QALYs).[15] We did not calculate costs per QALY, as quality of life measurements in young children mainly rely on proxy report and, as such, are limited to behavioral consequences of disease that are readily observable to the caregiver.[16] Because we based our economic evaluation on NNT, its results cannot be compared with those based on QALYs in other health care interventions and/or patient categories. On the other hand, ICERs based on NNT provides information on the costs made to cure 1 patient. Clinicians can use this information to decide whether the costs of treatment with trimethoprim-sulfamethoxazole in children with COM are counterbalanced by its clinical effectiveness.

Second, all children in our study received ototopical treatment, in addition to the study medication, when otorrhea was present. Because these antibiotic eardrops were used slightly more frequently in the placebo group, they might have influenced the cure rate in the placebo group at 12 weeks. This difference would, however, lead to an underestimation of the real treatment effect of trimethoprim-sulfamethoxazole.[11]

Third, serious reactions can occur during treatment with sulfonamides, such as agranulocytosis and Stevens-Johnson syndrome. However, the incidence of these reactions is very low. None of these serious reactions occurred in the 101 children included in this study.[11]

Fourth, resistance to trimethoprim-sulfamethoxazole might be a problem (as with any other antibiotic). Because of the well-known restrictive policy regarding antibiotic treatment in upper respiratory disease in the Netherlands, antibiotic resistance rates, including these to trimethoprim-sulfamethoxazole, are low. Unpublished data on the antibiotic resistance of the nasopharyngeal flora alongside our trial confirm this.

Fifth, our study population included children who had persistent symptoms of COM despite conventional management with topical medications, short-term systemic antibiotics, and/or ear surgery. Our results are therefore generalizable only to patients with similarly complicated COM. Sixth, in most cost-effectiveness studies, a sensitivity analysis is performed to account for differences in parameter estimates such as unit

costs.[14,17] Because the price of trimethoprim-sulfamethoxazole is not expected to change substantially in the years to come, we did not perform such an analysis.

CONCLUSION

In children with COM, direct and indirect costs of a 6- to 12-week course of high-dose oral trimethoprim-sulfamethoxazole are modest in the light of its short-term clinical benefit.

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Chapter 5

Microbiology of children with chronic mucosal otitis media

Chapter 5.1

Influence of Sampling Technique on Detection of Potential Pathogens in the Nasopharynx

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Archives of Otolaryngology - Head & Neck Surgery 2006;132:752-755.

ABSTRACT

Objectives: To determine the optimal approach for nasopharyngeal culture and to establish which approach children tolerate best.

Design: Cross-sectional study.

Patients: A cohort of 42 children with chronic suppurative otitis media.

Intervention: Paired nasopharyngeal samples were collected transorally and transnasally and cultured for potential aerobic pathogens.

Main Outcome Measures: The isolation rate of both samples and the amount of discomfort measured by the visual analog scale.

Results: Forty-six (87%) of 53 samples obtained transnasally were culture positive vs 40 (75%) of 53 samples obtained transorally ($P = .20$). *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis,* and *Staphylococcus aureus* were found more frequently with the transnasal than with the transoral approach: 34% vs 13% ($P = .003$), 62% vs 51% ($P = .20$), 30% vs 19% ($P = .15$), and 21% vs 11% ($P = .18$), respectively. Mean (SD) visual analog scale scores were 5.3 (1.0) and 3.4 (1.7) ($P<.001$) for the transnasal and transoral approaches, respectively.

Conclusions: Although the transoral approach is better tolerated in children, the isolation rate of the transnasal approach is higher, especially for *S pneumoniae*. The transnasal sampling technique should therefore be the preferred approach for detection of potential pathogens in the nasopharynx in children.

INTRODUCTION

The nasopharynx is the assembly point of clearance of secretions from the nasal cavity, paranasal sinuses, and middle ear.[1] Both in children and adults the nasopharynx is colonized by a variety of microorganisms, including commensals and potential pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae,* and *Moraxella catarrhalis*.[1-3] Detection of potential pathogens in the nasopharynx is important in understanding the etiology of middle ear disease, the evaluation of antibiotic resistance before and after treatment,[4-5] and the development of new vaccines.[4,6-7] Reported carriage varies strongly across studies. Differences in study populations, sampling and culture techniques, and the frequency of specimen collection may explain this variation.[2] Although variables in genetic background, socioeconomic conditions,[2] and culture techniques[8-9] have been studied extensively, little is known about the influence of different nasopharyngeal sampling techniques on the culture results, especially in children.

The nasopharynx can be approached through the nose and the mouth. In most studies, samples are obtained transnasally because this technique is presumed to be the best way to obtain a representative sample of the nasopharyngeal flora. However, transnasal sampling is unpleasant, and the transoral approach may be preferred, especially when samples have to be obtained repeatedly from children. So far, to our knowledge, comparisons of these 2 approaches in children have not been published. The purposes of this study are (1) to compare the isolation rate of paired nasopharyngeal samples obtained transorally and transnasally in children and (2) to establish which approach is tolerated best.

METHODS

Population characteristics

From October 11, 2004, until February 21, 2005, paired nasopharyngeal samples were collected from children visiting the Department of Pediatric Otolaryngology of the University Medical Center Utrecht. All children had chronic suppurative otitis media and participated in a trial on the effectiveness of prolonged treatment with a combination product of sulfamethoxazole and trimethoprim. Inclusion criteria for this study were age between 1 and 12 years and otorrhea for more than 3 months. Exclusion criteria were cholesteatoma, known immune deficiency other than for IgA or IgG, Down syndrome, craniofacial anomalies, cystic fibrosis, immotile cilia syndrome, allergy to sulfamethoxazole-trimethoprim, or continuous use of antibiotics for more than 6 weeks in the past 6 months. The medical ethics committee of the University Medical Center Utrecht approved the study protocol.

Collection of specimens

Both at baseline and during follow-up visits, study physicians obtained nasopharyngeal samples transnasally and transorally, using flexible, sterile, rayon-tipped swabs (Medical Wire & Equipment Co, Corsham, England). For the transnasal technique, the nasal cavity was inspected using a nasal speculum. A culture swab was inserted under the inferior turbinate along the floor of the nose until the nasopharynx was reached. When resistance was felt, the swab was rotated and subsequently removed. For the transoral technique, the throat was inspected under direct light and depression of the tongue. A culture swab was inserted in the oropharynx and, using the flexibility of the wire, curved upward behind the soft palate, where it was swept over the posterior wall of the nasopharynx. Contact of the swab with the pharyngeal tonsils and the mucosa of the oral cavity were avoided.

Microbiological investigation

The nasopharyngeal swabs were immediately stored in Stuart transport medium at room temperature. Swabs were transported to the microbiology laboratory and plated within 18 hours of sampling onto sheep blood (5%), *Haemophilus,* and MacConkey agar plates for the isolation of potential aerobic pathogens. The culture plates were incubated aerobically at 37°C (MacConkey agar) and less than 5% carbon dioxide (blood and *Haemophilus* agars). They were examined at 24 and 48 hours. Colonies suspected to be *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *Staphylococcus aureus*, *Streptococcus pyogenes,* or aerobic gram-negative bacteria were identified by previously described methods.[10]

Visual analog scale

For children older than 4 years, the 6-smiles visual analog scale (VAS) was used to establish which approach was tolerated best. Children were asked to point out which smile best described the feeling associated with sampling the nasopharynx through the nose and through the mouth. The first smile indicated no pain, whereas the sixth smile indicated worst possible pain. Finally, the children were asked which technique they felt was most unpleasant.

Statistical analysis

The total percentage of culture-positive samples obtained transnasally was compared with the percentage obtained transorally. The isolation rate of each microorganism for both samples was compared. Statistical significance was tested by the McNemar test. Means with standard deviations and median scores were calculated for the VAS scores of both nasopharyngeal approaches, and statistical significance was tested by the Wilcoxon signed rank test. We also performed repeated-measures analyses because some

children had samples obtained more than once. Since the results were similar, the results of the generally known analyses are presented.

RESULTS

In total, 53 paired nasopharyngeal samples were collected from 42 children at baseline or at one of the follow-up visits. The mean age of the children was 5.5 years (range, 1.3-11.7 years); 20 were boys, and 22 were girls.

Forty-six (87%) of the samples obtained transnasally were culture positive vs 40 (75%) of the samples obtained transorally ($P = .20$; Figure 5.1.1). Seven different species groups were detected by both approaches. Multiple microorganisms were isolated in 26 (49%) of the samples obtained transnasally and in 14 (26%) obtained transorally ($P = .004$).

S. pneumoniae was isolated significantly more frequently with the transnasal approach than with the transoral approach (34% vs 13%; $P = .003$; Figure 5.1.1). In 6 (11%) of the samples, both samples were positive for *S. pneumoniae*. For *H. influenzae*, *M. catarrhalis,* and *S. aureus,* both samples were positive in 22 (42%), 7 (13%), and 4 (8%) of the samples, respectively. These potential pathogens were also cultured more frequently with the transnasal approach than with the transoral approach: 62% vs 51% ($P = .20$), 30% vs 19% ($P = .15$), and 21% vs 11% ($P = .18$), respectively.

For detection of *S. pneumoniae*, the transnasal approach alone would have missed 1 of 19 positive *S. pneumoniae* culture results, and the transoral approach alone would have missed 12. For *H. influenzae, M. catarrhalis,* and *S. aureus,* the transnasal and transoral approaches would have missed 5 vs 11 of 38, 3 vs 9 of 19, and 2 vs 7 of 13 positive culture results, respectively. The other potential pathogens were isolated in less than 6% of the nasopharyngeal samples, and isolation rates were similar for the transnasal and transoral approaches.

Nineteen of the 22 children older than 4 years were evaluated with the VAS. Mean (SD) VAS scores were 5.3 (1.0) (median, 6.0; range, 3-6) and 3.4 (1.7) (median, 4.0; range, 1-6) for the transnasal and transoral approaches, respectively ($P<.001$). Sixteen children indicated that the transnasal approach was more unpleasant than the transoral approach, 2 children felt that both approaches were equally unpleasant, and only 1 child believed the transoral approach to be the most unpleasant.

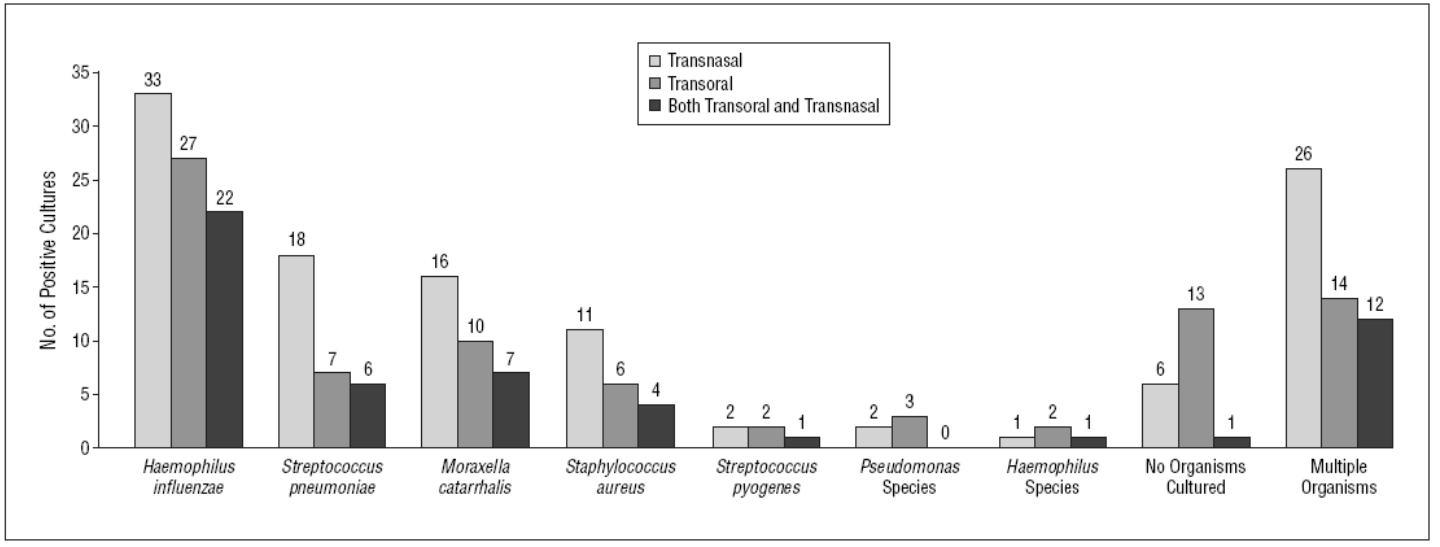

Figure 5.1.1. Number of positive nasopharyngeal cultures obtained by the transnasal and transoral approach and the combination of these approaches per species (N = 53).

DISCUSSION

In children, sampling the nasopharynx by a transnasal approach gave a higher detection rate of potential pathogens than by a transoral approach, especially for *S. pneumoniae*. The transoral technique, however, was better tolerated.

Chi et al1 performed a similar study in 99 healthy adults. In contrast to our findings, they found that the transoral technique gave the best detection of *S. pneumoniae*, *H. influenzae,* and *M. catarrhalis*. This discrepancy may be explained by the fact that nasopharyngeal colonization in their population of healthy adults may differ from that in ours of children with chronic suppurative otitis media. The use of different culture swabs may also have played a role. Chi and colleagues used a culture swab with a 60° bent metal shaft to swab the nasopharynx transorally. We used the flexibility of the shaft to curve the swab from the posterior oropharyngeal wall into the nasopharynx. This method is preferred in children, since their smaller dimensions of the oral and pharyngeal cavity do not allow easy passage of a bent shaft into the nasopharynx. Furthermore, our method can be performed quicker, which is advantageous in children, who are usually less cooperative than adults. On the other hand, it is possible that the samples we obtained transorally intentionally reflect the oropharyngeal rather than the nasopharyngeal flora. Previous studies comparing the culture results of different sampling sites in the nasopharynx and oropharynx support this explanation: higher detection rates of *S. pneumoniae* are found at nasal or nasopharyngeal sites than at oral or oropharyngeal sites.[11-18] For *H. influenzae,* reported carriage rates varied across studies, and similar to our findings, differences in yield between nasopharyngeal and oropharyngeal samples were relatively small.[11,17,19-20]

The different isolation rates of transnasally and transorally obtained samples may also indicate that swabs obtained transnasally are contaminated by flora of the nasal mucosa, which is known to have a high carriage rate of *S. pneumoniae* and *S. aureus*.[21] In clinical practice, this contamination is inevitable and in fact preferable in view of a better detection of potential upper respiratory tract pathogens.

It may be important to know whether the strains isolated by either approach differ genetically. Although the isolates obtained in this study were not characterized, other researchers[16-17,20] performed serotyping for *S. pneumoniae* isolates and found no important differences between serotypes cultured from the nose, nasopharynx, or oropharynx.

Our VAS scores indicate that children prefer the transoral approach over the transnasal approach. Although Hendley et al[14] previously suggested that transnasal sampling is not well tolerated by children, this is the first study, to our knowledge, to give evidence that the transoral sampling technique is tolerated best.

CONCLUSION

Although the transoral approach is better tolerated in children, the isolation rate of the transnasal approach is higher, especially for *S. pneumoniae*. The transnasal sampling technique should therefore be the preferred approach for detection of potential pathogens in the nasopharynx in children.

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Chapter 5.2

Effect of trimethoprim-sulfamethoxazole on nasopharyngeal carriage and resistance patterns in children with chronic otitis media

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Submitted

ABSTRACT

Objective: to examine the effects of a 6 to 12 week course of trimethoprim-sulfamethoxazole (TMP-SMX) on nasopharyngeal carriage and resistance patterns in children.

Methods: At baseline, 6 weeks, 12 weeks and 1 year follow-up nasopharyngeal swabs were obtained from 101 children with chronic otitis media who participated in a randomized placebo controlled trial on the effectiveness of TMP-SMX. Nasopharyngeal carriage and antibiotic susceptibility patterns were compared between the patients.

Results: At 6 weeks follow-up prevalences of *H.influenzae*, *M.catarrhalis* and *S. pneumoniae* were lower in the TMP-SMX group than in the placebo group; 30% vs 59% (RD-29 [95%CI-49;-9]), 0% vs 39% (RD-39 [95%CI-54;-24]), 7% vs 46% (RD-39 [95%CI-56;-22]), respectively. At 12 weeks follow-up the prevalences of these bacteria were still significantly lower in the TMP-SMX group. Prevalences of TMP-SMX resistant *H. influenzae* increased from 4% at baseline to 58% and 44% at 6 and 12 weeks follow-up. At 1 year follow-up, the prevalences of *H.influenzae*, *M.catarrhalis, S.pneumoniae* as well as that of TMP-SMX resistant *H.influenzae* returned to baseline.

Conclusion: Our data show that prolonged treatment with TMP-SMX reduces the nasopharyngeal carriage of potential respiratory pathogens and selects for TMP-SMX resistant *H.influenzae*. These effects are most pronounced with the shorter course and disappear if TMP-SMX is discontinued.

INTRODUCTION

Chronic active otitis media (COM) is a common infectious disease affecting children both in developing and industrialized countries.[2,19] It causes considerable morbidity and is a major global cause of hearing impairment in children.[3,11,17,19]

Since there is no consensus regarding the most effective management of COM in children we recently performed a randomized placebo controlled trial on the effectiveness of trimethoprim-sulfamethoxazole (TMP-SMX). We found that a 6 to 12 weeks course of TMP-SMX (18mg/kg, two times daily) reduced otorrhea by 72% at 6 weeks and 68% at 12 weeks. The treatment effect disappeared if TMP-SMX was discontinued. [18]

The question is whether this positive clinical effect of TMP-SMX treatment is associated with a reduction in carriage of potential pathogens in the nasopharyngeal flora or that TMP-SMX treatment selects for TMP-SMX resistant nasopharyngeal pathogens which may influence the clinical effect negatively. Since the nasopharynx is an important site for transmission of pathogens between children [10,15], changes in nasopharyngeal carriage and susceptibility patterns of potential pathogens can easily become widespread.[1,13] This may be an unwanted side effect of TMP-SMX treatment and may increase treatment costs.

The aim of this study is to establish the effect of TMP-SMX treatment on nasopharyngeal carriage of potential pathogens and TMP-SMX susceptibility of *Haemophilus influenzae* and *Moraxella catarrhalis* in children with COM who participated in a randomized placebo controlled trial on the effectiveness of TMP-SMX.

PATIENTS AND METHODS

Study population

Between February 2003 and June 2006, 101 children were included in a randomized controlled study on the clinical effectiveness of TMP-SMX treatment in COM.[18]

Children, aged between 1 and 12 years, with at least 3 months of continuous otorrhea were referred to the Otolaryngology department of the University Medical Center Utrecht, the Netherlands. Children with cholesteatoma, known immune deficiency other than for IgA or IgG subclasses, Down's syndrome, craniofacial anomalies, cystic fibrosis, primary ciliary dyskinesia, allergy to TMP-SMX, or with continuous use of antibiotics for more than 6 weeks in the past 6 months were excluded from the study. After obtaining written informed consent, children were randomly assigned to either oral TMP-SMX (18mg/kg, two times daily) or placebo for 6 weeks. When otorrhea was present in either ear at the first follow visit after 6 weeks, the study medication was continued for another 6 weeks. The study medication was discontinued at the 6 weeks follow-up visit if both

ears were free from otorrhea. Parents were instructed to restart the study medication if otorrhea recurred after the 6 weeks follow-up visit. All study medication was discontinued at the end of 12 weeks follow-up. The medical ethics committee of the University Medical Center Utrecht approved the study protocol.

Specimen collection and microbiology procedures

At baseline, 6 weeks, 12 weeks and 1 year follow-up nasopharyngeal swabs were obtained by the study physicians. A flexible, sterile, rayon-tipped swab (Medical Wire & Equipment Co.) was inserted under the inferior turbinate along the floor of the nose until the nasopharynx was reached. When resistance was felt the swab was rotated and subsequently removed. The nasopharyngeal swabs were immediately stored in Stuart's medium at room temperature. The swabs were transported to the microbiology laboratory and plated within 18 hours of sampling onto sheep blood (5%), Haemophilus and MacConkey agar plates for the isolation of potential aerobic pathogens. The culture plates were incubated aerobically at 37^0C (MacConkey agar) and < 5% carbon dioxide (blood and Haemophilus agars).

Colonies suspected to be *Streptococcus pneumoniae*, *Haemophilus influenzae, Moraxella catarrhalis,* were identified by previously described methods.[16] Susceptibility of *H.influenzae* and *M.catarrhalis* to TMP-SMX was performed by the disc-diffusion method of Kirby-Bauer. Strains were labeled susceptible, intermediate susceptible or resistant based on the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (NCCLS 1994).[14]

We were not able to test TMP-SMX susceptibility for *S.pneumoniae* because blood supplemented culture media rich in folic acid is needed to culture this organism. TMP-SMX is a folic acid antagonist interfering with the growth of *S.pneumoniae* and therefore decreasing the reliability of the susceptibility results.[9,12]

Statistical analysis

The characteristics of the study population and baseline distribution of the potential pathogens were described with summary statistics. Rate differences and 95% confidence intervals were calculated to compare the effects in the TMP-SMX and control group regarding nasopharyngeal carriage and antibiotic susceptibility patterns, at 6 weeks, 12 weeks and 1 year. Rate differences and 95% confidence intervals were also used to evaluate the differences in percentages within the groups over time. For analysis purposes, we considered both intermediate and fully resistant strains to be resistant. All analyses were performed according to the intention-to-treat principle.

RESULTS

Baseline characteristics were similar for the children in the TMP-SMX and the placebo group (Table 5.2.1). The mean age of the children was 4.8 years (SD 3.3) in the TMP-SMX group (n=50), and 4.4 years (SD 2.9) in the placebo group (n=51). In the 2 weeks before study entry, 3 children (6%) in the TMP-SMX group and 2 (4%) in the placebo group had used systemic antibiotics. In the first 12 weeks of follow-up the duration of study medication used by the children in the TMP-SMX and placebo group was similar; 9.4 (95%CI 8.6; 10.2) weeks in the TMP-SMX group and 9.6 (95%CI 8.7; 10.4) weeks in the placebo group.

Nasopharyngeal carriage after antibiotic treatment.

In the TMP-SMX group prevalences of *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* decreased from 65%, 35% and 35% at baseline to 30%, 0% and 7% (RD 35 [95%CI 15; 55], 35 [95%CI 21; 49], 28 [95%CI 12; 44]) at 6 weeks follow-up and 21%, 19% and 23% (RD 44 [95%CI 25; 63], 16 [95%CI -2; 34] and 12 [95%CI -7; 31]) at 12 weeks follow-up, respectively. At 1 year follow-up carriage of *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* returned to baseline. In the placebo group prevalences of *H. influenzae*, *M. catarrhalis* and *S. pneumoniae* did not change substantially with 66%, 48% and 45% at baseline to 59%, 39% and 46% (RD 7 [95%CI -14; 28], 9 [95%CI -12; 30] and -1 [95%CI -22; 20]) at 6 weeks follow-up and 59%, 37% and 43% (RD 7 [95%CI -13; 27], 31[95%CI 13; 49] and 2 [95%CI -19; 23]) at 12 weeks follow-up, respectively. Only at 6 and 12 weeks follow-up prevalences of *H.influenzae*, *M.catarrhalis* and *S.pneumoniae* were lower in the TMP-SMX group as compared to the placebo group; RD-29 [95%CI -49; -9], RD-39 [95%CI -54; -24], RD-39 [95%CI -56; -22] at 6 weeks follow-up and RD-38 [95%CI -57; -19], RD-18 [95%CI -36; 0] and RD-20 [95%CI -39; -1] at 12 weeks follow-up, respectively (Figure 5.2.1).

Table 5.2.1. Characteristics of patients with COM in the TMP-SMX and the placebo group

Characteristics	TMP-SMX (N=50)	Placebo (N=51)
	N(%)	N(%)
Male	28 (56)	26 (51)
Mean age in years (SD)	4.8 (3.3)	4.4 (2.9)
Day care or school attendance in year before study entry	45 (90)	47 (92)
Use of systemic antibiotics in year before study entry	48 (96)	46 (90)
Use of systemic antibiotics in the last 2 weeks before study entry	3 (6)	2 (4)

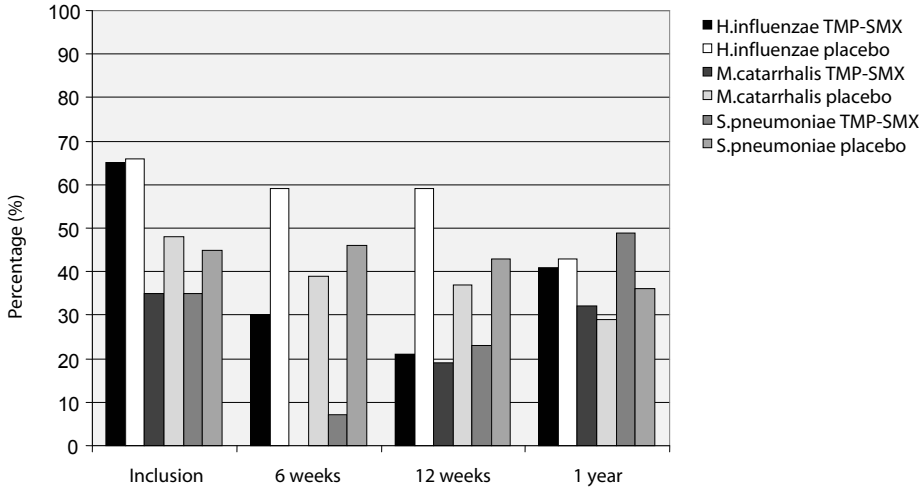

Figure 5.2.1. The effect of TMP-SMX on potential nasopharyngeal pathogens at inclusion and during follow-up
TMP-SMX: trimethroprim-sulfamethoxazole

Antimicrobial susceptibility of H.influenzae and M.catarrhalis isolates

At baseline, 4% of the children in the TMP-SMX group carried TMP-SMX resistant *H.influenzae*. At 6 and 12 weeks follow-up this percentage increased to 58% and 44% (RD 54 [95%CI 25; 83] and 40 [95%CI 7; 73]) respectively and returned to 6% at 1 year follow-up (RD 2 [95%CI -12; 16]). In the placebo group these percentages were 7%, 8%, 15% and 25% respectively (Fig2). The difference between the TMP-SMX group and the placebo was significant at 6 and 12 weeks follow-up with 42% versus 92% (RD -50 [95%CI -80; -20]) and 56% versus 84% (RD -28 [95%CI -63; -7]), respectively. In the TMP-SMX group, prevalences of *M.catarrhalis* were too low to study changes in TMP-SMX susceptibility (Figure 5.2.2).

DISCUSSION

This is the first randomized placebo controlled trial studying the effects of TMP-SMX on nasopharyngeal carriage and resistance patterns in children. Our results showed that treatment with TMP-SMX is associated with a substantial reduction of nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. The prevalence of TMP-SMX resistant *H. influenzae* increased during TMP-SMX treatment. After discontinuation of TMP-SMX, carriage of *H. influenzae*, M. *catarrhalis*, *S. pneumoniae* and TMP-SMX resistant *H. influenzae* returned to baseline.

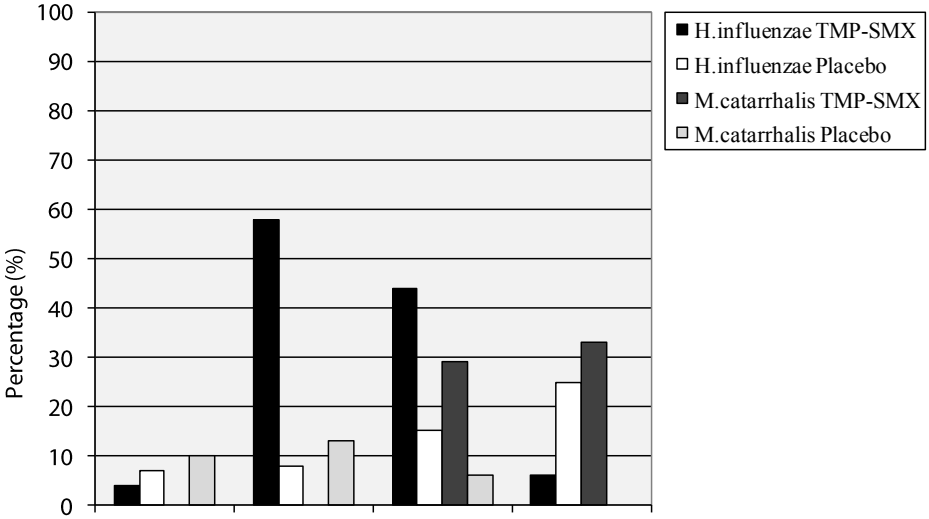

Figure 5.2.2. Proportion of children with TMP-SMX resistant isolates among positive *H.influenzae* and *M. catarrhalis* cultures at inclusion and during follow-up
TMP-SMX: trimethroprim-sulfamethoxazole

In clinical terms, our findings are important, because they show that changes in the nasopharyngeal flora and resistance are reversible, since prevalences return to baseline after discontinuation of the TMP-SMX therapy. It is unclear however, whether the resistant strains have really disappeared or decreased under detection level.

Previous studies also found a reduced carriage of potential respiratory pathogens during treatment with antibiotics[4,6,7,8] and a return to baseline values after discontinuation of therapy.[4] Moreover, most studies with a long follow-up after cessation of antibiotic treatment showed a return of the resistant nasopharyngeal flora to its state at enrolment as reported in this manuscript.[5,8]

To appreciate the results of our trial, some possible limitations should be discussed. First, since follow up visits were limited to 6 and 12 weeks and 1 year, we were not able to determine the exact time span in which the nasopharyngeal flora and TMP-SMX resistance returned to baseline after TMP-SMX therapy was discontinued. Second, we intended to study the effect of duration of TMP-SMX therapy (6 versus >6 weeks) on nasopharyngeal carriage. The numbers of potential pathogens within these subgroups however were too small to demonstrate such an effect. Third, since the prevalence of *M.catarrhalis* and *H.influenzae* were low during TMP-SMX therapy, we were not able to report on TMP-SMX susceptibility of *M.catarrhalis* and the resistance rates of *H.influenzae* should be interpreted with care. However, since the prevalence of *M.catarrhalis* and

H.influenzae in the nasopharyngeal flora remained low during TMP-SMX treatment, resistance to TMP-SMX appears not to be a major factor.

Fourth, we were unable to test TMP-SMX susceptibility for *Streptococcus pneumoniae* because the disc diffusion method for testing TMP-SMX susceptibility to *Streptococcus pneumoniae* is generally considered unreliable and is normally not performed in the clinical situation.[9,12]

In conclusion, prolonged treatment with TMP-SMX reduces the nasopharyngeal carriage of potential respiratory pathogens and selects for TMP-SMX resistant *H.influenzae*. These effects are most pronounced with the short course and disappear if TMP-SMX is discontinued.

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Chapter 5.3

Effect of long-term trimethoprim/sulfamethoxazole treatment on resistance and integron prevalence in the intestinal flora: a randomized, double-blind, placebo-controlled trial in children

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The Journal of Antimicrobial Chemotherapy 2009 May;63(5):1011-1016.

ABSTRACT

Objectives: The aim of this study was to test the hypothesis that trimethoprim/sulfamethoxazole selects for integron-positive and multidrug-resistant Enterobacteriaceae in the intestinal flora.

Methods: During 1 year of follow-up, antibiotic susceptibility and the presence of integrons were determined in faecal Enterobacteriaceae isolated from 99 children with chronic active otitis media, randomly assigned to treatment with trimethoprim/sulfamethoxazole or placebo (http://www.clinicaltrials.gov/; trial registration number NCT00189098).

Results: At 6 and 12 weeks of follow-up, 32 (91%) and 24 (67%) children in the trimethoprim/sulfamethoxazole group carried trimethoprim/sulfamethoxazole-resistant Enterobacteriaceae versus 10 (21%) and 8 (17%) children in the placebo group [rate differences (RDs): 70 (95% CI: 55; 85) and 50 (95% CI: 31; 69)], respectively. Multiresistance also increased during trimethoprim/sulfamethoxazole treatment. At 6 weeks of follow-up, the integron prevalence was 26 (79%) in the trimethoprim/sulfamethoxazole group and 10 (22%) in the placebo group [RD: 57 (95% CI: 39; 75)]. After 12 weeks the integron prevalence, and after 1 year the susceptibility levels, had returned to baseline values.

Conclusions: Initially, trimethoprim/sulfamethoxazole usage was strongly associated with the appearance of integron-positive (multi)drug-resistant Enterobacteriaceae in the intestinal flora. After prolonged exposure to trimethoprim/sulfamethoxazole, however, this population of Enterobacteriaceae was substituted by a population with non-integron-associated resistance mechanisms. After trimethoprim/sulfamethoxazole was discontinued, susceptibility rates to all antibiotics returned to baseline levels.

INTRODUCTION

The intestinal tract is an important reservoir of many bacterial pathogens[1] allowing transfer of antimicrobial resistance genes within and across bacterial species.[1,2] Selective pressure exerted by antimicrobial usage is considered crucial to the emergence and dissemination of antibiotic-resistant strains in the intestinal tract.[1]

Trimethoprim/sulfamethoxazole is widely used for long-term prophylaxis and treatment of infections of the urinary and respiratory tracts.[3–6] This widespread use is associated with increasing resistance rates to trimethoprim/sulfamethoxazole. Most trimethoprim/ sulfamethoxazole resistance genes in Enterobacteriaceae reside within class 1 integrons, genetic elements that frequently reside in horizontally transferable elements and play an important role in the dissemination of resistance genes.[7–9] Integrons are capable of recognizing, capturing and expressing multiple resistance genes in cassette structures and are strongly associated with multidrug resistance.[10–12] Class 1 integrons are the most common integrons among Enterobacteriaceae and usually contain a *sul1* gene encoding resistance to sulfamethoxazole, alongside the mobile gene cassettes. The aim of this study was to test the hypothesis that by oral use of trimethoprim/sulfamethoxazole, the sulfamethoxazole component will select for class 1 integron-positive strains, leading to the presence of clinically relevant concentrations of integron-positive multidrug-resistant Enterobacteriaceae in the intestinal flora.

PATIENTS AND METHODS

Patients

From February 2003 until June 2006, faecal samples were collected from 99 children with chronic active otitis media who participated in a randomized placebo-controlled trial on the effectiveness of sulfamethoxazole/trimethoprim (http://www.clinicaltrials.gov/; trial registration number NCT00189098).[13] Inclusion criteria were age of 1–12 years and a documented history of more than 3 months of continuous otorrhoea through either a tympanic membrane perforation or a tympanostomy tube. Children with cholesteatoma, known immunodeficiency other than for IgA or IgG subclasses, Down's syndrome, craniofacial anomalies, cystic fibrosis, primary ciliary dyskinesia, allergy to trimethoprim/ sulfamethoxazole or continuous use of antibiotics for 6 weeks in the past 6 months were excluded from the study. Children whose parents gave informed consent (including to this substudy) were randomly assigned to either trimethoprim/sulfamethoxazole to be given orally (18 mg/kg, twice daily) or a placebo for 6 weeks. The investigators remained blinded to the randomization until the end of the study. If otorrhoea was present in either ear at the first follow-up visit at 6 weeks, the study medication was continued for

a further 6 weeks. The study medication was discontinued at this first follow-up visit if both ears were free from otorrhoea. Parents were instructed to restart the study medication if symptoms of otorrhoea recurred between the follow-up visits at 6 and 12 weeks. At the 12 week follow-up visit, all study medication was discontinued.

Further details regarding the randomization procedure, allocation concealment and the results concerning the primary outcome, i.e. otomicroscopic signs of otorrhoea at 6 weeks, 12 weeks and 1 year of follow-up, have been published elsewhere.[13] To study the effect of trimethoprim/ sulfamethoxazole on the proportion of children with integron-positive and (multi)drug-resistant Enterobacteriaceae in their intestinal tract (secondary outcome), faecal samples were collected at study entry and 6 and 12 weeks and 1 year of follow-up. To determine whether susceptibility patterns of the isolates from the trial participants differed from those of children in the open population, we also collected faecal samples from 55 healthy children attending a daycare centre or primary school in the vicinity of Utrecht, The Netherlands. The medical Ethics Committee of the University Medical Center Utrecht approved the study protocol, including this substudy.

Microbiological investigation

Since resistance to sulfamethoxazole is a very sensitive screening criterion for the detection of integrons in Enterobacteriaceae, faecal samples from patients and controls were cultured for Gram-negative bacteria on MacConkey agar with sulfamethoxazole (512 mg/L). As a control for the adequacy of the culture conditions, all samples were cultured on MacConkey agar without sulfamethoxazole as well. From each plate, all morphologically different colonies were subcultured for further investigation with a minimum of three colonies per plate. Identification and susceptibility were tested using the Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD, USA). Susceptibility to the following antimicrobials was determined: trimethoprim/sulfamethoxazole, ciprofloxacin, trimethoprim, gentamicin, amoxicillin, amoxicillin/clavulanate, cefuroxime, ceftriaxone, nitrofurantoin, colistin, chloramphenicol and meropenem. Extended-spectrum β-lactamase (ESBL) production was determined for *Escherichia coli* and *Klebsiella* spp. using Etest (AB Biodisk, Solna, Sweden) ESBL. Additional testing for susceptibility to sulfamethoxazole was performed using the agar disc diffusion method. Breakpoints were those recommended by the CLSI.[14] All not fully susceptible isolates were grouped together with the resistant isolates. Multiresistance was defined as resistance to at least two antimicrobial classes.

All isolated Enterobacteriaceae resistant to sulfamethoxazole were tested for the presence of class 1 integrons by PCR amplification of the class 1 integrase-specific *int1* gene (GenBank accession no. M73819 [GenBank]), as described previously.[15] The primers were *Int1*-F (5′-TCTCGGGTAACATCAAGG-3′) and *Int1*-R (5′-AGGAGATCCGAAGACCTC-3′).

Statistical analysis

The power of the study was calculated according to the primary outcome, i.e. otomicroscopic signs of otorrhoea at 12 weeks of follow-up. Assuming a spontaneous recovery of otorrhoea of 25%, a treatment effect of trimethoprim/sulfamethoxazole of 50% (based on a retrospective study of children treated with trimethoprim/sulfamethoxazole for chronic active otitis media at our hospital) and using an *a* of 0.05 and a power of 0.80, it was calculated that each group should consist of 50 children.[13]

Rate differences with 95% confidence intervals were calculated at baseline and at the three follow-up visits to compare the children with chronic otitis media and the healthy control group for the prevalences of Enterobacteriaceae, antibiotic resistance and integrons. Duration of treatment with trimethoprim/sulfamethoxazole varied as the study medication was continued at 6 weeks of follow-up if otorrhoea was present and discontinued if the ear was dry. Therefore, subanalyses were performed for the results at 12 and 52 weeks of follow-up for those who had received trimethoprim/sulfamethoxazole from study entry until the follow-up visit at 6 weeks (TMP-SMX-I) and for those who continued or restarted trimethoprim/sulfamethoxazole between 6 and 12 weeks of follow-up (TMP-SMX-II).

RESULTS

Baseline characteristics of the trial participants and the healthy controls are summarized in Table 5.3.1. As expected, fewer children in the healthy control group had used systemic antibiotics in the previous year. Figure 5.3.1 shows the flow chart of the number of eligible patients and those that consented to participate, as well as the numbers of participants with complete follow-up and the dropouts. Of the 48 children in the trimethoprim/sulfamethoxazole group, 20 (42%) discontinued (TMP-SMX-I) and 28 (58%) children continued (TMP-SMX-II) the study medication between 6 and 12 weeks of follow-up.

Table 5.3.2 shows the number (%) of children with faecal samples from which Enterobacteriaceae were isolated in the trimethoprim/sulfamethoxazole, the placebo and the healthy control groups with positive cultures for Enterobacteriaceae at baseline and at the follow-up visits. The high detection rates at baseline and during follow-up in the placebo group reflect the adequacy of the culture conditions. The most frequently cultured species during the study were *E. coli* (90% of faecal samples), followed by *Klebsiella* spp. (8%). Non-fermenting Gram-negative bacteria were cultured from <10% of the faecal samples. The distribution of species did not change during the study and did not differ between the trimethoprim/sulfamethoxazole group and the placebo group.

During the study, antimicrobials involved in multidrug resistance were mainly sulfamethoxazole, trimethoprim/sulfamethoxazole, amoxicillin and amoxicillin/clavulanic

Table 5.3.1: Characteristics of patients with chronic active otitis media randomized to trimethoprim/sulfamethoxazole or placebo and healthy control children; number (%)

Characteristics	TMP-SMX (N=48)		Placebo (N=51)		Healthy controls (N=55)	
Male	28	(58)	27	(53)	28	(51)
Mean age in years (SD)	4.5	(3.4)	3.9	(2.8)	3.6	(2.3)
Daycare or school attendance in year before study entry	43	(90)	47	(92)	55	(100)
Use of systemic antibiotics in year before study entry	46	(96)	46	(90)	20	(36)
Use of systemic antibiotics in the last 2 weeks before study entry	2	(4)	2	(4)	1	(2)

Table 5.3.2: Number (%) of children with fecal samples from which Enterobacteriaceae were isolated

	Inclusion	T6 weeks	T12 weeks	T 1 year
Healthy controls	53 (96)	-	-	-
Placebo group	47 (92)	47 (100)*	47 (98)**	34 (85)
TMP-SMX group	47 (98)	35 (74)*	36 (84)**	36 (97)
-TMP-SMX-I			14 (82)	15 (94)
-TMP-SMX-II			22 (85)	21 (100)

*((RD -26 [95%CI -39; -13]).
**(RD -14 [95%CI -26; -2])

acid. Resistance to aminoglycosides and ciprofloxacin was found sporadically. No resistance was observed to meropenem and no ESBL-positive strains were identified.

At baseline, antibiotic resistance and multiresistance rates were similar for the trimethoprim/sulfamethoxazole group, the placebo group and the healthy control group.

At 6 weeks of follow-up, 32 (91%) Enterobacteriaceae-positive cultures were resistant to trimethoprim/sulfamethoxazole in the trimethoprim/sulfamethoxazole group versus 10 (21%) in the placebo group (Figure 5.3.2). Multidrug resistance was present in 34 (97%) Enterobacteriaceae-positive cultures in the trimethoprim/sulfamethoxazole group versus 23 (49%) in the placebo group (Figure 5.3.3).

At 12 weeks of follow-up, 24 (67%) Enterobacteriaceae-positive cultures were resistant to trimethoprim/sulfamethoxazole in the trimethoprim/sulfamethoxazole group versus 8 (17%) in the placebo group (Figure 5.3.2). Multidrug resistance was present in 26 (72%) Enterobacteriaceae-positive cultures in the trimethoprim/sulfamethoxazole group versus 26 (55%) in the placebo group (Figure 5.3.3). Within the trimethoprim/sulfamethoxazole group, the children who continued trimethoprim/sulfamethoxazole therapy between 6 and 12 weeks of follow-up (TMP-SMX-II) had higher resistance rates:

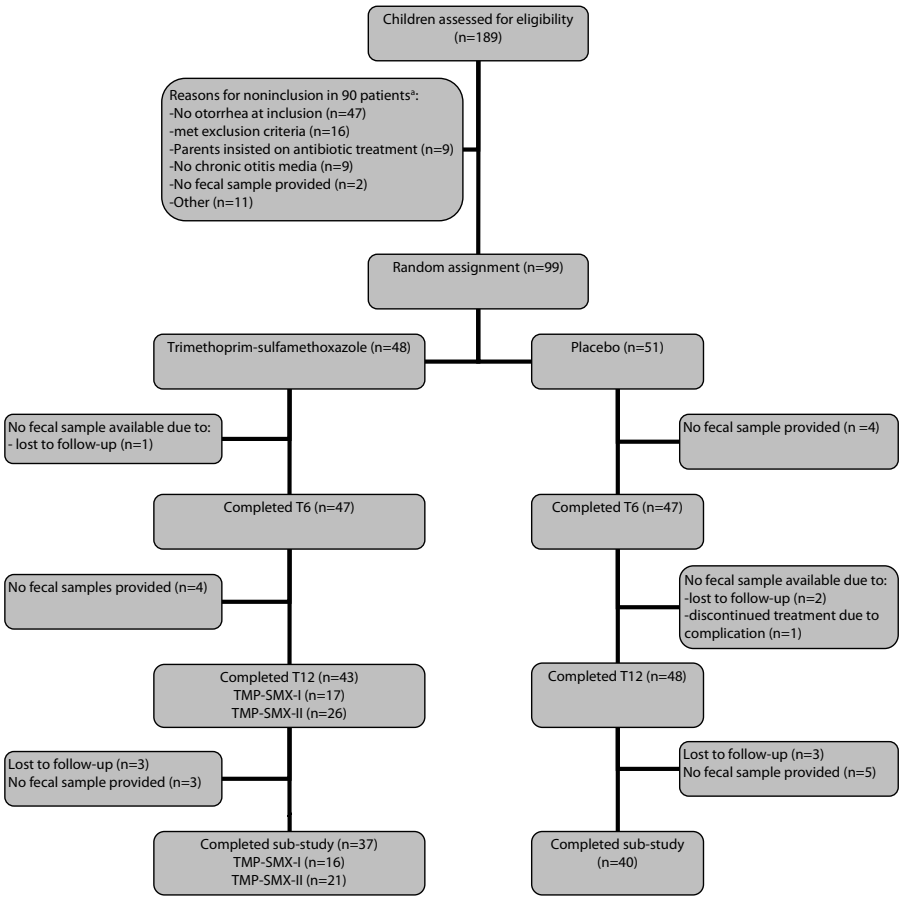

Figure 5.3.1. Flow of participants through the substudy of the trial.
[a]The number exceeds 90 because more than one reason could be indicated. TMP-SMX-I, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole from study entry up to the follow-up visit at 6 weeks; TMP-SMX-II, children with chronic active otitis media who were also treated with trimethoprim/sulfamethoxazole between 6 and 12 weeks of follow-up; T6, 6 weeks of follow-up; T12, 12 weeks of follow-up.

19 (86%) Enterobacteriaceae-positive cultures were resistant to trimethoprim/sulfamethoxazole in the TMP-SMX-II group versus 5 (36%) in the TMP-SMX-I group. Multidrug resistance was found in 20 (91%) Enterobacteriaceae-positive cultures of the TMP-SMX-II group and in 6 (43%) of the TMP-SMX-I group.

At 1 year of follow-up, the proportion of Enterobacteriaceae-positive cultures resistant to trimethoprim/sulfamethoxazole as well as multidrug resistance returned to baseline levels.

The susceptibility patterns for sulfamethoxazole and amoxicillin followed the same pattern as that for trimethoprim/sulfamethoxazole.

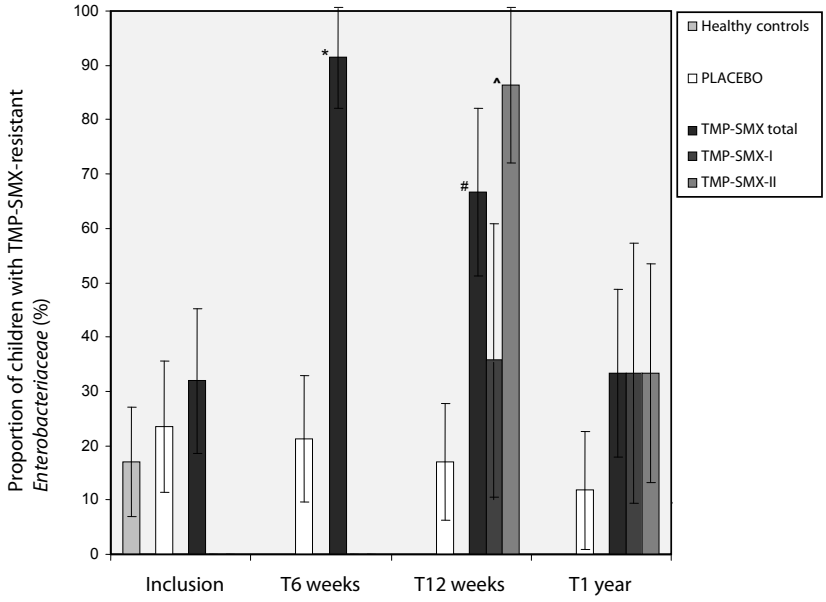

Figure 5.3.2. Proportion of children with trimethoprim/sulfamethoxazole-resistant Enterobacteriaceae at inclusion and during follow-up.
*$P < 0.05$; #$P < 0.05$; ^$P < 0.05$. TMP-SMX total, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole for 6–12 weeks (TMP-SMX-I + TMP-SMX-II); TMP-SMX-I, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole from study entry up to the follow-up visit at 6 weeks; TMP-SMX-II, children with chronic active otitis media who were also treated with trimethoprim/sulfamethoxazole between 6 and 12 weeks of follow-up; T6, 6 weeks of follow-up; T12, 12 weeks of follow-up; T1 year, 1 year of follow-up.

At baseline, the proportion of children with integron-positive Enterobacteriaceae was similar in the trimethoprim/sulfamethoxazole group, the placebo group and the healthy control group (Figure 5.3.4). At 6 weeks of follow-up, more children in the trimethoprim/sulfamethoxazole group than in the placebo group carried integron-positive Enterobacteriaceae: 26 (79%) and 10 (22%), respectively. At 12 weeks of follow-up, these percentages decreased to 32% in the trimethoprim/sulfamethoxazole group and 11% in the placebo group. Subanalysis of the data at 12 weeks for the TMP-SMX-I and TMP-SMX-II groups did not alter the results.

At 1 year of follow-up, integron prevalences returned to baseline in all groups.

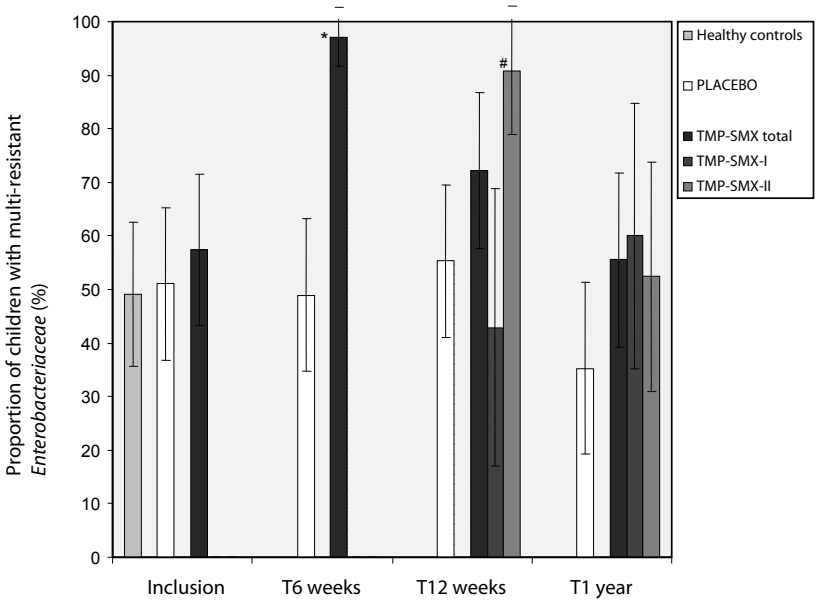

Figure 5.3.3. Proportion of children with multiresistant Enterobacteriaceae at inclusion and during follow-up.

*$P < 0.05$; #$P < 0.05$. TMP-SMX total, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole for 6–12 weeks (TMP-SMX-I + TMP-SMX-II); TMP-SMX-I, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole from study entry up to the follow-up visit at 6 weeks; TMP-SMX-II, children with chronic active otitis media who were also treated with trimethoprim/sulfamethoxazole between 6 and 12 weeks of follow-up; T6, 6 weeks of follow-up; T12, 12 weeks of follow-up; T1 year, 1 year of follow-up.

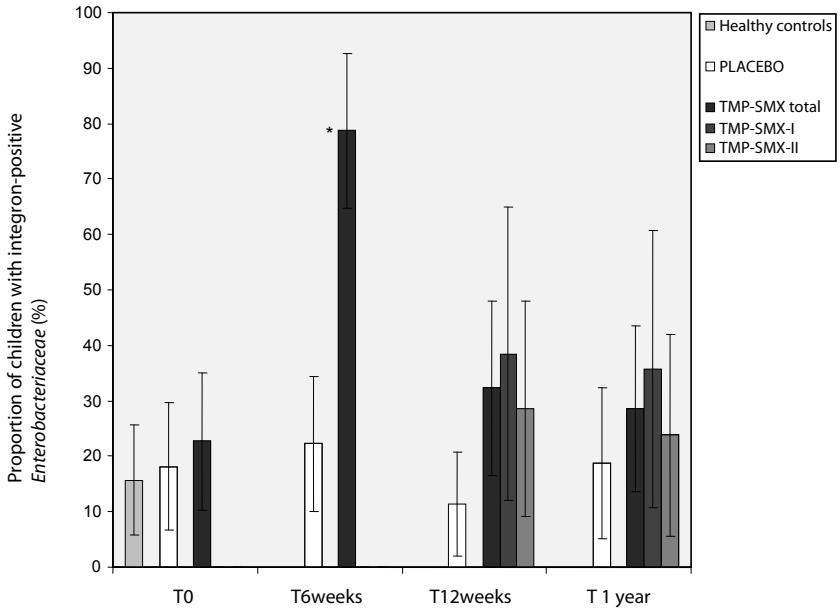

Figure 5.3.4. Proportion of children with integron-positive Enterobacteriaceae at inclusion and during follow-up.
*$P < 0.05$. TMP-SMX total, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole for 6–12 weeks (TMP-SMX-I + TMP-SMX-II); TMP-SMX-I, children with chronic active otitis media treated with trimethoprim/sulfamethoxazole from study entry up to the follow-up visit at 6 weeks; TMP-SMX-II, children with chronic active otitis media who were also treated with trimethoprim/sulfamethoxazole between 6 and 12 weeks of follow-up; T6, 6 weeks of follow-up; T12, 12 weeks of follow-up; T1 year, 1 year of follow-up.

DISCUSSION

To our knowledge, this is the first study that evaluates the effects of trimethoprim/sulfamethoxazole on the intestinal flora alongside a randomized placebo-controlled trial and the first study to report the effect of prolonged oral use of trimethoprim/sulfamethoxazole on the prevalence of integrons in Enterobacteriaceae in the intestinal flora.

So far, only a few prospective studies have investigated the effect of trimethoprim/sulfamethoxazole on Enterobacteriaceae in the intestinal tract.[16–22] Comparison of the results of these studies with that of ours is hampered by considerable differences in the carriership of trimethoprim/sulfamethoxazole-resistant strains at study entry, population characteristics, duration of treatment and follow-up.

Nonetheless, consistent with our findings, most studies found a decrease in the proportion of patients with Enterobacteriaceae isolated during trimethoprim/sulfamethoxazole treatment, although it was often more pronounced.[17–19,21] A possible explanation

for the less pronounced effect in our study was the relatively high proportion of carriage of trimethoprim/sulfamethoxazole-resistant strains among children at inclusion (around 20%).

The use of trimethoprim/sulfamethoxazole caused a temporary increase in the proportion of children with (multi-)resistant Enterobacteriaceae, a finding in accordance with previous observations.[20,22] Since the intestinal tract is an important reservoir of many bacterial pathogens, it can be assumed that these children are more prone to acquire an infection with (multi-)resistant Enterobacteriaceae. The recent identification of antimicrobial prophylaxis as a risk factor for antimicrobial resistance among children with recurrent urinary tract infections supports this assumption.[23]

The increased (multi)drug resistance rates at 6 weeks of follow-up were associated with an increased prevalence of integrons, supporting the hypothesis that by oral administration of trimethoprim/sulfamethoxazole, the sulfamethoxazole component selects for class 1 integron-positive multidrug-resistant Enterobacteriaceae in the intestinal flora. This association was also found between trimethoprim/sulfamethoxazole treatment and the occurrence of *sul* genes and increased resistance to (trimethoprim/)sulfamethoxazole in *E. coli* in urines from children with recurrent urinary tract infections.[24]

Surprisingly, at 12 weeks of follow-up, the proportion of children with integron-positive strains had returned to baseline levels despite continued trimethoprim/sulfamethoxazole therapy and while the strains retained high resistance levels. Apparently, between 6 and 12 weeks of follow-up, integron-negative Enterobacteriaceae substituted the integron-positive Enterobacteriaceae with non-integron-associated resistance mechanisms. This finding can be explained by the concept that low antibiotic concentrations may produce a substantial stress in the bacterial population leading to increased mutation rates and transfer of resistance genes stepwise, resulting in more diverse and effective adaptive responses.[25,26]

After trimethoprim/sulfamethoxazole was discontinued, resistance rates to all antibiotics returned to baseline levels consistent with the findings of the one study that also followed their participants after discontinuation of therapy.[20] Further research is necessary to establish whether the patients really lost their resistant strains or whether the resistant strains only decreased below detection levels to possibly re-emerge following a further antimicrobial course.

A possible limitation of this study is the limited sensitivity of the detection method of the integron-positive Enterobacteriaceae used in this study; more colonies per plate or higher inocula would have increased the sensitivity of the detection method. However, we think that the detection method used was adequate. The aim of this study was to determine the change in the proportion of children with integron-positive multidrug-resistant strains, and using this method a significant increase was found.

CONCLUSIONS

Trimethoprim/sulfamethoxazole usage was initially strongly associated with the appearance of integron-positive (multi)drug-resistant Enterobacteriaceae in the intestinal flora. After prolonged exposure to trimethoprim/sulfamethoxazole, however, this population of Enterobacteriaceae was substituted by a population with non-integron-associated resistance mechanisms. The presence of resistant Enterobacteriaceae in the intestinal tract during therapy most likely results in a higher risk of acquisition of infections caused by these (multi)resistant strains. Further studies are needed to determine both the presence and the duration of such increased risk after discontinuation of therapy.

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Chapter 6

General Discussion

GENERAL DISCUSSION

Active chronic mucosal otitis media (COM) is one of the most common chronic infectious diseases affecting children worldwide.[1-3] As it causes considerable morbidity and hearing loss, establishing its most effective treatment is important both from a medical as well as a societal point of view.[4-8] It is generally accepted that antibiotic eardrops should be the first step in treating COM[9-11] and surgery the last when optimal medical treatment has failed. What optimal medical treatment beyond eardrops entails, however has not been established.[11-18] We therefore initiated a randomized placebo controlled trial of a 6- to 12-week course of orally administered TMP-SMX in children with active chronic mucosal otitis media who had failed conventional management with antibiotic eardrops and/or short courses of systemic antibiotic treatment.[19] The results are presented in this thesis. In short, TMP-SMX cured 68% of children at 12 weeks follow-up, compared to 53% of children treated with a placebo. We consider these results to be positive, also in the light of TMP-SMX being inexpensive and well tolerated by young children. In this chapter, we will address several questions raised by our trial and make recommendations as to how to implement its results in the management of children with the management of children with COM.

Could the chronicity of symptoms of the children included in the trial have been avoided?

The children who participated in the trial had complicated active chronic mucosal otitis media; their ears had drained continuously for more than 3 months despite conventional management with eardrops and systemic antibiotics.[19] Prevention of such a complicated course of the condition requires on the one hand knowledge of factors that predispose children to such a prolonged course of COM and on the other hand an active approach early in the course of COM. Since it is thought that COM begins with an episode of acute otitis media[1, 20], it is likely that factors contributing to otitis media also play a role in the development of COM. Otitis media is a multifactorial condition resulting from a failed immune response of the child to microbial pressure in the nasopharynx and middle ear. Risk factors known to be involved in otitis media relate to these two core elements. (Figure 6.1)

In chapter 3.1 "Predictors of chronic suppurative otitis media in children" we studied the association between COM and risk factors including sex, age, bottle feeding, parental smoking, day-care attendance, previous upper respiratory tract infections or otitis media episodes and surgery for otitis media.[21] In our population treatment with tympanostomy tubes was most strongly associated with COM. Other independent factors were having older siblings and a low educational level of the mother. We also studied the association between COM and these factors in children with tympanostomy tubes

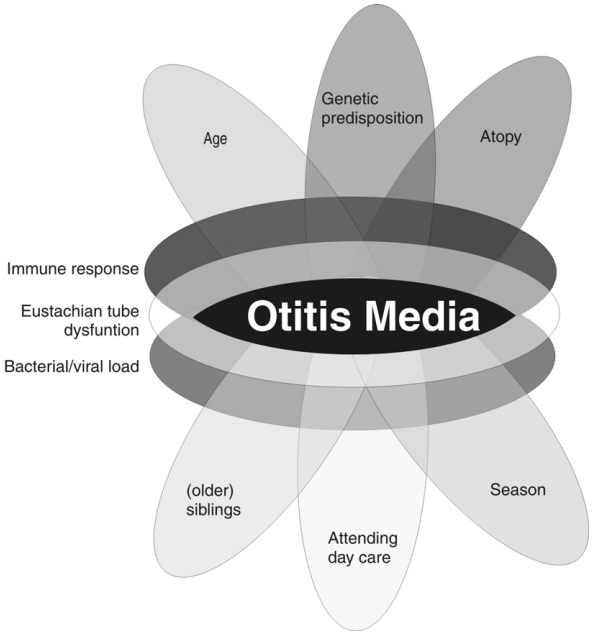

Figure 6.1. Risk factors for otitis media

and found that a history of recurrent acute otitis media predisposes these children to COM. Because we performed a case-control study comparing children with COM (cases) to children without COM (controls), we could only establish whether these risk factors were more or less common in children with COM. Cause and effect relationships and absolute risks could not be established with this study design.

In chapter 3.2 we studied the role of polymorphisms in the pathogen recognition receptor genes TLR2, TLR4, CD14 and MyD88 in COM. Such polymorphisms may lead to functional variations of these receptors of the innate immune system, altering the interaction with and susceptibility to pathogens. In contrast to previous studies, mostly performed in-vitro and in mice, that did find an association between these polymorphisms and an increased susceptibility to pathogens involved in COM, we did not find these polymorphisms to predispose children to active chronic mucosal otitis media.

The role of the adaptive immune response in COM was studied by relating the serum levels of IgA, IgM and IgG and subclasses of the participants of our trial to age specific normal levels. Figure 6.2 shows that the IgG and IgA levels of children with COM were within the normal range for age, and so were IgM subclasses.

In summary, with the data collected in this thesis we could not predict reliably which children are likely to develop active chronic mucosal otitis media.

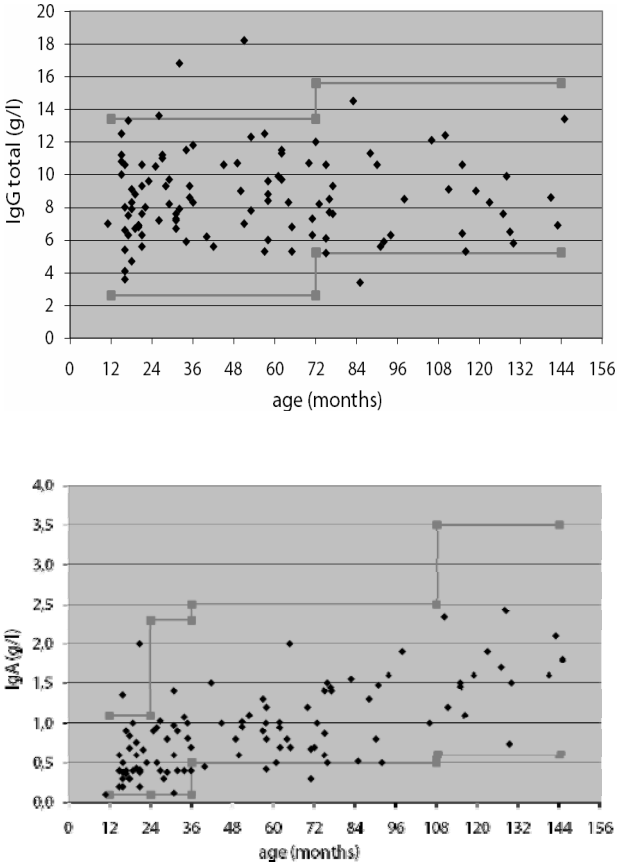

Figure 6.2. IgG and IgA levels in 101 children with COM participating in our trial on the effectiveness of TMP-SMX
Upper horizontal grey line: age related highest normal value for immunoglobulines
Lower horizontal grey line: age related lowest normal value for immunoglobulines

However, we did find, that children with recurrent acute otitis media who are selected for tympanostomy tubes, have a higher risk to developing COM as compared to children selected for tympanostomy tubes because of persistent otitis media with effusion. We recommend to discuss this information with parents of children that are candidates for tympanostomy tubes.

Why was the treatment effect at 6- and 12 weeks follow-up no longer present at 1 year follow-up?

Recovery in the placebo group increased from 26 (53%) children at 3 months follow-up to 37 (80%) at 1 year follow-up.[19] In the children treated with TMP-SMX these figures were remained more or less the same at 32 (68%) and 33 (75%), respectively. Apparently, the

difference between the two groups disappeared because the children initially treated with placebo improved with time, rather than the children initially treated with TMP-SMX relapsed. This suggests that with conventional management children with COM eventually get better after many months, whereas with a prolonged course of TMP-SMX children are cured after 6 weeks.

There are several explanations for the improvement of the children in the placebo group during the last 9 months of the study. First, after 12 weeks follow-up, the parents, general practitioner and local otolaryngologist or pediatrician of the participants were informed about the assigned treatment. From then on doctors were free to manage otorrhea in both groups according to their own preferences. As such, between 12 weeks and 1 year follow-up, 78% of the children in the TMP-SMX group, and 82% in the placebo group received antibiotic eardrops (RD 4%; 95%CI:-22% to 14%); for short courses of systemic antibiotics these figures were 62% and 47%, respectively (RD: 15%; 95% CI:-7% to 37%); and for prolonged courses of antibiotics 16% and 22%, respectively (RD: -6%; 95% CI:-22% to 10%). Thirty percent of the children in the TMP-SMX group and 24% in the placebo group (RD: 6%; 95% CI: -12% to 24%) underwent an ear-nose-throat operation between 12 weeks and 1 year. These non-randomized treatments during the last 9 months of the study could have influenced the high cure rate in the placebo group causing a dilution of the effect at 1 year follow-up.

These figures also suggest that even the children who are free from otorrhea in the short term are at risk for developing recurrent episodes of otorrhea requiring treatment. This calls for long-term follow up of children with COM.

Second, we included a group of children with a complicated course of COM. When in a sample group pre-treatment expression of a condition, i.e. otitis media, is extreme, i.e. complicated COM, then post-treatment symptoms are likely to be closer to the population mean, i.e. less severe COM. In other words, both groups are likely to improve with time, so the difference between them decreases. This phenomenon is also known as regression to the mean.

Will children with active chronic mucosal otitis media presenting in day to day otolaryngology practice benefit as much from TMP-SMX as the children in our trial?

This question concerns both the representativeness of the trial population as well as the validity of the study. The children in our study had a complicated course of COM, i.e. they had failed conventional management with eardrops, short courses of systemic antibiotics, tympanostomy tubes and middle ear and mastoid surgery. Most children presenting to general practitioners, pediatricians or otorhinolaryngologist with drain-

ing ears will have had symptoms for a much shorter course and have not yet received such extensive medical or surgical therapy. These children are likely to respond well to conventional management.[9-11] Our results are therefore applicable only to children with otorrhea persisting for more than 3 months despite these therapies.

Our results may have been affected by medication taken besides the study medication. All children used ototopical antibiotics when otorrhea was present during the first 12 weeks of the trial. Between 12 weeks and 1 year follow-up the children were managed by their own general physician or specialist according to their preferences; most of them were given eardrops and systemic antibiotics. This may have influenced the high cure rate in the placebo group at 12 weeks and 1 year follow-up, and as such we may even have underestimated the real treatment effect.

Therefore, we believe that the current trial results reflect the real treatment effect of TMP-SMX in children with a complicated course of COM.

Can we predict which children with chronic active mucosal otitis media will not benefit from TMP-SMX?

To answer this question we first studied how many of the children with otorrhea at 12 weeks follow-up also had otorrhea at 1 year follow-up. Of the 15 children of the TMP-SMX group who suffered from otorrhea at 12 weeks, 7 (47%) also had otorrhea at 1 year. This means that children who do not benefit from a prolonged course of antibiotics in the short-term, run an increased risk of continuous middle ear disease in the long-term.

Moreover, 7 (16%) children in the TMP-SMX group and 10 (22%) children in the placebo group with persistent otorrhea at 12 weeks follow-up were treated with a prolonged course of antibiotics between 12 and 52 weeks follow-up. Four (57%) and 6 (60%) were otorrhea free at 1 year follow-up, respectively. This means that a secondary prolonged course of antibiotics is beneficial in about half of the children. In the remaining children who have persistent otorrhea despite this management, other treatment options should be explored. So far, the literature is inconclusive as to what is most effective in that situation.

Furthermore, we also compared possible prognostic factors across the children with draining ears at 1 year follow-up and those whose ears were dry. An independent predictor for persistent or recurrent otorrhea at 1 year follow-up was having more than 2 siblings (OR 8.8; 95%CI 2.0 to 38.4). An independent protective factor was age <4 years (OR 0.2; 95%CI 0.1 tot 0.8). This may be explained by the potential of the young child to improve with age along with the natural maturation of the immune system and Eustachian tube function.

These results must be interpreted with caution in light of the statistical power of our study. The 101 children included in our trial allowed us to detect a clinically relevant effect of at least 25% on the primary outcome otorrhea at the follow-up visits. However,

the power was too low to detect significant effects in specific subgroups of children. This study is therefore, not conclusive concerning specific subgroups that are likely to benefit from TMP-SMX.

Did the choice of the antibiotic influence the trial results?

We have chosen for TMP-SMX because of our wide experience with this antibiotic in the management of upper respiratory tract infections and otitis media in children. It is inexpensive and well tolerated by young children. This positive experience is shared by others.[22, 23] On the other hand, the most common organism cultured in COM, *P. aeruginosa*, is known to be unsusceptible to TMP-SMX in vitro. This is in fact reflected by our own culture results; at inclusion 43% of the otorrhea cultures were positive for *P. aeruginosa*, at 6- and 12 weeks follow-up these numbers were 56% and 50%, respectively.[19] All children whose otorrhea sample was positive for *P. aeruginosa* at 6 and 12 weeks follow-up, also had a *P. aeruginosa* positive otorrhea sample at inclusion. Nevertheless, the number of children with discharging ears and a positive culture for *P. aeruginosa* treated with TMP-SMX decreased considerably during the first 12 weeks (from 22 children to 6).

Previous studies on the management of COM have applied different antibiotics, both culture directed and aimed at *P. aeruginosa* as well as broad-spectrum. Remarkably, the results of these studies were very similar in the short term (Table 6.1).[14-16, 18] Apparently, *P. aeruginosa* is a secondary rather than the causative microorganism in COM, implicating that antibiotic therapy need not necessary be aimed at *P. aeruginosa*. This illustrates the need for high quality studies on the etiology of COM.

Table 6.1. Studies on systemic antibiotic treatment for COM

	Antibiotic	Treatment duration	Follow-up	Success
Fliss (1990)	Mezlocilline vs. Ceftazidime (iv)	10-14 days	12 days	100 vs 100%
Legent (1994)	Ciprofloxacin vs. Augmentin (po)	9 days	10 days	58 vs 37%
Khanna (2000)	Culture specific antibiotic vs. TMP-SMX	14 days	2 weeks	85 vs 75%
Somekh (2000)	Ceftazidime vs. Aztreonam (iv)	14 days	90 days	73 vs 53%
Veen, van der (2007)	TMP-SMX (po) vs. placebo	6-12 weeks	6 weeks	72 vs 47%
			12 weeks	68 vs 53%
			1 year	75 vs 80%

Evidence based and common sense management of chronic mucosal otitis media

Taken the evidence of our trial, the literature and many years of experience at the Wilhelmina Children's Hospital practice, we recommend the following management algorithm for children with COM (Figure 6.3):

1. We advise to treat children with persistent symptoms of otorrhea for 2 weeks to 3 months with antibiotic eardrops.[9-11] When culture results indicate *P.aeruginosa*, quinolone eardrops should be considered.
2. When treatment with topical antibiotics fails and otorrhea persists, we recommend a short course of antibiotics in combination with antibiotic eardrops based on culture results.
3. When after 3 months of treatment otorrhea persists we advice a prolonged course of systemic oral antibiotics in combination with 10 days of antibiotic eardrops.[19] Considering the best evidence at this stage we advice TMP-SMX 18mg/kg twice daily during 6 weeks in combination with 10 days of topical antibiotics. At 6 weeks the children should be assessed for otomicroscopic signs of otorrhea. If both ears are dry, we advice to discontinue the antibiotic treatment. When signs of otorrhea improved in either ear, we advice to continue TMP-SMX 18mg/kg twice daily for another 6 weeks in combination with topical antibiotics.[19] When otorrhea is unaltered during TMP-SMX treatment, we recommend to switch to a different prolonged antibiotic course of 6 weeks, for example azithromycin (5 mg/kg, once per day, for 6–12 weeks). At 6 weeks venous samples should be tested for adverse reactions, by means of complete blood counts and hepatic and renal function tests.
3. When this medical treatment fails we advice, based on clinical experience, to take another otorrhea sample and to exclude or treat immunodefeciencies conform the protocol of the European Society for immunodefeciencies.[24] The treatment at this stage should be, in our opinion, a prolonged course of culture directed antibiotics in combination with 10 days of topical antibiotics.
4. If otorrhea still persists after this treatment we recommend, after exclusion of underlying middle ear disease by CT-scan of the mastoids, a tympanomastoidectomy combined with intravenous culture-directed antibiotics.

Implications for future research

Our study has identified several aspects of COM that deserve further attention.

Like acute otitis media, we believe that COM results from a failed immune response to bacteria ascending from the nasopharynx to the middle ear. This study does not reveal why in some children their defence mechanisms at the nasopharyngeal and the middle ear level fails. This requires further study of the interaction between the pathogens at these sites and the mucosal immune system of the host. Understanding the balance of

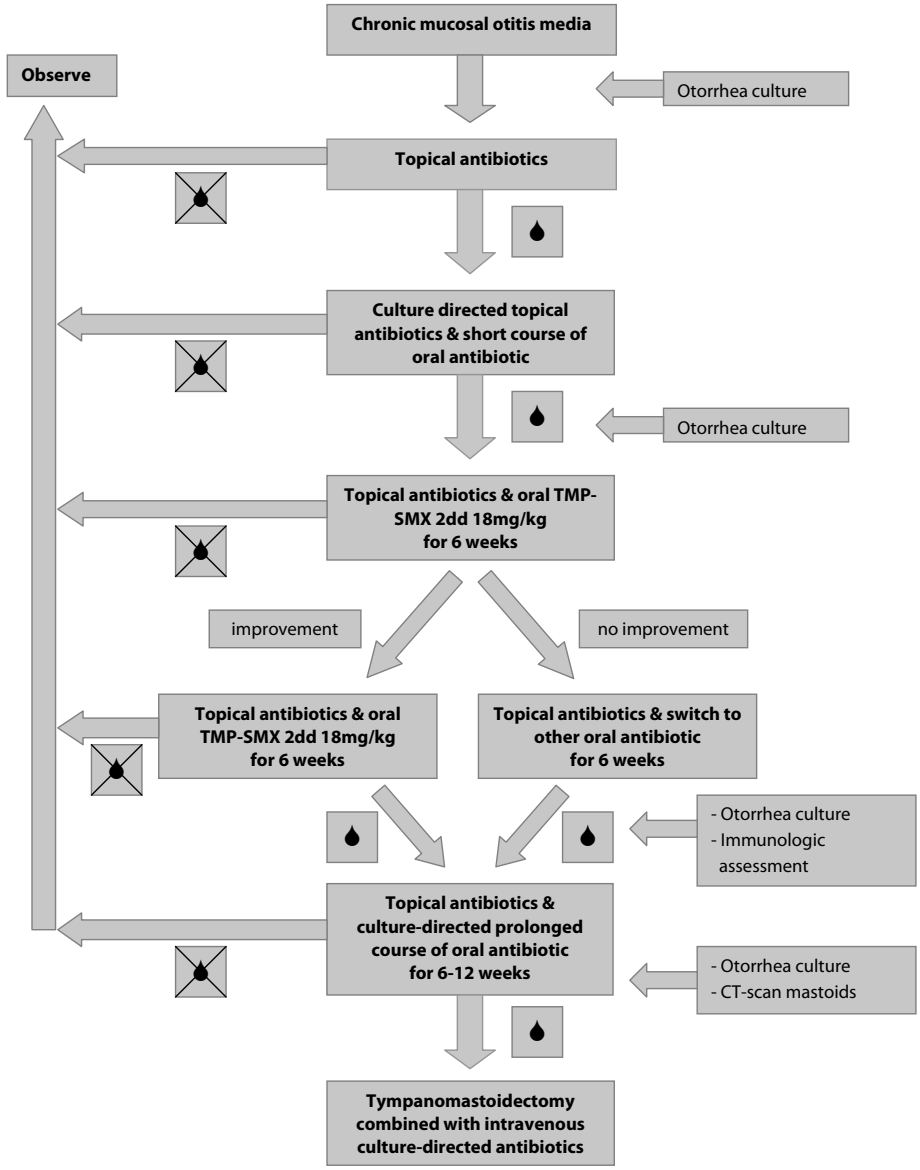

Figure 6.3. Recommended management of active chronic mucosal otitis media
♦ ; otomicroscopic signs of otorrhea in either ear
✖ ; no otomicroscopic signs of otorrhea
TMP-SMX; trimethoprim-sulfamethoxazole

this complex ecological system and its interaction with the host is pivotal to optimize vaccination strategies in various populations as well as to understand consequences of new vaccination strategies.

With our case-control study we could not calculate absolute risks to predict which children are at risk for developing COM. A long-term prospective cohort study could provide data to determine these absolute risks. The children participating in the Wheezing Illnesses Study LEidsche Rijn (WHISTLER), a prospective birth cohort on respiratory illnesses would provide an ideal population for this purpose.[25] With the knowledge of these absolute risk factors we could early treat and aim our interventions to the children at risk for COM.

Our choice for TMP-SMX at a dosage of 18mg/kg twice daily for 6 to 12 weeks was based on clinical beliefs about its effectiveness, tolerability and low costs. Although the trial results confirmed our beliefs, similar or even better results may have been achieved with other treatment regimens. Therefore further research of the most effective systemic antibiotic and its optimal dosage and duration in children with active chronic mucosal otitis media is needed.

Although we have shown that changes in the nasopharyngeal and fecal flora and resistance are reversible[26], further research is necessary to establish whether the patients really lost their resistant strains or whether the resistant strains only decreased below detection levels and possibly re-emerge during further antimicrobial treatment. Additionally, since the intestinal and nasopharyngeal tract are an important reservoir for the transfer of antimicrobial resistance between bacteria and individuals, the effects of a temporarily acquired resistance should also be studied on population level.

The place of tympanomastoidectomy in the management of chronic active mucosal otitis media is unknown. In The Netherlands this operation is still very popular in the management of chronic otorrhea. To answer the question at what stage in the management of COM a tympanomastoidectomy must be performed, a controlled study comparing tympanomastoidectomy with a prolonged course of antibiotics should be initiated.

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Chapter 7

Summaries

Chapter 7.1

Summary

SUMMARY

Chronic active mucosal otitis media (COM) is a common infectious disease, affecting children in both developing and industrialized countries. It causes considerable morbidity and is a major global cause of hearing impairment in children. Moreover, it may lead to serious extracranial and intracranial complications, such as mastoiditis and meningitis. An active approach in the management of COM is therefore important. Since there is no consensus regarding the most effective treatment, we initiated a randomized placebo controlled trial of a 6- to 12-week course of orally administered trimethoprim/sulfamethoxazole (TMP-SMX) in children with chronic active mucosal otitis media. Alongside this trial we studied the cost-effectiveness of this treatment, its effects on carriage and antibiotic susceptibility of potential pathogens, potential risk factors for COM, and the role of the innate and adaptive immune defense system in the development of COM.

In **Chapter 2**, we reviewed the evidence on the aetiology, pathogenesis and management of COM in children.

Various definitions of COM exist, varying from otorrhea through a perforation of the tympanic membrane or a tympanostomy tube for at least two weeks to symptoms persisting for more than 3 months. Knowledge concerning risk factors and etiologic factors for COM is mostly extrapolated from what is known on acute otitis media. This needs further study in COM. Regarding treatment, the evidence is scarce and there is no consensus on the optimal medical and/or surgical strategy in children with COM. Topical antibiotics, especially quinolones are effective in patients with otorrhea of short duration, but this needs further monitoring regarding adverse effects and effectiveness in patients with symptoms of longer duration. We concluded that there is a need for prospective and well-controlled studies to establish the role of both medical and surgical therapies in the management of COM.

In **Chapter 3** we studied the epidemiology of COM in children.

Using a case control design we first studied factors associated with COM. Factors independently associated with COM in children are previous tympanostomy tube insertion, more than 3 upper respiratory tract infections in the past 6 months, having parents with a low education level, and having older siblings. Factors independently associated with COM after tympanostomy tube insertion are more than 3 episodes of otitis media in the past year, attending day care, and having older siblings.

Second, we studied polymorphisms of pathogen recognition receptor genes as a risk factor for COM. Such polymorphisms may lead to functional variations of these receptors of the innate immune system, altering the interaction with and susceptibility to pathogens. Using a case-control design the genotype and haplotype frequencies of

TLR2 SNPs (-16934; Pro631His; Arg753Gln), TLR4 SNPs (Thr399Ile), CD14 SNPs (-651; -260) and MyD88 SNPs (1944) were studied in 90 children with COM and 375 controls. We found that neither the frequency of the wild type and mutant SNP alleles of the TLRs, CD14 and MyD88 genes, nor the genotype frequencies and haplotype frequencies of CD14 and TLR2 differed between the case and control group. We therefore concluded that with the data collected in these studies we could not predict reliably which children are likely to develop active chronic mucosal otitis media.

Chapter 4 presents the actual trial results. 101 children aged 1 to 12 years with a documented history of continuous otorrhea for at least 12 weeks were randomized to receive 6 to 12 weeks of orally administered TMP-SMX (18 mg/kg, 2 times per day) or placebo and were monitored for 1 year. At 6 and 12 weeks follow-up, cure rates were 72 and 68% in children treated with TMP-SMX and 47 and 53% in those treated with placebo. At 1 year, the cure rate was similar in both groups; 75% and 80%, respectively. Pure-tone hearing levels and health related quality of life improved during the study but did not differ between the TMP-SMX group and the placebo group. One child of the TMP-SMX group developed a skin rash and 9% of the TMP-SMX group and 2% of the placebo group had gastrointestinal complaints. Complications were uncommon and equally divided between the groups; 1 child in the TMP-SMX and one child in the placebo group developed mastoiditis.

A cost-effectiveness analysis of this treatment regimen was performed in **Chapter 4.2**. We calculated the costs to cure 1 patient (incremental cost-effectiveness ratio [ICER]). After 6 and 12 weeks of follow-up, the average extra costs to cure 1 child from otorrhea were 400 and 1,113 Euros, respectively. Since the clinical effect of trimethoprim-sulfamethoxazole disappeared after its discontinuation, we did not calculate an ICER at 1 year follow-up.

We concluded that TMP-SMX treatment in children with chronic mucosal otitis media is effective with the shorter course, but the effect disappears if the medication is discontinued. In light of its clinical benefit and tolerability, both the direct and indirect costs of a 6- to 12-week course of high-dose oral TMP-SMX appear to be modest.

In **Chapter 5** we studied the effect of prolonged treatment with TMP-SMX on carriage and antibiotic susceptibility of the microbial flora in children with COM.

In **Chapter 5.1** we established the optimal approach and tolerability for nasopharyngeal cultures by sampling the nasopharynx of a cohort of 42 children with COM both transorally as well as transnasally. The samples were cultured for potential aerobic pathogens. Eighty seven percent of the samples obtained transnasally were culture positive versus 75% obtained transorally (*P*=.20). *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis,* and *Staphylococcus aureus* were all found more frequently

with the transnasal than with the transoral approach. However, the tolerability was better with the transoral approach. We concluded that although the transoral approach is better tolerated in children, the isolation rate of the transnasal approach is higher and should therefore be the preferred approach for detection of potential pathogens in the nasopharynx in children with COM.

In **Chapter 5.2** we studied the effects of a prolonged course of TMP-SMX on carriage and antibiotic susceptibility of potential pathogens in the nasopharynx. Nasopharyngeal swabs were obtained transnasally from all children who participated in our trial at baseline, 6 weeks, 12 weeks and 1 year follow-up. We found that during TMP-SMX treatment prevalences of *H.influenzae*, *M.catarrhalis* and *S.pneumoniae* in the nasopharynx were lower in the TMP-SMX group than in the placebo group. In the TMP-SMX group, prevalences of TMP-SMX resistant *H. influenzae* increased from 4% at baseline to 58% and 44% at 6 and 12 weeks follow-up. At 1 year follow-up, the prevalences of *H.influenzae*, *M.catarrhalis, S.pneumoniae* as well as that of TMP-SMX resistant *H.influenzae* returned to baseline values. We therefore concluded that prolonged treatment with TMP-SMX reduces the nasopharyngeal carriage of potential respiratory pathogens, but selects for TMP-SMX resistant *H.influenzae*. These effects disappear if TMP-SMX is discontinued.

In **Chapter 5.3** we tested the hypothesis that TMP-SMX selects for integron-positive and multidrug-resistant Enterobacteriaceae in the intestinal flora. The intestinal flora is a main reservoir for pathogens and plays an important role in the emergence and spread of antimicrobial resistance. Most TMP-SMX resistance genes in Enterobacteriaceae reside within integrons; genetic bacterial elements associated with multidrug resistance. We found that at 6 and 12 weeks follow-up, 91% and 67% of the children in the TMP-SMX group carried TMP-SMX-resistant Enterobacteriaceae versus 21% and 17% of the children in the placebo group. Multiresistance also increased during TMP-SMX treatment. At 6 weeks follow-up, the integron prevalence was 79% in the TMP-SMX group and 22% in the placebo group. At 12 weeks the integron prevalence had returned to baseline values. At 1 year all antibiotic susceptibility levels had returned to baseline values. This indicates that the increased levels of (multi)drug-resistant Enterobacteriaceae during TMP-SMX treatment are only temporarily associated with high prevalences of integrons, hereafter integron resistance is substituted by non-integron-associated resistance mechanisms.

In **Chapter 6** we discuss the pros and cons of our trial and propose the following management algorithm for children with COM (Figure 7.1).

Ideally interventions for COM should be aimed at prevention. At present, these are not available. It is therefore crucial that creative options based on modern insights into the pathofysiology of COM are developed.

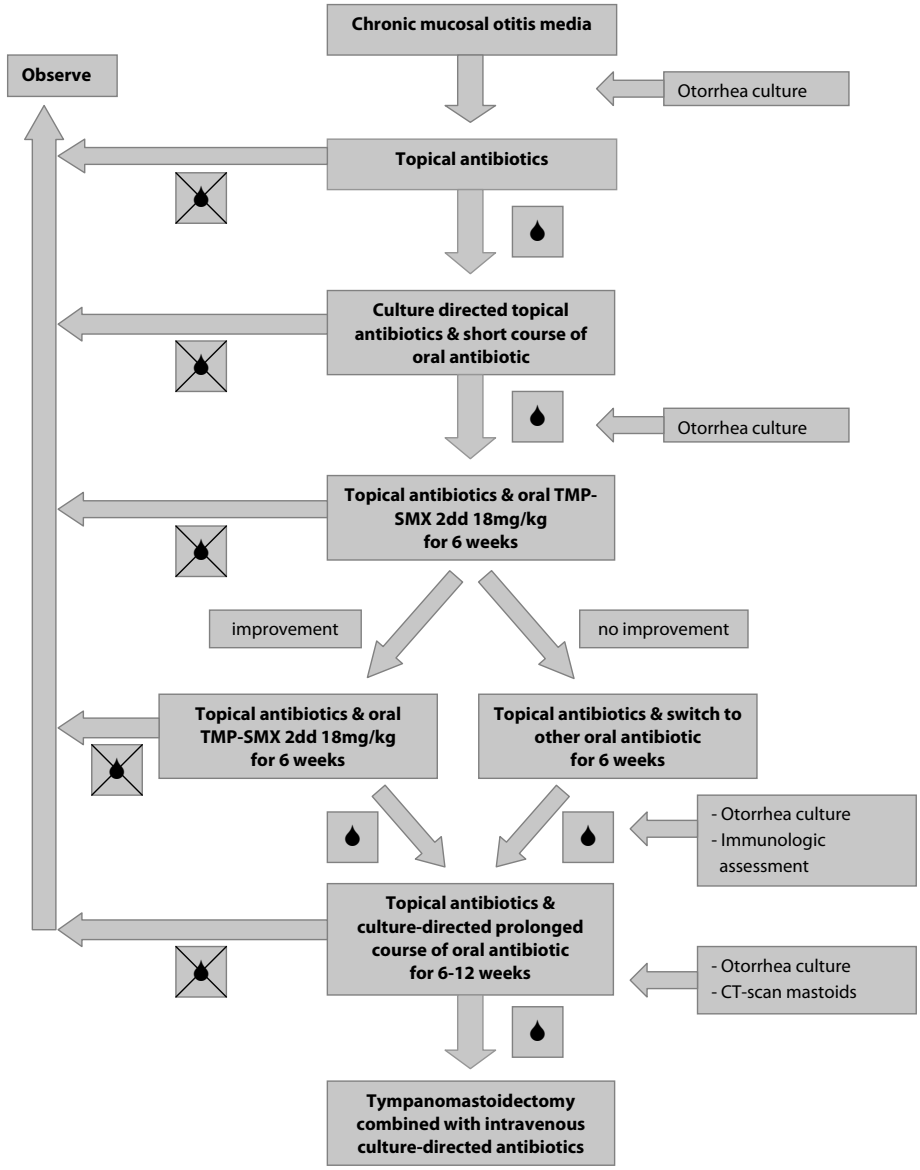


Figure 7.1. Recommended management of active chronic mucosal otitis media
♦ ; otomicroscopic signs of otorrhea in either ear
✖ ; no otomicroscopic signs of otorrhea
TMP-SMX; trimethoprim-sulfamethoxazole

Chapter 7.2

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Chronische otitis media (COM) is een van de meest frequente chronische infectieziekten bij kinderen, zowel in Westerse- als in ontwikkelingslanden. COM is een belangrijke oorzaak van gehoorverlies en kan tot ernstige extra- en intracraniële complicaties leiden zoals mastoïditis en meningitis. Adequate behandeling van kinderen met COM is dan ook belangrijk, maar consensus over wat dan de beste behandeling is ontbreekt. Al geruime tijd worden in het Wilhelmina Kinderziekenhuis van het UMC Utrecht kinderen met chronische otitis media die onvoldoende reageren op de gebruikelijke behandeling met antibiotische oordruppels en/ of korte kuren antibiotica, behandeld met co-trimoxazol in hoge dosering (18 mg/kg, 2 maal daags) gedurende minimaal 6 weken. Een retrospectieve analyse van de klinische gegevens van 48 kinderen met dergelijke klachten van chronische otitis media toonde een positief resultaat ten aanzien van de klachten van een loopoor. Dit was de aanleiding voor een placebo-gecontroleerde interventiestudie naar het effect van langdurige behandeling met co-trimoxazol bij kinderen met chronische otitis media. Naast de klinische effectiviteit onderzochten wij de kosteneffectiviteit van deze behandeling, de effecten op antibiotica resistentie, risicofactoren voor COM en de rol van de aangeboren en verworven afweer in de ontwikkeling van COM.

In **Hoofdstuk 2** gaven wij een overzicht van de literatuur over de oorzaken en behandeling van COM bij kinderen.

Clinici en onderzoekers blijken verschillende definities voor COM te hanteren; met name de duur van de otorroe varieerde van meer dan 2 weken tot meer dan 3 maanden. De risicofactoren voor COM bleken te zijn overgenomen uit studies bij kinderen met acute otitis media of otitis media met effusie. Het is onduidelijk of dit ook de daadwerkelijke risicofactoren voor COM zijn. Er bleek geen consensus te bestaan over de beste chirurgische of medicamenteuze behandeling bij kinderen met COM. Antibiotische oordruppels, met name die met quinolone, zijn veelbelovend, maar zowel de effectiviteit als de mogelijke bijwerkingen bij kinderen met een zeer langdurig beloop van COM moeten nog beter worden onderzocht. Wij concludeerden dat prospectieve en gecontroleerde studies nodig zijn om de plaats van zowel lokale en systemische medicatie als chirurgie in de behandeling van COM vast te stellen.

In **Hoofdstuk 3** wordt de epidemiologie van COM bij kinderen beschreven. Middels een patiënt-controle onderzoek werden factoren bestudeerd die geassocieerd zijn met COM. Factoren die onafhankelijk geassocieerd waren met COM betroffen; een voorgeschiedenis van trommelvliesbuisjes, meer dan 3 bovenste luchtweginfecties in de voorgaande 6 maanden, een laag opleidingsniveau van de ouders en het hebben van oudere broers

of zussen. Bij kinderen die trommelvliesbuisjes hadden, bleken meer dan 3 acute otitis media episodes in het afgelopen jaar, bezoek aan een kinderdagverblijf en het hebben van oudere broers of zussen geassocieerd te zijn met COM.

Wij bestudeerden ook genetische polymorfismen van receptoren die pathogene micro-organismen herkennen als risicofactor voor COM. Dergelijke polymorfismen kunnen leiden tot functionele varianten van die receptoren die behoren tot het aangeboren immuunsysteem. Zo'n functionele variant kan de interactie tussen de receptor en het pathogeen veranderen en zo ook de gevoeligheid voor infecties veroorzaakt door deze pathogene micro-organismen. In een patiënt-controle onderzoek werden de meest bekende polymorfismen van deze receptoren vergeleken bij 90 kinderen met COM en 375 controle personen. We konden geen verschil aantonen tussen deze 2 groepen. Wij concludeerden dat we met de ons beschikbare data niet betrouwbaar kunnen voorspellen welke kinderen COM zullen ontwikkelen.

In **Hoofdstuk 4.1** beschrijven we de resultaten van onze interventiestudie naar de effectiviteit van co-trimoxazol bij kinderen met COM. In totaal werden 101 kinderen tussen de 1 en 12 jaar oud met klachten van een loopoor gedurende meer dan 3 maanden gerandomiseerd; de helft werd gedurende 6-12 weken behandeld met co-trimoxazol (18 mg/kg, 2 maal per dag), de andere helft van de kinderen ontving gedurende dezelfde periode een placebo. Beide groepen werden gedurende 1 jaar gevolgd. Tijdens de controles na 6 en 12 weken werd bij respectievelijk 72 en 68% van de kinderen die behandeld waren met co-trimoxazol geen loopoor meer geconstateerd, terwijl bij de groep kinderen die een placebo hadden gekregen 47 en 53% geen loopoor meer had. Na 1 jaar was het verschil tussen beide groepen verdwenen; 75% van de co-trimoxazol groep had geen loopoor meer; in de placebo groep was dit 80%. Zowel het gehoor (luchtgeleidingsdrempels) als de kwaliteit van leven verbeterden tijdens de studie, maar verschilden niet tussen de co-trimoxazol groep en de placebo groep. In de met co-trimoxazol behandelde groep ontwikkelde één kind een huiduitslag en 9% van de kinderen maag-darmklachten t.o.v. geen huiduitslag en 2% maag-darmklachten in de placebo groep. Complicaties kwamen zelden voor en bleken gelijk verdeeld over beiden groepen; 1 kind in de co-trimoxazol groep ontwikkelde een acute mastoiditis en 1 kind in de placebo groep.

In **Hoofdstuk 4.2** beschrijven we de resultaten van de kosten-effectiviteit studie. We berekenden de kosten om 1 patiënt te genezen. Na 6 en 12 weken behandeling bedroegen de extra kosten om 1 kind te genezen van een loopoor respectievelijk €400 en €1.113. Aangezien het klinische effect na het staken van de behandeling verdween is de kosten effectiviteit na 1 jaar per definitie 0 of negatief en daarom hebben we deze niet berekend.

We concludeerden dat een behandeling met co-trimoxazol bij kinderen met COM effectief is op de korte termijn, maar dat het effect verdwijnt nadat de medicatie gestaakt wordt. In het licht van deze klinische effectiviteit en de geringe bijwerkingen van co-trimoxazol bij kinderen vinden wij de kosten van deze behandeling acceptabel.

In **Hoofdstuk 5** beschrijven we de effecten van langdurige behandeling met co-trimoxazol op het dragerschap en de ontwikkeling van resistentie in de bacteriële flora van kinderen met COM.

In **Hoofdstuk 5.1** bestudeerden we de optimale kweek methode voor de nasofarynx door bij een groep van 42 kinderen zowel een nasofaryngeale kweek via de mond als via de neus af te nemen. De monsters werden gekweekt op aerobe pathogenen. 87% van de kweken die via de neus werden afgenomen was positief t.o.v. 75% van de monsters die via de mond werden afgenomen (p=0.20). De bacteriën *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis,* en *Staphylococcus aureus* werden vaker met de methode via de neus dan die via de mond gevonden. De methode via de mond werd door de kinderen echter als minder vervelend ervaren. We concludeerden dat hoewel kinderen de kweekmethode via de mond beter verdragen, de opbrengst van de kweekmethode via de neus beter is en dat deze benadering daarom de voorkeur heeft bij onderzoek naar potentiële bacteriële pathogenen in de nasofarynx bij kinderen met COM.

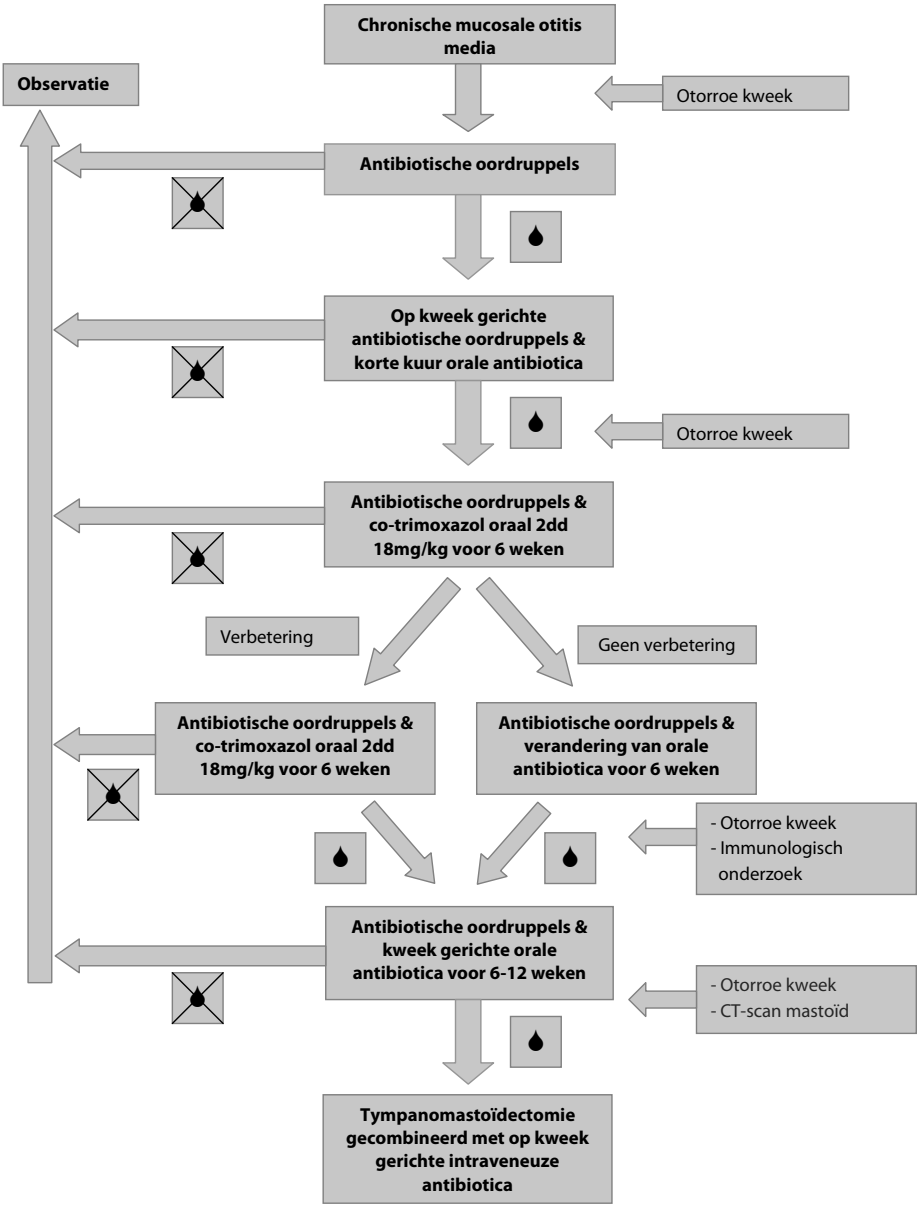
In **Hoofdstuk 5.2** worden de effecten van een langdurige behandeling met co-trimoxazol op het nasofaryngeaal dragerschap en de ontwikkeling van resistentie in de nasofaryngeale flora van kinderen met COM beschreven. Nasofaryngeale kweken werden bij alle deelnemers van onze studie via de neus afgenomen tijdens inclusie en tijdens de controles na 6 en 12 weken en 1 jaar. Prevalenties van *H.influenzae*, *M.catarrhalis* en *S.pneumoniae* waren ten tijde van de behandeling lager in de met co-trimoxazol behandelde groep dan in de placebo groep. In de co-trimoxazol groep nam de prevalentie van co-trimoxazol resistente *H.influenzae* toe van 4% bij inclusie tot 58% en 44% ten tijde van de controles na 6 en 12 weken. Ten tijde van het controle bezoek na 1 jaar waren zowel de prevalenties van *H.influenzae*, *M.catarrhalis en S.pneumoniae* als de prevalentie van co-trimoxazol resistente *H.influenzae* weer op het niveau van inclusie. Een langdurige behandeling met co-trimoxazol leidt dus enerzijds tot een afname van potentiële pathogenen in de nasofaryngeale flora, maar anderzijds tot een toename van co-trimoxazol resistente *H.influenzae*. Deze effecten verdwijnen als de behandeling met co-trimoxazol wordt gestaakt.

In **Hoofdstuk 5.3** worden de resultaten beschreven van een studie naar het effect van langdurig gebruik van co-trimoxazol op integron positieve en multiresistente Enterobacteriaceae in de darm flora van kinderen met COM. De darm flora is een groot reservoir voor pathogenen en speelt een belangrijke rol in het ontstaan en de versprei-

ding van resistentie tegen antibiotica. De meeste co-trimoxazol resistente genen van Enterobacteriaceae zijn gelegen in integronen; genetische elementen die geassocieerd zijn met multiresistentie. Bij de controles na 6 en 12 weken was 91 en 67% van de met co-trimoxazol behandelde kinderen drager van co-trimoxazol resistente Enterobacteriaceae, terwijl dit in de placebo groep respectievelijk 21 en 17% van de kinderen bedroeg. Multiresistentie nam ook toe tijdens de behandeling met co-trimoxazol. Bij de controle na 6 weken bedroeg de prevalentie van integronen 79% in de met co-trimoxazol behandelde groep en 22% in de placebo groep. Bij de controle na 12 weken was de prevalentie van integronen gedaald naar de waarde ten tijde van inclusie. De antibiotica resistentie van de Enterobacteriaceae was bij de controle na 1 jaar weer gedaald tot het niveau bij de aanvang van de studie. Dit geeft aan dat de verhoogde resistentie tijdens de behandeling met co-trimoxazol slechts tijdelijk geassocieerd is met hoge prevalenties van integronen, op den duur spelen blijkbaar andere resistentie mechanismen een rol.

In **Hoofdstuk 6** geven we een beschouwing op de resultaten beschreven in dit proefschrift. Tevens hebben we een algoritme ontwikkeld dat gebruikt kan worden bij de behandeling van COM bij kinderen (Figuur 7.2).

Idealiter zouden interventies op de preventie van COM gericht zijn, maar helaas zijn dergelijke preventieve maatregelen op dit moment nog niet voor handen. Voor de toekomst is het dan ook belangrijk dat op basis van de huidige pathofysiologische inzichten innovatieve en creatieve preventieve oplossingen ontwikkeld worden.



Figuur 7.2: Aanbeveling behandeling Chronische mucosale otitis media
♦ ; otomicroscopisch tekenen van otorroe in een van beide oren
🐞 ; otomicroscopisch geen tekenen van otorroe

Dankwoord

DANKWOORD

Natuurlijk was dit proefschrift nooit tot stand gekomen zonder de hulp en steun van de vele betrokken collega's, vrienden en dierbaren. Ik zou van deze gelegenheid gebruik willen maken om een aantal mensen in het bijzonder te bedanken.

Allereerst wil ik alle kinderen en hun ouders bedanken, die deel hebben genomen aan het COCO project. Dankzij jullie enthousiasme en trouwe deelname is het COCO project een groot succes geworden.

Alle KNO-artsen en kinderartsen die de ouders en hun kinderen hebben geïnformeerd, geënthousiasmeerd en verwezen, wil ik hartelijk bedanken voor het mogelijk maken van dit onderzoek.

Professor Schilder, beste Anne. Zonder jou was dit proefschrift nooit tot stand gekomen! Jouw bezielende begeleiding, onuitputtelijke drive en streven naar perfectie heeft het COCO-project tot een succes gemaakt. Jij hebt mij wetenschappelijk leren schrijven en de kansen geboden om mij in de wetenschap en de KNO-heelkunde te ontwikkelen. Daarvoor zal ik je altijd dankbaar blijven!

Professor Sanders, beste Lieke. Ondanks je drukke agenda kon ik altijd laagdrempelig met je overleggen en afspreken om artikelen te bespreken. Dit ging altijd gepaard met veel interesse en enthousiasme. Daarnaast dank voor het kritisch lezen van mijn stukken.

Doctor Rovers, beste Maroeska. Jij was altijd makkelijk benaderbaar en jouw kritische blik op mijn eerste versie van een manuscript tot aan een artikel heeft uiteindelijk mede tot dit proefschrift geleid. Je veelzijdigheid, mogelijkheid om wel dingen tegelijkertijd te doen en je gemeenschappelijke drive en samenwerking met Anne heb ik als zeer prettig ervaren.

Nelly van Eden, zonder jouw toewijding en secretariële kwaliteiten was het COCO project nooit succesvol geworden. Daarnaast was je ook nog eens een hele fijne kamergenoot.

Alie McArthur-Noom bedankt voor de uitgevoerde microbiologische bepalingen.

Tevens wil ik mijn dankbaarheid tonen aan de opleiders van de KNO van de afgelopen jaren. Professor Hordijk, u heeft mij destijds het vertrouwen en de kans gegeven om naast deze promotie ook met de opleiding te kunnen starten. Dit stel ik nog steeds zeer op prijs. Wijlen Professor Albers, voor zijn interesse en enthousiasme. Doctor van Olphen

en Professor Grolman, dank voor jullie interesse in mijn onderzoek en de begeleiding tijdens mijn opleiding.

Staf KNO en (ex)arts-assistenten KNO van het UMC Utrecht en de Gelre Ziekenhuizen. Dank voor de samenwerking in de afgelopen jaren en de interesse die jullie altijd hadden in de voortgang van mijn proefschrift. Jullie zijn een gezellige en fijne groep om mee samen te werken.

Monique Verhoeff bedankt voor het opzetten en de prettige overdracht van het COCO-project.

De co-auteurs die nog niet eerder genoemd zijn wil ik bedanken voor hun energie en bijdrage aan de artikelen. In alfabetische volgorde Prof. Dr. M.J.M. Bonten, Drs. C.W.B. Boonacker, Prof. Dr. C.K. van der Ent, Dr. A.C.Fluit, Dr. N. van Heerbeek, Dr. M.A. Leverstein-van Hall, Dr. Ir. G.T. Rijkers, Dr. H.J.T. Ruven, Drs. T.K. Timmers, Prof. Dr. G.J. van der Wilt, Prof. Dr. Ir. G.A. Zielhuis.

Paranimfen Casper en Sung-Wook. Jullie maken een belangrijk onderdeel uit van mijn leven en ik ben er dan ook trots op dat jullie hier vandaag naast mij willen staan. Casper, sinds je mijn zus kent heb ik een broer en goede vriend erbij. Vanaf dat moment zitten we op één lijn. Je openheid, gezelligheid en de positieve manier waarop je in het leven staat spreken me ontzettend aan. Poedersneeuw voor altijd! Sung-Wook, onze vriendschap gaat terug naar onze allereerste dag van geneeskunde. Ik kan het met jou overal over hebben, we vullen elkaar goed aan en we hebben al heel wat samen meegemaakt. Die gezelligheid en het respect voor elkaar zijn voor mij een groot goed. Ben benieuwd wat we samen allemaal nog mee gaan maken.

"Schoonfamilie" Margriet, Eveline, Ruud, Dieneke, Lilian en Marlon. Ook jullie hebben me de afgelopen jaren volop gesteund, dank hiervoor.

Lieve vrienden en vriendinnen. De meeste van jullie ken ik al een hele lange tijd. Bedankt voor de interesse, de steun en afleiding tijdens mijn promotietraject. De drukte heeft er soms voor gezorgd dat we elkaar veel te weinig zagen, maar echte vrienden blijven voor altijd.

Lieve mama, jouw vertrouwen en luisterend oor zijn prachtige eigenschappen die mij er altijd doorheen gesleept hebben. Je interesse is warm. Dat we nog maar vaak "spinazie" zullen eten. Papa, ik bewonder je energie om door te gaan en kansen te pakken. Ik

heb veel van je geleerd en als ik je nodig heb, ben je er altijd. Zonder jullie had ik hier vandaag niet gestaan.

Ingeborg, bedankt voor je interesse, gezelligheid en afleiding de afgelopen jaren. Je positieve kijk op het leven en je lach zijn eigenschappen die ik zeer waardeer. Ik had me geen betere zus kunnen wensen.

Tot slot, Annemieke. Lieve Miek, dank voor al je hulp, inspanningen en de ruimte die je me hebt gegeven de afgelopen jaren. Je bent een fantastische, evenwichtige en vrolijke vrouw. Jij hebt me de afgelopen jaren ontlast op die momenten dat ik het qua drukte op het werk even nodig had. Dit proefschrift had zonder die hulp niet kunnen plaats vinden. Ik hoop dat ik voor jouw promotie hetzelfde kan doen. Met ons gezamenlijk positivisme belooft het een mooie toekomst te worden.

Curriculum Vitae

CURRICULUM VITAE

Erwin Lammet van der Veen is geboren op 9 april 1981 te Warnsveld en groeide samen met zijn zus op in Woerden en Veenendaal. In 1999 behaalde hij het VWO diploma aan het Christelijk Lyceum Veenendaal. In datzelfde jaar startte hij met de studie geneeskunde aan de Universiteit Utrecht. Tijdens zijn co-schappen deed hij een keuze co-schap radiologie met specialisatie hoofd/hals gebied. In 2005 liep hij zijn wetenschappelijke stage en semi-arts stage bij de afdeling keel-, neus- en oorheelkunde van het Universitair Medisch Centrum Utrecht. Het promotieonderzoek resulterend in dit proefschrift startte hij na zijn arts-examen in 2005 onder leiding van Prof. dr. A.G.M. Schilder, Prof. dr. E.A.M. Sanders en Dr. M.M. Rovers. In 2006 begon hij met de opleiding tot keel-, neus- en oorarts in het Universitair Medisch Centrum Utrecht (opleider Prof. dr. G.J. Hordijk, Prof. dr. F.W.J. Albers, thans dr. A.F. van Olphen en Prof. dr. W. Grolman). Voor het perifere gedeelte van zijn opleiding heeft hij in de Gelre Ziekenhuizen te Apeldoorn gewerkt (opleider dr. P.P.G. van Benthem).

List of Publications

LIST OF PUBLICATIONS

van der Veen EL, Schilder AG, Timmers TK, Rovers MM, Fluit AC, Bonten MJ, Leverstein-van Hall MA. Effect of long-term trimethoprim/sulfamethoxazole treatment on resistance and integron prevalence in the intestinal flora: a randomized, double-blind, placebo-controlled trial in children. J Antimicrob Chemother. 2009 May;63(5):1011-6.

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isbn: **978-90-8559-943-2**