

**Incidence, microbiology and management  
of **acute otitis media and ear discharge** in primary care**



**Saskia Hullegie**



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PhD thesis, Utrecht University, the Netherlands

Cover design: Querian Eijgensteijn

Lay-out: ProefschriftMaken.nl

Printing: ProefschriftMaken.nl

ISBN: 978-94-6510-020-3

DOI: <https://doi.org/10.33540/2445>

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The research described in this thesis was primarily funded by the Netherlands Organisation for Health Research and Development (ZonMw) – Rational Pharmacotherapy 5th Open Call – grant number 84801 5006.

Financial support by SBOH, employer of GP trainees, and the Julius Center for Health Sciences and Primary Care for this publication of this thesis is gratefully acknowledged.



# **Incidence, microbiology and management of acute otitis media and ear discharge in primary care**

De incidentie, microbiologie en het beleid van  
otitis media acuta met loopoor in de eerste lijn  
(met een samenvatting in het Nederlands)

## **Proefschrift**

ter verkrijging van de graad van doctor aan de  
Universiteit Utrecht  
op gezag van de  
rector magnificus, prof. dr. H.R.B.M. Kummeling,  
ingevolge het besluit van het College voor Promoties  
in het openbaar te verdedigen op

woensdag 18 september 2024 des middags te 4.15 uur

door

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geboren op 23 augustus 1991  
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CHAPTER 1

1



## General Introduction

Based on:

Hullegie S, Schilder AGM, Damoiseaux RAMJ, Venekamp RP.

Een acuut loopoor [Acute ear discharge, a reason for consultation]. Ned Tijdschr Geneeskd. 2022 Aug 30;166:D6772. Dutch. PMID: 36300442.



## Acute otitis media (and ear discharge)

### Etiology, epidemiology and clinical picture

Acute otitis media (AOM) - an infection of the middle ear with the presence of middle ear effusion (MEE) and signs of an acute infection - is one of the most common childhood infections and a leading cause of doctor consultations and antibiotic prescribing worldwide.<sup>1-3</sup>

An episode of AOM is often preceded by a viral upper respiratory tract infection (URTI).<sup>4-6</sup> In children, the Eustachian tube does not yet function optimally, causing otopathogens to ascend easier from the nasopharynx to the middle ear, and middle ear fluid – created during an upper respiratory infection - to drain less easily.<sup>7</sup>

AOM has a peak incidence in the first year of life and the global incidence in childhood is 100-150 per 1000 person-years.<sup>8</sup> In adults the incidence is considerably lower: less than 5 per 1000 person-years.<sup>9</sup> Approximately 15%-20% of children with AOM present with ear discharge due to a spontaneous tear or perforation of the eardrum (AOMd).<sup>10,11</sup> The WHO defines ear discharge that exists for less than two weeks as acute and for longer than 2 weeks as chronic. However, many clinical guidelines apply a threshold of six weeks or longer. Chronic ear discharge is usually a manifestation of chronic inflammation of the ear mucosa and mastoid.

AOMd could be accompanied by ear pain and/or fever. In contrast to widespread beliefs, acute ear discharge as a presenting symptom of AOM does not mean that the infection is improving. Children with AOMd have similar levels of ear pain and feel worse at first presentation than those without ear discharge.<sup>10,11</sup> Also, children with AOMd have a higher disease burden with higher rates of ear pain and/or fever at 3–7 days and more AOM recurrences and hearing problems at 3 months compared with children without ear discharge.<sup>10,11</sup>

*Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* are the most common bacteria associated with AOM globally.<sup>12</sup> *Streptococcus pyogenes* is thought to be more prevalent in children with AOMd and the differences in clinical picture and disease course between AOM without ear discharge and AOMd might be attributed to differences in causative pathogens, but data are sparse.<sup>13-16</sup>

**Example A from daily practice**

Patient A is a 18 month aged girl without any relevant medical history. She visits the general practitioner (GP) because of acute ear discharge present for three days. She is agitated, cries a lot and had difficulty sleeping, but has no fever. Otoscopy shows otorrhea in the left ear which hampers visualisation of the eardrum. The right eardrum appears normal and intact. The GP explains to her mother that she is suffering from an acute middle ear infection with ear discharge due to a spontaneous perforation of the eardrum and opts for a watchful waiting approach. However, the child does not recover and after three days she revisits the GP. Otoscopy features are similar to the previous visit and the GP suggests an oral antibiotic. However, mother is reluctant to start these as her other child suffered from vomiting and diarrhoea following previous oral antibiotics. Moreover, she has read a news article about the problem of resistance resulting from antibiotic use.

**Differential diagnosis**

Based on the clinical presentation it is sometimes difficult to distinguish between AOMd and an acute presentation of otitis externa. In young children who present with acute ear discharge and other signs and symptoms of AOM such as a preceding URTI, pain and fever, AOMd is the most likely diagnosis. Otitis externa - an inflammation of the skin of the ear canal - is uncommon in children, but could occur after swimming in contaminated water (“swimmer’s ear”) and is usually accompanied by itching.<sup>17</sup> Fever, a preceding URTI and a history of prior AOM are often lacking.

**Diagnostic work-up**

Medical history and ear examination by otoscopy are essential for the diagnosis of AOM, both in primary and secondary care. Consequently, remote consultation is insufficient.

Alarm symptoms for a complicated disease course include severe illness, altered consciousness, neck stiffness, severe headache, vomiting, retro auricular redness, swelling or tenderness, and a protruding ear.<sup>18</sup>

Microbiological testing is indicated if first-line treatment fails. Otomicroscopy is an important diagnostic tool in secondary care, which provides a more detailed view and makes suction possible for removing fluid/debris from the ear canal and for a detailed picture of the skin of the ear canal, eardrum and the status of the ear canal. Diagnostic imaging (CT scan) is indicated if a cholesteatoma or complication (mastoiditis) is suspected.

**Example B from daily practice**

Patient B is a 38-year-old man with a medical history of ulcerative colitis for which he receives treatment with a TNF- $\alpha$  blocker and recurrent episodes of otitis externa for which he was treated in the past with triamcinolone ear drops. He calls the doctor's office on Friday afternoon because he is not feeling well, has pain in his right ear, hearing loss and yellowish fluid running out of his ear since that morning. He sees the assistant, who consults the GP who opts for a repeat prescription of triamcinolone ear drops. However, his symptoms worsen and two days later, the patient calls the General Practice Center and is advised (by phone) to take ibuprofen 400mg three times a day in addition to paracetamol, which he is already taking. He starts feeling worse and develops a severe headache. On Monday he visits his own GP who notices he is severely ill and requires support from his wife to walk into the consulting room. On physical examination, there is fever (T39.1 C°) with no obvious neck stiffness. The patient is confused and incoherent. Inspection in the right ear shows otorrhoea. Given the fever, confusion and incoherent speech, the GP consults the neurologist and refers the patient to the emergency department where, after laboratory examination, lumbar puncture and consultation with the ENT doctor, he was diagnosed as having acute bacterial meningitis secondary to otitis media.

**Management*****Pharmaceutical treatment***

An individual patient data meta-analysis has shown that antibiotics have a beneficial effect (number needed to treat (NNT) = 3) on pain and fever in children with AOM and ear discharge.<sup>10</sup> The current Dutch AOM guideline therefore recommends GPs to consider oral antibiotics in these children.<sup>19</sup> If the ear discharge is still persistent after 7 days while initially being left untreated, initiation of either oral amoxicillin (cotrimoxazole in case of penicillin allergy) or topical antibiotics for 7 days are advocated. In the absence of improvement within 48 hours, either amoxicillin/clavulanate should be started or the patient should be referred to the ENT surgeon. Furthermore, if there are risk factors for a complicated disease course, such as the use of immunosuppressive agents (Patient B), oral antibiotics should be started immediately.

Oral antibiotics do, however, expose children to common side effects such as diarrhoea, vomiting and rash. The routine use of antibiotics also adds to the development of antimicrobial resistance. Furthermore, evidence is accumulating that early life exposure to oral antibiotics puts young children at risk for developing asthma, overweight and inflammatory bowel disease later in life, possibly due to microbiome disturbances.<sup>20–25</sup> Alternative treatment strategies are therefore warranted. A Dutch trial has shown that in children with acute ear discharge in the presence of tympanostomy tubes antibiotic-

corticosteroid eardrops are clinically much more effective and less costly than oral antibiotics and initial observation.<sup>26</sup> Due to the existence of a perforation in the eardrum of children with AOMd, topical antibiotics may be an effective treatment strategy. In daily practice medical doctors already prescribe topical antibiotics (ear drops) for AOMd, however evidence to substantiate this routine practice is lacking.

The risk of inner ear damage (ototoxicity) from antibiotic-containing ear drops in the case of a not intact eardrum is still under debate. Ototoxicity has been demonstrated in animal experimental studies in which ear drops, in particular aminoglycosides, are applied directly to the round window.<sup>27</sup> A consensus statement of the Dutch ENT Society considered that the estimated risks of hearing damage from an (ongoing) infection of the middle ear are higher than the risk of ototoxicity related to the use of ear drops and that swelling of the middle ear mucosa – which occurs during an infection – protects the inner ear.<sup>28</sup> The statement advises to stop using the antibiotics ear drops if the ear is dry for 24 hours.

### ***Follow-up, referral criteria and complications***

It is important to actively schedule a follow-up visit for patients with acute ear discharge given the risk of a chronic disease course (chronic suppurative otitis media, CSOM), an underlying cholesteatoma, permanent hearing loss or a persistent eardrum perforation. Referral to the ENT doctor for additional diagnostic work-up and management is indicated when there is persistent ear discharge despite treatment, frequent recurrences or a persistent ear drum perforation six weeks after the onset of ear discharge.<sup>29</sup>

If suppurative complications of AOM - extracranial or intracranial - are suspected, an urgent referral to secondary care should be made. Extracranial complications include mastoiditis, facial nerve paralysis, petrositis, labyrinthitis and a Bezold's abscess.<sup>18</sup> Intracranial complications include meningitis, brain or epidural abscess, subdural empyema, sinus thrombosis or hydrocephalus/encephalitis. Although these (extra- and intracranial) complications are rare, early recognition, referral and treatment are crucial given their potentially life-threatening nature. Symptoms of these complications include retro-auricular swelling and/or redness, protruding ear, signs of sepsis, focal neurological signs, decreased consciousness, meningeal irritation, petechiae, persistent or severe headache.<sup>29</sup> Symptoms of cholesteatoma include hearing loss and intermittent ear discharge.<sup>30</sup> Chronic mastoiditis may be largely symptomless with phases of pain and ear discharge. In advanced stages, it can lead to facial nerve paralysis, meningitis, brain abscess or sinus thrombosis.

## Aim and outline of this thesis

Since evidence about the causative pathogens and most optimal management of AOMd is currently scarce, the aim of this thesis is to provide insight in the incidence, microbiology and management of AOMd in primary care. The focus is mainly on children as AOM is primarily a childhood disease. However, AOM can also occur in adults, so this thesis also aims to gain further insight in the incidence and current management of AOM in adults as data on this topic is scarce.

**Chapter 2** describes the incidence of otitis media. In the first part (**chapter 2.1**) the impact of the COVID-19 pandemic on the incidence of childhood otitis media is investigated, whereas in the second part (**chapter 2.2**) the incidence and management of AOM in adults is explored.

**Chapter 3** aims to synthesize the global evidence on the prevalence and antimicrobial resistance (AMR) status of bacteria in children with AOMd after the introduction of pneumococcal conjugate (PCV) vaccination.

**Chapter 4** presents the rationale and design (**chapter 4.1**) and results (**chapter 4.2**) of a primary care-based, open, individually randomised, controlled, non-inferiority trial comparing antibiotic-corticosteroid eardrops and oral antibiotics in children with AOMd.

**Chapter 5** reports on a study assessing the differential impact of antibiotic-corticosteroid eardrops and oral antibiotics on the gut microbiota composition and antibiotic resistance genes in children, which was nested in our trial of antibiotic treatments for children with AOMd

Finally, in **chapter 6**, the thesis' main findings and their implications for future primary-care based research will be discussed.

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CHAPTER 2

2

## **Incidence of otitis media**

## CHAPTER 2.1

# 2.1

# **Impact of the COVID-19 pandemic on the incidence of childhood otitis media in the Netherlands**

Based on:

Hullegie S, Schilder AGM, Marchisio P, de Sévaux JLH, van der Velden AW, van de Pol AC, Boeijen JA, Platteel TN, Torretta S, Damoiseaux RAMJ, Venekamp RP. A Strong Decline in the Incidence of Childhood Otitis Media During the COVID-19 Pandemic in the Netherlands. *Front Cell Infect Microbiol*. 2021 Nov 1;11:768377. doi: 10.3389/fcimb.2021.768377.

# Abstract

## Introduction

Recent reports have highlighted the impact of the COVID-19 pandemic on the incidence of infectious disease illnesses and antibiotic use. This study investigates the effect of the pandemic on childhood incidence of otitis media (OM) and associated antibiotic prescribing in a large primary care-based cohort in the Netherlands.

## Methods

Retrospective observational cohort study using routine health care data from the Julius General Practitioners' Network (JGPN). All children aged 0-12 registered in 62 practices before the COVID-19 pandemic (1 March 2019 - 29 February 2020) and/or during the pandemic (1 March 2020 - 28 February 2021) were included. Data on acute otitis media (AOM), otitis media with effusion (OME), ear discharge episodes and associated antibiotic prescriptions were extracted. Incidence rates per 1,000 child years (IR), incidence rate ratios (IRR) and incidence rate differences (IRD) were compared between the two study periods.

## Results

OM episodes declined considerably during the COVID-19 pandemic: IR pre-COVID-19 vs COVID-19 for AOM 73.8 vs 27.1 [IRR 0.37]; for OME 9.6 vs 4.1 [IRR 0.43]; and for ear discharge 12.6 vs 5.8 [IRR 0.46]. The absolute number of AOM episodes in which oral antibiotics were prescribed declined accordingly (IRD pre-COVID-19 vs COVID-19: -22.4 per 1,000 child years), but the proportion of AOM episodes with antibiotic prescription was similar in both periods (47% vs 46%, respectively).

## Conclusion

GP consultation for AOM, OME and ear discharge declined by 63%, 57% and 54% respectively in the Netherlands during the COVID-19 pandemic. Similar antibiotic prescription rates before and during the pandemic indicate that the case-mix presenting to primary care did not considerably change. Our data therefore suggest a true decline as a consequence of infection control measures introduced during the pandemic.

## Introduction

On 11 March 2020, the WHO<sup>1</sup> declared a global pandemic of COVID-19 which enforced many countries to introduce generic infection control measures such as wearing face masks, hand washing, social distancing, working from home and closure of schools/day-care centers. Other than reducing SARS-CoV-2 transmission, these generic measures have likely affected transmission of other respiratory viruses.<sup>2,3</sup> Since otitis media (OM) is generally preceded by a viral upper respiratory tract infection (URTI),<sup>4</sup> changes in transmission dynamics of these viruses may have had an impact on the incidence of OM. Several reports from the early phase of the COVID-19 pandemic have indeed suggested a decline in doctor consultations for OM in children.<sup>5-10</sup> The question is whether the infection control measures or the sudden COVID-19 related changes in health care access and delivery are responsible for these changes. We will address this question by investigating the effect of the COVID-19 pandemic on OM consultations and associated antibiotic prescribing in children in a large primary care cohort in the Netherlands where the general practitioner (GP) is the first point of call for the management of OM for all children and practices could be contacted for medical advice throughout the pandemic.<sup>11</sup>

## Methods

### Design and study population

In this retrospective observational cohort study, data were obtained from the Julius General Practitioners' Network (JGPN). Its database contains anonymously extracted routine health care data from electronic records from 62 general practices in the Utrecht area.<sup>12</sup> All children aged 0-12 registered 1 March 2019 - 29 February 2020 (pre-COVID-19 pandemic) and/or 1 March 2020 - 28 February 2021 (COVID-19 pandemic) were included. The data requested was subject to approval by the independent scientific committee of JGPN, under the obligation to make the results publicly available. The Medical Research Ethics Committee Utrecht has reviewed the study protocol and declared that official ethical approval is not required since this research is outside the scope of the Dutch Medical Research Involving Human Subjects Act (protocol no 21-562/C).

### Data extraction

From the electronic health records, GP consultations of OM (International Classification of Primary Care [ICPC] code H04 (ear discharge); H71 (acute otitis media) H72 (otitis media with effusion)) and H01 (ear pain) were extracted. A new OM episode started if there was no OM-related GP consultation for 28 days. For each episode, the

start date, the child's age at the start of the episode, the number and type of consultations, antibiotic prescriptions and complications (mastoiditis, ICPC code H74.02) were extracted. OM treated with antibiotics was defined as an OM episode with an oral or topical antibiotic prescription according to the Anatomical Therapeutic Chemical (ATC) classification. Since episodes and antibiotic prescriptions are not directly linked in the JGPN database, antibiotic prescriptions within two days before and after the start and stop date of the episode were captured. The full list of ATC codes used in this study can be found in Supplementary Table 1. Additionally, data on acute upper respiratory tract infections (URTI, ICPC code R74) were extracted.

### **Implementation of infection control measures in the Netherlands**

On March 15<sup>th</sup> 2020, the prime minister of the Netherlands introduced social distancing, working from home, and the closure of restaurants/bars, sport facilities and schools/daycare. Primary schools and daycare centres reopened 11<sup>th</sup> of May 2020<sup>13</sup>, but a 1.5 meter distance rule remained in place and wearing non-medical face-masks was introduced (both not obligatory for children up to 12 year of age). The second lockdown, including closure of schools and daycare centres, started the December 14<sup>th</sup>, 2020 and ended 9 February 9<sup>th</sup>, 2021.

### **Analysis**

We calculated the total number of OM episodes pre-COVID-19 and during the COVID-19 pandemic. Incidence rates (IR) were calculated per 1000 person-years by dividing the number of OM episodes by the total number of person-years in that specific time period. In stratified analyses, children were split into the following age groups: <2 year, 2-6 year and  $\geq 6$ -12 years. Differences in overall OM episodes and those treated with antibiotics between the two time periods were expressed as rate ratios (IRR) and rate differences (IRD) with accompanying 95% confidence intervals (CI). All statistical analyses were performed with SPSS (version 26.0, Chicago, IL, USA) and MedCalc for Windows, version 19.4 (MedCalc Software, Ostend, Belgium).

## **Results**

### **Study population**

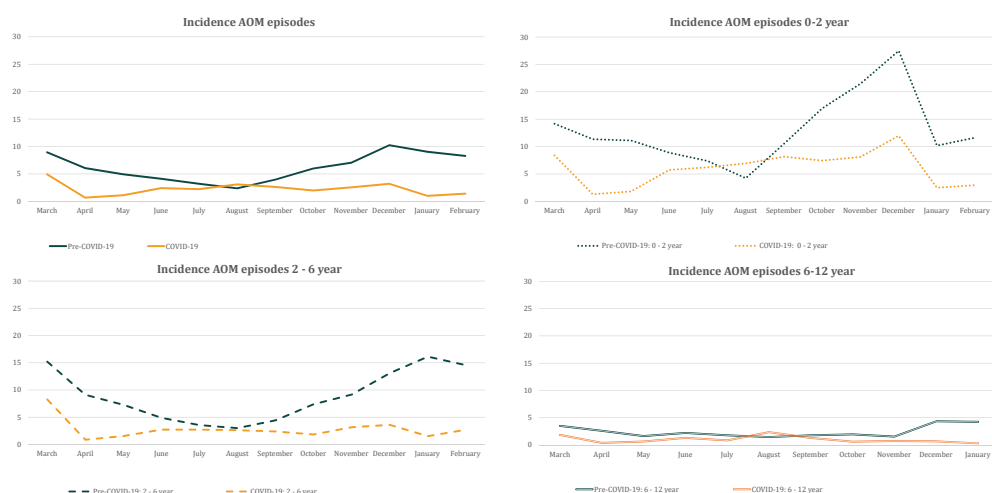
In the pre-COVID-19 period, electronic health record data of 67,245 children aged 0-12 years were available (time point: 1 September 2019) whereas data of 67,134 children were available during the pandemic (time point: 1 September 2020). Sex and age distribution were similar across periods: 51% male, 16% aged <2 years, 33% 2-6 years and 51%  $\geq 6$ -12 years.



## Overall OM episodes

OM episodes declined considerably during the COVID-19 pandemic (Table 1). The IR per 1,000 child-years pre-COVID-19 vs COVID-19 for AOM were 73.8 vs 27.1 [IRR 0.37, 95% CI 0.35-0.39], for OME 9.6 vs 4.1 [IRR 0.43, 95% CI 0.37-0.49], for ear discharge 12.6 vs IR 5.8 [IRR 0.46, 95% CI 0.41-0.52] and for ear pain 18.1 vs 11.8 [IRR 0.65, 95% CI 0.60-0.71]. Age-specific analyses revealed similar results, except for a less pronounced decline in OME episodes in children aged 0-2 years [IRD -0.66, 95% CI -2.50-1.18].

Figure 1 shows the monthly incidences of AOM episodes per 1,000 child months for various age groups before and during the pandemic and illustrates the absence of the usual winter peak in AOM incidence during the COVID-19 pandemic, especially in young children. Supplementary Figure 1 shows the monthly incidence of OME episodes per 1,000 child months for various age before and during the pandemic.



**Figure 1. Incidence of AOM episodes per 1,000 child months (total and according to age)**

Figure 2 illustrates the timing of implementation of generic infection control measures together with the monthly incidences of AOM and OME per 1,000 child months from March 2019 to March 2021. AOM and OME incidences decrease sharply during the COVID-19 peaks as well as during closure of schools and daycare centers. Acute upper respiratory tract infections show a similar pattern (Supplementary Figure 2).

Table 1. Number of Otitis Media Episodes and Episodes with Antibiotic Prescription Pre COVID-19 era and COVID-19 era, including rate ratios and rate difference

Episodes of	Pre COVID-19 era <sup>a</sup>						COVID-19 era <sup>a</sup>						Rate Ratios and Rate Differences					
	N	N	IR	% AB	% AB	% AB	N	N	IR	% AB	% AB	% AB	IR	IRR	IRD <sup>e</sup>	IRD episodes with	IRD episodes with	
	patients	episodes	episodes <sup>b</sup>	oral <sup>c</sup>	top <sup>d</sup>	top <sup>d</sup>	patients	episodes	episodes <sup>b</sup>	oral <sup>c</sup>	top <sup>d</sup>	top <sup>d</sup>	episodes	episodes	episodes	AB oral <sup>f</sup>	AB top <sup>g</sup>	
AOM	67245	4959	73.7	47.4	10.7	6.7	67134	1822	27.1	46.2	14.5	0.37	-46.61	(0.35; 0.39)*	-22.4	(-24.1; -20.80)*	-3.9	
Age 0 - 2	10896	1574	144.5	61.6	6.7	10243	681	66.5	62.1	4.1	4.1	0.46	-77.97	(0.42; 0.50)*	-47.6	(-54.1; -40.7)*	-7.0	
Age 2 - 5	21731	2354	108.3	44.0	11.5	21859	743	34.0	41.0	15.5	0.31	-74.33	(0.29; 0.34)*	-33.7	(-36.9; -30.38)*	-7.2		
Age 6 - 12	34618	1031	29.8	33.8	14.8	35032	398	11.4	28.4	30.7	0.38	-18.42	(0.34; 0.43)*	-6.8	(-8.9; -5.40)*	-0.9		
OME	67245	648	9.6	8.5	10.2	67134	277	4.1	7.2	12.6	0.43	-5.51	(0.37; 0.49)*	-0.5	(-0.77; -0.27)*	-0.46		
Age 0 - 2	10896	54	5.0	16.7	7.4	10243	44	4.3	18.2	4.5	0.87	-0.66	(0.57; 1.31)	-0.04	(-0.81; 0.72)	-0.17		
Age 2 - 5	21731	297	13.7	9.4	10.1	21859	110	5.0	7.3	7.3	0.37	-8.63	(0.29; 0.46)*	-0.9	(-1.46; -0.38)*	-1.0		
Age 6 - 12	34618	297	8.6	6.1	10.8	35032	123	3.5	3.3	20.3	0.41	-5.07	(0.33; 0.51)*	-0.4	(-0.70; -0.14)*	-0.2		
Ear discharge	67245	847	12.6	25.4	40.3	67134	388	5.8	14.2	48.2	0.46	-6.82	(0.41; 0.52)*	-2.38	(-0.64; -0.21)*	-2.3		
Age 0 - 2	10896	222	20.4	36.9	27.0	10243	78	7.6	28.2	19.2	0.37	-12.76	(0.29; 0.49)*	-5.4	(-2.86; -1.90)*	-4.0		
Age 2 - 5	21731	388	17.9	27.1	41.0	21859	158	7.2	15.8	46.8	0.40	-10.63	(0.33; 0.49)*	-3.7	(-7.27; -3.49)*	-3.9		
Age 6 - 12	34618	237	6.8	11.8	51.5	35032	152	4.3	5.3	64.5	0.63	-2.51	(0.33; 0.49)*	-0.58	(-4.71; -2.66)*	-0.7		
													(0.51; 0.78)*	(-0.92; -0.24)*		(-1.56; 0.11)		

<sup>a</sup>Pre COVID-19 era = March 2019 – 29 February 2020, COVID-19 era = 1 march 2020 - 28 February 2021

<sup>b</sup> Incidence rate = total episodes per 1000 persons years

<sup>c</sup>Percentage of episodes with an oral antibiotic prescriptions

<sup>d</sup>Percentage of episodes with a topical antibiotic prescriptions

<sup>e</sup>Incidence Rate ratios (IRR) and Incidence Rate differences (IRD) per episode (Pre COVID-19 era vs COVID-19 era), including 95% confidence interval

<sup>f</sup> IRD episodes with an oral antibiotic prescription per 1000 persons years (Pre COVID-19 era vs COVID-19 era)

<sup>g</sup> IRD episodes with a topical antibiotic prescription per 1000 persons years (Pre COVID-19 era vs COVID-19 era) \* p value < 0,05

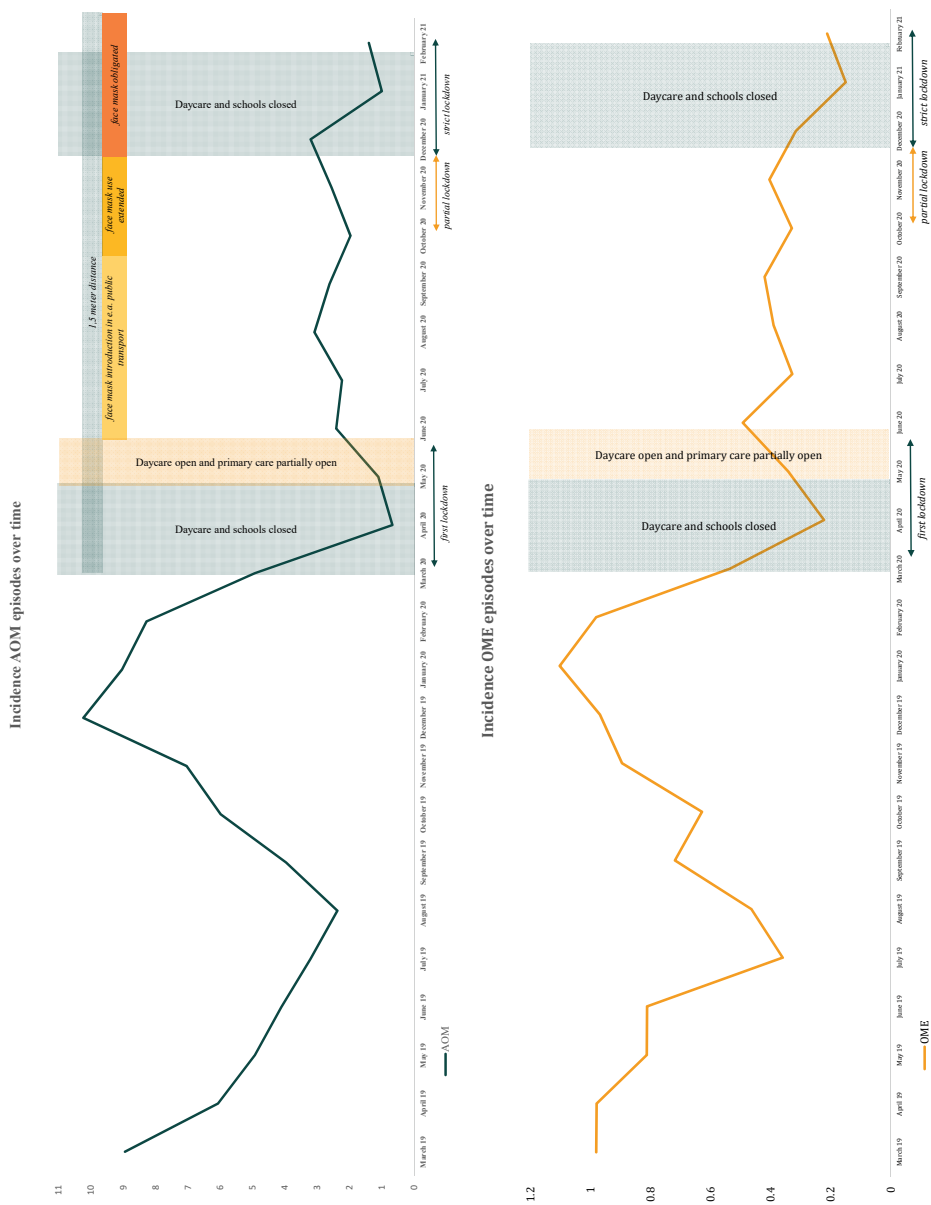


Figure 2. Incidence AOM and OME episodes per 1000 childmonths and government restrictions. 2A: AOM, 2B: OME

## **OM episodes treated with antibiotics**

Similar to the overall OM episodes, the absolute number of AOM episodes in which oral antibiotics were prescribed declined accordingly (IRD pre-COVID-19 vs COVID-19: -22.4 per 1,000 child years), but the proportion of AOM episodes with antibiotic prescription was similar in both periods (47% vs 46%, respectively) (Table 1).

## **Type of consultation**

The numbers of OM-episodes are based on ICPC-codes, which consist of both face-to-face and telephone GP consultations. The proportion of OM episodes (AOM, OME, ear discharge combined) which were coded based on only telephone consultation(s) only, increased over time [pre-COVID-19 vs COVID-19: 7.4% vs 22.3%].

## **Complications**

The incidence of acute mastoiditis remained low throughout the study period; IR per 1,000 child year pre-COVID-19 vs COVID-19: 0.15 vs 0.10 [RR 0.70, 95% 0.23-2.04].

## **Discussion**

This large retrospective cohort study showed that GP consultation for AOM, OME and ear discharge declined by 63%, 57% and 54% respectively in the Netherlands during the COVID-19 pandemic.

Previous studies in other countries have reported a similar trend in childhood OM incidence during the first COVID-19 peak.<sup>5-8,10</sup> Under normal circumstances, OM typically shows a seasonal pattern with a winter peak coinciding with the increase in URTI incidence.<sup>14</sup> Our study demonstrates the absence of the usual winter peak in OMA and OME during COVID-19 which is comparable with the reports of bronchiolitis from Belgium.<sup>15</sup> The observed reduction in childhood OM might be attributed to the generic infection control measures, or changes in health care access and delivery. Although primary care services in the Netherlands remained accessible during the pandemic, the measures could have lead to a higher threshold for consulting the GP, particularly early in the pandemic.

We found no evidence of an increase in the proportion of childhood OM episodes treated with antibiotics despite a substantial reduction in GP consultations for OM. This suggests that the observed decline in doctor consultations for OM was not related to OM severity and therefore not primarily attributed to a higher threshold to consultation. In line with our findings, a previous study in Scotland reported that the COVID-19

lockdown led to a decline in pediatric emergency care consultations without an associated increase in severity.<sup>16</sup>

The major strengths of our study are its large sample size using well-documented electronic routine primary care-based health care data. The longitudinal nature of our study allowed us to compare the same study population within the same practices during two full years, i.e. one full year pre-COVID-19 and a complete year during the COVID-19 pandemic. Some methodological limitations need to be considered. First, misclassification might have occurred. Particularly during the COVID-19 pandemic, a substantial proportion of OM diagnoses were based on telephone consultation only. A previous study from our group found that around 50% of parent-reported OM episodes led to a GP diagnosis of OM.<sup>17</sup> Misclassification in OM diagnoses as a result of an increase in telephone consultation during the COVID-19 pandemic would however have led to an overestimation of OM episodes during the pandemic and an underestimation of the observed decline over time. Besides that, despite the increase in telephone consultations, the ICPC code ‘ear pain’ has showed a decrease as well, which is another argument for a true decline of OM during the pandemic. Moreover, misclassification might have occurred for the ICPC code ‘ear discharge’ since we were unable to determine whether this related to an acute onset of ear discharge or chronic suppurative otitis media. Second, we were unable to reliably extract data on specialist referrals and data about out of hours primary care were not available. Therefore, our results regarding complications should be interpreted with caution. Reassuringly, a previous study from Italy did not find a significant difference in OM-related complications during the first COVID-19 wave.<sup>6</sup> Finally, we were not able to link reductions in childhood AOM episodes to changes in causative viruses and bacteria over time. Such data would have allowed us to better explain our observations.

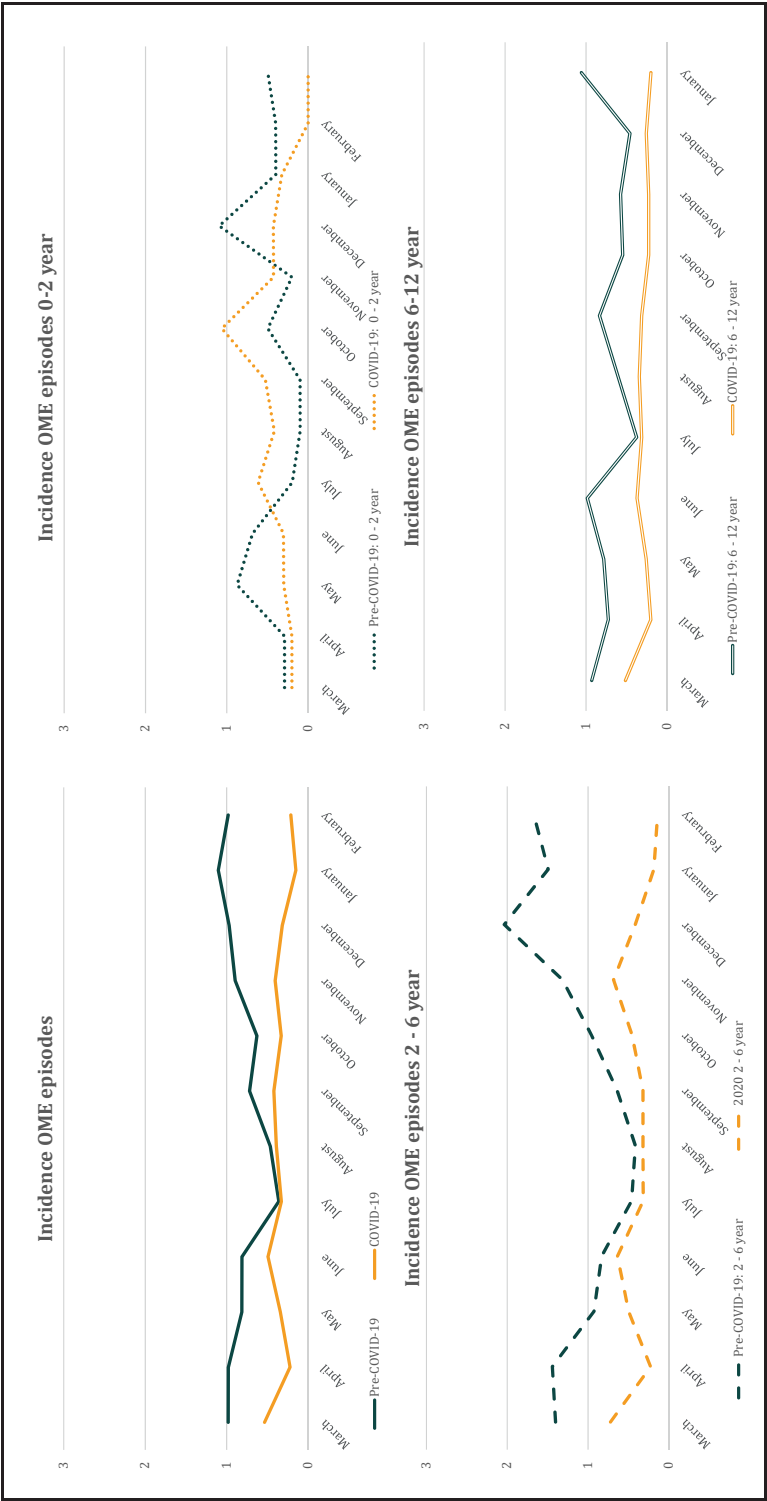
## Conclusion

GP consultation for AOM, OME and ear discharge declined by 63%, 57% and 54% respectively in the Netherlands during the COVID-19 pandemic. Similar antibiotic prescription rates before and during the pandemic indicate that the case-mix presenting to primary care did not change considerably. Our data therefore suggests a true decline as a consequence of infection control measures introduced during the pandemic.

## Supplementary Material

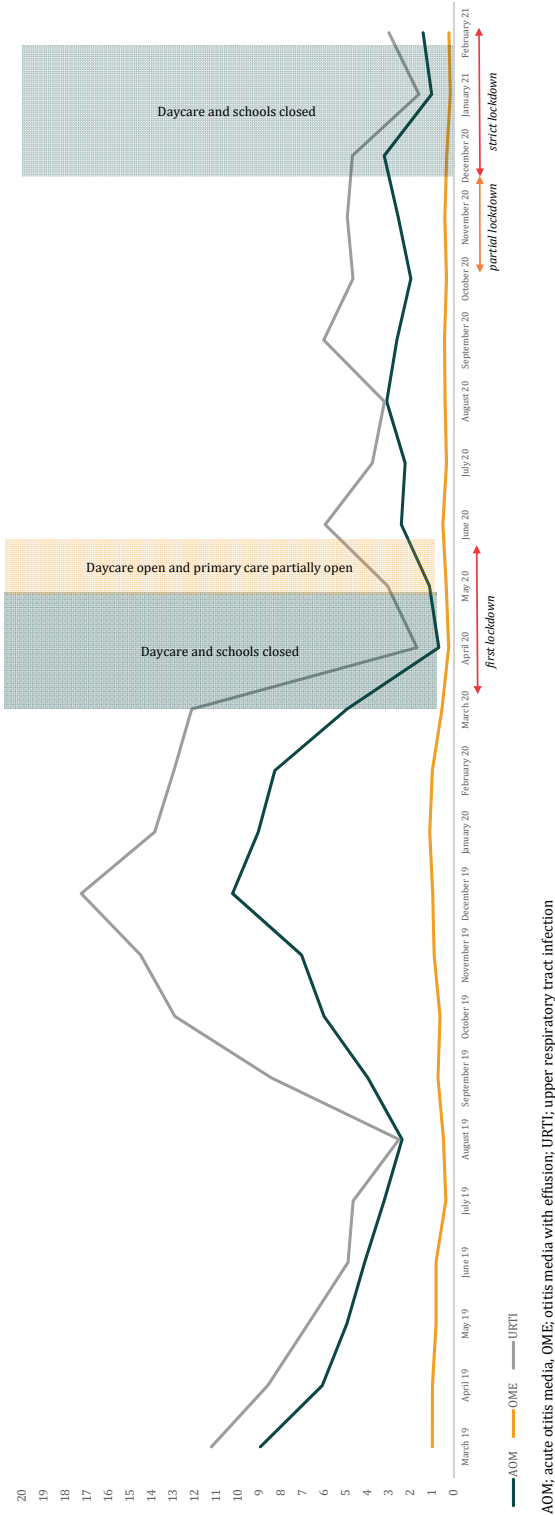
**Supplementary Table 1. Oral and topical antibiotics with Anatomical Therapeutic Chemical (ATC) codes**

Oral antibiotics	ATC codes
amoxicillin	J01CA04
amoxicillin/clavulanic-acid	J01CR02
co-trimoxazole	J01EE01
clarithromycin	J01FA09
azithromycin	J01FA10
Topical antibiotics	ATC codes
tobramycin	J01GB01
ofloxacin	S01AE01/S02AA16/S01AX11
dexamethasone/tobramycin	S01CA01
dexamethasone/framycetine/gramicidin	S02CA06
hydrocortisone/colistin/bacitracin	S02CA03



Supplementary Figure 1. Incidence of OME episodes per 1,000 childmonths (total and according to age)

Incidence AOM, OME and URTI episodes over time



AOM; acute otitis media, OME; otitis media with effusion; URTI; upper respiratory tract infection

Supplementary Figure 2. Incidence AOM, OME and URTI episodes per 1000 childmonths and government restrictions

AOM; acute otitis media, OME; otitis media with effusion; URTI; upper respiratory tract infection



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## CHAPTER 2.2

# 2.2

# **Incidence and management of acute otitis media in adults**

Based on:

Rijk MH, Hullegie S, Schilder AGM, Kortekaas MF, Damoiseaux RAMJ, Verheij TJM, Venekamp RP. Incidence and management of acute otitis media in adults: a primary care-based cohort study. *Fam Pract.* 2021 Jul 28;38(4):448-453. doi: 10.1093/fampra/cmaa150

## **Abstract**

### **Introduction**

Although primarily considered a childhood disease, acute otitis media (AOM) also occurs in adults. Data on the burden of this condition in adults are, however, scarce.

### **Objective**

To explore the primary care incidence and current management of AOM in adults.

### **Methods**

All patients aged 15 and older included in the routine health care database of the Julius General Practitioners' Network were followed from 2015 to 2018 (contributing to a total of 1261575 person-years). We extracted data on AOM episodes, AOM-related consultations, co-morbidities and antibiotic and analgesic prescriptions.

### **Results**

Five thousand three hundred and fifty-eight patients experienced one or more AOM episodes (total number of AOM episodes: 6667; mean 1.2 per patient). The overall AOM incidence was 5.3/1000 person-years and was fairly stable over the study period. Incidence was particularly high in atopic patients (7.3/1000 person-years) and declined with age (from 7.1 in patients 15-39 years of age to 2.7/1000 person-years in those aged 64 years and older). Oral antibiotics, predominantly amoxicillin, were prescribed in 46%, and topical antibiotics in 21% of all episodes.

### **Conclusion**

Over the past years, the incidence of AOM in adults in primary care has been stable. Oral antibiotic prescription rates resemble those in children with AOM, whereas a remarkably high topical antibiotic prescription rate was observed. Future prognostic research should inform on the need and feasibility of prospective studies into the best management strategy in this condition.

## Introduction

Although young children are particularly prone to develop acute otitis media (AOM), it can also occur in adults. Factors associated with the relatively high incidence in children are immaturity of the immune system and the Eustachian tube<sup>1–3</sup>, along with other host and environmental factors such as atopic diathesis and exposure to tobacco smoke.<sup>4,5</sup> Complications of AOM are rare in high-income countries, but include serious – and potentially lethal – disease entities such as meningitis and brain abscesses.<sup>1</sup>

To date, direct data on the burden of AOM in adults are scarce; a 2012 systematic review therefore provided a modelling-based incidence of AOM in this population.<sup>6</sup> A recent publication on AOM in primary and emergency care settings among a predominantly male cohort of USA veterans reported a mean incidence rate of 2.7/1,000 person-years.<sup>7</sup> Furthermore, evidence on prognosis and management of AOM is lacking, which leaves general practitioners (GPs) with uncertainty how to best treat adult AOM patients in everyday practice. As a result, GPs may base their treatment decisions on the available clinical guidelines of AOM, which solely derive from research in children.<sup>8–10</sup>

To gain more insight in the burden of AOM among adults, we set out to explore the incidence and current management of AOM in adults in Dutch primary care using 2015 to 2018 routine primary health care data.

## Methods

### Design and patient population

For this retrospective cohort study, we used pseudonymized routine health care data extracted from the Julius General Practitioners' Network (JGPN) database.<sup>11</sup> This database covers over 70 primary care practices with around 200 GPs and holds data on patient demographics, consultations, disease episodes (using the International Classification of Primary Care (ICPC)<sup>12</sup>) and prescriptions (using Anatomical Therapeutic Chemical (ATC) codes<sup>13</sup>). We included all patients aged 15 and older enlisted in the participating practices between 2015 and 2018.

### Data extraction

For all eligible patients, we extracted data on year of birth, gender, diagnosis of atopic diathesis (ICPC R96 (asthma) and/or R97 (allergic rhinitis) and/or S87 (constitutional eczema)), type 1 and 2 diabetes mellitus (T90, which has been associated with increased susceptibility for infections<sup>14</sup>), and use of systemic corticosteroids (ATC H02) or other immunosuppressive drugs (ATC L04). In addition, we extracted data on AOM-related

prescriptions and number of AOM-related consultations. For oral antibiotics, we only included ATC codes of amoxicillin, amoxicillin/clavulanate, co-trimoxazole, and clarithromycin, since these are mentioned in the ‘AOM in children’ clinical practice guideline issued by the Dutch College of General Practitioners.<sup>8</sup> We also included azithromycin, since it has been listed as second-line option until the 2014 update of the ‘AOM in children’ guideline, and its prescription might have continued into our study period.

### **Outcome measures**

Our main outcome of interest was GP-diagnosed AOM (ICPC H71). A new AOM episode was defined as an AOM-related primary care consultation after a period of at least 28 days without any AOM-related consultation.

Secondary outcome measures included the proportion of AOM episodes in which (oral and topical) antibiotics and analgesics were prescribed, and the number of consultations per AOM episode. Since disease episodes and prescriptions are not directly linked in the JGPN database, an AOM-related prescription was defined as an antibiotic or analgesic prescribed between 7 days before and after an AOM episode.

### **Statistical analysis**

Overall and year-specific AOM incidence rates with corresponding 95% confidence intervals (CIs) were calculated by dividing the number of AOM episodes by the total number of person-years for all adults and stratified according to age (15-39, 40-64, and  $\geq 65$  years), gender, comorbidity, and use of systemic corticosteroids or other immunosuppressive drugs prior to an AOM episode. Additionally, we stratified incidence rates in atopic patients according to age to assess whether age modified the observations.

Next, we calculated the number of (oral and topical) antibiotics and analgesics per 100 AOM episodes and the mean number of consultations per AOM episode, all with corresponding 95% CIs. To assess whether management differed across patient groups, we stratified the results according to the specified age groups, gender, comorbidity, and use of immunosuppressive drugs. In an exploratory analysis, we estimated the consultation rates for patients who did and did not receive an immediate (oral or topical) antibiotic prescription. In line with the explorative nature of our study and due to the large number of subjects, we aimed to describe the observed trends in our database rather than performing formal tests to assess any statistically significant differences for the various outcome measures. Statistical analyses were performed using SPSS version 25 (SPSS Inc., Chicago, IL) and Rothman’s Episheet version 11 June 2008.



## Results

### Study population

The size of the cohort varied from 324 054 adults in 2015 to 351 495 in 2018 contributing to a total follow-up time of 1 261 575 person-years. Overall, 52.1% of the patients was female, 45.5% was aged between 15 and 39 years, 37.6% between 40 and 64 years, and 16.9% was 65 or older. On average, 21.4% of the population had an atopic diathesis and 6.0% had diabetes. Systemic corticosteroids or other immunosuppressants were used during 0.7% of all person-years.

**Table 1. Baseline characteristics of included AOM episodes from 2015 to 2018**

	2015 - 2018	2015	2016	2017	2018
Total, n	6667	1689	1665	1695	1618
Age, mean (SD)	38.3 (16.6)	38.0 (16.6)	38.2 (16.3)	38.5 (16.7)	38.7 (16.7)
Age, median (IQR)	36 (23)	35 (23)	36 (23)	36 (24)	37 (22)
Age, n (%)					
15-39	3918 (58.8)	1008 (59.7)	1011 (60.7)	977 (57.6)	922 (57.0)
40-64	2166 (32.5)	539 (31.9)	520 (31.2)	558 (32.9)	549 (33.9)
≥65	583 (8.7)	142 (8.4)	134 (8.0)	160 (9.4)	147 (9.1)
Gender, n (%)					
Male	2748 (41.2)	703 (41.6)	665 (39.9)	706 (41.7)	674 (41.7)
Female	3919 (58.8)	986 (58.4)	1000 (60.1)	989 (58.3)	944 (58.3)
Comorbidities, n (%)					
Atopic diathesis	1983 (29.7)	459 (27.2)	502 (30.2)	523 (30.9)	499 (30.8)
Diabetes	313 (4.7)	73 (4.3)	73 (4.4)	93 (5.5)	74 (4.6)
Use of immunosuppressive drugs <sup>a</sup> , n (%)	52 (0.8)	16 (0.9)	9 (0.5)	17 (1.0)	10 (0.6)

Abbreviations: AOM, acute otitis media; SD, standard deviation; IQR, interquartile range. <sup>a</sup> Defined as use of systemic corticosteroids or immunosuppressants at the day prior to AOM episode.

### AOM episodes

A total of 5358 patients (58.9% female) experienced one or more AOM episodes (total number of AOM episodes: 6667; range 1 to 13; median 1 episode per patient; Table 1).

### Incidence rates

The overall AOM incidence rate was 5.3/1000 person-years (95% CI: 5.2 to 5.4). Yearly incidence rates revealed a more or less stable pattern (Figure 1).

Incidence of AOM declined with age and was higher in female patients (Table 2). The presence of an atopic diathesis was associated with a higher AOM incidence rate (Table 2); this observation was independent of age (Table S1).

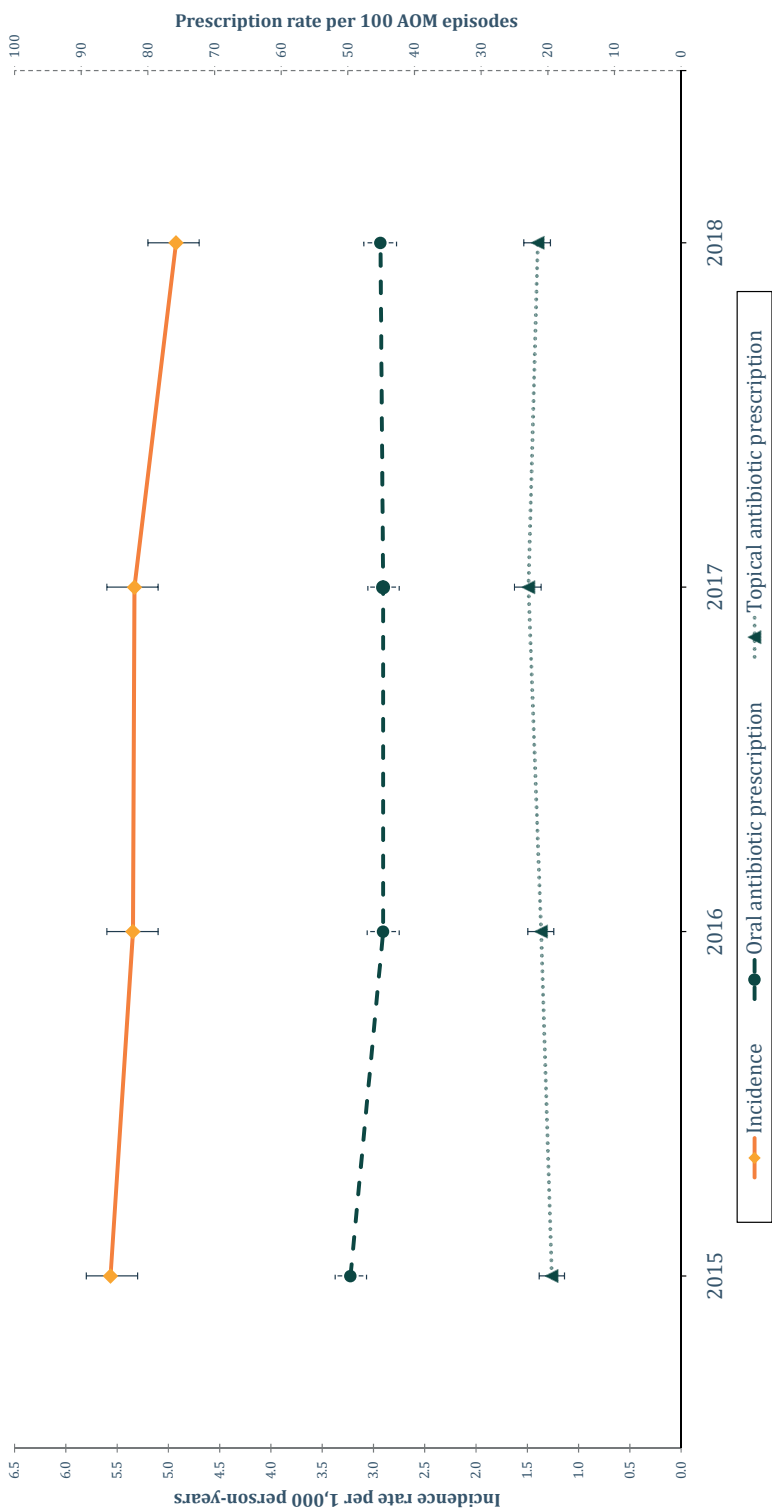


Figure 1. Annual incidence and prescription rates for AOM episodes from 2015 to 2018

**Table 2. Incidence, oral antibiotic prescription and consultation rates for AOM in adults from 2015 to 2018**

	Incidence rate <sup>a</sup> (95% CI)	Oral antibiotic prescription rate <sup>b</sup> (95% CI)	Consultation rate <sup>c</sup> (95% CI)
Age, years			
15-39	7.1 (6.8 to 7.3)	44.1 (42.6 to 45.7)	1.5 (1.4 to 1.5)
40-64	4.4 (4.2 to 4.6)	49.4 (47.3 to 51.5)	1.6 (1.5 to 1.6)
≥65	2.7 (2.5 to 2.9)	46.1 (42.1 to 50.2)	1.7 (1.6 to 1.8)
Gender			
Male	4.5 (4.4 to 4.7)	43.5 (41.7 to 45.4)	1.5 (1.4 to 1.5)
Female	6.0 (5.8 to 6.2)	47.8 (46.2 to 49.3)	1.5 (1.5 to 1.6)
Atopic diathesis			
Yes	7.3 (7.0 to 7.6)	44.7 (42.5 to 46.9)	1.5 (1.5 to 1.6)
No	4.7 (4.6 to 4.9)	46.6 (45.2 to 48.0)	1.5 (1.5 to 1.6)
Diabetes			
Yes	4.1 (3.7 to 4.6)	46.6 (41.2 to 52.2)	1.5 (1.4 to 1.7)
No	5.4 (5.2 to 5.5)	46.0 (44.8 to 47.2)	1.5 (1.5 to 1.6)
Use of immunosuppressive drugs <sup>d</sup>			
Yes	5.5 (4.1 to 7.3)	57.7 (44.0 to 70.5)	1.5 (1.2 to 1.9)
No	5.3 (5.2 to 5.4)	45.9 (44.7 to 47.1)	1.5 (1.5 to 1.6)

Abbreviations: AOM, acute otitis media; CI, confidence interval. <sup>a</sup> Incidence rate per 1,000 person-years. <sup>b</sup> Prescription rates per 100 AOM episodes. <sup>c</sup> Consultation rates per AOM episode. <sup>d</sup> Defined as use of systemic corticosteroids or immunosuppressants at the day prior to AOM episode.

## Prescriptions

Oral antibiotics were prescribed in 46.0% of AOM episodes (95% CI: 44.8 to 47.2); 84.0% were immediate prescriptions. In 5.1% of these episodes, a second course of oral antibiotics was prescribed (Table 3). Amoxicillin was most commonly prescribed (83.8%), followed by amoxicillin/clavulanate (Figure 2). Antibiotic prescription rates were stable over time (Figure 1). No substantial differences in oral antibiotic prescription rates across various subgroups were observed (Table 2).

Overall, topical antibiotics were prescribed in 21.2% of AOM episodes (95% CI: 20.2 to 22.2). Topical antibiotic prescription rates increased with age, ranging from 18.7% (95% CI: 17.5 to 20.0) in patients aged 15-39 years to 31.7% (95% CI: 28.0 to 35.6) in patients aged 65 years and older.

Twelve percent of the episodes in which no immediate oral antibiotic prescription strategy was applied resulted in an oral antibiotic prescription during reconsultation versus 9% of those initially treated with topical antibiotics (Table 3).

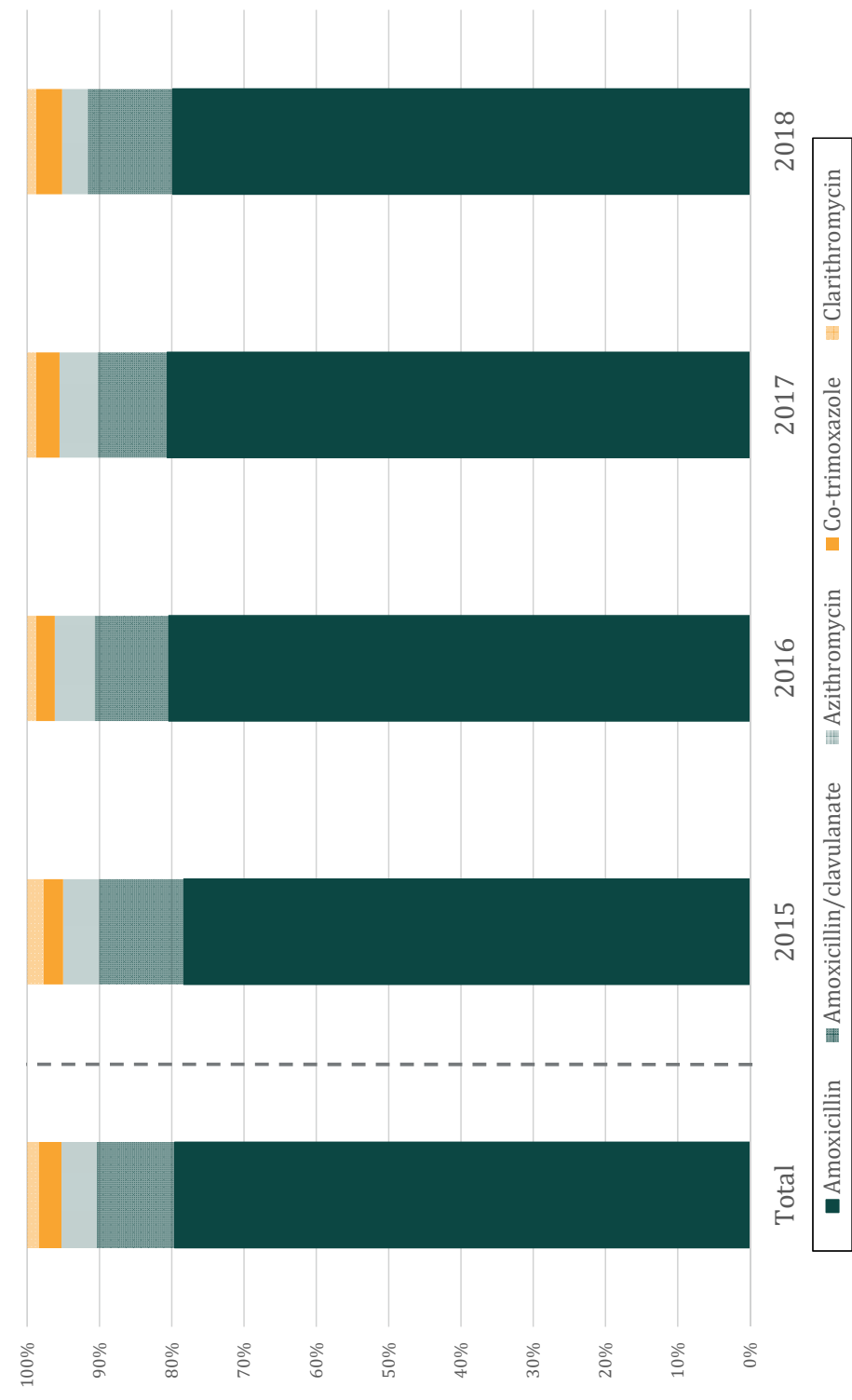


Figure 2. Types of oral antibiotics prescribed for AOM episodes from 2015 to 2018

Analgesics were prescribed in 8.4% of AOM episodes (95% CI: 7.8 to 9.1), mainly non-steroidal anti-inflammatory drugs (NSAIDs, 4.4%). Cyclooxygenase-2 inhibitors and opioids were prescribed in 2.3% and 1.6%, respectively.

## Consultation rates

The overall consultation rate in our cohort was 1.5 per AOM episode (95% CI: 1.5 to 1.6). Consultation rates increased with age (Table 2) and were higher in patients receiving immediate oral and topical antibiotics than in those who did not (Table 3).

**Table 3. Consultations and additional prescriptions for AOM based on immediate antibiotic prescription (2015 – 2018)**

	Consultation rate <sup>a</sup> (95% CI)	Oral antibiotics during reconsultation, %
Oral antibiotics at first consultation		
Yes	1.6 (1.6 to 1.6)	5.1
No	1.5 (1.4 to 1.5)	12.0
Topical antibiotics at first consultation		
Yes	1.6 (1.5 to 1.7)	9.3
No	1.5 (1.5 to 1.5)	-

Abbreviations: AOM, acute otitis media; CI, confidence interval. <sup>a</sup> Consultation rates per AOM episode.

## Discussion

### Summary

Our primary care-based cohort study of routine health care data showed that the incidence rate of AOM in adults is around 5/1000 person-years. The incidence of AOM declined with age and was higher in patients with an atopic diathesis. Oral antibiotics, predominantly amoxicillin, were prescribed in half of the AOM episodes, and topical antibiotics in one-fifth of episodes.

### Strengths and limitations

To the best of our knowledge, our study is one of the first to explore the incidence and management of AOM in adults in primary care. Major strengths are the size of the cohort and data completeness. Since characteristics of all disease episodes in Dutch primary care are systematically registered using ICPC- and ATC-codes, and registration in a primary care practice is mandatory for all Dutch citizens, the JGPN database encompasses recent, well-documented electronic primary health care data. Moreover, the database includes data from primary care practices from both urban and rural areas. The prevalence rates of comorbidities in our cohort closely resemble those of national figures<sup>15</sup>, which further underlines the generalizability of our data.

Some limitations deserve further attention. Most importantly, the use of routine health care data may introduce misclassification of AOM diagnosis and comorbidities. This is particularly relevant to cases for which topical antibiotics were prescribed. These episodes might reflect adults with AOM and ear discharge due to a spontaneous rupture of the ear drum, which is generally considered a more severe expression of AOM.<sup>16</sup> There is however no evidence to support the use of topical antibiotics in AOM and ear discharge.<sup>17</sup> Other possible explanations might be misclassification of ICPC coding or disease. When the ear canal is filled with ear discharge it is difficult to distinguish AOM from acute otitis externa, in particular in adults where acute otitis externa is more common.<sup>8,18</sup>

Furthermore, we were not able to extract data on AOM complications requiring specialist referrals since a direct link between ICPC-code and referrals were missing in our database. In addition, exposure to tobacco smoke is a well-known risk factor for AOM in children.<sup>4,5</sup> We were, however, not able to extract valid data on patient's smoking history, due to a substantial number of missing data.

In contrast to the World Allergy Organization's definition of atopy<sup>19</sup>, we did not include proven immunoglobulin E (IgE) sensitization (through elevated IgE levels or a positive skin prick test) in our definition of atopic diathesis, since these tests are not routinely performed in primary care. This may, however, have led to an overestimation of atopic diathesis in our study population.

Inherent to its observational design, our study may suffer from confounding. This is particularly relevant for the observed higher incidence rate in patients with atopic diathesis. Although this observation was consistent across different age groups, we were unable to correct for other (unknown) confounding factors that might have influenced this observation. Confounding by indication particularly applies to our analysis in which we determined the consultation rates of patients who did and did not receive an immediate antibiotic prescription. The observation, i.e. higher consultation rates in those receiving immediate antibiotics, should therefore be interpreted with caution.

## **Comparison with existing literature**

The AOM incidences observed in our study are in line with those modelled by Monasta et al.<sup>6</sup> for Western European adults, although they reported a slight increase in incidence in those aged 65 years and older. Incidences are also similar to the Dutch 2000-2002 rates which ranged from 8.2/1000 person-years at ages 15-24 years to 1.7 at ages over 74.<sup>15</sup>

Approximately half of AOM episodes were treated with oral antibiotics, predominantly amoxicillin. These rates are similar to those in Dutch children, with antibiotic prescribing rates ranging from 55% to 59%.<sup>20,21</sup> The antibiotic prescription rate among US veterans was higher, i.e. 75%,<sup>7</sup> which may be attributed to the difference in study setting and in antibiotic prescribing habits between the USA and the Netherlands.

The very low analgesic prescription rates observed in our study are in agreement with those observed in childhood AOM.<sup>22</sup> The actual use of analgesics in AOM is probably much higher since paracetamol and most NSAIDs are available over-the-counter.

### **Implications for practice and further research**

In conclusion, we found that AOM incidence among adults has been stable over the past years at around 5/1000 person-years and that approximately half and one-fifth of the episodes was treated with oral and topical antibiotics, respectively. The observed prescribing of azithromycin and topical antibiotics, to the degree these were actually prescribed for AOM, implies a need for GP-targeted interventions to promote the appropriate use of antibiotics for AOM.

This explorative study serves as a first step in unravelling the burden of AOM among adults in primary care. To determine the need and feasibility of prospective studies into the best management strategy for AOM in adults, further research should focus on factors associated with the prognosis of AOM in terms of symptom duration, complications and need for specialist referrals and/or hospitalization in this patient group.

**Supplementary table 1. Incidence rates for AOM in atopic and non-atopic patients stratified according to age**

	Incidence rate <sup>a</sup> (95% CI)	
	Atopic	Non-atopic
Age, years		
15-39	9.0 (8.5 to 9.5)	6.5 (6.2 to 6.7)
40-64	6.3 (5.9 to 6.8)	3.9 (3.7 to 4.1)
≥65	4.3 (3.7 to 5.0)	2.3 (2.1 to 2.6)

Abbreviations: AOM, acute otitis media; CI, confidence interval. <sup>a</sup> Incidence rate per 1,000 person-years.



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CHAPTER 3

3

# **Systematic review of prevalence and antimicrobial resistance of bacteria in children with acute otitis media and ear discharge**

Based on:

Hullegie S, Venekamp RP, van Dongen TMA, Hay AD, Moore MV, Little P, Schilder AGM, Damoiseaux RAMJ. Prevalence and Antimicrobial Resistance of Bacteria in Children With Acute Otitis Media and Ear Discharge: A Systematic Review. *Pediatr Infect Dis J*. 2021 Aug 1;40(8):756-762. doi: 10.1097/INF.0000000000003134.

## Abstract

### Introduction

Of children with acute otitis media (AOM), 15%-20% present with acute onset ear discharge due to a spontaneous perforation of the tympanic membrane (AOMd). This review aims to quantify the prevalence and antimicrobial resistance (AMR) status of bacteria in children with AOMd in the PCV era.

### Methods

Systematic searches were performed in PubMed, EMBASE and Cochrane Library from inception to June 7, 2019. Two reviewers extracted relevant data and assessed risk of bias independently. All English studies reporting any prevalence and/or AMR data of bacterial middle ear isolates from children with AOMd were included. Risk of bias was assessed using the Joanna Briggs Institute Critical Appraisal checklist.

### Results

Of 4,088 unique records retrieved, 19 studies (10,560 children) were included. Overall quality was judged good. *S. pneumoniae* (median 26.1%, range 9.1%-47.9%), *H. influenzae* (median 18.8%, range 3.9%-55.3%), *Staphylococcus aureus* (median 12.3%, range 2.3%-34.9%) and *Streptococcus pyogenes* (median 11.8%, range 1.0%-30.9%) were the most prevalent bacteria. In 76.0% (median, range 48.7%-100.0%, 19 studies, 1,429 children) any bacterium was identified. AMR data were sparse and mainly limited to *S. pneumoniae*. We found no evidence of a clear shift in the prevalence of bacteria and AMR over time.

### Conclusion

In children with AOMd, *S. pneumoniae* and *H. influenzae* are the two predominant bacteria, followed by *S. aureus* and *S. pyogenes* in the post PCV era. AMR data are sparse and no clearly change over time was observed. Ongoing surveillance of the microbiology profile in children with AOMd is warranted to guide antibiotic selection and to assess the impact of children's PCV status.

## Introduction

Acute otitis media (AOM) is one of the most common childhood infections and a leading cause of doctor consultations and antibiotic prescribing worldwide.<sup>1,2</sup> Around 15%-20% of children with AOM present with acute onset ear discharge due to a spontaneous perforation of the tympanic membrane (AOMd).<sup>3,4</sup> In contrast to widespread beliefs, children with AOMd have similar levels of ear pain and feel less well at presentation than those without ear discharge (AOMwd). Also, children with AOMd have a higher disease burden with higher rates of ear pain and/or fever at 3-7 days and more AOM recurrences and hearing problems at 3 months compared to children without ear discharge.<sup>3,4</sup> Antibiotics are more effective in children with AOMd than in those with AOMwd; number needed to treat to achieve resolution of ear pain and/or fever at days 3 to 7: 3 versus 8, respectively.<sup>3</sup> AOM guidelines therefore recommend clinicians to consider immediate antibiotic prescribing in children with AOMd<sup>5,6</sup>, in contrast to AOMwd, for which a watchful waiting approach is recommended for otherwise healthy children with non-severe unilateral disease.<sup>5,6</sup>

It has been suggested that the differences in clinical picture and disease course between AOMwd and AOMd might be attributed to differences in causative pathogens. A 2016 systematic review including 38 published reports of microbiology of children with AOMwd found that *Streptococcus pneumoniae* (average detection rate of 27.8%), *Haemophilus influenzae* (23.1%), and *Moraxella catarrhalis* (7.0%) are the most common bacteria associated with AOMwd globally.<sup>7</sup> *Streptococcus pyogenes* is thought to be more prevalent in children with AOMd,<sup>8-10</sup> but data are conflicting.<sup>9-11</sup> The routine administration of pneumococcal conjugate vaccines (PCV) during infancy has led to a change in childhood AOM epidemiology.<sup>12-14</sup> This review aims to provide an overview of the prevalence and antimicrobial resistance (AMR) of bacteria in children with AOMd in the post PCV era.

## Methods

Our review protocol was published on PROSPERO (CRD42018100523).<sup>15</sup> The review was reported according to the most recent PRISMA statement.<sup>16</sup>

### Primary objective

To provide an up-to-date overview of the prevalence of bacteria and their AMR profile in children with AOMd in the post PCV era.

## Secondary objectives

To explore, in children with AOMd, i) whether the prevalence and AMR rates of bacteria varied over time; ii) PCV status of participating children impacted our results and; iii) how the definition of AMR as applied in the individual studies impacted our results.

## Data sources and search strategy

Systematic searches of PubMed, EMBASE and the Cochrane Library were performed from inception to June 7, 2019. A broad search strategy was designed using a combination of any key word relevant to ‘acute otitis media’ and ‘antibiotic resistance or resistant bacteria or individual pathogens’ as well as ‘acute otitis media’ and ‘antibiotics’, with database-specific syntaxes (Table, Supplemental Digital Content 1).

## Patient and public involvement

Patients were not involved in the development or conduct of this review.

## Study selection

Two reviewers (SH, RPV) independently screened titles and abstracts of unique records for eligibility using pre-specified criteria. The same reviewers independently reviewed the full texts of potentially eligible papers. Any disagreements were resolved by discussion.

All studies reporting any prevalence or AMR data of bacterial middle ear isolates from children (0-16 years) with AOMd were included. Non-English studies, animal studies, studies conducted before the year 2000 (i.e. before routine implementation of PCV in infancy), studies focussing on complicated AOM (>25% of sample consisting of otitis prone children, children with recurrent AOM, treatment failure or hospitalized children) and those from which the full text could not be retrieved were excluded. To extent the yield of relevant studies, the reference lists of included studies were reviewed to identify any additional articles.

## Data extraction and Quality appraisal

Two review authors (SH, RPV) independently extracted the following data from the included studies using a standardized data extraction form: year of conduction, study design, study population (country, age, number of participants), prevalence and AMR data for the following bacterial isolates: *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. pyogenes*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, methods of sampling and antibiotic sensitivity testing, and participants’ PCV status.

AMR was primarily defined as non-susceptibility to antibiotics (resistant and intermediate resistant strains combined).



Quality of included studies was assessed by two reviewers (SH, RPV) independently using the Joanna Briggs Institute Critical Appraisal checklist.<sup>17</sup> Any disagreements were resolved by discussion.

## Data synthesis and analysis

All statistical analysis were conducted with Rothman's Episheet.<sup>18</sup> In descriptive analysis, prevalence (median and range) of bacterial middle ear isolates and their AMR rates to most commonly prescribed antibiotics for AOM (penicillin, amoxicillin, amoxicillin-clavulanic acid, trimethoprim/sulfamethoxazole, erythromycin, cephalosporin, quinolones, ampicillin). Forrest plots were used to summarize these findings. Total prevalence rates of individual bacteria were calculated by combining cultures where the bacterium was identified as a single isolate and those where the bacterium was identified together with other bacteria (mixed infection).

We assessed clinical and statistical heterogeneity across studies. Where studies were sufficiently homogeneous, we aimed to calculate pooled prevalences as summary statistic.

In a sensitivity analysis, we excluded studies with less than 50 participants to assess the robustness of research findings. In a further sensitivity analysis, we restricted our AMR definition by analysing resistance strains only (instead of combining resistant and intermediate resistant strains).

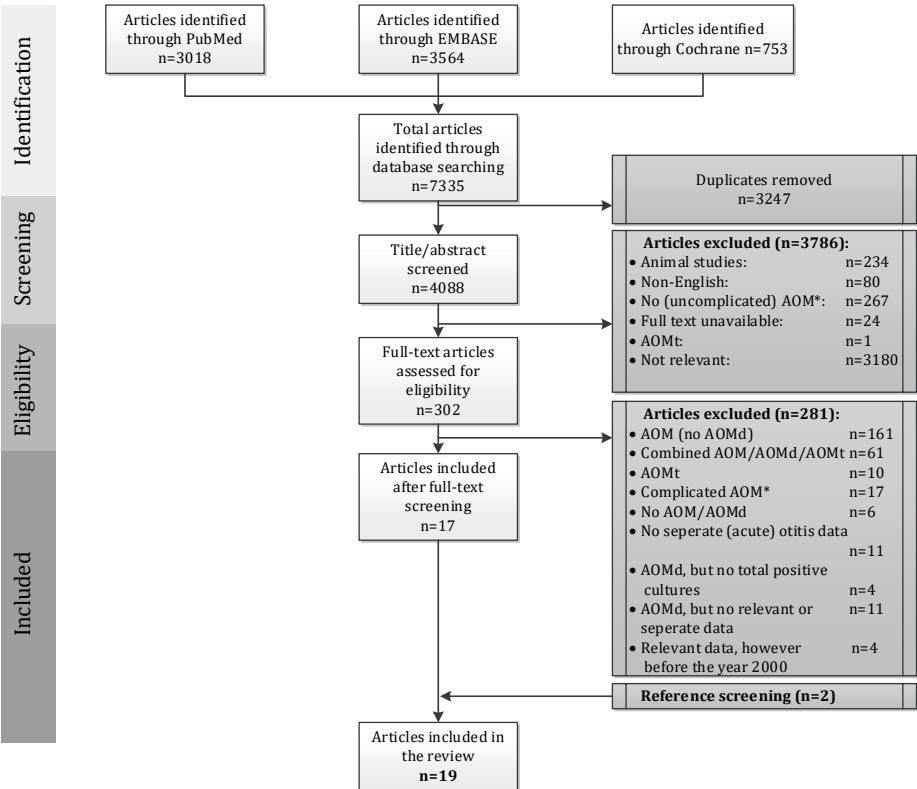
## Results

### Search results

The literature search yielded 7,335 records. Removing duplicates left 4,088 unique records. After title and abstract screening, 302 potentially relevant articles remained (Fig 1). Of these, 285 were excluded for various reasons (Fig 1), leaving 17 studies suitable for inclusion. A further two studies were retrieved from reviewing reference lists; these were not identified in our initial search strategy since the term 'acute' was not mentioned in the titles and abstracts. This left 19 studies<sup>4,10,11, 19-34</sup> including 10,560 children (range 16 to 5,580) suitable for inclusion in this review (Fig 1).

### Study characteristics

Main study characteristics are presented in Table 1: nine were conducted in Europe,<sup>4, 10, 25, 27-30, 32, 34</sup> seven in Asia,<sup>19-21, 23, 24, 26, 33</sup> two in South-America,<sup>22, 31</sup> and one in North-America.<sup>11</sup> The studies were conducted from 2000 to 2017 with five studies conducted after 2011. All studies were observational and most (74%) had a prospective cohort design. Three studies reported both culture and polymerase chain reaction



\*complicated AOM: treatment failure, >25% recurrent AOM or otitis prone children or hospitalized

Figure 1. Flow chart included studies.

(PCR) results<sup>19, 32, 34</sup> while the remaining 16 studies reported culture results only. Seventeen studies used standard microbiological techniques for isolation and identification, including the use of chocolate and blood agar whereas methods were unclear in two studies.<sup>4, 31</sup> Prevalence and AMR rates of bacteria could be extracted from 18 (95%) and 12 (63%) studies, respectively. Most studies (10/19) included only children who did not receive previous antibiotic treatment. In seven studies, no information about antibiotic use was reported. In one study, 23% of the children received antibiotics in the previous month<sup>34</sup> and in the remaining study 12.4% of the children received antibiotics at the moment of swabbing.<sup>28</sup>

Eleven studies provided information about the PCV status of participants: one study reported pre and post vaccination data<sup>10</sup>, in two studies children were not vaccinated<sup>23, 27</sup> whereas the PCV level of participants varied between 4.4% and 95% in eight studies.<sup>11, 19, 25, 28-31, 34</sup>

Study	Country	Design <sup>a</sup>	Year	Children(n)	Age (mo.)	Pathogens <sup>c</sup>	Cultures(n)	Cultures pos (n,%)	Susceptibility <sup>e</sup>	Sample method <sup>f</sup>	Pathogen
Ubukata 2018	Japan	Prospective	2016-2017	318	0-180	1:2:3:4:6	318	258 (81.1%)	No data	Culture/PCR	Bacterium
Naziat 2018	Bangladesh	Prospective	2014-2015	981	0-168	1:2:3:4:5:6	891	452 (50.7%)	1:2	Culture	Bacterium
Ling Ding 2018	China	Retrospective	2013-2015	228	0-156	1:2:3:4:5:6	228	181 (79.4%)	4	Culture	Bacterium
Rosenblut 2017	Chile	Prospective	2009-2010	17	4-59	1:2:3:6	17	15 (88%)	No data	Culture	Bacterium
Clveti 2017	Spain	Prospective	2011-2014	487	2-96	1:2:3:6	521	481 (92.1%)	1:2	Culture/PCR	Bacterium
Sonsuwan 2016	Thailand	Prospective	2007-2008	40	3-60	1:2:3:4:5:6	53	53 (100%)	1:4	Culture	Bacterium
Ding 2015	China	Prospective	2011-2013	229	0-216	1:2:3:4:6	229	159 (69%)	1	Culture	Bacterium
Linden 2015	Germany	Prospective	2008-2011	944	2-60	1:2:3:4:6	963	341 (35%)	No data	Culture	Bacterium
Lee 2014	Korea	Retrospective	2001-2010	215	0-192	1:4:5	215	156 (73%)	5	Culture	Bacterium
Setchanova 2013	Bulgaria	Retrospective	1994-2011 <sup>b</sup>	168	0-168	1:2	n.a. <sup>d</sup>	168	1:2	Culture	Bacterium
Rodrigues 2013	Portugal	Prospective	2010-2011	113	3-158	1:2:3:6	113	55 (49%)	No data	Culture	Bacterium
Marchisio 2013	Italy	Retrospective	2001-2011	458	0-72	1:2:3:4:6	705	487 (69%)	1:2:3:4:6	Culture	Bacterium
Grevers 2012	Germany	Prospective	2008-2010	76	3-60	1:2:6	76	36 (47%)	1:2	Culture	Bacterium
Stamboulidis 2011	Greece	Prospective	2000-2008	5580	0-168	1:2:3:6	5580	2409 (43%)	1	Culture	Bacterium
Sierra 2011	Colombia	Prospective	2008-2009	16	3-60	1:2:3	16	13 (81%)	No data	Culture	Bacterium
Neumark 2011	Sweden	Prospective	2007-2009	68	24-192	1:2:3:6	68	41 (60%)	No data	Culture/PCR	Bacterium
Junejo 2011	Pakistan	Prospective	2007-2009	484	0-180	1:2:4:5:6	484	307 (63%)	1:2:4:5:6	Culture	Bacterium
Smith 2010	UK	Prospective	2003-2006	38	6-120	1:2:4:5:6	38	22 (58%)	No data	Culture	Bacterium
Brook 2009	USA	Retrospective	1993-2006 <sup>b</sup>	100	5-144	1:2:3:6	125	109 (87%)	1	Culture	Bacterium

<sup>a</sup> All studies were either cohort, cross-sectional or database studies

<sup>b</sup> Setchanova reported separate data from 1994-2004 (n=49) and 2006-2011 (n=79). Brook reported separate data from 1993-1998 (n=60) and 2001-2006 (n=50). Only the data after 2000 is used in this review.

<sup>c</sup> 1 = *S. pneumoniae*, 2 = nontypeable *H. influenzae*, 3 = *M. catarrhalis*, 4 = *S. aureus*, 5 = *P. aeruginosa*, 6 = *S. pyogenes*

<sup>d</sup> Only *S. pneumoniae* or *H. influenzae* positive strains were studied; prevalence data not available

<sup>e</sup> Culture results extracted

## Quality appraisal

Overall quality of included studies was judged good (Figure, Supplemental Digital Content 2). However, data analysis was judged inadequate in 14 studies; in most of these studies antimicrobial susceptibility was not reported for all isolates. Data reporting was unclear in one study.<sup>26</sup>

## Prevalence of bacteria

*S. pneumoniae* (median 26.1%, range 9.1%-47.9%; 18 studies, 2,191 children), *H. influenzae* (median 18.8%, range 3.9%-55.3%; 17 studies, 2,185 children), and *S. aureus* (median 12.3%, range 5.3%-34.9%; 13 studies, 592 children) were the three most prevalent bacteria, followed by *S. pyogenes* (median 11.8%, range 1.0%-30.9%; 16 studies, 1,053 children) (Table 2). The prevalence of positive cultures (any bacterium identified) was 76% (median, range 48.7%-100%, 17 studies, 3643 children). Pooled prevalences were not calculated due to substantial heterogeneity across studies.

The prevalence of bacteria did not clearly change over time (Figure, Supplemental Digital Content 3). Excluding the three studies with less than 50 participants revealed similar results as our main analysis. There was no clear evidence of a shift in pathogens when stratifying results according to PCV status (Figure, Supplemental Digital Content 4).

## Antimicrobial resistance

AMR data were mainly reported for *S. pneumoniae* with very limited data reported for the remaining bacteria (Table, Supplemental Digital Content 5). Non-susceptibility rates of *S. pneumoniae* to commonly used antibiotics varied widely between countries. Non-susceptibility rates of pneumococcus to penicillin ranged from 0%-65.8% (median 10.0%; 8 studies). Albeit being highly sensitive to quinolones (median non-susceptibility rate 0.9%, range 0%-5.5%; median; 3 studies), non-susceptibility rates to other antibiotics varied widely; amoxicillin: median 16.7% (range 0%-64.8%; 4 studies), trimethoprim/sulfamethoxazole: median 27.3% (range 0%-93.5%; 5 studies), erythromycin: median 36.5% (range 10.5%-99.1%; 6 studies) and cephalosporins: median 5.4% (range 0%-63.0%; 6 studies).

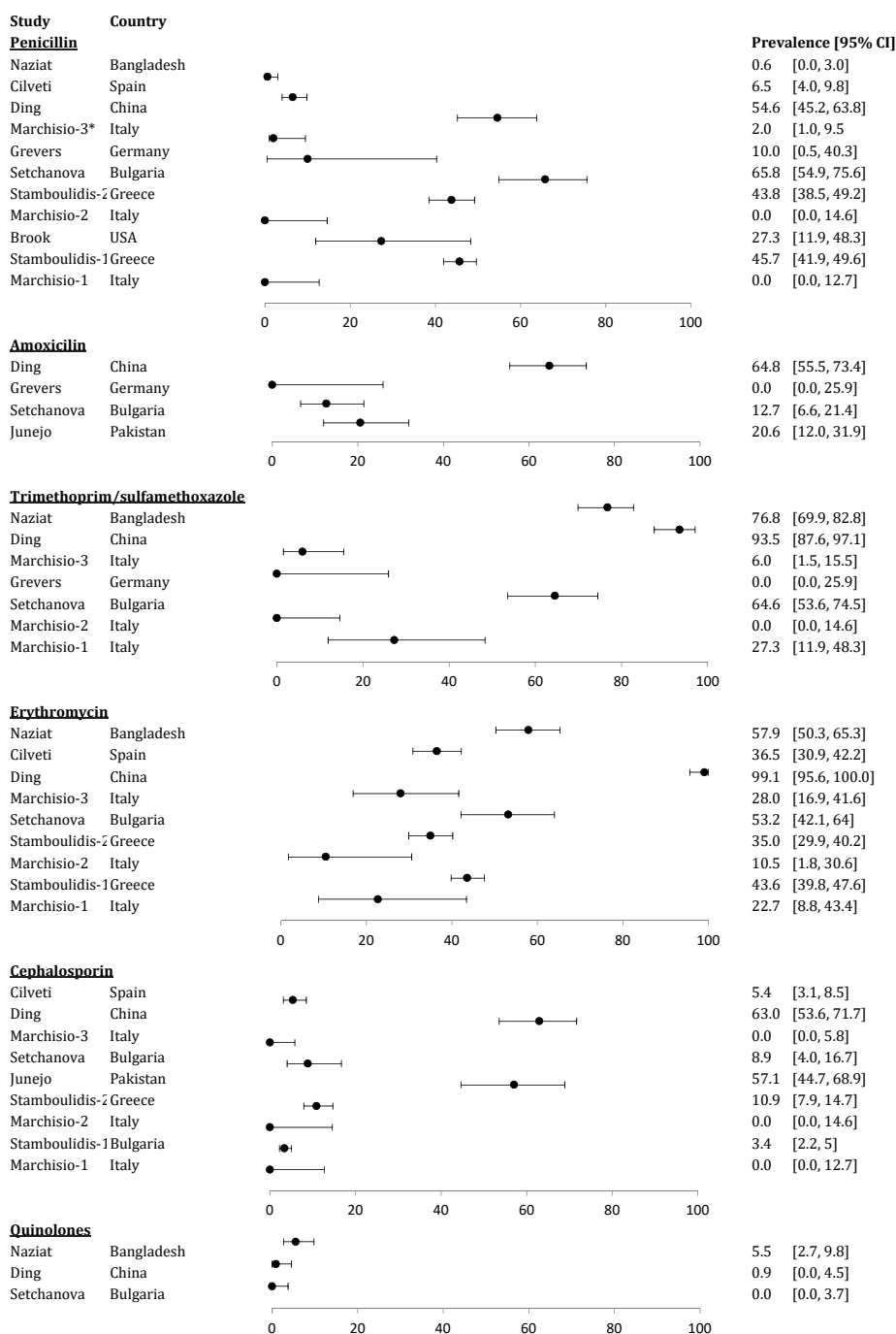
Non-susceptibility rates of *S. pneumoniae* did not clearly change over time (Fig 2). The limited data available did not permit us to assess the impact of children's PCV status on AMR.

When restricting the AMR definition to resistance strains only, antibiotic resistance rates of *S. pneumoniae* to the various antibiotics were considerably lower (Table, Supplemental Digital Content 6)

**Table 2. Prevalence rates of otopathogens**

Study	Samples			S. pneumoniae			H. influenzae			M. catarrhalis			S. aureus			P. aeruginosa			S. pyogenes			Any bacterium		
	n	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI		
Ubukata 2018 <sup>a</sup>	318	71	22.3	18.0-27.2	176	55.3	49.8-60.7	4	1.3	0.4-3.0	17	5.3	3.2-8.3				13	4.1	2.3-6.7	258	81.1	76.5-85.2		
Naziat 2018	891	164	18.4	16.0-21.1	187	21.0	18.4-23.8	4	0.4	0.1-1.1	83	9.3	7.5-11.4	38	4.3	3.1-5.7	9	1.0	0.5-1.8	452	50.7	47.4-54.0		
Ling Ding 2018	228	83	36.4	30.3-42.8	9	3.9	1.9-7.1	1	0.4	0.0-2.1	37	16.2	11.9-21.4	10	4.4	2.2-7.7	4	1.8	0.6-4.2	181	79.4	73.8-84.3		
Rosenblut 2017	17	5	29.4	11.7-53.7	8	47.1	24.8-70.3	1	5.9	0.3-25.8							2	11.8	2.0-33.7	15	88.2	66.3-98.0		
Gilveti 2017	521	208	39.9	35.8-44.2	251	48.2	43.9-52.5				35	6.7	4.8-9.1				71	13.6	10.9-16.8	480	92.1	89.6-94.2		
Sonsuwan 2016	53	5	9.4	4.7-25.6	19	35.8	23.8-49.4	1	1.9	0.1-9.0	14	26.4	15.9-39.5	6	11.3	4.7-22.1	3	5.7	1.5-14.6	53	100.0	94.5-100.0		
Ding 2015	229	108	47.2	40.8-53.6	17	7.4	4.5-11.4	1	0.4	0.0-2.1	43	18.8	14.1-24.2				4	1.7	0.6-4.2	159	69.4	63.2-75.1		
Linden 2015	963	88	9.1	7.4-11.1	63	6.5	5.1-8.2	8	0.8	0.3-1.6	97	10.1	8.3-12.1				113	11.7	9.8-13.9	819	85.0	82.7-87.2		
Lee 2014	215	59	27.4	21.8-33.7							75 <sup>b</sup>	34.9	28.8-41.4	9	4.2	2.1-7.5				156	72.6	66.3-78.2		
Rodrigues 2013	113	28	24.8	17.5-33.3	11	9.7	5.2-16.3	15	13.3	7.9-20.5							17	15.0	9.3-22.5	55	48.7	39.5-57.9		
Marchisio 2013	705	112	15.9	13.3-18.7	265	37.6	34.1-41.2	8	1.1	0.5-2.1	49	7.0	5.2-9.0				90	12.8	10.5-15.4	487	69.1	65.6-72.4		
Grevers 2012	76	10	13.2	6.9-22.2	14	18.4	10.9-28.3	0	0.0	0.0-3.9	8	10.5	5.0-19.0	2	2.6	0.4-8.4	13	17.1	9.9-26.8	36	47.4	36.3-58.6		
Stamboulidis 2011-2 <sup>c</sup>	1061	373	35.2	32.3-38.1	459	43.3	40.3-46.3	35	3.3	2.3-4.5							328	30.9	28.2-33.7					
Stamboulidis 2011-1 <sup>c</sup>	1548	741	47.9	45.4-50.4	650	42.0	39.5-44.5	67	4.3	3.4-5.4							342	22.1	20.1-24.2					
Sierra 2011	16	7	43.8	21.5-68.0	5	31.30	12.4-56.3													13	81.3	57.0-95.0		
Neumark 2011 <sup>d</sup>	68	12	17.6	9.9-28.1	5	7.4	2.7-15.5	6	8.8	3.7-17.4							6	8.8	3.7-17.4	41	60.3	48.3-71.4		
Junejo 2011	484	63	13.0	10.2-16.2	22	4.50	2.9-6.7				114	23.6	19.9-27.5	7	1.40	0.6-2.8	19	3.9	2.5-6.0	307	63.4	59.1-67.6		
Smith 2010	38	5	13.2	5.0-26.8	3	7.9	2.0-20.0				7	18.4	8.4-33.1	2	5.3	0.9-16.3	7	18.4	8.4-33.1	22	57.9	41.9-72.7		
Brook 2009 <sup>c</sup>	64	22	34.4	23.5-46.6	12	18.8	10.6-29.7	6	9.4	3.9-18.5	9 <sup>c</sup>	14.1	7.1-24.2				5	7.8	2.9-16.5	58	90.6	81.5-96.1		

<sup>a</sup> Rates consist of samples positive for both PCR and culture<sup>b</sup> The separate MRSA and MSSA data are combined<sup>c</sup> Data collection: Stamboulidis 2011-1;2000-2003; Stamboulidis 2011-2; 2005-2008; Brook 2009; 2001-2006<sup>d</sup> Combined PCR and culture data



**Figure 2. Prevalence rates of *S. pneumoniae*, *H. influenzae*, *S. aureus* and no bacterium according to year.**

\*Marchisio, Setchanova, Stamboulidis and Brook provided non-susceptibility data over time.

## Discussion

This systematic review of studies conducted in the post PCV era showed that, in children with AOMd, any bacterium is isolated in more than three quarter of middle ear fluid samples and that *S. pneumoniae*, *H. influenzae*, *S. aureus*, and *S. pyogenes* are the most prevalent bacteria.

A 2016 literature review found that *S. pneumoniae* (average detection rate: 27.8%) and *H. influenzae* (23.1%) are also the predominant bacteria in children with AOMwd globally;<sup>7</sup> *S. aureus* and *S. pyogenes* are however more common in AOMd than in AOMwd.<sup>7, 35, 36</sup> Also, a bacterium is more frequently isolated in children with AOMd than in those with AOMwd (any bacterium identified in 76% (range 48.7%-100%) versus 62% (range 25%-95%),<sup>7</sup> respectively). These findings add to the growing body of evidence that AOMwd and AOMd might be regarded as different parts of the spectrum of the AOM disease entity.

Theoretically, prevalences of bacteria isolated in AOMd and AOMwd may differ due to the sampling technique; middle ear fluid from children with AOMd is obtained from visible ear discharge in the external ear canal and may be contaminated with commensal bacteria. In AOMwd, tympanocentesis is required to obtain a middle ear fluid sample from children; with this procedure contamination with commensal bacteria is less likely. With *S. aureus* being a common component of the microbiota in the ear canal, one may argue that this leads to an overestimation of this bacterium in AOMd. There is, however, increasing evidence that *S. aureus* should be regarded as an important upper respiratory tract pathogen originating from the nasopharyngeal niche.<sup>37,38</sup> This is further substantiated by a recent study in children with ventilation tubes who developed acute ear discharge; it found a high correlation between the abundances of *S. aureus* in nasopharynx and in otorrhoea samples.<sup>39,40</sup>

In our study, non-susceptibility rates of *S. pneumoniae* to penicillin in AOMd varied between 0% and 65.8% (median 10.0%). A pooled analysis including 10 studies of children with AOMwd showed an average non-susceptibility rate of 18.5%.<sup>36</sup> We found no clear evidence of a shift in AMR over time in AOMd which is in agreement with a recent review of studies involving children with AOMwd,<sup>35</sup> but the small sample means inferences must be cautious. Besides that, AMR data should be interpreted in the context of PCV status, availability and adherence to local AOM guidelines, and general antibiotic since this may substantially impact AMR.

To our knowledge, we are the first to systematically synthesize prevalences of bacteria and their AMR profile in children with AOMd. To capture only data relevant to our

study population of interest, i.e. children with AOMd, and to avoid contamination with chronic suppurative otitis media cases, we excluded all studies that did not provide data for children with AOMd only or in which the diagnosis was not explicitly described. We prospectively registered our study protocol.<sup>15</sup> While conducting this review, we broadened the scope of our review by also including data on the prevalence of bacteria in children with AOMd. Since we designed very broad literature search syntaxes - including the names of the individual bacteria of interest - and reviewed all reference lists of relevant studies, we consider it unlikely that we missed any relevant data.

Some important limitations deserve further attention. First, while large numbers of studies have been published on the prevalence of bacteria in children with AOMwd,<sup>7, 41</sup> relatively few studies have focused on children with AOMd. Large differences between studies (e.g. number of participants, design, country and setting of conduct) resulted in substantial clinical and statistical heterogeneity across studies which did not allow us to calculate summary statistics. Second, most studies relied on conventional culture to identify bacteria. This has likely resulted in an underestimation of the prevalence rate of bacteria since PCR techniques are more accurate than culture in detection of bacteria in middle ear fluid.<sup>7, 36, 42</sup> Third, the absence of evidence of a shift in microbiology profiles over time in our review should be interpreted in the context of the limited available information on children's PCV status and the few data of recent years. Previous studies of childhood AOMwd showed that the introduction of more-valent PCVs has led to a shift in otopathogens from vaccine-type pneumococci to non-vaccine-type pneumococci and other otopathogen including non-typeable *H. Influenza* and *S. Aureus* and impacted AMR patterns.<sup>12-14, 43</sup> However, the data from included studies in this review is too limited to draw any meaningful conclusion regarding the shift of bacteria from the early post-PCV to the late post-PCV years. Fourth, this review did not focus on viruses. Virus alone can cause AOM (around 5% of middle ear fluid samples of children with AOMwd contain only viruses)<sup>44</sup> and evidence is accumulating that the interplay between viruses and bacteria in the upper respiratory tract may play an important role.<sup>45</sup> In our sample of studies, no one did report data on viruses. Future studies should focus on the interplay between viruses and bacteria during upper respiratory tract infections and the progression to AOM to initiate new (preventive) interventions.

Finally, we excluded children with complicated AOM, including those with treatment failure, from our analysis to maximize generalizability of our review findings to children with AOMd presenting to primary care and limit the potential impact of previous antibiotic exposure to the microbiology profile as much as possible. As a consequence, we were unable to link the microbiology data to the risk of severe intra- or extracranial



suppurative complications and/or hospitalizations. Future research is needed to bridge this knowledge gap.

## Conclusion

In children with AOMd *S. pneumoniae* and *H. influenzae* are the two predominant bacteria, followed by *S. aureus* and *S. pyogenes*, in the post PCV era. Antimicrobial resistance data were sparse and mainly limited to *S. pneumoniae*. No clearly change over time was observed. The limited data available did not permit us to assess the impact of children's PCV status, and therefore ongoing surveillance of the microbiology profile is warranted.

## Supplemental Digital Content

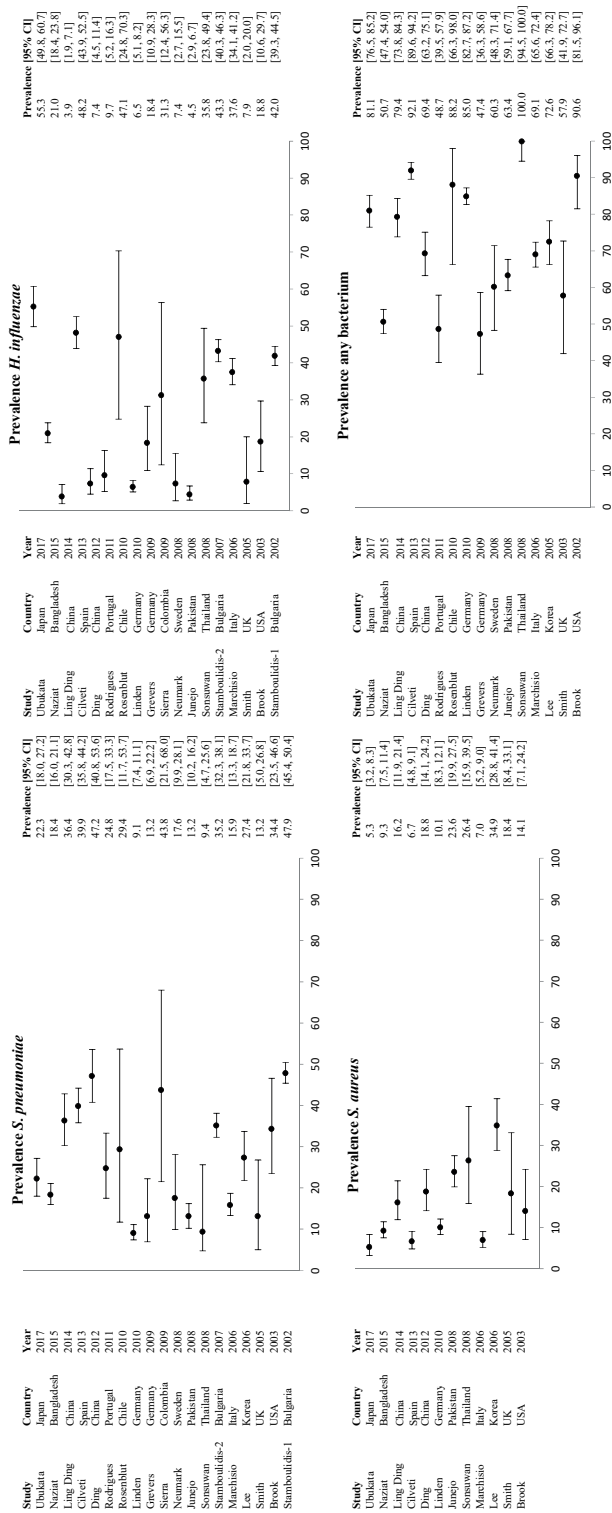
	Appropriate sample frame	Adequate sampling of study subjects	Adequate sample size	Detailed description of study subjects and setting	Sufficient data analysis	Valid methods of identification of the condition	Standard way of measuring the condition	Appropriate statistical analysis	Adequate response rate
<b>Yes</b>	Study population: children with AOMd not or less than 10% exposed to recent Abx (2weeks).	Reporting of sampling complete.	50 or more participants.	Sufficient details of study sample reported.	Pathogen tested and reported	Collection of ear discharge swabs well described (type of swab, transport medium).	Standard lab procedures and well described.	Outcome reporting: correct and complete.	No or less than 10% loss to follow up
<b>Unclear</b>	Study population: children with AOMd; exposure to recent Abx not described or less than 25% of the study population exposed to Abx.	Reporting of sampling methods incomplete.	n.a.	Limited details of study sample reported.	Susceptibility or prevalence data not reported for all isolated	Limited details of collection of ear discharge swabs	Limited details of lab procedures.	Outcome reporting: limited.	10-20 % loss to follow up
<b>No</b>	Study population: children with AOMd; at least 25% of the study population was exposed to recent Abx.	Reporting of sampling methods not mentioned.	Less than 50 participants.	No details of study sample reported.	Focussing on one pathogen only	No details of collection of ear discharge swabs	No details of lab procedures.	Outcome reporting: incorrect or incomplete.	More than 20% loss to follow up

**Figure, Supplemental Digital Content 1. Quality assessment of included studies** <http://links.lww.com/INF/E354>

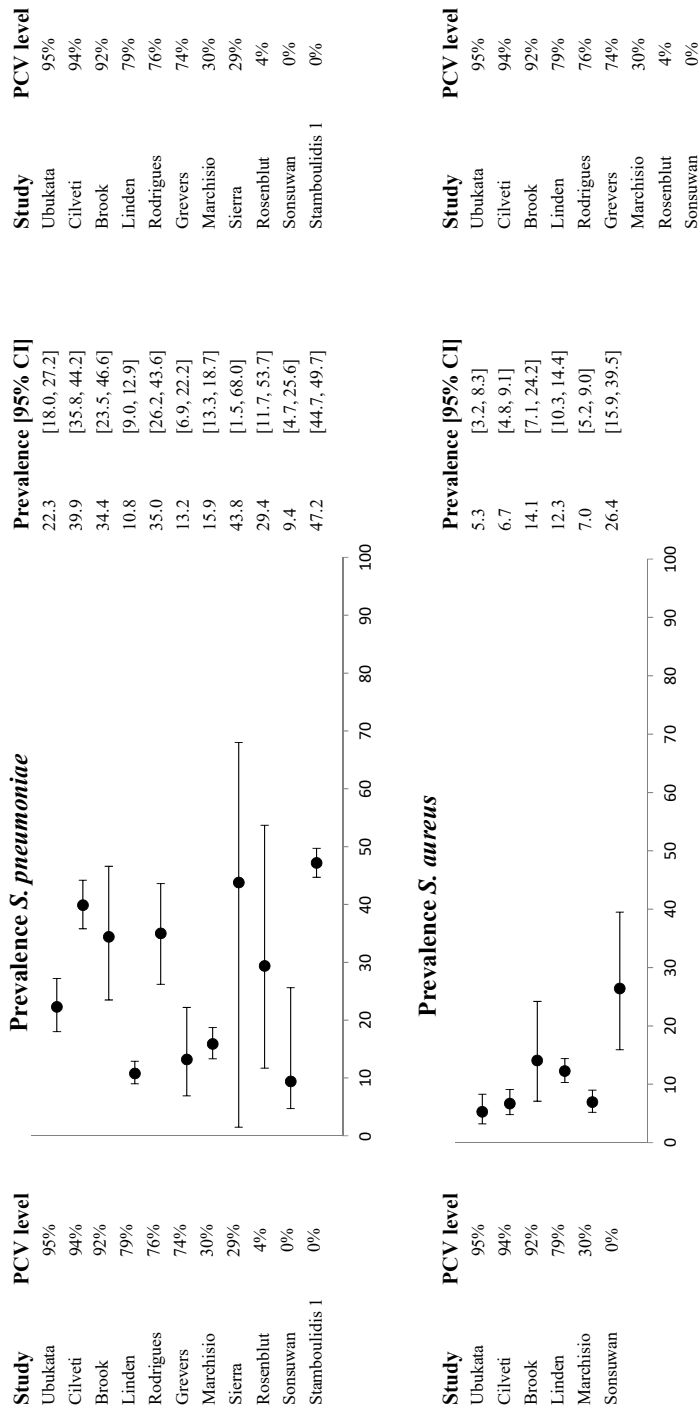
Table, Supplemental Digital Content 2. Search strategy

<http://links.lww.com/INF/E355>

Search Strategy: (#1 AND #2) OR (#1 AND #3)		
	PubMed	Embase
#1	acute otitis media[Title/Abstract] OR AOM[Title/Abstract] OR acute suppurative otitis media[Title/Abstract] OR (acute[Title/Abstract] AND (middle ear[Title/Abstract] OR draining ear[Title/Abstract] OR ear discharge [Title/Abstract] OR otorrhea [Title/Abstract] OR otorrhea [Title/Abstract]))	'acute otitis media':ab,ti OR 'AOM':ab,ti OR 'acute suppurative otitis media':ab,ti OR 'acute':ab,ti AND ('middle ear':ab,ti OR 'draining ear':ab,ti OR 'ear discharge':ab,ti OR 'otorrhea':ab,ti OR 'otorrhea':ab,ti))
		Cochrane Library ( 'acute otitis media' OR 'AOM' OR 'acute suppurative otitis media' OR 'acute' AND ('middle ear' OR 'draining ear' OR 'ear discharge' OR 'otorrhea' OR 'otorrhea'))
#2	antimicrobial resistance[Title/Abstract] OR anti-microbial resistance[Title/Abstract] OR bacterial resistance[Title/Abstract] OR antimicrobial susceptibility[Title/Abstract] OR antibiotic susceptibility[Title/Abstract] OR resistant organism[Title/Abstract] OR resistant organisms[Title/Abstract] OR resistant otopathogen[Title/Abstract] OR resistant otopathogens[Title/Abstract] OR resistant pathogen[Title/Abstract] OR resistant pneumonias[Title/Abstract] OR Streptococcus pneumoniae[Title/Abstract] OR Haemophilus influenzae[Title/Abstract] OR Moraxella catarrhalis[Title/Abstract] OR H. influenzae[Title/Abstract] OR Staphylococcus aureus[Title/Abstract] OR Pseudomonas aeruginosa[Title/Abstract] OR P. aeruginosa[Title/Abstract]	'antimicrobial resistance':ab,ti OR 'anti-microbial resistance':ab,ti OR 'bacterial resistance':ab,ti OR 'antimicrobial susceptibility':ab,ti OR 'antibiotic susceptibility':ab,ti OR 'resistant organism':ab,ti OR 'resistant organisms':ab,ti OR 'resistant otopathogen':ab,ti OR 'resistant otopathogens':ab,ti OR 'resistant pathogen':ab,ti OR 'resistant pneumonias':ab,ti OR 'Streptococcus pneumoniae':ab,ti OR 'Haemophilus influenzae':ab,ti OR 'H. influenzae':ab,ti OR 'Moraxella catarrhalis':ab,ti OR 'M. catarrhalis':ab,ti OR 'Staphylococcus aureus':ab,ti OR 'S. aureus':ab,ti OR 'Pseudomonas aeruginosa':ab,ti OR 'P. aeruginosa':ab,ti
		'antimicrobial resistance' OR 'anti-microbial resistance' OR 'bacterial resistance' OR 'antimicrobial susceptibility' OR 'antibiotic susceptibility' OR 'resistant organism' OR 'resistant organisms' OR 'resistant otopathogen' OR 'resistant otopathogens' OR 'resistant pathogen' OR 'resistant pneumonias' OR 'Streptococcus pneumoniae' OR 'Haemophilus influenzae' OR 'H. influenzae' OR 'Moraxella catarrhalis' OR 'M. catarrhalis' OR 'Staphylococcus aureus' OR 'S. aureus' OR 'Pseudomonas aeruginosa' OR 'P. aeruginosa')
#3	antibiotic[Title/Abstract] OR antibiotics[Title/Abstract] OR antimicrobial[Title/Abstract] OR antimicrobials[Title/Abstract] OR anti-microbial[Title/Abstract] OR anti-microbials[Title/Abstract] OR anti-infective[Title/Abstract] OR otological[Title/Abstract] OR otological drops[Title/Abstract] OR ear drops[Title/Abstract] OR eardrops[Title/Abstract]	'antibiotic':ab,ti OR 'antibiotics':ab,ti OR 'antimicrobial':ab,ti OR 'antimicrobials':ab,ti OR 'anti-microbials':ab,ti OR 'anti-infective':ab,ti OR 'otological':ab,ti OR 'otological drops':ab,ti OR 'ear drops':ab,ti OR 'eardrops':ab,ti
		( 'antibiotic' OR 'antibiotics' OR 'antimicrobial' OR 'antimicrobials' OR 'anti-microbial' OR 'anti-microbials' OR 'anti-infective' OR 'otological' OR 'otological' OR 'antimicrobial drops' OR 'ear drops' OR 'eardrops' )



Figure, Supplemental Digital Content 3. Prevalence rates of *S. pneumoniae*, *H. influenzae*, *S. aureus* and no bacterium according to year  
<http://links.lww.com/INF/E356>



Figure, Supplemental Digital Content 4: Prevalence rates *S. pneumoniae*, *H. influenzae*, *S. aureus* and no bacterium according to PCV level  
<http://links.lww.com/INF/E357>

Table, Supplemental Digital Content 5. Non-susceptibility rates of bacteria to antibiotics <http://links.lww.com/INF/E357>

Study	Year of conducton	S. pneumoniae			H. influenzae			S. aureus			P. aeruginosa			S. pyogenes			M. catharralis		
		n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Non-susceptible to penicillin																			
Naziat 2018	2014-2015	1 / 164	0.60	0.0-3.0															
Ling ding 2017	2013-2015							33 / 37	89.2	75.9-96.5									
Cilveti	2011-2014	18 / 280	6.5	4.0-9.8															
Ding 2015	2011-2013	59 / 108	54.6	45.2 - 63.8															
Lee 2014	2001-2010										1 / 1	100.0	20.7-100.0						
Setchanova 2013-2	2006-2011	52 / 79	65.8	54.9-75.6															
Marchisio 2013-3*	2008-2011	1 / 50	2.0	1.0-9.5	0 / 143	0.0	0.0-2.1	5 / 17	29.4	11.7-53.7				1 / 49	2.0	0.1-9.7	0 / 4	0.0	0.0-52.7
Marchisio 2013-2*	2005-2007	0 / 19	0.0	0-14.6	0 / 35	0.0	0.0-8.2	1 / 4	25.0	1.3-75.8				0 / 14	0.0	0.0-19.3			
Marchisio 2013-1*	2001-2004	0 / 22	0.0	0-12.7	1 / 42	2.4	0.1-11.2	1 / 12	8.3	0.4-34.7				0 / 20	0.0	0.0-13.9	0 / 1	0.0	0.0-79.3
Grevers 2012	2008-2010	1 / 10	10.0	0.5-40.3															
Stamboulidis 2011-2	2005-2008	144 / 329	43.8	38.5-49.2															
Stamboulidis 2011-1	2000-2008	296 / 647	45.7	41.9-49.6															
Brook 2009	2001-2006	6 / 22	27.3	11.9-48.3															
Non-susceptible amoxicillin																			
Ding 2015	2011-2013	70 / 108	64.8	55.5-73.4															
Setchanova 2013-2	2006-2011	10 / 79	12.7	6.6-21.4															
Grevers 2012	2008-2010	0 / 10	0.0	0.0-25.9	2 / 21	9.5	1.6-28.1												
Junejo 2011	2007-2009	13 / 63	20.6	12.0-31.9	17 / 22	77.3	56.6-91.2	96 / 114	84.2	76.6 - 90.1	7 / 7	100.0	65.2-100.0	7 / 19	36.8	17.8-59.7			
Non-susceptible to amoxicillin-clavulanic acid																			
Cilveti	2011-2014				10 / 264	3.8	1.9-6.6												
Sonsuwan 2016	2007-2008				0 / 19	0.0	0.0-14.6												
Non-susceptible to trimethoprim/sulfamethoxazole																			
Naziat 2018	2014-2015	126 / 164	76.8	69.9-82.8	76 / 187	40.6	33.8-47.8												
Ling ding 2017	2013-2015							1 / 37	2.7	0.0-12.6									
Ding 2015	2011-2013	101 / 108	93.5	87.6 - 97.1															
Sonsuwan 2016	2007-2008				10 / 19	52.6	30.6-73.9	1 / 14	7.1	0.4-30.5				0 / 2	0.0	0.0-77.6			
Lee 2014	2001-2010																		
Setchanova 2013-2	2006-2011	51 / 79	64.6	53.6-74.5															
Marchisio 2013-3*	2008-2011	3 / 50	6.0	1.5-15.5	11 / 143	7.7	4.1-13.0	1 / 17	5.9	0.3-25.8				3 / 49	6.1	1.6-15.8	0 / 4	0.0	0.0-52.7
Marchisio 2013-2*	2005-2007	0 / 19	0.0	0.0-14.6	0 / 35	0.0	0.0-8.2	0 / 4	0.0	0.0-52.7				0 / 14	0.0	0.0-19.3			
Marchisio 2013-1*	2001-2004	6 / 22	27.3	11.9-48.3	3 / 42	7.1	1.8-18.2	0 / 12	0.0	0.0-22.1				2 / 20	10.0	1.7-29.3	0 / 1	0.0	0.0-79.3
Grevers 2012	2008-2010	0 / 10	0.0	0.0-25.9	7 / 21 <sup>c</sup>	33.3	15.9-55.1												

Table, Supplemental Digital Content 5. Non-susceptibility rates of bacteria to antibiotics <http://links.lww.com/INF/E357> (continued)

Study	Year of conduction	<i>S. pneumoniae</i>			<i>H. influenzae</i>			<i>S. aureus</i>			<i>P. aeruginosa</i>			<i>S. pyogenes</i>			<i>M. catarrhalis</i>		
		n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
<b>Non-susceptible to erythromycin</b>																			
Naziat 2018	2014-2015	95 / 164	57.9	50.3-65.3	172/187	92.0	87.4-95.3												
Ling ding 2017	2013-2015							23 / 37	62	45.9-76.6									
Çilveti	2011-2014	102 / 280	36.5	30.9-42.2															
Ding 2015	2011-2013	107 / 108	99.1	95.6-100.0				3 / 14	21.4	5.8-48.0									
Sonsuwan 2016	2007-2008																		
Lee 2014	2001-2010										1	100.0	71.7-100.0						
Setchanova 2013-2	2006-2011	42 / 79	53.2	42.1-64.0															
Marchisio 2013-3 <sup>a</sup>	2008-2011	14 / 50	28.0	16.9-41.6	3 / 143	2.1	0.5-5.6	5 / 17	29.4	11.7-53.7				7 / 49	14.3	6.5-26.2	0 / 4	0.0	0.0-52.7
Marchisio 2013-2 <sup>a</sup>	2005-2007	2 / 19	10.5	1.8-30.6	0 / 35	0.0	0.0-8.2	1 / 4	25.0	1.3-75.8				1 / 14	7.1	0.4-30.5			
Marchisio 2013-1 <sup>a</sup>	2001-2004	5 / 22	22.7	8.8-43.4	4 / 42	9.5	3.1-21.4	1 / 12	8.3	0.4-34.7				4 / 20	20.0	6.7-41.5	0 / 1	0.0	0.0-79.3
Stamboulidis 2011-2 <sup>c</sup>	2005-2008	115 / 329	35.0	29.9-40.2															
Stamboulidis 2011-1 <sup>c</sup>	2000-2008	268 / 614	43.6	39.8-47.6															
<b>Non-susceptible to cephalosporin</b>																			
Çilveti	2011-2014	15 / 280	5.4	3.1-8.5															
Ding 2015	2011-2013	68 / 108	63.0	53.6 - 71.7															
Sonsuwan 2016	2007-2008				0 / 19	0.0	0.0-14.6												
Lee 2014	2001-2010										1 / 8	12.5	0.6-48.0						
Setchanova 2013-2 <sup>b</sup>	2006-2011	7 / 79	8.9	4.0-16.7															
Marchisio 2013-3 <sup>a</sup>	2008-2011	0 / 50	0.0	0.0-5.8	2 / 143	1.4	0.2-4.5	3 / 17	17.6	4.7-40.9				0 / 49	0.0	0.0-5.9	0 / 4	0.0	0.0-52.7
Marchisio 2013-2 <sup>a</sup>	2005-2007	0 / 19	0.0	0.0-14.6	0 / 35	0.0	0.0-8.2	1 / 4	25.0	1.3-75.8				0 / 14	0.0	0.0-19.3			
Marchisio 2013-1 <sup>a</sup>	2001-2004	0 / 22	0.0	0.0-12.7	0 / 42	0.0	0.0-6.9	1 / 12	8.3	0.4-34.7				0 / 20	0.0	0.0-13.9	0 / 1	0.0	0.0-79.3
Junejo 2011 <sup>b</sup>	2007-2009	36 / 63	57.1	44.7-68.9	2 / 22	9.1	1.6-26.9	90 / 114	78.9	70.7-85.7	4 / 7	57.1	21.6-87.7	9 / 19	47.4	26.1-69.3			
Stamboulidis 2011-3	2005-2008	36 / 329	10.9	7.9-14.7															
Stamboulidis 2011-1	2000-2008	22 / 647	3.4	2.2-5.0															
<b>Non-susceptible to quinolones</b>																			
Naziat 2018	2014-2015	9 / 164	5.5	2.7-9.8	0 / 187	0.0	0.0-1.6												
Ling ding 2017	2013-2015							0 / 37	0.0	0.0-9.4									
Ding 2015	2011-2013	1 / 108	0.9	0.0 - 4.5															
Sonsuwan 2016	2007-2008				0 / 19	0.0	0.0-14.6												
Setchanova 2013-2	2006-2011	0 / 79	0.0	0.0-3.7															
Junejo 2011	2007-2009										2 / 7	28.6	5.1-67.0						

Table, Supplemental Digital Content 5. Non-susceptibility rates of bacteria to antibiotics <http://links.lww.com/INF/E357> (continued)

Study	Year of conduction	S. pneumoniae			H. influenzae			S. aureus			P. aeruginosa			S. pyogenes			M. catharralis		
		n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Non-susceptible to ampicillin																			
Sonsuwan 2016	2007-2008				5 / 19	26.3	10.3-49.0												
Marchisio 2013-3 <sup>a</sup>	2008-2011	1 / 50	2.0	0.1-9.5	23 / 143	16.1	10.7-23.0	13 / 17	76.5	52.5-92.0				0 / 49	0.0	0-5.9	4 / 4	100.0	47.3-100.0
Marchisio 2013-2 <sup>a</sup>	2005-2007	1 / 19	5.2	0.3-23.3	2 / 35	5.7	1.0-17.6	2 / 4	50.0	9.4-90.6				0 / 14	0.0	0.0-19.3	0 / 0	0.0	0.0-79.3
Marchisio 2013-1 <sup>a</sup>	2001-2004	0 / 22	0.0	0.0-12.7	1 / 42	2.4	0.1-11.2	7 / 12	58.3	30.2-82.8				0 / 20	0.0	0.0-13.9	1 / 1	100.0	20.7-100.0
Grevers 2012	2008-2010	0 / 10	0.0	0.0-25.9	4 / 21	19.0	6.4-39.8												

<sup>a</sup> Marchisio only reported resistance data

<sup>b</sup> Setchanova and Grevers reported data of several cephalosporins; we extracted their data of ceftiaxone.

<sup>c</sup> Grevers reported for H. Influenzae and Stamboulidis reported for erythromycin only resistance data and no intermediate or sensitive data



Table, Supplemental Digital Content 6. Resistance rates of bacteria to antibiotics: <http://links.lww.com/INF/E359>

Study	Year of conduction	S. pneumoniae			H. influenzae			S. aureus			P. aeruginosa			S. pyogenes			M. catharraditis		
		n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Resistance to penicillin																			
Ding 2015																			
Marchisio 2013-3	2011-2013	11 / 108	10.2	5.5-17.0															
Marchisio 2013-3	2008-2011	1 / 50	2.0	1.0-9.5	0 / 143	0.0	0.0-2.1	5 / 17	29.4	11.7-53.7				1 / 49	2.0	0.1-9.7	0 / 4	0.0	0.0-52.7
Marchisio 2013-2	2005-2007	0 / 19	0.0	0-14.6	0 / 35	0.0	0.0-8.2	1 / 4	25.0	1.3-75.8				0 / 14	0.0	0.0-19.3			
Marchisio 2013-1	2001-2004	0 / 22	0.0	0-12.7	1 / 42	2.4	0.1-11.2	1 / 12	8.3	0.4-34.7				0 / 20	0.0	0.0-13.9	0 / 1	0.0	0.0-79.3
Grevers 2012																			
Stamboulidis 2011-2	2008-2010	1 / 10	10.0	0.5-40.3															
Stamboulidis 2011-1	2005-2008	44 / 329	13.4	10.0-17.4															
Brook 2009	2000-2008	28 / 647	4.3	3.0-6.1															
2001-2006	2001-2006	1 / 22	4.5	0.2-20.4															
Resistance to amoxicillin																			
Ding 2015																			
Grevers 2012	2011-2013	45 / 108	41.7	32.6-51.1															
Grevers 2012	2008-2010	0 / 10	0.0	0.0-25.9	2 / 21	9.5	1.6-28.1												
Junejo 2011	2007-2009	5 / 63	7.9	3.0-16.7	7 / 22	31.8	56.6-91.2	63 / 114	55.3	46.1-64.2	7 / 7	100.0	65.2-100.0	2 / 19	10.5	1.8-30.6			
Resistance to trimethoprim/sulfamethoxazole																			
Ding 2015																			
Marchisio 2013-3	2011-2013	89 / 108	82.4	74.4-88.7															
Marchisio 2013-3	2008-2011	3 / 50	6.0	1.5-15.5	11 / 143	7.7	4.1-13.0	1 / 17	5.9	0.3-25.8				3 / 49	6.1	1.6-15.8	0 / 4	0.0	0.0-52.7
Marchisio 2013-2	2005-2007	0 / 19	0.0	0.0-14.6	0 / 35	0.0	0.0-8.2	0 / 4	0.0	0.0-52.7				0 / 14	0.0	0.0-19.3			
Marchisio 2013-1	2001-2004	6 / 22	27.3	11.9-48.3	3 / 42	7.1	1.8-18.2	0 / 12	0.0	0.0-22.1				2 / 20	10.0	1.7-29.3	0 / 1	0.0	0.0-79.3
Grevers 2012	2008-2010	0 / 10	0.0	0.0-25.9	7 / 21	33.3	15.9-55.1												
Resistance to erythromycin																			
Ding 2015																			
Setchanova 2013	2011-2013	107 / 108	99.1	95.6-100.0															
1994-2011	1994-2011	60 / 128	46.9	38.3-55.5															
Marchisio 2013-3	2008-2011	14 / 50	28.0	16.9-41.6	3 / 143	2.1	0.5-5.6	5 / 17	29.4	11.7-53.7				7 / 49	14.3	6.5-26.2	0 / 4	0.0	0.0-52.7
Marchisio 2013-2	2005-2007	2 / 19	10.5	1.8-30.6	0 / 35	0.0	0.0-8.2	1 / 4	25.0	1.3-75.8				1 / 14	7.1	0.4-30.5			
Marchisio 2013-1	2001-2004	5 / 22	22.7	8.8-43.4	4 / 42	9.5	3.1-21.4	1 / 12	8.3	0.4-34.7				4 / 20	20.0	6.7-41.5	0 / 1	0.0	0.0-79.3
Stamboulidis 2011-2																			
2005-2008	2005-2008	115 / 329	35.0	29.9-40.2															
Stamboulidis 2011-1	2000-2003	268 / 614	43.6	39.8-47.6															
Resistance to cephalosporin																			
Ding 2015																			
Marchisio 2013-3	2011-2013	37 / 108	34.3	25.8-43.6															
Marchisio 2013-3	2008-2011	0 / 50	0.0	0.0-5.8	2 / 143	1.4	0.2-4.5	3 / 17	17.6	4.7-40.9				0 / 49	0.0	0.0-5.9	0 / 4	0.0	0.0-52.7
Marchisio 2013-2	2005-2007	0 / 19	0.0	0.0-14.6	0 / 35	0.0	0.0-8.2	1 / 4	25.0	1.3-75.8				0 / 14	0.0	0.0-19.3			
Marchisio 2013-1	2001-2004	0 / 22	0.0	0.0-12.7	0 / 42	0.0	0.0-6.9	1 / 12	8.3	0.4-34.7				0 / 20	0.0	0.0-13.9	0 / 1	0.0	0.0-79.3

Table, Supplemental Digital Content 6. Resistance rates of bacteria to antibiotics: <http://links.lww.com/INF/E359> (continued)

Study	Year of conduction	<i>S. pneumoniae</i>			<i>H. influenzae</i>			<i>S. aureus</i>			<i>P. aeruginosa</i>			<i>S. pyogenes</i>			<i>M. catharralis</i>		
		n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI	n	%	95% CI
Chen 2013	2011-2012	2 / 32	6.3	1.1-19.1															
Junco 2011	2007-2009	7 / 63	11.1	5.0-20.7	1 / 22	4.5	0.2-20.4	60 / 114	52.6	43.5-61.7	2 / 7	28.6	5.1-67.0	3 / 19	15.8	4.2-37.2			
Stamboulidis 2011-3	2005- 2008	8 / 329	2.4	1.1-4.6															
Stamboulidis 2011-1	2000-2003	3 / 647	0.5	0.1-1.3															
<b>Resistance to quinolones</b>																			
Ding 2015	2011-2013	1 / 108	0.9	0.0-4.5															
Junco 2011	2007-2009										1 / 7	0.143	0.7-53.0						
<b>Resistance to ampicillin</b>																			
Marchisio 2013-3	2008- 2011	1 / 50	2.0	0.1-9.5	23 / 143	16.1	10.7-23.0	13 / 17	76.5	52.5-92.0				0 / 49	0.0	0-5.9	4 / 4	100.0	47.3-100.0
Marchisio 2013-2	2005- 2007	1 / 19	5.2	0.3-23.3	2 / 35	5.7	1.0-17.6	2 / 4	50.0	9.4-90.6				0 / 14	0.0	0.0-19.3	0 / 0	0.0	0-79.3
Marchisio 2013-1	2001- 2004	0 / 22	0.0	0.0-12.7	1 / 42	2.4	0.1-11.2	7 / 12	58.3	30.2-82.8				0 / 20	0.0	0.0-13.9	1 / 1	100.0	20.7-100.0
Grevers 2012	2008- 2010	0 / 10	0.0	0.0-25.9	4 / 21	19.0	6.4-39.8												

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CHAPTER 4





**Randomised trial of topical versus oral antibiotics  
for children with acute otitis media and ear  
discharge**

## CHAPTER 4.1

# 4.1

# **Topical or oral antibiotics for children with acute otitis media presenting with ear discharge: study protocol of a randomised controlled non-inferiority trial**

Based on:

Hullegie S, Venekamp RP, van Dongen TMA, Mulder S, van Schaik W, de Wit GA, Hay AD, Little P, Moore MV, Sanders EAM, Bonten MJM, Bogaert D, Schilder AG, Damoiseaux RAMJ. Topical or oral antibiotics for children with acute otitis media presenting with ear discharge: study protocol of a randomised controlled non-inferiority trial. *BMJ Open*. 2021 Dec 16;11(12):e052128. doi: 10.1136/bmjopen-2021-052128.

## **Abstract**

### **Introduction**

Around 15-20% of children with acute otitis media present with ear discharge due to a spontaneous tear or perforation of the eardrum (AOMd). Current guidance recommends clinicians to consider oral antibiotics as first-line treatment in this condition. The opening in the eardrum however should allow topical antibiotics to enter the middle ear directly. Local administration of antibiotics does not expose children to systemic side effects and may put less selective resistance pressure on bacteria. Evidence on the effectiveness of this approach in children with AOMd is lacking.

### **Methods and analysis**

A primary care-based, open, individually randomised, controlled, non-inferiority trial. The trial aims to recruit 350 children aged 6 months to 12 years with AOMd and ear pain and/or fever. Participants will be randomised to 7 days of hydrocortisone-bacitracin-colistin eardrops five drops three times daily or amoxicillin oral suspension 50 mg per kilogram body weight per day, divided over three doses. Parents will keep a daily diary of AOM symptoms, adverse events and complications for 2 weeks. In addition, they will record AOM recurrences, healthcare utilisation, and societal costs for 3 months. The primary outcome is the proportion of children without ear pain and fever at day 3. Secondary outcomes include ear pain and fever intensity/severity; days with ear discharge; eardrum perforation at 2 weeks; adverse events during first 2 weeks; costs; and cost-effectiveness at 2 weeks and 3 months. The primary analyses will be intention-to-treat and per-protocol analyses will be conducted as well.

### **Ethics and dissemination**

The medical research ethics committee Utrecht, The Netherlands has given ethical approval (17-400/G-M). Parents/guardians of participants will provide written informed consent. Study results will be submitted for publication in peer reviewed medical journals and presented at relevant (inter)national scientific meetings.

## Introduction

Acute otitis media (AOM) is one of the most common childhood infections and an important reason for doctor consultations and antibiotic prescribing.<sup>1,2</sup> Approximately 15%-20% of children with AOM present with ear discharge due to a spontaneous tear or perforation of the eardrum (AOMd).<sup>3,4</sup> Contrary to widespread beliefs, children with AOMd have similar levels of ear pain and are more unwell at presentation than those without ear discharge.<sup>3,4</sup> These children also have a poorer prognosis with higher rates of ear pain and/or fever at 3-7 days and more AOM recurrences and hearing problems at 3 months than children presenting with AOM without ear discharge.<sup>3,4</sup> They also benefit more from oral antibiotics than those with AOM without ear discharge: number needed to treat to achieve resolution of ear pain and/or fever at days 3-7: 3 versus 8, respectively.<sup>3</sup> Based on this evidence, current guidelines recommend general practitioners (GPs) to consider an immediate oral antibiotic prescribing strategy for children with AOMd.<sup>5,6</sup> Oral antibiotics, however, expose children to systemic side effects such as diarrhoea, vomiting and rash<sup>7</sup> and routine use of oral antibiotics in common infections such as AOM contributes to emergence of antimicrobial resistance.<sup>8,9</sup> Alternative treatment strategies for AOM are therefore urgently needed.

In children with AOMd, the perforation should allow topical antibiotics to enter the middle ear directly. Topical antibiotic treatment does not expose children to systemic side effects and may put less selective resistance pressure on commensal microbes.<sup>10,11</sup> We have shown that in children with acute ear discharge in the presence of ventilation tubes (grommets) antibiotic-corticosteroid eardrops are clinically much more effective and less costly than oral antibiotics.<sup>12,13</sup> Topical antibiotics may therefore also be an effective treatment strategy in children with AOMd. So far, evidence to support this hypothesis is lacking.<sup>14,15</sup> Our trial aims to provide this key evidence.

## Objective

The aim of this randomised controlled trial is to establish whether treatment with antibiotic-corticosteroid eardrops is non-inferior to treatment with oral antibiotics in children aged 6 months to 12 years presenting to their GP with AOM with acute ear discharge due to a spontaneous tear or perforation of the eardrum (AOMd).

The objectives are to determine the:

- effectiveness of antibiotic-corticosteroid eardrops versus oral antibiotics in terms of:
  - the proportion of children without ear pain and fever at day 3;
  - severity and duration of ear pain, fever, ear discharge;
  - time to resolution of total symptoms;
  - middle ear effusion (MEE) and eardrum perforation at 2 weeks
  - otitis media (OM)-specific quality of life (QoL) at 2 weeks and 3 months;
  - antibiotic consumption during the first 2 weeks and at 3 months and AOM recurrences at 3 months;
  - adverse events during the first 2 weeks.
- costs and cost-effectiveness of antibiotic-corticosteroid eardrops versus oral antibiotics;
- prevalence of bacteria and viruses in otorrhoea and nasopharyngeal samples of children with AOMd before and after treatment and the antimicrobial susceptibility profile of the bacteria;
- impact of the treatment regimens on antimicrobial resistance genes in the human gut.

## Methods and analyses

### Study design and setting

An open, individually randomised, controlled, non-inferiority trial in 350 children aged 6 months to 12 years presenting to their GP with AOMd. Children will be randomly allocated to 7 days treatment with either: 1) antibiotic-corticosteroid (hydrocortisone-bacitracin-colistin) eardrops or 2) oral antibiotics (amoxicillin suspension). Follow-up will be 3 months.

At trial commencement, in December 2017, we anticipated a 2-year trial recruitment period. Approximately 250 GPs in the region of Utrecht, The Netherlands, agreed to recruit children to the trial. Due to the COVID-19 related infection control measures, we anticipate a relatively low AOM incidence during trial recommencement. To meet the required sample size, we will recruit additional general practices to the trial. Further details on the trial status are outlined in the ‘current study status’ section below.

### Participants

Children aged 6 months to 12 years presenting to their GP with recent onset AOMd in one or both ears and either ear pain or fever or both. Children with grommets in place and those with a pre-existing perforation of the eardrum are excluded. For detailed inclusion and exclusion criteria, see Box 1.

**Box 1. Full list of inclusion and exclusion criteria**

**Inclusion criteria:**

Children **aged 6 months to 12 years** whose parents are consulting the GP with **AOM and ear discharge** in one or both ears ( $\leq 7$  days duration) *and* either parent-reported **ear pain** in the previous 24 hours **or fever** (child's body temperature of  $\geq 38.0^{\circ}\text{C}$  in the previous 24 hours as reported by parents or as measured by the GP during consultation) or both.

**Exclusion criteria:**

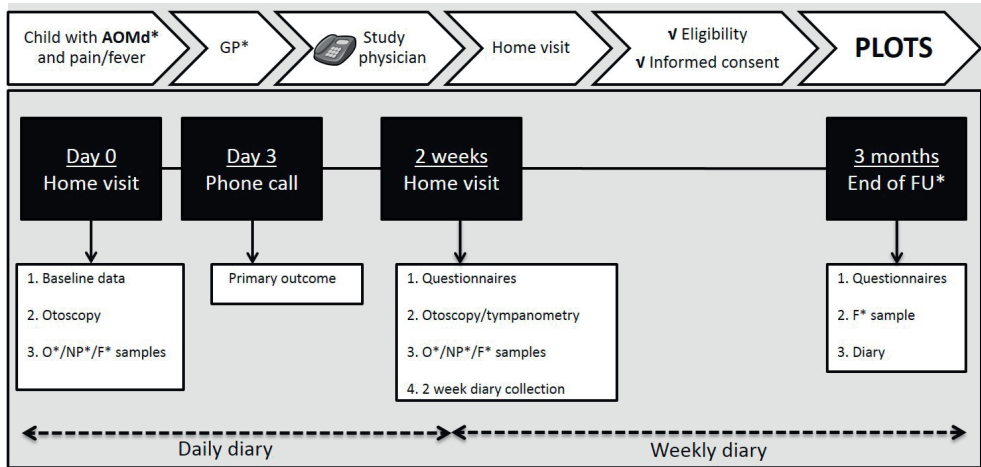
Children will be excluded from participation if they;

1. are systemically very unwell and require immediate oral antibiotics or immediate hospitalisation (e.g. child has signs and symptoms of serious illness and/or complications such as mastoiditis/meningitis);
2. are at high risk of serious complications including children with known immunodeficiency other than partial IgA or IgG2 deficiencies, craniofacial malformation such as cleft palate, children with Down syndrome, previous ear surgery other than grommet insertion;
3. have grommets in place;
4. have a pre-existing perforation of the eardrum;
5. had a prior AOM episode (with or without ear discharge) in previous 28 days;
6. used oral antibiotics or topical antibiotics in previous 2 weeks;
7. have a known allergy or sensitivity to oral amoxicillin or hydrocortisone-bacitracin-colistin;
8. have already participated in this trial.

AOM presenting with ear discharge due to a spontaneous tear or perforation of the eardrum (AOMd) is defined as the presence of acute-onset of otoscopically confirmed otorrhoea together with other symptoms of an acute infection such as ear pain and/or fever, and/or irritability.

**Inclusion and baseline assessments**

Figure 1 depicts a flow diagram of study procedures. Participating GPs inform parents of potentially eligible children about the trial, take consent for sharing their contact details with the research team at the UMC Utrecht and provide a study information letter. Members of the research team contact parents by phone to provide detailed information about the trial. If parents provisionally agree to participate and if the child meets the eligibility criteria, a home visit by the trial doctor is scheduled for the same day.



**Figure 1. Flow diagram of main study procedures**

Legend: AOMd; acute otitis media with ear discharge, GP; general practitioner, O; otorrhoea, NP; nasopharynx, F; faeces.

At this visit, the trial doctor takes written informed consent from parents/guardians, collects baseline demographic and disease-specific data, records otoscopic findings and takes otorrhoea, nasopharynx and faeces samples. Parents will complete an OM-specific QoL questionnaire on behalf of their child.

**Study group assignment**

An independent data manager generates a computer-generated randomisation sequence with stratification according to age (<2 versus ≥2 years) and laterality (uni- versus bilateral AOM at baseline). At the conclusion of the baseline home visit, the trial doctor accesses a trial randomisation website for concealed study-group assignment. The assignment will be balanced in a 1:1 ratio for the two study groups:

- 1) hydrocortisone-bacitracin-colistin (Bacicoline-B®) eardrops, five drops, three times per day in the discharging ear(s) for 7 days; or
- 2) amoxicillin, 50 mg per kilogram of body weight per day, divided over three doses administered orally for 7 days.

Hydrocortisone-bacitracin-colistin eardrops are the most widely used commercially available eardrops in the Netherlands that do not contain a potentially ototoxic aminoglycoside. The combination of antibiotics in these eardrops cover the spectrum of bacteria most often found in AOM.<sup>16, 17</sup> We have chosen an eardrop that also contains a corticosteroid since the available evidence from children with grommets suggests that topical treatment with a combination of antibiotics and a corticosteroid might be more effective than topical antibiotics alone in resolving acute ear discharge.<sup>18, 19</sup> Hydro-



cortisone-bacitracin-colistin eardrops were also used in our previous trial in children with acute ear discharge in the presence of ventilation tubes (grommets).<sup>12</sup> Parents of children assigned to the antibiotic-corticosteroid eardrops group will be shown how to remove any visible ear discharge with a tissue and apply the drops while tilting their child's head to one side, and to apply tragal pressure (tragal pumping).<sup>20</sup>

Amoxicillin is listed as first-line antibiotic for AOM in children in most European guidelines, including the Netherlands.<sup>6, 21</sup> Based on the current antimicrobial resistance profiles in the Netherlands, the clinical practice guideline refers to the Dutch Paediatric Formulary which recommends a dosage of 50 mg per kilogram of body weight per day, divided over three doses for 7 days.<sup>6, 22</sup>

The study team will notify the GP and local pharmacist about the result of the randomisation. During follow-up, parents and GPs are encouraged to manage AOM recurrences according to current Dutch clinical practice guidance<sup>6</sup>, but any treatment decisions will be up to the GPs' discretion. Being a pragmatic trial, no restrictions in concurrent treatment will be applied and any concurrent treatments will be captured in the daily symptom diary.

### Follow-up data collection

Participants will be followed for 3 months. Parents will keep a daily diary of AOM-related symptoms including fever recordings and ear pain scores, use of study and other medication, adverse events and complications of AOM for 2 weeks. Thereafter, they will keep a weekly diary recording AOM recurrences, GP consultations, prescribed and over-the-counter (OTC) medication, hospital admissions, and societal costs for AOM for 3 months. A telephone call will be scheduled at day 3 to answer any remaining questions about the study, to optimise compliance to the diary and to capture data on our most critical parent-reported outcomes. A follow-up visit at the child's home will be scheduled at 2 weeks to check diary data for accuracy, perform otoscopy and tympanometry, and sample otorrhoea (where possible), nasopharynx and faeces samples. Parents will complete OM-specific QoL and productivity loss questionnaires at 2 weeks and at 3 months. Parents will also send a faeces sample to the laboratory for analysis at 3 months.

### Validated questionnaires

Parents report the presence and severity of their child's symptoms using a validated seven point Likert scale.<sup>23, 24</sup> OM-specific QoL of the child will be assessed using the parent-reported OM-6 questionnaire, a 6-item questionnaire recording ear-related problems in the previous period.<sup>25</sup> An adapted version of the iMTA Productivity Cost Questionnaire (iPCQ) is used to capture parental productivity losses due to AOM.<sup>26</sup>

## Temperature measurement

Parents measure their child's temperature twice daily (morning and evening) with a tympanic membrane thermometer in the unaffected ear.<sup>27</sup> In children aged below 2 years and in those with bilateral ear discharge, temperature is measured rectally. To standardise measurements, a study thermometer will be provided. The definition of 'no fever' at day 3 (primary outcome) is a temperature recording below 38.0°C both in the morning and evening.<sup>6</sup>

## Eardrum perforation

Otосcopy will be used to assess the integrity of the eardrum at 2 weeks. In case of inconclusive otосcopy results, tympanometry results will be used.

## MEE assessment

A diagnostic algorithm combining tympanometry and otосcopy will be used to diagnose MEE at 2 weeks.<sup>28</sup>

## Collection and analyses of otorrhoea and nasopharynx samples

The otorrhoea and nasopharynx samples are collected using a flexible applicator swab with flocked nylon fiber tip.<sup>29</sup> The swabs will be immediately transported to the microbiology laboratory of the UMC Utrecht where they will be stored at -80°C until further analysis.

## Collection and analyses of faeces samples

The 2-week and 3-month faeces samples are collected using the OMNIgene•GUT (OMR-200)®, a trademark of DNA Genotek Inc. Ottawa Canada. Parents will send the 3 month- faeces samples by mail. If faeces samples cannot be collected during the baseline and 2-week home visit, we will provide the parents with a collection kit and transport envelop, so parents can collect and send the faeces samples by mail at their earliest convenience. Samples will be stored at -80°C at the microbiology laboratory of the UMC Utrecht until analysis for detection and quantification of the dynamics of bacterial genes that confer resistance to the antibiotics used in our trial.<sup>30</sup>

## Primary and secondary outcomes

The primary outcome is the proportion of children without ear pain (ear pain score 0 on the 0-6 Likert scale) and fever (body temperature of 38.0°C or higher) at day 3 (72 hours after randomisation).

Secondary outcomes are the proportion of children with at most mild ear pain (ear pain score less than 3 on the 0-6 Likert Scale) at day 3; mean ear pain score over days 0-3, number of days with ear pain (ear pain score 1 or higher on the 0-6 Likert scale);

mean body temperature over days 0-3; number of days with fever (body temperature of 38.0°C or higher) during the first 2 weeks; the proportion of children with parent-reported ear discharge at day 3; number of days with parent-reported ear discharge at day 3 and during the first 2 weeks; proportion of children with otoscopically confirmed ear discharge at 2 weeks; time to resolution of total symptoms (time to all of pain, fever, discharge, being unwell, sleep disturbance, and distress/crying being rated 0 or 1 on the Likert scale); MEE and proportion of children with otoscopically confirmed eardrum perforation at 2 weeks; OM-specific quality of life at baseline, 2 weeks and 3 months; antibiotic consumption during the first 2 weeks and at 3 months; number of AOM recurrences at 3 months; number of adverse events during the first 2 weeks; costs and cost-effectiveness at 2 weeks and 3 months; the prevalence of viruses and bacteria in otorrhoea and nasopharynx samples at baseline and 2 weeks; the antimicrobial susceptibility profiling of the bacteria and the impact of the treatment regimens on antimicrobial resistance genes in the human gut; microbiome profile of nasopharynx at baseline and 2 weeks.

### Sample size calculation

The main aim is to demonstrate that antibiotic-corticosteroid eardrops are non-inferior to oral antibiotics in relieving ear pain and fever at day 3. The proportion of children without ear pain and fever at day 3 is expected to be 65% in the oral antibiotics group and around 35% if placebo or no treatment would be trialled.<sup>3</sup> Our parent panel assisted in defining the non-inferiority margin by advising on the maximum difference in primary outcome that they would regard as unimportant. Following these discussions, the clinically acceptable non-inferiority margin is set at 15%; i.e. 50% of the difference (30%) observed between oral antibiotics and placebo or no treatment in earlier trials.<sup>3</sup> Taking 50% of such a difference is also a widely-accepted method to determine the non-inferiority margin.<sup>31, 32</sup> Testing significance at a one-sided 0.025 level ( $\alpha$ ) and using a power of 80% ( $\beta$  0.20), each treatment arm should include at least 159 children to demonstrate that the upper limit of the one-sided 97.5% confidence interval (CI) (or equivalently a two-sided 95% CI) of the difference in treatment effect for the primary outcome does not exceed the predefined non-inferiority margin of 15%. To allow for a maximum of 10% loss to follow-up, we aim to randomise 350 children.

### Statistical analysis

Primarily, all analyses will be performed according to the intention-to-treat principle. Per-protocol analyses will also be conducted as well because of its importance in non-inferiority trials.<sup>33</sup> All analyses will be performed blinded with respect to study-group

assignment and analysis and presentation of results will be in accordance with the CONSORT guidelines.<sup>34, 35</sup>

## **Clinical effectiveness**

We will use descriptive statistics to describe the baseline characteristics trial population; we will present means and standard deviations for normally distributed continuous variables, medians and inter-quartile ranges for non-normally distributed continuous variables, and numbers with percentages for categorical variables.

The primary outcome will be analysed with binomial logistic regression model including treatment group and effectiveness of oral antibiotics versus antibiotic-corticosteroid eardrops will be expressed as relative risk and absolute risk difference with accompanying 95% CIs. This latter will enable us to judge whether non-inferiority has been demonstrated, in particular whether the upper limit of the two-sided 95% CI exceeds the predefined non-inferiority margin of 15%. In adjusted analyses, stratification factors and other important prognostic factors (baseline ear pain score, duration of ear pain prior to enrolment) will be added to the model. Subgroup effects according to age (<2 versus  $\geq 2$  years) and laterality (uni- versus bilateral AOM at baseline) will be evaluated by including an interaction term (treatment\*age) in the model.

In sensitivity analyses, we will impute for missing baseline and outcome data using multiple imputation techniques.<sup>36, 37</sup> In further sensitivity analysis, we will assess whether results differ when defining the absence of fever for the primary outcome as parental fever score 0 or 1 on the 0-6 Likert scale at day 3 (instead of the child's body temperature recordings as specified above).

In secondary analysis, we will use log binomial regression analyses for dichotomous variables, Poisson regression analyses for count variables, and linear regression analyses for continuous variables, where applicable corrected for repeated measurements. For these analyses, the comparison between treatment groups will be expressed as risk ratios, rate ratios, and mean differences, respectively; all with 95% CIs. Kaplan-Meier curves will be plotted for duration of symptoms and log-rank tests for differences between groups.

## **Cost-effectiveness analysis (CEA)**

A societal perspective will be used for this analysis, i.e. medical and non-medical costs will be taken into account. We will use a short time horizon for all analyses and therefore, all costs will be presented undiscounted.

First, effectiveness will be assessed: the main clinical effectiveness outcome will be symptom (ear pain and fever) resolution. Similar to our previous trials in this field, we will not use quality-adjusted life-years (QALYs) as the nature of the condition (self-limiting in the vast majority of the children and of relatively short duration) does not impact importantly on QALYs.<sup>12</sup>

Second, costs will be calculated; all costs will be estimated at the patient level by multiplying resource use with cost estimates per unit of resources use. Cost prices will be estimated according to guidelines for economic evaluation in health care research or taken from standard reference lists, as far as possible.<sup>38, 39</sup> Costs of medication use will be retrieved from the Dutch formulary and a pharmacist's fee will be added for every prescription.<sup>38, 40</sup> Costs of over-the-counter and complementary medicines will be calculated per day, based on current average retail prices. Costs of consulting a GP or a medical specialist, and hospitalisations will be based on current Dutch guidelines for pharmaco-economic evaluation<sup>38</sup> or charges if no other estimates are available. Costs of diagnostic tests will be derived from the Dutch diagnostic formulary<sup>41</sup>, where relevant increased by a technician's charge. Costs of surgical procedures will be based on a previous Dutch costing study.<sup>42</sup> Costs associated with absence from work will be retrieved from the completed *iPCQ*.<sup>26</sup> The hourly cost estimate for childcare will be derived from the Dutch National Institute for Family Finance Information (NIBUD).<sup>43</sup> Travel expenses will be calculated for healthcare visits following the Dutch guideline for pharmaco-economic evaluation.<sup>38</sup> Overall costs will be compared across the treatment groups, and where relevant, differences will be calculated, including 95% CIs. Finally, we will compare differences in costs between treatment groups to differences in clinical effects between groups by calculating incremental cost-effectiveness ratios (ICERs). ICERs will indicate the incremental cost per day with ear pain and fever avoided when comparing antibiotic-corticosteroid eardrops with oral antibiotics, both in the short- (14 days) and long-term (3 months). Uncertainty will be addressed in a probabilistic sensitivity analysis by means of bootstrapping. Results will be presented using incremental cost-effectiveness planes and cost-effectiveness acceptability curves.

## Ethics and dissemination

The study is conducted according to the principles of the Declaration of Helsinki (10<sup>th</sup> version, October 2013), in accordance with the Medical Research Involving Human Subjects Act (WMO) and the principles of Good Clinical Practice. The medical research ethics committee Utrecht, The Netherlands has approved the protocol (protocol number 17-400/M-G). The trial doctor will take written informed consent from both parents/guardians. Regular trial audits including checks on source data verification,

accuracy, validity and completeness of informed consent forms and captured data will be performed by a clinical research associate of Julius Clinical, an independent clinical research organization. We have not established a data safety monitoring board and refrain from conducting any interim analysis for safety or superiority/futility since we neither expect any safety issues nor large differences in treatment failures between the two active treatment groups given the difference (30%) observed between oral antibiotics and placebo or no treatment in previous trials.<sup>2, 12</sup> However, in accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The data management department of the Julius Center for Health Sciences and Primary Care of the UMC Utrecht will be responsible for handling and storage of the data using innovative software applications like SLIM and Research Online. Upon completion of the trial, data will be stored for a minimum of 15 years at a central data drive at the Julius Center and will only be made available for use by third parties upon request and approval of the principle investigator (professor R.A.M.J. Damoiseaux).

### **Dissemination plan**

We will publish study results in peer reviewed scientific journals and present at relevant (inter)national scientific meetings. We will work with our parent panel to help interpret the findings of the trial and harness their resources for dissemination to the lay public.

### **Patient and public involvement**

For this trial, we have established a panel of eight parents. This panel provided input to the design of the trial including the sample size calculation by advising on the clinically acceptable non-inferiority margin and the outcome measures by proposing additional outcomes of interest such as being unwell and sleep disturbance, and commented on the recruitment strategy and patient information letter. One of the parent panel members is a co-applicant on the grant application and co-author on this paper. The parent panel will be actively involved throughout all critical stages of the trial through regular parent panel meetings. They will work with us throughout the recruitment phase, will be involved in reporting the trial results, and will be ultimately key to pull the evidence into mainstream clinical practice.

### **Trial registration**

This trial is registered with the Netherlands National Trial Register (NTR6723).

### **Current study status**

The first participant was enrolled in the trial on 13 December 2017. On 8 August 2018 with 34 participants being enrolled, trial recruitment was put on hold due to supply issues of hydrocortisone-bacitracin-colistin eardrops. These drops are available again

since early 2021. Our funding body, the Netherlands Organisation for Health Research and Development (ZonMw), has approved the trial to re-open in September 2021. We expect data collection to be completed by the end of 2023 with trial results being available by March 2024.

## Discussion

This trial is one the first to compare the effectiveness of topical with oral antibiotics in children with AOMd. The only other trial in this field is conducted in UK primary care (Runny Ear Study; REST). This study has been designed in close collaboration with members of the Dutch study team to enable future meta-analysis by harmonising design, outcomes and outcome measure instruments.<sup>14, 15</sup> The UK-based trial has stopped prematurely due to problems with the electronic health record system platform which resulted in low recruitment (n=22) and failure to reach the predefined sample size.<sup>44</sup>

With obvious theoretical advantages of topical over oral antibiotic treatment and the lack of direct evidence, further research is needed to establish whether with AOMd can effectively be treated with topical antibiotics. Our trial will not only provide this key evidence, but also establish the impact of the two antibiotic treatment strategies on microbiome composition and antimicrobial resistance. The pragmatic, open-label design of our trial enhances the applicability of the findings to daily practice and is most suited to address key secondary outcomes such as antibiotic consumption during the first 2 weeks and cost-effectiveness in everyday practice. This would be much more difficult to determine realistically in a blinded study where children in both groups would receive oral suspension and ear drops. The lack of blinding might, however, introduce bias caused by the awareness of treatment assignment which may be particularly problematic in trials with subjective outcomes. However, both study groups receive an active treatment, our parent panel had no strong beliefs or preferences for one treatment over the other, and a recent meta-epidemiological study found no evidence for a difference in estimated treatment effect between blinded and non-blinded trials.<sup>45</sup> Another limitation of our study is, that we do not capture data on the prevalence of longer term complications such as the presence of otoscopically confirmed MEE or chronic suppurative otitis media at three months. We, however, will collect information about ENT-related specialist referrals, hospitalisation and/or surgery during the three months follow-up period, which provide information on the occurrence of AOM sequelae in the short- and long-term.

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## CHAPTER 4.2

# 4.2

# **Topical or oral antibiotics in childhood acute otitis media and ear discharge: a randomised controlled non-inferiority trial**

Based on:

Hullegie S, Damoiseaux RAMJ, Hay AD, Zuithoff NPA, van Dongen TMA, Little P, Schilder AGM, Venekamp RP. Topical or oral antibiotics in childhood acute otitis media and ear discharge: a randomized controlled non-inferiority trial. *Fam Pract.* 2024 Jun 24;cmæ034. doi: 10.1093/fampra/cmæ034

## **Abstract**

### **Introduction**

Current guidance suggests oral antibiotics can be considered for children with acute otitis media and ear discharge (AOMd), but there is an absence of evidence regarding the relative effectiveness of antibiotic-corticosteroid eardrops.

### **Aim**

To establish whether antibiotic-corticosteroid eardrops are non-inferior to oral antibiotics in children with AOMd.

### **Design and setting**

Open randomised controlled non-inferiority trial set in Dutch primary care.

### **Methods**

Children were randomised to hydrocortisone-bacitracin-colistin eardrops or amoxicillin oral suspension for 7 days. Primary outcome was proportion of children with resolution of ear pain and fever at day 3.

### **Results**

Between December 2017 and March 2023, 58 of a planned 350 children were recruited due to slow accrual for various reasons. Children assigned to eardrops (n=26) had lower resolution rates of ear pain and fever at 3 days compared to those receiving oral antibiotics (n=31): 42% vs 65%; adjusted risk difference 20.3%, 95% confidence interval -5.3% to 41.9%), longer parent-reported ear discharge (6 vs 3 days;  $p=0.04$ ), and slightly higher mean ear pain scores (Likert scale 0-6) over days 1-3 (2.1 vs 1.4,  $p=0.02$ ), but received fewer oral antibiotic courses in three months (11 for 25 children vs 33 for 30 children), and had less GI upset and rash (12% vs 32% and 8% vs 16%, respectively).

### **Conclusion**

Early termination stopped us from determining non-inferiority of antibiotic-corticosteroid eardrops. Our limited data, requiring confirmation, suggest that oral antibiotics may be more effective than antibiotic-corticosteroid eardrops in resolving symptoms and shortening the duration of ear discharge.

## Introduction

Of children with acute otitis media (AOM), 15-20% present with acute ear discharge due to a spontaneous perforation of the eardrum.<sup>1,2</sup> These children experience equal levels of ear pain, suffer from more frequent AOM recurrences and hearing problems and benefit more from antibiotic treatment than those with AOM who do not present with ear discharge.<sup>1,2</sup> They also seem to benefit more from antibiotic treatment with a number needed to treat (NNT) of 3 versus 8, respectively.<sup>1</sup> Current guidelines suggest clinicians can consider oral antibiotics for children presenting in primary care with AOM and ear discharge (AOMd)<sup>3,4</sup>, but this must always be balanced against the risk of side effects and the significant public health danger of antibiotic resistance.<sup>5</sup>

We have previously shown that treatment with antibiotic-corticosteroid eardrops is superior to oral antibiotics in children with ventilation tubes presenting with acute ear discharge.<sup>6</sup> The question is whether this strategy is also effective in children without ventilation tubes who present with acute ear discharge as one of the symptoms of AOM<sup>7</sup> arguing that the spontaneous perforation of the eardrum would provide an entry for topical antibiotics to act in the middle ear. Such approach would contribute to antibiotic stewardship and avoid systemic antibiotic side effects.

We therefore conducted a randomised controlled trial comparing treatment with oral antibiotics and antibiotic-corticosteroid eardrops in children presenting in primary care with AOMd.

## Methods

### Design and setting

From December 2017 to February 2023, an open, individually randomised controlled non-inferiority trial was conducted in 52 primary care practices in the region Utrecht, the Netherlands, including 225 general practitioners (GPs). Trial recruitment was put on hold from 8 August 2018 to November 2021 due to supply issues of one of the trial treatments (hydrocortisone-bacitracin-colistin (Bacicoline-B®) eardrops) and due to the COVID-19 pandemic. The trial's rationale and details of its design have been reported in detail elsewhere.<sup>8</sup> The trial was reported according to the Consolidated Standards of Reporting Trials (CONSORT) guideline.<sup>9</sup>

### Participants

Children aged 6 months to 12 years presenting to their GP with AOMd in one or both ears and either ear pain or fever or both, were eligible for trial participation.

AOMd is defined as the presence of acute-onset of otoscopically confirmed ear discharge together with other symptoms of an acute infection such as ear pain and/or fever, and/or irritability.

The following children were excluded: ventilation tubes in place or a pre-existing perforation of the eardrum, systemically very unwell, having received antibiotics during the previous two weeks and having had an episode of AOM in the previous 28 days, known immunodeficiency, craniofacial malformation, Down's syndrome, previous ear surgery other than ventilation tubes, allergy to oral amoxicillin or hydrocortisone-bacitracin-colistin ear drops, and having already participated in the trial during a previous AOMd episode.

## **Randomisation**

An independent data manager generated a computer-generated randomisation sequence with stratification according to age ( $<2$  versus  $\geq 2$  years) and laterality (uni- versus bilateral AOM at baseline). After completing informed consent and baseline assessments, the trial doctor accessed a trial randomisation website for concealed study-group assignment. Assignment was balanced in a 1:1 ratio for the two study groups.

## **Procedures**

### ***Treatment groups***

Children randomised to the oral antibiotic group were prescribed amoxicillin suspension, 50 mg per kilogram of body weight per day, divided over three doses administered orally for 7 days. Those randomised to eardrops were prescribed hydrocortisone-bacitracin-colistin eardrops, five drops, three times per day in the discharging ear(s) for 7 days. Parents were instructed to clean the ear of any visible ear discharge with a tissue and apply the drops while tilting their child's head to one side.

During follow-up, any further treatment decisions were be up to the GPs' discretion.

### ***Recruitment***

GPs informed parents of potentially eligible children about the trial, took consent to share their contact details with the study team at the UMC Utrecht and provided a study information leaflet. Upon receipt of details, the trial doctor contacted parents by phone to provide detailed information about the study and scheduled a home visit on the same day for those who provisionally agreed to participate and whose child met the eligibility criteria.



**Data collection****Baseline**

At the home visit, the trial doctor obtained written informed consent from parents or guardians, checked the inclusion and exclusion criteria, collected demographic and disease-specific data and recorded otoscopic findings. Otoscopy was performed with a Welch Allyn MacroView Otoscope. Parents completed the Otitis media-6 (OM-6) questionnaire, an OM-specific QoL questionnaire on behalf of their child.<sup>10</sup>

**2-week follow-up**

For the first 2 weeks of follow-up, parents kept a daily diary (paper or online) of AOM-related symptoms, treatment adherence, adverse events and complications of AOM for 2 weeks. Parents measured their child's temperature two times per day (morning and evening) with a tympanic membrane thermometer in the unaffected ear. In children aged below 2 years and in those with bilateral ear discharge, body temperature was measured rectally.

At 2 weeks, the trial doctor scheduled a follow-up home visit, where they verified the diary data and recorded otoscopy and tympanometry findings. Tympanometry was performed with a Interacoustics M10 tympanometer. A diagnosis of OME in one or both ears was based upon MOMES diagnostic algorithm which combines tympanometry and otoscopy findings.<sup>11</sup> Parents completed the OM-specific QoL (OM-6) questionnaire.<sup>11,13</sup>

**3-month follow-up**

After 2 weeks, parents kept a weekly diary (paper or online) recording AOM recurrence, GP consultations, medication use, hospital admissions, and societal costs for AOM for 3 months. At 3 months parents completed the OM-6 questionnaire, returned paper versions of the weekly diary and the questionnaire to the study team by mail; online versions were entered directly in the online database (Research Online).

**Outcomes**

The primary outcome was the proportion of children free from ear pain (ear pain score 0 on a 0-6 Likert scale<sup>12,13</sup>) and fever (body temperature lower than 38.0°C<sup>4</sup>) at day 3, i.e. 72 hours after randomisation.

Secondary outcomes are described in Box 1.

**Box 1. Secondary outcomes**

Ear pain	the mean ear pain score over the first 3 days; the proportion of children with at most mild ear pain at day 3 (score less than 3 on the 0-6 Likert scale); the number of days with ear pain score 1 or higher on the 0-6 Likert scale during the first 2 weeks.
Fever	the mean temperature over the first 3 days the number of days with fever (at least one recording of a body temperature of 38.0°C or higher per day) during the first 2 weeks.
Ear discharge	the proportion of children with parent-reported ear discharge at day 3; the number of days with parent-reported ear discharge during the first 2 weeks; the proportion of children with otoscopically confirmed ear discharge at 2 weeks; the number of days with parent-reported ear discharge at 3 months.
Time to resolution of total symptoms	time to all of pain, fever, ear discharge, unwell, disturbed sleep, and distress/crying being rated 0 or 1 on the Likert scale for two consecutive days.
Eardrum perforation	the proportion of children with an eardrum perforation at 2 weeks based on combined otoscopy and tympanometry findings.
OME	the proportion of children with OME (using the MOMES algorithm) in one or both ears at 2 weeks.
Antibiotic consumption	the total number of oral and ototopical antibiotics used during the first 2 weeks and at 3 months follow-up.
Adverse events	the adverse events during the first 2 weeks.
OM-specific quality of life	the OM specific quality of life assessed using the parent-reported OM-6 questionnaire at baseline, 2 weeks and 3 months.
AOM recurrences	the number of AOM recurrences during 3 months follow-up.

**Sample size calculation**

The clinically acceptable non-inferiority margin was set at 15%; that is, 50% of a 30% difference between oral antibiotics and placebo or no treatment as observed in earlier trials.<sup>14, 15</sup> To demonstrate that the upper limit of a two-sided 95% confidence interval (CI) of the difference in treatment effect for the primary outcome does not exceed the predefined non-inferiority margin of 15% with 80% power, a minimum of 159 children per group was needed. To allow for 10% attrition, we aimed to randomise 350 children.

**Statistical analysis**

All analyses were performed according to the intention-to-treat (ITT) principle. Because of its importance in non-inferiority trials, per-protocol analysis was also performed for our primary outcome.<sup>14</sup>

Descriptive statistics were used to describe the baseline characteristics trial population. The primary outcome was analysed with a logistic regression model, to reduce small sample size bias, the model was estimated with Firth's correction. In addition to treatment group assignment, age and laterality were included as potential confounders. Risk differences were derived from the regression model using the method described

by Austin: bootstrapping techniques were performed to calculate accompanying 95% CIs.<sup>16</sup> In sensitivity analysis, we assessed whether results differed when defining absence of fever for the primary outcome as parental fever score 0 or 1 (on the 0-6 Likert scale) at day 3 instead of the child's body temperature recordings as specified above.

For the dichotomous secondary outcomes, crude RRs and RDs with 95% CIs were calculated. The mean ear pain score and the mean fever score were analysed with a linear regression model. A residual covariance (i.e. GEE type) matrix was included to adjust for repeated measurements over time.<sup>17</sup> In these analyses adjustments for time, age, laterality and baseline pain score were made. The validity of the model assumptions (i.e. homoscedasticity and normality) was evaluated by assessing residuals.

Differences in dichotomous secondary outcomes were analysed with Chi-square tests and differences in duration of symptom outcomes were analysed using negative binomial regression. Kaplan-Meier curves were plotted for the duration of ear discharge and log-rank tests were used to test between group differences.

All statistical analyses were performed with IBM SPSS Statistics (version 27.0), RStudio (version 2023.06.0) and with SAS (version 9.4).

## Results

### Participants

58 of a planned 350 children were included in the trial; 27 were assigned to antibiotic-corticosteroid eardrops and 31 to oral antibiotics (Fig. 1). The primary outcome was assessed in 57 children (98%). Daily parental diaries were available for the same 57 children and weekly for 55 (95%) children. Treatment adherence was 88% in the eardrops group versus 97% in oral antibiotic group.

Baseline characteristics of the participants were well-balanced between the groups (Table 1). The median age of included children was 28 months and 40% was younger than 2 years. At the baseline visit parents reported ear pain in 95% of the children, and the trial doctor measured a body temperature of  $\geq 38.0^{\circ}\text{C}$  in 30% of the children and diagnosed bilateral AOM in 18 children (31%). Bilateral ear discharge was present in 7% (n=2) of the children in the eardrops group versus 13% (n=4) in the oral antibiotic group.

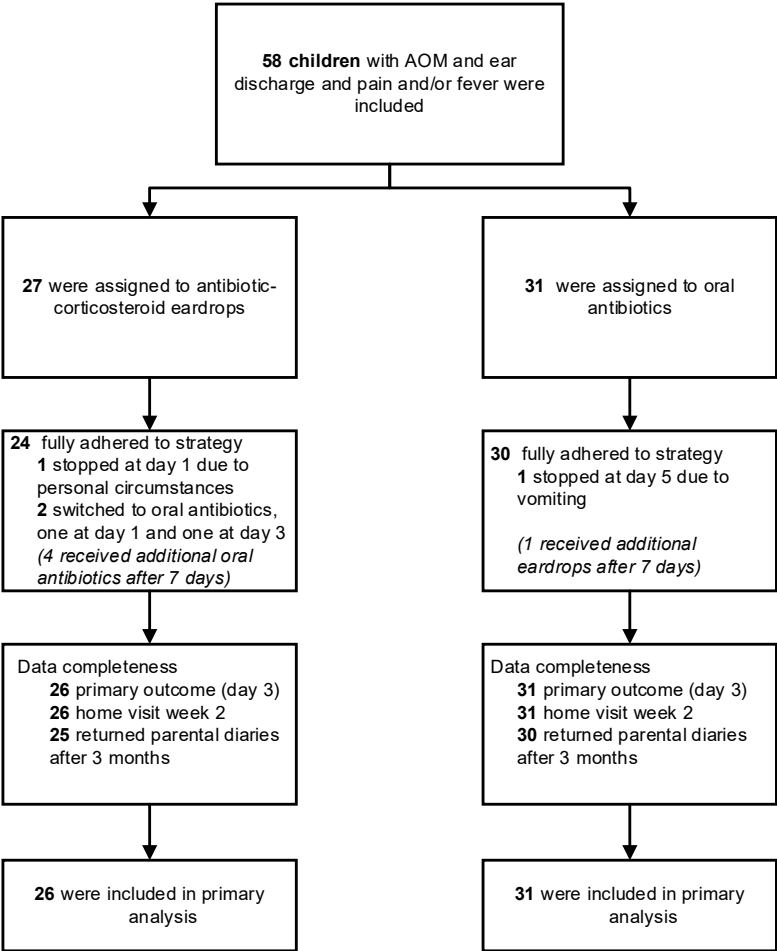


Figure 1. Flowchart

Table 1. Baseline

Characteristic	Antibiotic-corticosteroid eardrops (n=27)	Oral antibiotics (n=31)	All children (n= 58)
<b>Age</b>			
months, median [IQR]	28 [13 to 64]	28 [17 to 52]	28 [15 to 55]
< 2 year, n (%)	11 (40.7)	12 (38.7)	23 (39.7)
Sex, male, n (%)	13 (48.1)	14 (45.2)	27 (46.6)
<b>Medical history, n (%)</b>			
Previous AOMd <sup>a</sup>	14 (51.9)	16 (51.6)	30 (51.7)
Recurrent URTI (>6 in 1 year)	15 (55.6)	17 (54.8)	32 (55.2)
Atopic constitution	9 (33.3)	11 (35.5)	20 (34.5)
<b>Symptoms prior to enrolment</b>			
Duration of AOMd <sup>b</sup> – days, median	2 [1 to 3.5]	3 [1-5]	3 [1 to 4]
<b>Symptoms at baseline</b>			
Ear pain, n (%)	26 (96.3)	29 (93.5)	55 (94.8)
Fever ( $\geq 38^{\circ}\text{C}$ ) <sup>c</sup> , n (%)	8 (29.6)	9 (29.0)	17 (29.8)
Parent/GP reported fever <sup>d</sup> , n (%)	16 (59.3)	17 (54.8)	33 (56.9)
<b>Physical examination, n (%)</b>			
Temperature, $^{\circ}\text{C}$ , mean $\pm$ SD	37.6 $\pm$ 0.84	37.7 $\pm$ 0.95	37.6 $\pm$ 0.89
Parent/GP reported temperature <sup>d</sup> , $^{\circ}\text{C}$ , mean $\pm$ SD	38.1 $\pm$ 0.98	38.1 $\pm$ 0.89	38.1 $\pm$ 0.93
Bilateral AOM	9 (33.3)	9 (29.0)	18 (31.0)
Bilateral AOMd	2 (7.4)	4 (12.9)	6 (10.3)
<b>Risk factors, n (%)</b>			
Pneumococcal vaccination	25 (92.6)	30 (96.8)	55 (94.8)
Household smoking	0 (0)	4 (12.9)	4 (6.9)

<sup>a</sup>Previous AOMd: a previous episode of acute otitis media and ear discharge in the medical history. <sup>b</sup>Duration of AOMd (3 missings; group 1: n = 26; group 2: n=20; total: n=55). <sup>c</sup>One missing baseline temperature group 2 (n=30). <sup>d</sup>Fever/temperature: based on the combination of the baseline visit temperature and the temperature measured by either the GP or the parents within the 24 hours preceding the baseline visit.

## Primary outcome

Of the children assigned to eardrops 42% were free from ear pain and fever at day 3 versus 65% of those assigned to oral antibiotics; adjusted absolute risk difference: 20.3%, 95% CI -5.3% to 41.9% (Table 2). This exceeds the predefined non-inferiority margin of 15%.

Per-protocol analysis showed a crude relative risk of 0.71 (95% CI 0.43 to 1.18) and risk difference of 18.7% (95% CI -7.4 to 44.8). Estimates from sensitivity analysis were similar as all participants scoring 1 on the Likert Scale (n=4) experienced pain at day 3, resulting in the same proportions.

**Table 2. Primary outcome**

Outcomes	Antibiotic-corticosteroid eardrops (n=26)	Oral antibiotics (n=31)	Antibiotic-corticosteroid eardrops vs. oral antibiotics		
	n (%)	n (%)	Relative risk (95% CI)	Risk difference, % (95% CI)	Adjusted <sup>±</sup> risk difference, % (95% CI)
Primary outcome					
Children without ear pain and fever at day 3 - n (%)	11 (42.3)	20 (64.5)	0.66 (0.39 to 1.10)	22.2 (-3.2 to 47.6)	20.31 (-5.3 to 41.9)

<sup>±</sup>Adjusted for: age (<2 versus ≥2 years) and laterality (uni- versus bilateral AOM)

## Secondary outcomes

Results for the secondary outcomes are summarised in Table 3. The proportion of children with mild ear pain at day 3, the proportion of children with otoscopically confirmed ear discharge, eardrum perforation and MEE at 2 weeks were similar in both groups. The mean ear pain score over the first 3 days (Likert scale 0-6) was 2.1 in the eardrops group versus 1.4 in the oral antibiotic group ( $p=0.02$ ).

58% of children assigned to eardrops had parent-reported ear discharge at day 3 versus 19% of those assigned to oral antibiotics ( $p<0.05$ ). Mean duration of parent-reported ear discharge was 6 days in children receiving eardrops compared to 3 days for those receiving oral antibiotics (Kaplan-Meier curves, Figure 2).

Time to resolution of total symptoms was 5 days in the topical antibiotic group versus 4 days in the oral antibiotic group ( $p=0.04$ ). The total number of oral antibiotic courses in three months was 11 for 25 children in the eardrops group versus 33 for 30 children in the oral antibiotic group.

The OM-6 specific quality of life improved during follow-up in both groups (Supplementary Table S1).

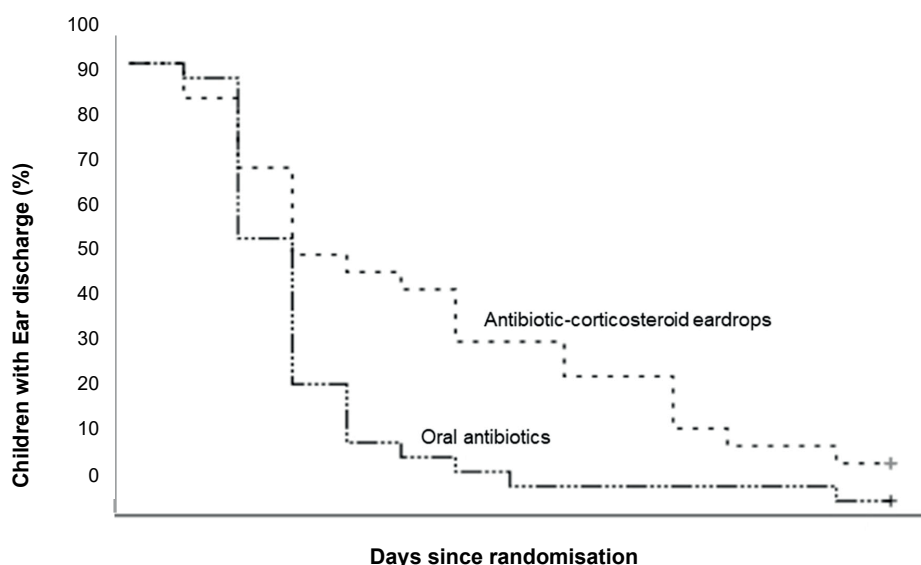
## Adverse events

Sixty-two percent of the children in the eardrops group experienced discomfort during administration of the study medication; this percentage was nineteen in the oral antibiotic group. For gastro-intestinal upset and body rash these percentage were 12% versus 32% and 8% versus 16%, respectively. No serious adverse events, were reported during the three months of follow-up.

**Table 3. Secondary outcomes**

Secondary Outcomes	Antibiotic-corticosteroid eardrops (n=26)	Oral antibiotics (n=31)	Effect size	
Scores			Mean differences (95% CI)	p-value <sup>1</sup>
Mean ear pain score over the first 3 days - (Likert Scale, $\pm$ SD)	2.1 (0.21)	1.4 (0.20)	0.67 (0.13 to 1.21)	0.016
Mean body temperature over the first 3 days - °C ( $\pm$ SD)	37.2 (0.08)	36.9 (0.07)	0.32 (0.13 to 0.51)	0.001
Proportions			Risk Ratios (95% CI)	p-value <sup>2</sup>
Children with at most mild ear pain at day 3 ( <i>Likert Scale</i> <3) - n (%)	22 (84.6)	27 (87.1)	0.97 (0.79 to 1.20)	1.000
Children with parent-reported ear discharge at day 3 - n (%)	15 (57.7)	6 (19.4)	2.98 (1.35 to 6.57)	0.003
Children with otoscopically confirmed ear discharge at 2 weeks - n (%)	1 (4.0)	3 (9.7)	0.41 (0.05 to 3.73)	0.620
Children with MEE <sup>3</sup> at 2 weeks - n (%)	22 (84.6)	26 (83.9)	1.01 (0.81 to 1.26)	1.000
Children with otoscopically confirmed eardrum perforation at 2 weeks - n (%)	2 (7.7)	4 (12.9)	0.60 (0.12 to 3.00)	0.678
Duration of symptoms			Rate Ratios (95% CI)	p-value <sup>4</sup>
No. of days with ear pain during the first 2 weeks ( <i>Likert</i> $\geq$ 1) mean (SD)	6 (3.3)	4 (2.4)	1.42 (1.05-1.93)	0.025
No. of days with fever during the first 2 weeks - mean (SD)	2 (2.4)	1 (1.5)	1.99 (1.06-3.73)	0.032
No. of days with parent-reported ear discharge at day 3 - mean (SD)	3 (1.0)	3 (0.8)	1.18 (0.87-1.59)	0.283
No. of days with parent-reported ear discharge during the first 2 weeks - mean (SD)	6 (3.8)	3 (2.3)	1.63 (1.17-2.27)	0.004
No. of days with parent-reported ear discharge at 3 months - mean (SD)	8 (6.2)	5 (4.6)	1.60 (1.07-2.38)	0.021
Time to resolution of total symptoms - mean (SD) <sup>5</sup>	5 (2.8)	4 (2.2)	1.36 (1.02-1.82)	0.037
Other				
No. of AOM recurrences at 3 months (symptom-free period of $\geq$ 28 days - n <sup>6</sup> )	0	2		
No. of AOM recurrences at 3 months (symptom-free period of $\geq$ 14 days) - n <sup>6</sup>	3	2		

<sup>1</sup>Mean ear pain score and mean body temperature were analysed with a linear regression model, adjusted for: age (<2 versus  $\geq$ 2 years) and laterality (uni- versus bilateral AOM); <sup>2</sup>Between-group differences in proportions were tested with Chi-Square tests and risk ratios were calculated; <sup>3</sup>MEE: middle ear effusion; <sup>4</sup>Between-group differences (means) were tested using negative binomial regression analyses; <sup>5</sup> Time to resolution of total symptoms (time to all of pain, fever, discharge, being unwell, sleep disturbance, and distress/crying being rated 0 or 1 on the Likert scale; <sup>6</sup>AOM recurrences has been defined as the occurrence of new AOM related symptoms after a symptom-free period of either 28 days or 14 days



No. of Children

#### Antibiotic-corticosteroid eardrops

No. of Children

##### Antibiotic-corticosteroid eardrops

Ear discharge	26	24	20	15	14	13	10	10	8	8	5	4	4	3	3
No Ear discharge	0	2	6	11	12	13	16	16	18	18	21	22	22	23	23

##### Oral antibiotics

Ear discharge	31	30	19	9	5	4	3	2	2	2	2	2	2	2	1
No Ear discharge	0	1	12	22	26	27	28	29	29	29	29	29	29	29	30

**Figure 2. Kaplan-Meier Curver for the duration of ear discharge after randomization as reported by parents in a diary. Log Rank: 5.811,  $p = 0.016$**

**Table 4. Treatment-related and serious adverse events**

	Antibiotic-corticosteroid eardrops (n=26)	Oral antibiotics (n=31)
Adverse event within 2 weeks	n (%)	n (%)
Local discomfort during administration	16 (61.5)	6 (19.4)
Difficulties during administration	15 (57.7)	3 (9.7)
Gastrointestinal discomfort	3 (11.5)	10 (32.3)
Rash - local (ear)	5 (19.2)	5 (16.1)
Rash - body	2 (7.7)	5 (16.1)
Dizziness	1 (3.8)	0 (0)
Serious adverse events	0 (0)	0 (0)



## Discussion

### Summary

Due to early termination of the trial non-inferiority of antibiotic-corticosteroid ear-drops to oral antibiotics could not be determined in children with AOMd. In our small group of 58 children, we found that those assigned to eardrops had lower resolution rates of ear pain and fever at 3 days, longer parent-reported ear discharge, and slightly higher mean ear pain scores over days 1-3 compared to those receiving oral antibiotics, but they received fewer oral antibiotic courses in three months and had less GI upset and rash.

### Strengths and limitations

This is the first report on the comparative effectiveness evidence of antibiotic-corticosteroid eardrops versus oral antibiotic treatment in children with AOM presenting with ear discharge. The pragmatic design of the trial and high rate of data-completeness support applicability of its findings to routine daily practice.

Some limitations deserve further attention. Accrual to our trial was affected by a temporary closure due to study medication supply issues. When this was resolved the COVID-19 pandemic complicated trial recruitment and accrual did not recover after the pandemic restrictions were lifted. This phenomenon has affected many trials world-wide<sup>31</sup>. Further, our non-blinded design, could potentially have introduced detection bias. However, detection bias is unlikely to have significantly impacted our findings since we compared two active treatments and – based on our parent panel input – parents do not have strong preferences for one over the other treatment. Also, a double dummy design would have hampered the applicability of trial results to everyday practice.

We chose hydrocortisone-bacitracin-colistin eardrops because they are widely used in the Netherlands and France, do not contain a potentially ototoxic aminoglycoside, cover the most important pathogens involved in AOM and have been proven effective in children with ventilation tubes who present with acute ear discharge.<sup>6</sup> They are however not available in many countries. Despite absence of evidence, we believe that any combination of antibiotic-corticosteroid eardrops with a similar antimicrobial profile, like a quinolone-containing eardrops plus dexamethasone, would have yielded comparable results.

### Comparison with existing literature

We initiated this trial after establishing superiority of antibiotic-corticosteroid ear-drops over oral antibiotics in children with ventilation tubes who present with acute ear discharge.<sup>6</sup> Our current findings in a small sample of children without ventilation

tubes who present with AOMd indicate that these findings cannot be extrapolated to this patient population. While there is a patent passage between ear canal and the middle ear in children with ventilation tubes, the spontaneous eardrum perforation in children with AOMd may close too early to allow antibiotic-corticosteroid eardrops to completely resolve the middle ear inflammation.

During the preparation of our trial we collaborated with the UK based team developing the REST (Runny Ear Study, trial registry number ISRCTN12873692) addressing the same topic.<sup>19</sup> We harmonised design and outcomes to enable future meta-analysis. This trial however was also terminated early due to issues with its electronic health record system platform and no formal statistical analysis was performed on its sample of 22 children.<sup>19</sup>

### **Implications for research and practice**

We were unable to determine non-inferiority of antibiotic-corticosteroid eardrops to oral antibiotics, but our findings in a small group of children, requiring confirmation, suggest that that oral antibiotics may be more effective in resolving symptoms and shortening the duration of ear discharge than antibiotic-corticosteroid eardrops in children with AOMd. That must be balanced against the findings that eardrops are associated with reasonable symptom control, fewer total oral antibiotic courses and less systemic side effects in case there is non-inferiority. Since we were unable to demonstrate non-inferiority of antibiotic-corticosteroid eardrops to oral antibiotics in children with AOMd, current guidelines' recommendation that clinicians can consider oral antibiotics in this group of children are not unreasonable, but must be balanced against the major public health threat of antibiotic resistance.

Supplementary Table 1. Disease specific quality of life assessed with the otitis media-6 questionnaire at baseline, at 2 weeks and 3 months follow up

Questionnaire	Baseline (n=57)			Week 2 (n=56)			Month 3 (n=48)			Change score*		Difference in change score±		Change score *		Difference in change score ±	
	Range of scores <sup>o</sup>	Eardrops mean (SD)	Oral Abx mean (SD)	Eardrops mean (SD)	Oral Abx mean (SD)	Eardrops mean (SD)	Oral Abx mean (SD)	Eardrops mean (SD)	Oral Abx mean (SD)	Δ T0-T2 (SD)	Eardrops Δ T0-T2 (SD)	Eardrops vs oral Abx Δ T0-T2 (p)	Eardrops Δ T2-T3 (SD)	Oral Abx Δ T2-T3 (SD)	Eardrops vs oral Abx Δ T2-T3 (p)	Eardrops vs oral Abx Δ T2-T3 (p)	
Physical suffering	1 to 7	5.2 (1.68)	5.9 (1.26)	5.1 (1.68)	4.6 (1.63)	1.9 (1.29)	2.2 (1.44)	0.1 (1.68)	1.3 (1.39)	-1.19 (p=0.006)	3.4 (1.66)	2.6 (2.39)	0.76 (p =0.207)				
Hearing loss	1 to 7	3.2 (1.83)	3.0 (2.05)	3.1 (1.90)	3.7 (1.8)	2.0 (1.40)	2.1 (1.37)	0.0 (1.71)	-0.7 (1.69)	0.71 (p = 0.127)	1.1 (1.52)	1.7 (1.69)	-0.57 (p = 0.234)				
Speech impairment	1 to 7	2.1 (1.23)	1.8 (1.20)	2.2 (1.41)	2.0 (1.13)	1.7 (1.34)	1.6 (0.69)	-0.2 (0.82)	-0.2 (1.43)	0.07 (p = 0.831)	0.5 (1.07)	0.6 (0.97)	-0.08 (p = 0.793)				
Emotional distress	1 to 7	4.8 (1.77)	5.4 (1.23)	4.3 (1.67)	4.3 (1.3)	1.9 (0.97)	1.9 (1.03)	0.5 (1.6)	1.1 (1.14)	-0.63 (p=0.097)	2.4 (1.64)	2.4 (1.72)	-0.01 (p = 0.988)				
Activity limitations	1 to 7	4.6 (1.79)	4.7 (1.42)	4.1 (1.62)	3.9 (1.3)	1.9 (1.09)	1.9 (0.93)	0.5 (1.42)	0.8 (1.42)	-0.33 (p=0.385)	2.3 (1.69)	2.2 (1.73)	0.15 (p = 0.764)				
Caregivers concerns	1 to 7	4.7 (1.42)	4.7 (1.37)	4.1 (1.3)	3.8 (1.4)	2.1 (1.45)	2.1 (1.20)	0.4 (1.29)	0.9 (1.54)	-0.55 (p = 0.149)	2.1 (1.59)	1.8 (1.92)	0.29 (p = 0.581)				
Mean symptom score	1 to 7	4.1 (1.24)	4.3 (0.95)	3.9 (1.33)	3.7 (1.11)	1.9 (1.11)	2.0 (0.99)	0.19 (0.98)	0.54 (0.91)	-0.36 (p = 0.164)	2.0 (1.22)	1.9 (1.47)	0.11 (p= 0.773)				
Total score	6 to 42	24.3 (7.43)	25.5 (5.68)	22.8 (7.54)	22.2 (6.67)	11.4 (6.68)	11.7 (5.96)	1.5 (5.50)	3.3 (5.49)	-1.73 (p = 0.246)	11.6 (7.63)	11.2 (8.80)	0.42 (p = 0.864)				
Visual analog score	0 to 10	4.4 (2.21)	4.4 (1.89)	4.8 (1.55)	5.1 (1.92)	7.8 (2.09)	7.8 (2.09)	0.4 (2.32)	0.7 (1.92)	-0.32 (p = 0.585)	3.0 (2.56)	3.8 (2.23)	-0.78 (p = 0.283)				

Oral Abx; oral antibiotics; <sup>o</sup>1-7: higher scores indicating more of a problem \*Change score between time points, a positive value indicates clinical improvement; a negative value, deterioration. A score < 0.5 indicates trivial change; 0.5 - 0.9 small change, 1.0 - 1.4 moderate change; and > 1.5 large change; ± using independent t-test

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CHAPTER 5

5

# **Impact of topical and systemic antibiotics on gut microbiota composition and antimicrobial resistance genes in children with acute otitis media and ear discharge**

Based on:

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# Abstract

## Introduction

Despite a surge in research into the impact of antimicrobial treatment on the microbiome, little is known about the magnitude and duration of the impact of antibiotic exposures to off-target microbes and antibiotic resistance genes (ARGs). Nested in a trial of antibiotic treatments for children with acute otitis media and ear discharge (AOMd), we evaluated the impact of topical and systemic antibiotics on gut microbiota composition and diversity and abundance of ARGs in their faeces.

## Methods

Faecal samples were collected from 51 patients prior to topical treatment with hydrocortisone-bacitracin-colistin eardrops or systemic treatment with amoxicillin suspension for 7 days, and at 2 weeks and 3 months after treatment. The total DNA extracted from the faecal samples was subjected to shotgun metagenomic sequencing. The gut microbiota and resistomes were characterised using read-based mapping approaches. Alpha and beta diversity measures were used to identify differences between the samples and multivariable association analyses to identify differentially abundant species across the samples.

## Results

At the phylum level, the gut microbiomes of children with AOMd were similar in the topical and systemic antibiotic groups at all timepoints. At the species level, the alpha and beta-diversity were also similar in both groups at all time points (Wilcoxon,  $P > 0.05$  and PERMANOVA  $P > 0.05$ , respectively). Within each treatment group over time, the alpha diversity of samples remained the same (Wilcoxon,  $P > 0.05$ ). The median ARG abundance were similar in the antibiotic treatment groups at all time points and within each treatment groups over time.

## Conclusion

Nested in a trial of topical and systemic antibiotics for children with AOMd, both antibiotic treatments had a similar impact on the gut microbiota composition at the phylum level, the alpha and beta diversity at the species level and the faecal ARG abundance. Future trials of antibiotics should routinely collect microbiological samples to further investigate their off-target impact to ultimately reveal which antibiotic treatment minimizes the disruption of the human microbiota diversity and spread of ARGs.



## Introduction

The gut is the largest reservoir of bacteria in the human body and plays an important role in health and disease.<sup>1,2</sup> The human gut microbiota comprises a complex microbial ecosystem including hundreds of species. The high density of species in this ecosystem facilitates horizontal transfer of antimicrobial resistance genes (ARGs) to pathogens.<sup>3</sup> Albeit dynamic during the first three years of life, the composition is relatively stable in healthy adults,<sup>4,5</sup> though it can quickly change due to diet, age, environment, and medication use including antibiotics.<sup>6,7</sup> Antibiotic use has been associated with alterations of the gut microbiota by reducing the microbiota diversity and stimulating the spread of ARGs.<sup>5,8,9</sup> In turn, microbial dysbiosis has been associated with negative health outcomes<sup>10</sup> such as the increased risk of the development of metabolic disease and celiac disease,<sup>11,12</sup> whereas the emergence of antimicrobial resistance poses a serious threat to public health worldwide.<sup>13</sup>

The majority of antibiotic prescriptions in humans are issued in primary care and acute otitis media (AOM) is among the prime reasons for antibiotic use in children.<sup>14,15</sup> Despite a surge in research into the impact of antimicrobial treatment on the microbiome,<sup>10,16,17</sup> little is known about the magnitude and duration of the impact of antibiotic exposures to off-target microbes (i.e., bacteria not targeted by treatment) and ARGs.<sup>10</sup> Better understanding of these mechanisms can be used to inform evidence-based treatment decisions by balancing the pros and cons of the various treatments. Nested in a recent randomized controlled trial (RCT) of topical and systemic (oral) antibiotics for children with AOM and ear discharge due to a spontaneous perforation of the eardrum (AOMd),<sup>18</sup> we collected faecal samples of participating children at baseline (prior to treatment), and at 2 weeks and 3 months follow-up. The aim of this study is to evaluate and compare the impact of topical and systemic antibiotics on the gut microbiota composition and the diversity and abundance of ARGs in faeces of these children.

## Methods

### Study design

The current study is nested in a recent RCT of topical and systemic antibiotics for children with AOMd. Details of the trial design have been reported elsewhere.<sup>18</sup> In short, children aged 6 months to 12 years presenting to their general practitioner (GP) with AOM and either ear pain or fever or both and acute-onset of otoscopically confirmed ear discharge in one or both ears were eligible for trial participation. Children were randomly allocated to either topical treatment with antibiotic-corticosteroid eardrops (hydrocortisone-bacitracin-colistin, Daleco Pharma B.V., five drops three times daily)

or systemic treatment with antibiotics (amoxicillin suspension, Sandoz B.V., 50mg/kg body weight per day, divided over three doses) for 7 days. Children who received antibiotics during the previous two weeks were excluded.<sup>18</sup>

### **Sample collection**

At baseline (prior to treatment), and at 2 weeks and 3 months follow-up, faecal samples of children participating in the trial were collected using the OMNIgene GUT (OMR-200) system (DNA Genotek, Ottawa, Canada). This system allows for easy self-collecting at home and transportation and storage of faeces at ambient temperature for up to 60 days.<sup>19,20</sup> Samples were transported by either the study team (if the faeces was available during the home visit) or by mail to the microbiology laboratory of the UMC Utrecht (timeframe baseline and faeces collection lab: median 2 days, IQR (1-5 days)) and then stored at -80°C until further analysis.

### **DNA isolation and sequencing**

For DNA isolation, an aliquot of ~50 mg of faecal matter was defrosted, and DNA was isolated using the Agowa Mag DNA extraction kit (LGC genomics, Berlin, Germany). Libraries were prepared according to the DNA Nano library preparation protocol by Illumina, starting by shearing 100ng of gDNA with the Covaris S2 system using the 350bp settings stated in the Illumina protocol. After the library preparation libraries were checked with the Fragment Analyzer system dsDNA 910 Reagent Kit (35-1500bp) (Cat. DNF-910-K1000) and with Qubit dsDNA HS Assay Kit (Cat. Q32854). Libraries with a major peak of 350 bp and a DNA concentration of 4 ng/ul were taken forward for sequencing. Sample libraries were pooled in equimolar amounts and sequenced on a Novaseq 6000 (Illumina) with a S2 flowcell using the 150bp paired-end protocol.

### **Analysis of sequence data**

#### ***QC and human read removal***

Sequencing adapter removal and quality trimming was performed with fastp (v.0.23.2) using the default parameters.<sup>21</sup> The quality trimmed reads were then mapped against the human genome assembly (GRCh38: GCA\_000001405.15) with bowtie 2 (v.2.4.1)<sup>22</sup> and SAMtools (v.1.5)<sup>23</sup> and BEDTools (v.2.26.0)<sup>24</sup> were used to retain the reads that did not map to the human genome assembly.

#### ***Community and antibiotic resistance gene profiling***

Community profiling was performed using a read mapping-based approach with MetaPhlAn4 (v.4.0.6)<sup>25</sup> employing the ChocoPhlAn database (v. vOct22\_CHOCOPhlAnSGB\_202212). Antibiotic resistance gene profiling was performed using ShortBRED (v.0.9.4).<sup>26</sup> Unique gene markers were identified using ShortBRED-Identify with the

ResFinder antibiotic resistance gene database<sup>27</sup> and the reference database Uniref90<sup>28</sup>. ShortBRED-Quantify was then used to map the metagenomic reads to the markers and obtain the relative abundance of each gene (RPKM: reads per kilobase of reference sequence per million sample reads).

### ***Statistical analysis***

All statistical analyses were performed using R (v.4.2.1) (R Core Team (2022)). Primary analyses were performed according to the intention-to-treat principle, including all randomised children from which faecal samples were collected. In sensitivity analyses, we excluded all samples of participants who received any additional (systemic or topical) antibiotic treatment during the 3 months follow-up period from the analyses.

The within sample (Alpha) diversity of the community and ARG abundances was measured using the Shannon diversity index implemented in the diversity function of the R package vegan (v.2.6-4).<sup>29</sup> The metaMDS function of vegan was used to generate Bray-Curtis dissimilarity matrices and perform Non-metric Multidimensional Scaling (NMDS) analyses on the matrices to measure beta diversity. Differences between the treatment groups were determined using permutational analysis of variance (PERMANOVA) analyses implemented in the adonis function of vegan with 10,000 permutations. Multivariable association analyses were performed using MaAsLin2 (v.1.12.0)<sup>30</sup> to identify bacteria and ARGs that were significantly associated with either of the treatment groups. Age and sex were included as fixed effects and sample ID as a random effect in the model.

### ***Data availability***

Raw sequencing reads will be deposited in the European Nucleotide Archive.

### ***Code availability***

All code used in this manuscript can be found at <https://github.com/rosssmcinnnes/>.

## **Results**

### **Study participants**

The trial was stopped early due to slow accrual with 58 of a planned 350 children included; of these 27 were assigned to topical treatment with antibiotic-corticosteroid eardrops and 31 to systemic treatment with antibiotics. Faecal samples were collected from 51 of the 58 (88%) included children; 24 (89%) assigned to topical antibiotics and 27 (87%) to systemic antibiotics. The distribution of faecal samples at the different time points according to study group assignment are summarized in Table 1.

**Table 1. Faeces collection in the topical eardrop and oral antibiotic group at each time point including a distinction between all children (A) and filtered data (B), excluding children who received additional antibiotic treatment during the follow-up.**

Timepoint	All children			Filtered data		
	T0	T2	T3	T0	T2	T3
Topical antibiotics (n)	23	22	21	23	14	11
Systemic antibiotics (n)	25	27	19	25	24	15
Total (n)	48	49	39	48	38	26

Baseline characteristics of study participants were well-balanced between the groups (Table 2). In 10 children who were assigned to topical antibiotics one or more additional (systemic or topical) antibiotic treatment was prescribed during follow-up versus in 5 children assigned to systemic antibiotics (Supplementary table 1).

**Table 2. Baseline all children**

Characteristic	Antibiotic-corticosteroid eardrops (n=24)	Systemic antibiotics (n=27)	All children (n= 51)
<u>Age</u>			
months, median [IQR]	27 [12 to 53]	27 [17 to 44]	27 [13 to 44]
< 2 year, n (%)	11 (45.8)	11 (40.7)	22 (43.1)
Sex, male, n (%)	10 (41.7)	14 (51.9)	24 (47.1)
<u>Medical history, n (%)</u>			
Previous AOMd <sup>a</sup>	13 (54.2)	14 (51.9)	27 (52.9)
Recurrent URTI (>6 in 1 year)	14 (58.3)	15 (55.6)	29 (56.9)
Atopic constitution	8 (33.3)	10 (37.0)	18 (35.3)
<u>Symptoms prior to enrolment</u>			
Duration of AOMd <sup>b</sup> – days, median	2 [1 to 3]	3 [1-5]	3 [1 to 4]
<u>Symptoms at baseline</u>			
Ear pain, n (%)	23 (95.8)	25 (92.6)	48 (94.1)
Fever ( $\geq 38^{\circ}\text{C}$ ) <sup>c</sup> , n (%)	7 (29.2)	8 (29.6)	15 (29.4)
Parent/GP reported fever <sup>d</sup> , n (%)	14 (58.3)	14 (51.9)	28 (54.9)
<u>Physical examination, n (%)</u>			
Temperature, $^{\circ}\text{C}$ , mean $\pm$ SD	37.6 $\pm$ 0.79	37.6 $\pm$ 0.97	37.6 $\pm$ 0.89
Parent/GP reported temperature <sup>d</sup> , $^{\circ}\text{C}$ , mean $\pm$ SD	38.4 $\pm$ 1.02	38.3 $\pm$ 0.58	38.3 $\pm$ 0.83
Bilateral AOM	8 (33.3)	6 (22.2)	14 (27.5)
Bilateral AOMd	2 (8.3)	1 (3.7)	3 (5.9)
<u>Risk factors, n (%)</u>			
Pneumococcal vaccination	23 (95.8)	26 (96.3)	49 (96.1)
Household smoking	0 (0)	4 (14.8)	4 (7.8)

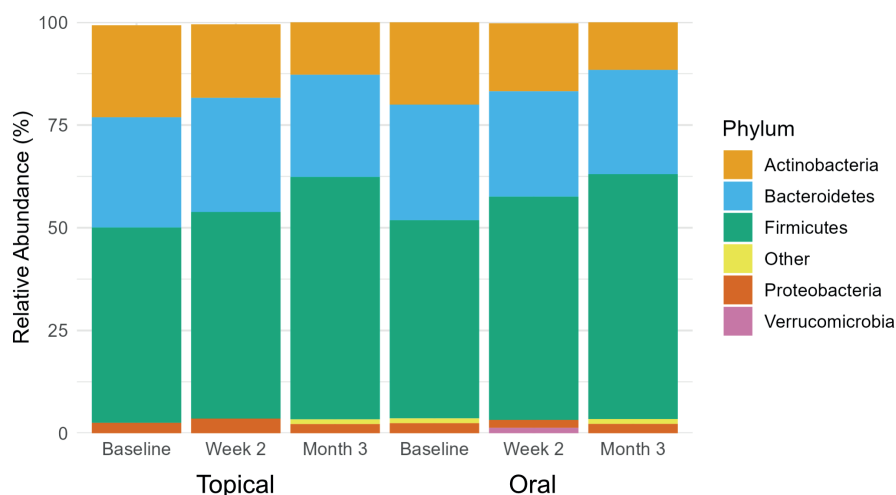
<sup>a</sup>Previous AOMd: a previous episode of acute otitis media and ear discharge in the medical history.

<sup>b</sup>Duration of AOMd (3 missings; group 1: n = 23; group 2: n=25; total: n=48). <sup>c</sup>One missing baseline temperature group 2 (n=26). <sup>d</sup>Fever/temperature: based on the combination of the baseline visit temperature and the temperature measured by either the GP or the parents within the 24 hours preceding the baseline visit.

## Impact of antibiotic treatments on gut microbiome and resistome

### *Gut microbiome community structure*

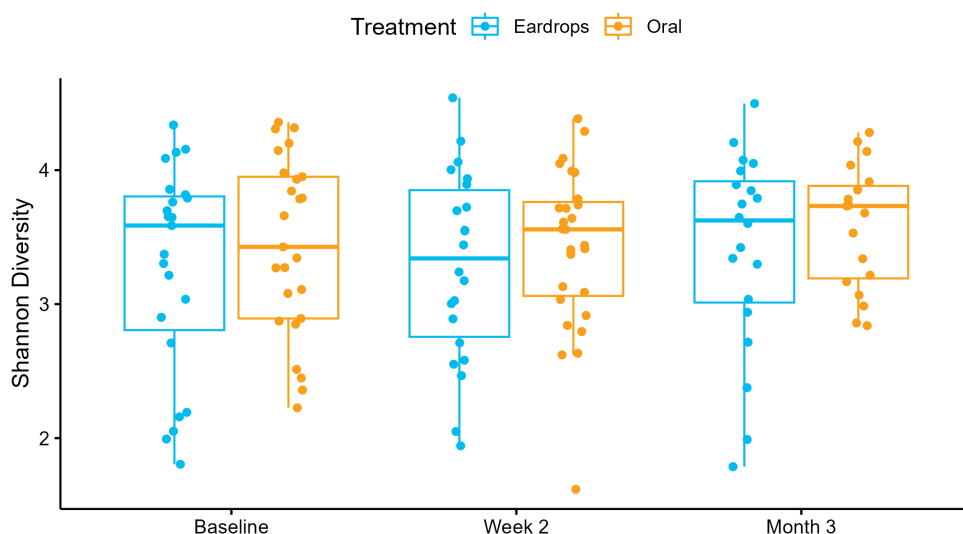
At the phylum level, the gut microbiomes of children with AOMd were similar in the topical and systemic antibiotic groups at all timepoints (Figure 1). Five different phyla had a relative abundance of greater than 1% in at least one of the time points. The most abundant phylum in both treatment groups was Firmicutes comprising a mean 52.0% (standard deviation (SD): 17.3) of the topical antibiotic group versus 53.6% (SD: 14.4) of the systemic antibiotic group. Bacteroidetes had a mean abundance of 26.6% (SD: 13.2) versus 26.5% (SD: 14.2) whereas these numbers were 17.9% (SD: 20.2) versus 16.4% (SD: 17.0) for Actinobacteria, respectively. Proteobacteria and Verrucomicrobia were found at a much lower abundance within the samples, with a mean abundance of 2.8% (SD: 5.5) versus 2.2% (SD: 2.8) and 0.5% (SD: 1.2) versus 1.0% (SD: 2.8), respectively. The abundances of the different phyla were dynamic during the 3 months follow-up period; the level of Actinobacteria decreased by around half in both treatment groups: from 22.4% (SD: 25.3) to 12.8% (SD: 13.8) and 20.0% (SD: 20.1) to 11.6% (SD: 11.4) in the topical and systemic antibiotics groups, respectively. Conversely, we observed a stepwise increase in the mean relative abundance of Firmicutes over time, from 47.5% (SD: 16.4) to 59.0% (SD: 15.6) and 48.2% (SD: 14.1) to 59.6% (SD: 11.8) in both groups, respectively. The level of Bacteroides was relatively stable in both groups, from 26.8% (SD: 13.4) to 24.9% (SD: 12.9), and 28.1% (SD: 16.7) to 25.4% (SD: 12.6), respectively.



**Figure 1. Relative abundance of Phyla within the gut microbiome of children undergoing treatment for acute otitis media.**

Phylum abundances were measured at three different timepoints; baseline, two weeks post treatment and 3 months post treatment for both treatment arms (Eardrops and Oral). The data presented is the mean abundance of the samples at each time point. Phyla with a mean relative abundance less than 1% were grouped into the “Other” category.

The alpha-diversity of the samples are presented in Figure 2. At the species level, the sample diversity were similar in both study groups at baseline, 2 weeks or 3 months following treatment (Wilcoxon,  $P > 0.05$ ). Moreover, the diversity of the samples within each treatment group was the same over time (Wilcoxon,  $P > 0.05$ ).

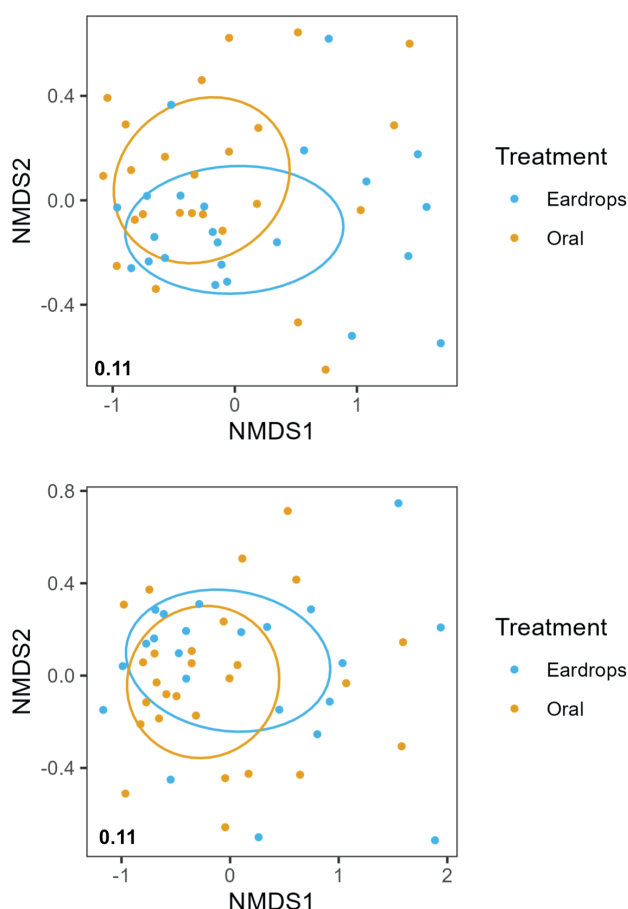


**Figure 2. Alpha-diversity of species present within the gut microbiome of children undergoing treatment for acute otitis media.**

Gut microbiome samples were collected from both treatment arms (Eardrops and Oral) before treatment, two weeks post treatment and 3 months post treatment. The alpha diversity was measured using the Shannon diversity index. The box plot represents the lower, middle, and upper quartiles of these data. Significance was determined using the Wilcoxon test.

The beta-diversity diversity, visualised using non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity matrices generated from species-level markers, was similar in both antibiotic treatment groups at baseline (PERMANOVA,  $P > 0.05$ ) (Figure 3A), and at 2 weeks (Figure 3B) and 3 months follow-up (PERMANOVA  $P > 0.05$ ). Although the beta-diversity observed in samples collected from males or females were similar at the various time points, the beta-diversity of samples from different age groups did significantly differ at baseline, and at 2 weeks and 3 months follow-up (PERMANOVA,  $P < 0.05$ ).

We did not identify any species that were significantly over- or underrepresented in any of the antibiotic treatment groups ( $P > 0.05$ ).



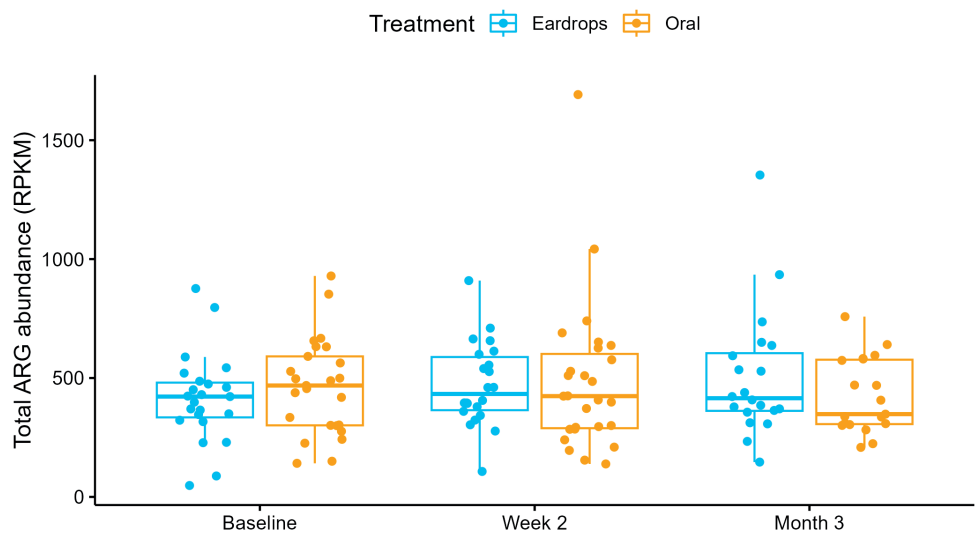
**Figure 3.** Beta-diversity of species present within the gut microbiome of children undergoing treatment for acute otitis media.

Non-metric multidimensional scaling was performed on Bray-Curtis dissimilarity matrices generated from species-level relative abundances. A. Samples prior to treatment. B. Samples two weeks post treatment. Data was stratified by treatment type (Eardrops or Oral). 50% confidence interval ellipses are indicated. Stress is indicated within the plots. Significance was measured by PERMANOVA with 10,000 permutations.

### *The gut resistome*

The abundance of the ARGs in the gut microbiome samples according to study group assignment is illustrated in Figure 4. The median ARG abundance at baseline was 421.9 RPKM (IQR: 334.4 to 480.6) in the topical antibiotics group and 468.5 RPKM (IQR: 300.9 to 590.9) in the systemic antibiotics group. At 2 weeks, the median ARG abundance were 433.0 RPKM (IQR: 364.7 to 588.3) and 423.8 RPKM (IQR: 288.9 to 601.3) in the topical and systemic antibiotics groups, respectively. At 3 months, the median abundance were 415.0 RPKM (IQR: 361.8 to 604.5) and 348.0 RPKM (IQR:

306.1 to 577.0), respectively. The median ARG abundance was similar in both antibiotic treatment groups at all time points and within the treatment groups over time.



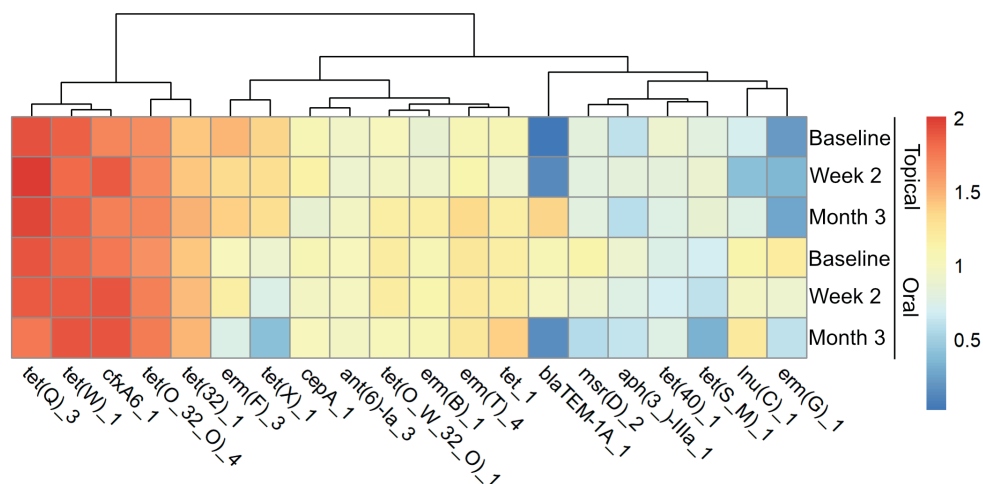
**Figure 4. Total antibiotic resistance gene abundance within the gut microbiome of children undergoing treatment for acute otitis media.**

The summed abundance RPKM (reads per kilobase of reference sequence per million sample reads) of antibiotic resistance genes found in gut microbiome samples collected before treatment, two weeks, and three months post treatment. Samples have been grouped by treatment type, Eardrops or Oral. The box plot represents the lower, middle, and upper quartiles of these data. Significance was determined using the Wilcoxon test.

A total of 117 ARGs were identified across all gut microbiome samples. The most abundant ARG found in any of the samples was *tet(Q)\_3*. Similarly, the tetracycline resistance genes were the most abundant class of ARGs, with 9 out of the 20 most abundant genes belonging to this class (Figure 5). Other clinically important ARGs included the beta-lactamase gene *bla<sub>TEM</sub>*, and the aminoglycoside resistance genes *ant(6)-Ia* and *aph(3\_-IIIa)*. The most abundant ARGs also contained four different erythromycin genes highlighting their prevalence and abundance in the gut microbiome. None of the ARGs were significantly over- or underrepresented in any of the antibiotic treatment groups.

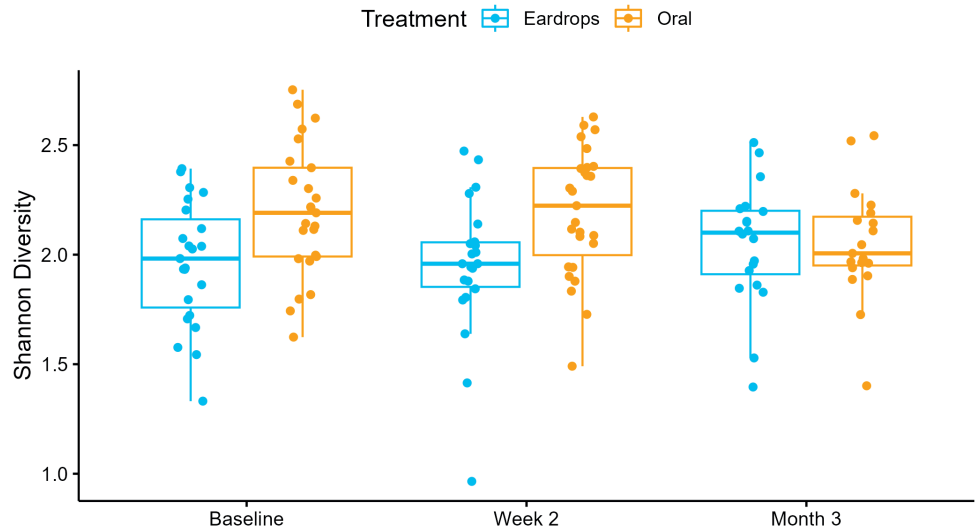
The Shannon diversity of the samples measured by the relative abundance of ARGs is illustrated in Figure 6. The alpha-diversity of the ARGs was similar in both treatment groups at all time points and within the treatment groups over time (Wilcoxon,  $P > 0.05$ ).





**Figure 5.** The 20 most abundant antibiotic resistance genes present in the gut microbiome samples of children undergoing treatment for acute otitis media.

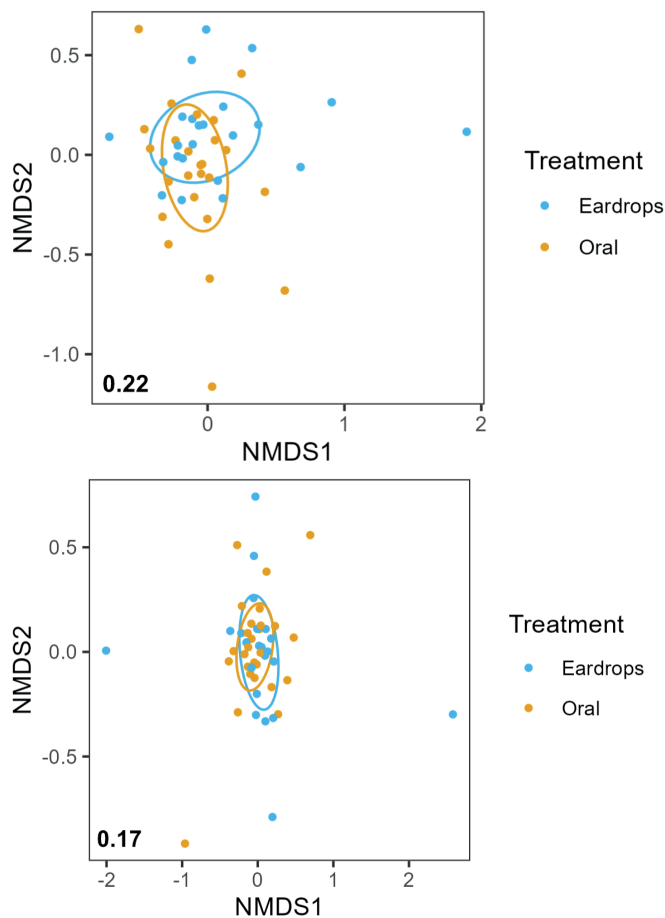
The log10 + 1 transformed mean abundance RPKM (reads per kilobase of reference sequence per million sample reads) of the 20 most abundant antibiotic resistance genes (ARGs) found in the gut microbiome samples. ARGs are clustered based on Euclidean distances.



**Figure 6.** Alpha-diversity of antibiotic resistance genes present within the gut microbiome of children undergoing treatment for acute otitis media.

Gut microbiome samples were collected from both treatment arms (Eardrops and Oral) before treatment, two weeks post treatment and 3 months post treatment. The alpha diversity was measured using the Shannon diversity index. The box plot represents the lower, middle, and upper quartiles of these data. Significance was determined using the Wilcoxon test.

The beta-diversity of the gut microbiome samples was visualised using non-metric multidimensional scaling (NMDS) on Bray-Curtis dissimilarity matrices generated from ARG abundances. The beta-diversity was similar in the antibiotic treatment groups at baseline and 2 weeks (Figure 7AB) and at 3 months (PERMANOVA,  $P > 0.05$ ). The beta-diversity observed in samples collected from males or females were similar at all time points (PERMANOVA,  $P > 0.05$ ). Although there was no impact on the beta-diversity of the samples at the baseline and 2 weeks follow-up, the age of the patient did affect the beta-diversity of the samples at 3 months follow-up (PERMANOVA,  $P < 0.05$ ).



**Figure 7. Beta-diversity of antibiotic resistance genes present within the gut microbiome of children undergoing treatment for acute otitis media.**

Non-metric multidimensional scaling was performed on Bray-Curtis dissimilarity matrices generated from antibiotic resistance gene abundances. A. Samples prior to treatment. B. Samples two weeks post treatment. Data was stratified by treatment type (Eardrops or Oral). 50% confidence interval ellipses are indicated. Stress is indicated within the plots. Significance was measured by PERMANOVA with 10,000 permutations.

### ***Sensitivity analysis***

Findings from the sensitivity analyses were comparable to those of the primary analyses and can be found in the Supplementary file.

## **Discussion**

### **Main findings**

This study nested in a trial of topical or systemic antibiotics for children with AOMd showed that both antibiotic treatments had a similar impact on the gut microbiota composition at the phylum level, the alpha and beta diversity at the species level and the faecal ARG abundance.

### **Comparison with existing literature**

In line with previous literature on gut microbiota composition of healthy humans, Firmicutes and Bacteroides were the most abundant phyla in the gut microbiome of our study population, followed by Actinobacteria and Proteobacteria.<sup>1,31</sup>

Comparative studies evaluating the impact of topical and systemic antibiotics on the gut microbiome are lacking, except from one small animal study (n=9).<sup>32</sup> We found no significant differences in sample diversity at species level and median ARG abundance between and within the antibiotic treatment groups over time. Recent systematic reviews about the impact of antimicrobial treatment on the gut microbiota composition suggest that systemic antibiotics may have a profound influence for some time, but observed effects vary substantially across included studies, findings are inconsistent across different studies investigating the same antibiotic<sup>16,17,33</sup> and observed effects vary substantially across included studies and only few studies included children. The key factors affecting study findings are the study design, the timing and method of sampling, use of different antibiotic classes and treatment duration, and prior exposure to antibiotics.

A review of 78 studies assessing the impact of various systemic antibiotics on the diversity and composition of the human microbiota (including 52 gut microbiota studies) using 16S rRNA gene sequencing found that 88.5% (69/78) did report changes in alpha diversity after antibiotic exposure<sup>16</sup>, which contrasts with our findings. However, the observed effect of beta-lactam antibiotics on the alpha diversity was inconsistent across studies, while only 3 of the 7 studies reported a difference in beta diversity. Many of the affected taxa did remain altered for a prolonged period of time. Ten studies included in another review investigated the impact of amoxicillin on gut microbiome.<sup>17</sup> Amoxicillin use was associated with an increase/overgrowth of Enterobacteriaceae, while changes in

the composition of anaerobic population varied greatly across studies, and microbiota composition returned to normal within two to four weeks in most of the studies. Only one study<sup>34</sup> included in the systematic review of Zimmerman et al.,<sup>33</sup> used shotgun metagenomic sequencing to investigate the effect of different systemic antibiotics (amoxicillin, ciprofloxacin, clindamycin, and minocycline) on the gut microbiota. In line with our study, no change in alpha-diversity (Shannon diversity index) was found after amoxicillin exposure. Compared to the other antibiotics, exposure to oral amoxicillin had the least noticeable impact on the gut microbiota composition. However, amoxicillin had the largest impact on the faecal ARG abundance a week after exposure to treatment.<sup>34</sup> The longer-term impact of amoxicillin on faecal ARG abundance was, however, not described in this study.

### **Strengths and limitations**

This is the first comparative study to evaluate the off-target effects of topical and systemic antibiotics, thereby providing unique data about any differential impact of these antibiotic regimens on the gut microbiota composition and the diversity and abundance of ARGs in faeces of children with AOMd. Another strength of this study is the use of shotgun metagenomic sequencing, which provide a more comprehensive and less biased view than traditional culture-based techniques. The pragmatic design of this trial strengthens the external validity of the findings. Some limitations deserve attention. First, our trial was prematurely ended with 58 children instead of the planned 350 children included due to slow accrual. However, we were able to collect faecal samples in most of these children (n=51). Only 4 out of 52 studies included in a recent systematic review about the impact of systemic antibiotics on the gut microbiota composition had a larger sample size.<sup>16</sup> Second, while children who were exposed to antibiotic treatment within two weeks prior to randomisation were excluded from trial participation, the overall magnitude of prior antibiotic exposure was unknown in our study which hampers interpretation of the data. Our study was, however, conducted in the Netherlands, a country with a relatively low antibiotic prescribing rate. Furthermore, faecal samples were obtained at 2 weeks and 3 months following antibiotic treatment and not immediately after treatment exposure ended (day 7). A recent RCT on the effects of early-life antibiotics on the gut microbiome found the most pronounced effect immediately after treatment,<sup>35</sup> which might have gone undetected in our study. Finally, we only collected samples of children treated with antibiotics, so we could not compare topical and systemic antibiotic treatment to placebo or no treatment.

### **Conclusion**

Nested in a trial of topical and systemic antibiotics for children with AOMd, both antibiotic treatments had a similar impact on the gut microbiota composition at the phylum level, the alpha and beta diversity at the species level and the faecal ARG

abundance. Future trials of antibiotics should routinely collect microbiological samples to further investigate their off-target impact to ultimately reveal which antibiotic treatment minimizes the disruption of the human microbiota diversity and spread of ARGs.

# Supplementary Material

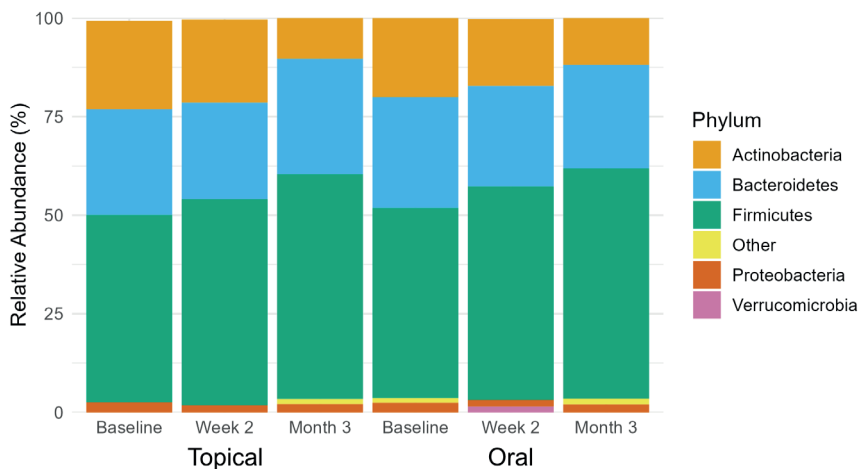
**Supplementary Table 1. Additional antibiotic prescription during follow-up.**

Type of antibiotic prescription	At 2 weeks		At 3 months (incl. the first 2 weeks)	
	Topical group	Oral group	Topical group	Oral group
	n	n	n	n
oral antibiotics	6	1	9	4
topical antibiotics	2	1	3	2
total antibiotics	8	2	10 <sup>a</sup>	5 <sup>b</sup>

<sup>a</sup> 2 subjects received additional topical and oral antibiotic treatment, so in total 10 subjects in the topical group received additional antibiotics during follow-up.

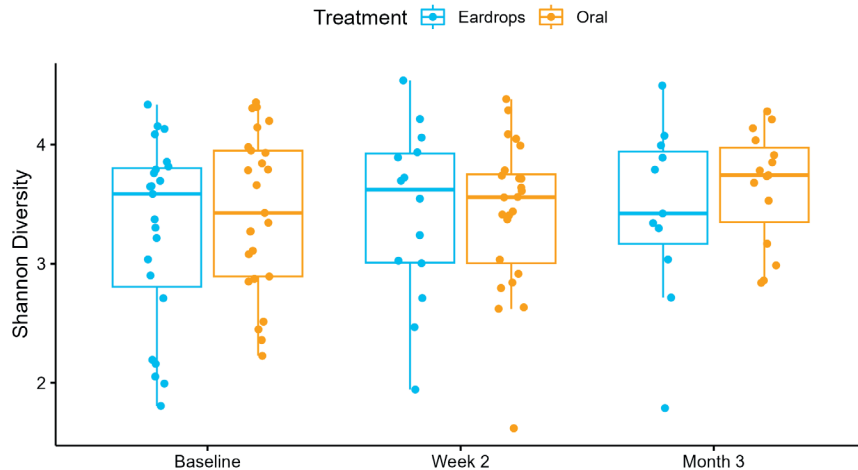
<sup>b</sup> 1 subject received additional topical and oral antibiotic treatment, so in total 5 subjects in the oral group received additional antibiotics during follow-up

## Supplementary figures – filtered data



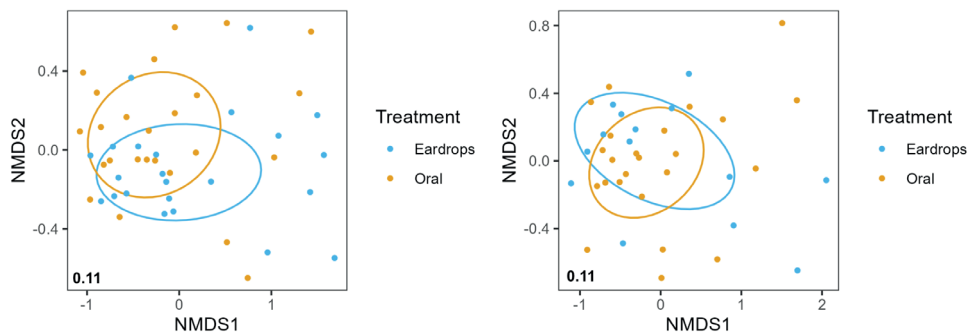
**Supplementary figure 1. Relative abundance of Phyla within the gut microbiome of children undergoing treatment for acute otitis media.**

Phylum abundances were measured at three different timepoints; baseline, two weeks post treatment and 3 months post treatment for both treatment arms (Eardrops and Oral). The data presented is the mean abundance of the samples at each time point. Phyla with a mean relative abundance less than 1% were grouped into the “Other” category.<sup>2</sup>



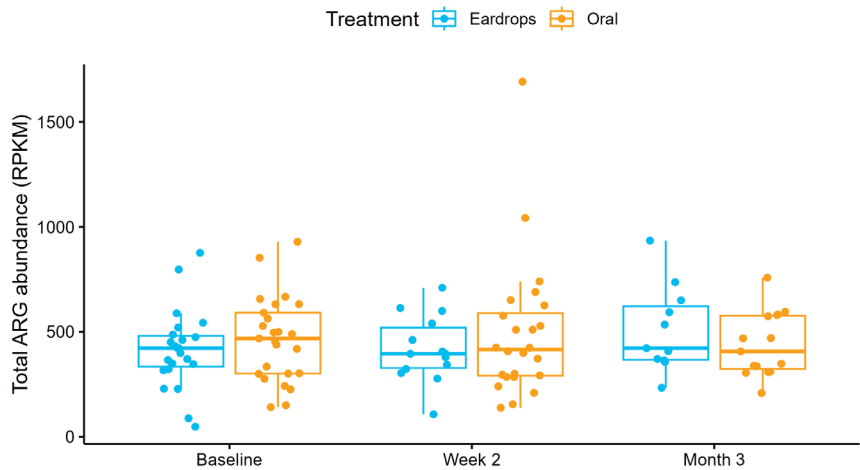
**Supplementary figure 2. Alpha-diversity of species present within the gut microbiome of children undergoing treatment for acute otitis media.**

Gut microbiome samples were collected from both treatment arms (Eardrops and Oral) before treatment, two weeks post treatment and 3 months post treatment. The alpha diversity was measured using the Shannon diversity index. The box plot represents the lower, middle, and upper quartiles of these data. Significance was determined using the Wilcoxon test.



**Supplementary figure 3. Beta-diversity of species present within the gut microbiome of children undergoing treatment for acute otitis media.**

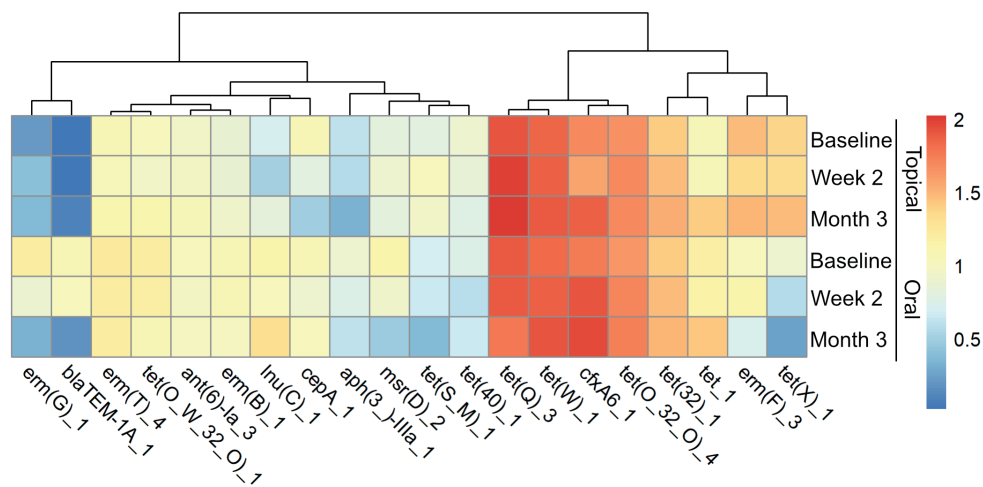
Non-metric multidimensional scaling was performed on Bray-Curtis dissimilarity matrices generated from species-level relative abundances. A. Samples prior to treatment. B. Samples two weeks post treatment. Data was stratified by treatment type (Eardrops or Oral). 50% confidence interval ellipses are indicated. Stress is indicated within the plots. Significance was measured by PERMANOVA with 10,000 permutations.



**Supplementary figure 4. Total antibiotic resistance gene abundance within the gut microbiome of children undergoing treatment for acute otitis media.**

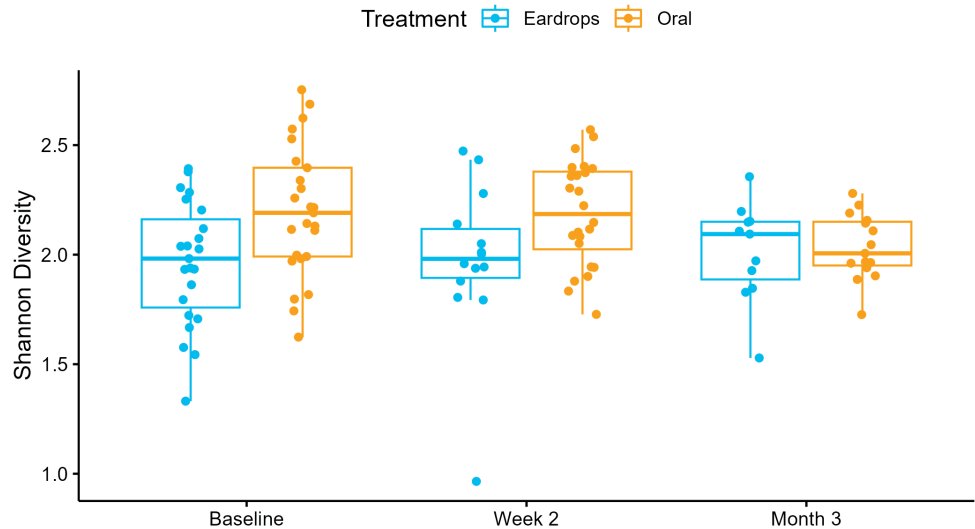
The summed abundance RPKM (reads per kilobase of reference sequence per million sample reads) of antibiotic resistance genes found in gut microbiome samples collected before treatment, two weeks, and three months post treatment. Samples have been grouped by treatment type, Eardrop or Oral. The box plot represents the lower, middle, and upper quartiles of these data. Significance was determined using the Wilcoxon test.





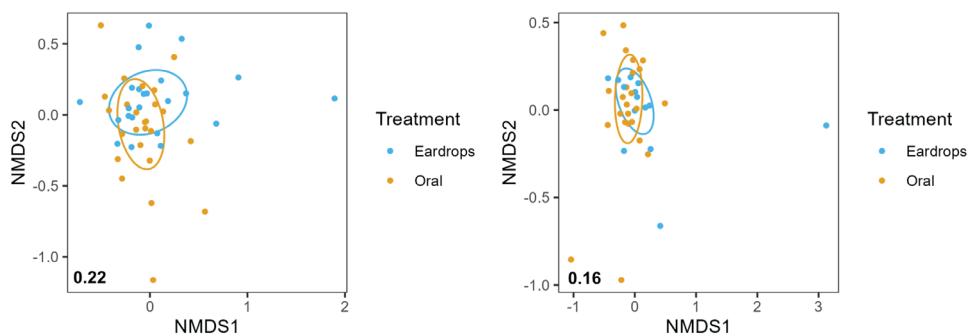
**Supplementary figure 5. The 20 most abundant antibiotic resistance genes present in the gut microbiome samples of children undergoing treatment for acute otitis media.**

The  $\log_{10} + 1$  transformed mean abundance RPKM (reads per kilobase of reference sequence per million sample reads) of the 20 most abundant antibiotic resistance genes (ARGs) found in the gut microbiome samples. ARGs are clustered based on Euclidean distances.



**Supplementary figure 6. Alpha-diversity of antibiotic resistance genes present within the gut microbiome of children undergoing treatment for acute otitis media.**

Gut microbiome samples were collected from both treatment arms (Eardrops and Oral) before treatment, two weeks post treatment and 3 months post treatment. The alpha diversity was measured using the Shannon diversity index. The box plot represents the lower, middle, and upper quartiles of these data. Significance was determined using the Wilcoxon test.



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**CHAPTER 6**

6

## **General discussion**







## General Discussion

In this final chapter I will discuss my thesis' main findings, including the trial's recruitment challenges, in the context of the wider body of literature and will reflect on their implications for future primary-care based research.

### Incidence and microbiology of otitis media

During the COVID-19 pandemic, we observed a decline in the incidence of GP-diagnosed otitis media (OM) episodes in children in the Netherlands (chapter 2). A similar decline was observed in many other countries.<sup>1-3</sup> In our Dutch study, we also found that antibiotic prescription rates for OM during the pandemic were similar to before, suggesting that the OM case-mix presenting to primary care did not change substantially and that infection control measures introduced during the pandemic caused a true decline in childhood OM incidence. Worldwide similar reductions in other respiratory tract infections (RTIs) were reported.<sup>1-7</sup> System changes, including social distancing, handwashing, and daycare and school closure implemented during the pandemic caused a reduction of circulating viruses.<sup>8-10</sup> After easing of these measures, an increase of infections and changes in pathogens occurred.<sup>11-14</sup> French researchers collected middle ear fluid samples from 852 children with acute otitis media presenting with ear discharge due to a spontaneous perforation of the eardrum (AOMd) between 2015 to January 2023.<sup>15</sup> They found that *Group A Streptococcus (GAS)* was the second most common pathogen, after *H. influenzae*. Especially towards the end of their study period GAS became increasingly prevalent. This is in line with the observed rise in GAS infections across Europe and in the United States after the COVID-19 pandemic restrictions were lifted.<sup>16,17</sup> Recently, a surge in lower RTIs in children caused by *Mycoplasma pneumonia* has been observed which lead to temporary recommendations to use azithromycin rather than amoxicillin as first-line antibiotic in some regions in the Netherlands. The widespread implantation of vaccines such as pneumococcal conjugate vaccines has also led to a shift in otopathogens to non-vaccine serotypes and other bacteria.<sup>18</sup> In all, these phenomena underline the need for continued surveillance of the microbiology profile of common infections such as AOM to tailor antibiotic treatment and optimise its management.

Our systematic review presented in chapter 3 identified that *S. pyogenes* and *S. aureus* were more common in children with AOMd than in children with AOM who do not present with acute ear discharge. As such, AOM and AOMd may be two different entities along the OM spectrum warranting a different disease management. This is supported by other studies reporting that children with AOMd experienced similar levels of ear pain at first presentation, but encountered a higher disease burden with higher rates of

ear pain and/or fever at 3–7 days and more AOM recurrences and hearing problems at 3 months than those without ear discharge.<sup>19,20</sup> Also, oral antibiotics are more effective than placebo or no treatment in reducing the duration of ear pain and fever in children with AOMd than in those with ear discharge (number needed to treat to benefit: 3 versus 8 children, respectively).

The use of antibiotics should, however, always be balanced against the possible harms, including systemic side effects and the significant public health danger of antibiotic resistance. Despite a surge in research into the impact of antimicrobial treatment on the microbiome<sup>21–23</sup>, little is known about the magnitude and duration of the impact of antibiotic exposures to off-target microbes (i.e., bacteria not targeted by treatment) and antibiotic resistance genes (ARGs).<sup>22</sup> Nested in our trial of antibiotic treatments for children with AOMd (chapter 4), we collected faeces samples to compare the impact of topical and oral antibiotics on the gut microbiota composition and the diversity and abundance of antimicrobial resistance genes in children with AOMd (chapter 5). Both antibiotic treatments had a similar impact on the gut microbiota composition at the phylum level. Moreover, a similar impact was observed on the sample diversity and median antimicrobial resistance genes abundance between and within the antibiotic treatment groups over time. Recent systematic reviews about the impact of antimicrobial treatment on the gut microbiota composition suggest that systemic antibiotics may have a profound influence for some time, but observed effects vary substantially across included studies, findings are inconsistent across different studies investigating the same antibiotic<sup>23–25</sup> and only few studies included children. The key factors affecting study findings are the study design, the timing and method of sampling, use of different antibiotic classes and treatment duration, and prior exposure to antibiotics. A review of 78 studies<sup>25</sup> assessing the impact of systemic antibiotics on the diversity and composition of human microbiota (including 52 gut microbiota studies) found that 88.5 % (69/78) did report changes in alpha diversity after antibiotic exposure, which contrasts with our study which showed no significant impact of systemic antibiotics (amoxicillin suspension) over time. However, the observed effect of beta-lactam antibiotics on the alpha diversity was inconsistent across studies, while only 3 of the 7 studies reported a difference in beta diversity.<sup>25</sup> Many of the affected taxa remained, however, altered for a prolonged period of time. Given the limited evidence-base, future trials of antimicrobial treatment should consider routinely collecting microbiological samples to investigate the off-target impact of antibiotic exposure and to ultimately reveal which antibiotic treatment minimizes the disruption of the human microbiota diversity and spread of antimicrobial resistance genes. In our trial, we also collected middle ear fluid samples and nasopharyngeal samples, however these samples have not been analysed yet. This analysis will be valuable to further balance any benefits of oral antibiotic use against the possible harms.

## Clinical trials in primary care: the past, the present and the future

Our randomised controlled trial (RCT) which is at the heart of my thesis has been initiated following a trial from our group demonstrating that antibiotic-corticosteroid eardrops were clinically superior to oral antibiotics in children with ventilation tubes presenting with acute ear discharge.<sup>26,27</sup> It was hypothesised that for children with AOMd, topical antibiotics may also be a possible alternative to oral antibiotics assuming that the spontaneous perforation of the eardrum would allow direct entry of the antibiotic into the middle ear. The trial was however prematurely ended. With the limited data available we were unable to demonstrate non-inferiority of antibiotic-corticosteroid eardrops compared to oral antibiotics in children with AOMd (chapter 4.2). By contrast, our data suggest that oral antibiotics may be more effective in resolving symptoms and shortening the duration of ear discharge than antibiotic-corticosteroid eardrops in children with AOMd. Therefore, I believe that current recommendation<sup>28</sup> to consider oral antibiotics in this group of children are not unreasonable.

The trial's data also, again, underline the importance of conducting experiments within a specific group, rather than extrapolating results from related conditions. While a ventilation tube creates a passage between ear canal and the middle ear allowing for topical treatment, our results suggest this is not the case in children with AOMd. The spontaneous tear or perforation of the eardrum in these children may be too small or close too early to allow antibiotic-corticosteroid eardrops to enter the middle ear and treat the infection.

## Recruitment challenges

We encountered a range of operational challenges that affected the progress of our trial. In the first year (2018), there were supply issues of the study medication, i.e. the hydrocortisone–bacitracin–colistin eardrops, which made us pause the study. This was followed by the COVID-19 pandemic, which held up the restart of the study until November 2021. The COVID-19 pandemic not only had an impact on the incidence of AOM but also put a considerable strain on healthcare resources including primary care, which resulted in slow accrual and early termination of the trial in March 2023.

Our trial provided valuable learnings in terms of trial design and conduct in primary care. Challenges in recruiting to time and target occur worldwide in clinical studies.<sup>29–34</sup> A 2007 survey of 78 Dutch primary care studies, showed that only 46% achieved their recruitment targets within the specified time frame. Studies that targeted incident cases, like our trial, had even a considerably lower success rate, with only 28% being successfully completed in time.<sup>30</sup>

Factors negatively affecting trial recruitment include a lower than anticipated incidence rate, interruption of daily practice, investing extra time of the clinician, treatment

preference, and patient burden.<sup>29,31</sup> Strategies to optimise recruitment include minimising time constraints by delegating as much work as possible to the study team, financial incentives for participating clinicians, implementing an internal pilot-study to identify potential pitfalls, and telephone reminders to non-responders.<sup>31,32,35,36</sup> Building on our previous success in recruiting to trials of children in primary care<sup>26,37–39</sup>, we implemented several of these strategies in our trial. First, all study related procedures, including the informed consent procedure and follow-up measurements, were performed by the trial doctor to minimise time and effort for the GP and avoid interruption of daily practice. Second, we reimbursed GPs for their time to identify and pre-screen trial candidates and take consent to share contact details with our study team. Third, we co-designed the trial with our parent panel to optimise the recruitment processes and patient information materials. Fourth, we pursued collaboration with GP trainees of the UMC Utrecht to also include patients. However, the participation of the GP trainees was optional. Including participation in clinical studies within the GP training curriculum may encourage trainees to engage with trials during and beyond their training. Finally, throughout our trial we looked to expand the number of participating GPs and sent regular newsletters about the trial progress. Despite embedding these strategies, we failed to meet the desired sample size.

### **The future is now?**

From now on, an internal pilot study including stop-go criteria to proceed, and if so how, should be considered to increase the likelihood that the trial's desired sample size will be met. Furthermore, it might be valuable to collaborate nationally to complete large primary care-based RCTs successfully. In recent years, the General Practice Research Consortium Netherlands (GPRC-NL) has been established which aims to improving the research infrastructure for future primary care-related research in the Netherlands.<sup>40</sup> This will undoubtedly foster collaboration and improve recruitment to clinical trials in the near future. Moreover, it is equally important for future projects to acquire sufficient budget to hire operational management expertise given the growing complexity in rules and legislations related to the conduct of studies involving investigational medicinal products. Furthermore, it is important that researchers continue to explore novel approaches for trial recruitment, such as integrating research in routine care. A recent Dutch diagnostic incidental case study in primary care showed that the introduction of a novel inclusion pathway, involving a linkage between standard care and research through the integration of their study in the diagnostic referral system of the GP, strongly improved recruitment rate.<sup>41</sup> In our study this could be a 'recruitment reminder pop-up' in the GP registration system, which might elevate the awareness of the study. However, this has been proposed before and has gained little traction. Additionally, raising awareness of the study by promoting it on healthcare websites such as [www.thuisarts.nl](http://www.thuisarts.nl) and [www.moetiknaardedokter.nl](http://www.moetiknaardedokter.nl) could be beneficial.

Finally, it can be debated whether traditional RCTs will remain the preferred approach for evaluating the effectiveness of interventions in primary care. Advances in technology foster the development of promising innovations like digital clinical trials which uses technology to improve recruitment and retention, data collection and analytics<sup>42</sup> or digital clinical trial platforms, like the Eureka Research Platform in the US.<sup>43</sup> They enable remote recruitment strategies, digital data collection and analysis which in turn reduce workload and costs. Moreover, the use of observational real-world evidence and big data hold promise.<sup>44–46</sup> Such approaches, can provide results that are more generalizable to everyday practice and are particular powerful to improve treatment of rare conditions compared to conventional trial methods.<sup>47–49</sup> However, this transition comes with important methodological challenges, including minimizing the risk of bias and controlling for confounding.<sup>48</sup> Furthermore, the use of such designs in evaluating the effectiveness of interventions to relieve symptoms in common infections, such as in our trial, is often hampered by the lack of information on the course of symptoms over time in the primary care electronic health records. As emphasized in recent literature, observational real-world data is more likely to supplement findings from clinical trials in the future rather than completely replace them, highlighting the complementary nature of these approaches.<sup>46–48,50</sup>

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Appendices



**Summary**

**Nederlandse samenvatting**

**List of publications**

**Dankwoord/Acknowledgements**

**About the author**



## Summary

Acute otitis media (AOM) is one of the most common childhood infections and a leading cause of doctor consultations and antibiotic prescriptions worldwide. Approximately 15-20% of children with AOM present and ear discharge due to a spontaneous tear or perforation of the eardrum (AOMd). In contrast to widespread beliefs, acute ear discharge as a presenting symptom of AOM does not mean that the infection is improving. Children with AOMd have similar levels of ear pain and feel worse at first presentation than those without ear discharge. Also, children with AOMd have a higher disease burden with higher rates of ear pain and/or fever at 3-7 days and more AOM recurrences and hearing problems at 3 months compared with children without ear discharge. Despite, evidence about the causative pathogens and most optimal management of this condition was scarce. The main aim of this thesis was therefore to provide insight in the incidence, microbiology and management of AOMd in primary care.

**Chapter 2.1** reports on a large retrospective primary care-based cohort study designed to estimate the impact of the COVID-19 pandemic on the incidence of childhood otitis media in the Netherlands. We extracted routine electronic health records data on AOM, otitis media with effusion (OME), ear discharge episodes and associated antibiotic prescriptions from all children aged 0-12 years registered to general practices of the Julius General Practitioners' Network (JGPN) before (1 March 2019 - 29 February 2020) and/or during the COVID-19 pandemic (1 March 2020 - 28 February 2021). General practitioner (GP) consultations for AOM, OME and ear discharge in children declined by 63%, 57% and 54% respectively during the COVID-19 pandemic. Antibiotic prescription rates for these conditions during the pandemic were similar to before, suggesting that the case-mix presenting to primary care did not change substantially. This implies that infection control measures introduced during the pandemic resulted in a true decline in OM incidence in children.

**Chapter 2.2** explores the primary care incidence and current management of AOM in adults. To this end, all patients aged 15 and older included in the routine electronic health care database of the JGPN were followed from 2015 to 2018. We extracted data on AOM episodes, AOM-related consultations, comorbidities, and antibiotic and analgesic prescriptions. In total, 6,667 AOM episodes among 5,358 adult patients (mean 1.2 AOM episode per patient) were identified, resulting in an overall AOM incidence of 5.3/1,000 person-years. This incidence rate was fairly stable over time. Incidence was particularly high in atopic patients (7.3/1000 person-years) and declined with age (from 7.1 in patients 15-39 years of age to 2.7/1000 person-years in those aged 64 years and older). Oral antibiotics, predominantly amoxicillin, were prescribed in 46%, and topical antibiotics in 21% of all episodes. The observed irrational prescribing of azithromycin

and topical antibiotics implies a need for GP-targeted interventions to promote the appropriate use of antibiotics for AOM in adults.

**Chapter 3** aims to synthesize the global evidence on the prevalence and antimicrobial resistance (AMR) status of bacteria in children with AOMd after the widespread introduction of pneumococcal conjugate vaccines (PCV). Systematic searches of PubMed, EMBASE and Cochrane Library from inception to June 2019 were conducted to identify all English studies reporting any prevalence and/or AMR data of bacterial middle ear isolates from children with AOMd. Of 4,088 unique records retrieved, 19 studies (10,560 children) conducted between 2000 and 2017 were included. Overall quality, following the Joanna Briggs Institute Critical Appraisal checklist, was judged high. *Streptococcus pneumoniae* (median 26.1%, range 9.1%–47.9%), *Haemophilus influenzae* (median 18.8%, range 3.9%–55.3%), *Staphylococcus aureus* (median 12.3%, range 2.3%–34.9%) and *Streptococcus pyogenes* (median 11.8%, range 1.0%–30.9%) were the most prevalent bacteria. In 76.0% (median, range 48.7%–100.0%, 19 studies, 1,429 children) any bacterium was identified. AMR data were sparse and mainly limited to *S. pneumoniae*. We found no evidence of a clear shift in the prevalence of bacteria and AMR over time, which should be interpreted in the context of the limited available information on children's PCV status and the lack of recent data. Ongoing surveillance of the microbiology profile in children with AOMd is therefore warranted to guide antibiotic selection.

**Chapter 4** reports the rationale and design (chapter 4.1), and results (chapter 4.2) of our trial comparing topical and oral antibiotics for children with AOMd.

In 2016, we obtained funding from the Netherlands Organisation for Health Research and Development (ZonMw) for conducting a primary care-based, open, individually randomised, controlled, non-inferiority trial in children aged 6 months to 12 years with AOMd and ear pain and/or fever comparing hydrocortisone-bacitracin-colistin eardrops and amoxicillin suspension. The primary outcome of interest was the proportion of children without ear pain and fever at day 3. The first participant was enrolled in the trial on 13 December 2017. On 8 August 2018 with 34 participants being enrolled, trial recruitment was put on hold due to supply issues of hydrocortisone–bacitracin–colistin eardrops. These drops were available again since early 2021 and the funding body, ZonMw, approved the trial to reopen in September 2021. Due to slow accrual the trial was prematurely ended with 58 of a planned 350 children included; 27 were assigned to antibiotic-corticosteroid eardrops and 31 to oral antibiotics. Children assigned to eardrops (n=26) had lower resolution rates of ear pain and fever at 3 days compared to those receiving oral antibiotics (n=31): 42% vs 65%; adjusted risk difference 20.3%, 95% confidence interval -5.3% to 41.9%), longer parent-reported ear discharge (6 vs 3

days;  $p=0.04$ ), and slightly higher mean ear pain scores (Likert scale 0-6) over days 1-3 (2.1 vs 1.4,  $p=0.02$ ), but received fewer oral antibiotic courses in three months (11 for 25 children vs 33 for 30 children), and had less GI upset and rash (12% vs 32% and 8% vs 16%, respectively).

In conclusion, we were unable to determine non-inferiority of antibiotic-corticosteroid eardrops to oral antibiotics, but our findings in a small group of children, requiring confirmation, suggest that that oral antibiotics may be more effective in resolving symptoms, and shortening the duration of ear discharge than antibiotic-corticosteroid eardrops in children with AOM and ear discharge. Current guidelines' recommendation that clinicians can consider oral antibiotics in this group of children therefore seem not unreasonable, but must be balanced against the major public health threat of antibiotic resistance.

**Chapter 5** reports on a study assessing the differential impact of topical and systemic antibiotics on the gut microbiota composition and antibiotic resistance genes, which was nested in our trial of antibiotic treatments for children with AOMd. Faecal samples were collected from 51 patients prior to treatment (hydrocortisone-bacitracin-colistin eardrops or amoxicillin suspension for 7 days), and 2 weeks and 3 months after treatment. The total DNA extracted from the faecal samples was subjected to shotgun metagenomic sequencing. Alpha and beta diversity measures were used to identify any differences between the samples and multivariable association analyses were used to identify differentially abundant species across the samples. At the phylum level, the gut microbiota composition of children with AOMd was similar regardless of study group assignment. At the species level, the alpha and beta-diversity were also similar in both groups at all time points (Wilcoxon,  $P > 0.05$  and PERMANOVA  $P > 0.05$ , respectively). Within each treatment group over time, the alpha diversity of samples remained the same (Wilcoxon,  $P > 0.05$ ). The median antibiotic resistance genes abundance was similar in both antibiotic treatment groups at all time points and within each treatment groups over time. Our study indicates that topical and systemic antibiotics for children with AOMd had a similar impact on the gut microbiota composition at the phylum level, the alpha and beta diversity at the species level and the faecal antibiotic resistance genes abundance. Future trials of antibiotics should routinely collect microbiological samples to further investigate their off-target impact to ultimately reveal which antibiotic treatment minimizes the disruption of the human microbiota diversity and spread of antibiotic resistance genes.

**In chapter 6** I discuss my thesis' main findings, including the trial's recruitment challenges, in the context of the wider body of literature and I reflect on their implications for future primary-care based research. During the course of the trial we encountered

various operational challenges that affected the progress of our trial, including supply issues of our study medication and the impact of the COVID-19 pandemic. Challenges in recruiting to time and target occur worldwide in clinical studies. Factors negatively affecting trial recruitment include a lower than anticipated incidence rate, interruption of daily practice, investing extra time of the clinician, treatment preference, and patient burden. Building on our previous experiences from successful trials in primary care, we implemented several strategies to enhance the recruitment rate. Despite, we failed to reach our desired sample size. From now on, an internal pilot study including stop-go criteria to proceed, and if so how, should be considered to increase the likelihood that the trial's desired sample size will be met. Moreover, it will be instrumental to collaborate nationally to complete large primary care-based RCTs successfully. Furthermore, it is important that researchers continue to explore novel approaches for inclusion, such as integrating research in routine care. Finally, it can be debated whether traditional RCTs will remain the preferred approach for studies evaluating the effectiveness of interventions in primary care. The use of observational real-world evidence and big data hold promise. However, this transition comes with important methodological challenges, including minimizing the risk of bias and controlling for confounding. As emphasized in recent literature, observational real-world data is more likely to supplement findings from clinical trials in the future rather than completely replace them, highlighting the complementary nature of these approaches.



## Nederlandse Samenvatting

Otitis media acuta (OMA), ofwel acute middenoorontsteking, is één van de meest voorkomende kinderinfecties en een belangrijke oorzaak van doktersbezoeken en antibioticumvoorschriften wereldwijd. Ongeveer 15-20% van de kinderen met een OMA presenteert zich met een acuut loopoor door een spontane perforatie van het trommelveel. In tegenstelling tot wat vaak gedacht wordt, is een acuut ontstaan loopoor geen teken dat een OMA episode op zijn retour is. Kinderen met een OMA en een acuut loopoor hebben dezelfde mate van oorpijn en voelen zich bij de eerste presentatie vaak slechter dan kinderen zonder een loopoor. Ook hebben kinderen met OMA en een loopoor een hogere ziektelast met meer oorpijn en/of koorts na 3-7 dagen en meer OMA-recidieven en gehoorproblemen na 3 maanden vergeleken met kinderen zonder een loopoor. Er is weinig literatuur beschikbaar over de meest voorkomende otopathogenen en de meest optimale behandeling van kinderen met OMA en een loopoor. Het hoofddoel van dit proefschrift was daarom om inzicht te krijgen in de incidentie, microbiologie en behandeling van OMA met een loopoor in de eerste lijn.

In **hoofdstuk 2.1** worden de resultaten beschreven van een groot retrospectief cohort-onderzoek in de eerste lijn naar de impact van de COVID-19-pandemie op de incidentie van otitis media bij kinderen in Nederland. Daartoe is routine zorgdata verzameld over OMA, otitis media met effusie (OME), looporen en bijbehorende antibioticumvoorschriften van alle kinderen in de leeftijd van 0-12 jaar die vóór (1 maart 2019 - 29 februari 2020) en tijdens de COVID-19-pandemie (1 maart 2020 - 28 februari 2021) ingeschreven waren bij huisartsenpraktijken verbonden aan het Julius Huisartsen Netwerk (JHN). Huisartsbezoeken voor OMA, OME en looporen bij kinderen daalden tijdens de COVID-19 pandemie met respectievelijk 63%, 57% en 54%. Het aantal antibioticumvoorschriften voor otitis media was tijdens de pandemie vergelijkbaar met voorheen, wat erop wijst dat de mix van patiënten in de eerstelijnszorg niet substantieel was veranderd. Dit suggereert dat de tijdens de COVID-19 pandemie ingevoerde maatregelen om besmettingen te voorkomen resulteerden in een daadwerkelijke daling van de otitis media incidentie bij kinderen.

**Hoofdstuk 2.2** beschrijft een onderzoek naar de incidentie en de behandeling van OMA bij volwassenen in de eerste lijn. Hiertoe werd gebruikt gemaakt van de routine zorgdata van alle patiënten van 15 jaar en ouder die van 2015 tot 2018 ingeschreven waren bij huisartsenpraktijken verbonden aan JHN. We hebben gegevens verzameld over OMA-episodes, OMA-gerelateerde consulten, co-morbiditeiten en antibioticumvoorschriften. In totaal werden 6.667 OMA-episodes bij 5.358 volwassen patiënten (gemiddeld 1.2 OMA-episode per patiënt) geïdentificeerd, resulterend in een totale OMA-incidentie van 5.3/1000 persoonsjaren. Dit incidentiecijfer was redelijk stabiel over de bestudeerde ja-

ren. De incidentie was met name hoog bij atopische patiënten (7.3/1000 persoonsjaren) en daalde met de leeftijd (van 7.1 bij patiënten van 15-39 jaar tot 2.7/1000 persoonsjaren bij patiënten van 64 jaar en ouder). Orale antibiotica, voornamelijk amoxicilline, werden voorgeschreven bij 46%, en lokale antibiotica bij 21% van alle episoden. De niet-conform de richtlijn voorschriften van azitromycine en lokale antibiotica voor OMA bij volwassenen impliceert ruimte voor gerichte interventies om het juist gebruik van antibiotica bij deze aandoening te bevorderen.

**Hoofdstuk 3** heeft als doel om de beschikbare literatuur aangaande de prevalentie en antibiotica resistentie van bacteriën bij kinderen met OMA en een loopoor na de introductie van pneumokokkenvaccinatie samen te vatten. Hiertoe werd een uitgebreide systematische zoekstrategie binnen de elektronische databases PubMed, EMBASE en Cochrane Library verricht. Van de 4.088 gevonden unieke studies zijn uiteindelijk 19 studies (10.560 kinderen), uitgevoerd tussen 2000 en 2017, geïncludeerd in ons literatuuronderzoek. De algemene kwaliteit van de studies werd, volgens de Critical Appraisal checklist van het Joanna Briggs Institute, als goed beoordeeld. *Streptococcus pneumoniae* (mediaan 26.1%, range 9.1%–47.9%), *Haemophilus influenzae* (mediaan 18.8%, range 3.9%–55.3%), *Staphylococcus aureus* (mediaan 12.3%, range 2.3%–34.9%) en *Streptococcus pyogenes* (mediaan 11.8 %, range 1.0%–30.9%) waren de meest voorkomende bacteriën. In 76.0% (mediaan, range 48.7%–100.0%, 19 onderzoeken, 1 429 kinderen) werd een bacterie geïdentificeerd. Antibiotica resistentie gegevens waren schaars en beperkten zich voornamelijk tot *S. pneumoniae*. Er werd geen bewijs gevonden voor een duidelijke verschuiving in de prevalentie van bacteriën en antibiotica resistentie over het verloop van de tijd. Deze bevinding dienen echter wel geplaatst te worden binnen de context van de beperkte beschikbare gegevens over de pneumokokkenvaccinatie status van kinderen en het gebrek aan recente studies. Het verdient aanbeveling om het microbiologische profiel van kinderen met OMA en een loopoor te blijven monitoren om ook in de toekomst wetenschappelijk onderbouwde beslissingen ten aanzien van een passend antibioticumgebruik te kunnen (blijven) nemen.

**Hoofdstuk 4** beschrijft de rationale en design (hoofdstuk 4.1) en de resultaten (hoofdstuk 4.2) van onze trial waarin we de effectiviteit van lokale en orale antibiotica voor kinderen met OMA en een loopoor vergelijken. In 2016 hebben we financiering gekregen van ZonMw voor het uitvoeren van een open, individueel gerandomiseerde, gecontroleerde, non-inferioriteitsstudie in de eerste lijn waarin behandeling met hydrocortison-bacitracine-colistine oordruppels vergeleken werd met een amoxicilline drankje bij kinderen van 6 maanden tot 12 jaar met OMA en een loopoor en oorpijn en/of koorts. De primaire uitkomst was het percentage kinderen zonder oorpijn en koorts op dag 3. In december 2017 werd de eerste deelnemer in de studie ingesloten en op 8 augustus 2018, toen er 34 kinderen waren ingesloten, werd de studie tijdelijk opgeschort van-

wege leveringsproblemen van de hydrocortison-bacitracine-colistine oordruppels. Deze oordruppels waren sinds begin 2021 weer beschikbaar en ZonMw ging akkoord met de herstart van de studie per september 2021. Vanwege vertraagde inclusie werd de studie vroegtijdig beëindigd. Op dat moment waren 58 van de beoogde 350 kinderen geïnccludeerd; 27 werden via loting toegewezen aan lokale antibiotica en 31 aan de orale antibiotica. Kinderen die antibiotica-corticosteroid oordruppels kregen, waren minder vaak vrij van oorpijn en koorts na 3 dagen vergeleken met degenen die een antibioticum drankje kregen (n=31): 42% versus 65%; gecorrigeerd risicoverschil 20,3%, 95% betrouwbaarheidsinterval -5,3% tot 41,9%), hadden een langere duur van het loopoor (6 versus 3 dagen;  $p=0,04$ ) en een iets hogere gemiddelde oorpijn score (Likert-schaal 0-6) over dag 1-3 (2,1 versus 1,4,  $p=0,02$ ), maar kregen minder orale antibioticumkuren gedurende de drie maanden follow-up (11 kuren in 25 kinderen versus 33 kuren in 30 kinderen), en hadden minder vaak maag-darmklachten en huiduitslag (12% versus 32% en 8% versus 16%, respectievelijk). Concluderend waren we niet in staat om non-inferioriteit aan te tonen van antibiotica-corticosteroid oordruppels ten opzichte van orale antibiotica. Onze bevindingen bij een kleine groep kinderen, die bevestiging behoeven, suggereren echter dat orale antibiotica effectiever zijn in het verminderen van symptomen en het verkorten van de duur van het loopoor. De huidige richtlijn aanbeveling aan huisartsen om orale antibiotica bij deze groep kinderen te overwegen lijkt op basis van onze studie niet onredelijk, maar dient worden afgewogen tegen de risico's van een toename van de antibiotica resistentie.

**Hoofdstuk 5** beschrijft een studie waarin de impact van lokale en orale antibiotica op het darmmicrobioom en antibiotica resistentiegenen wordt onderzocht bij de kinderen met OMA en een loopoor die aan onze studie uit hoofdstuk 4 hebben deelgenomen. Bij 51 deelnemers werden vóór de behandeling (hydrocortison-bacitracine-colistin oordruppels of amoxicilline suspensie gedurende 7 dagen) en 2 weken en 3 maanden na de behandeling fecesmonsters verzameld. Het totale DNA dat uit de fecesmonsters werd geëxtraheerd werd onderworpen aan shotgun-metagenomic sequencing. Op fyllumniveau waren de darmmicrobiota van kinderen met OMA en een loopoor vergelijkbaar tussen beide groepen. Ook op speciesniveau waren de alfa- en bèta-diversiteit op alle tijdstippen vergelijkbaar tussen de behandelingsgroepen (respectievelijk Wilcoxon,  $P > 0,05$  en PERMANOVA  $P > 0,05$ ). Bovendien was binnen elke behandelingsgroep de alfa-diversiteit van de monsters over de tijd vergelijkbaar (Wilcoxon,  $P > 0,05$ ). Er was op geen enkel tijdstip een significant verschil in de abundantie van de antibioticaresistentiegenen tussen de behandelingsgroepen, noch enig significant verschil binnen de groepen over de tijd. Onze studie suggereert dan ook dat lokale en orale antibiotica bij kinderen met OMA en een loopoor een vergelijkbare impact hebben op de samenstelling van de darm microbiota op stamniveau, op de alfa en bèta-diversiteit op groepsniveau, en op de abundantie van de antibioticaresistentiegenen. Toekomstige onderzoeken naar

antibiotische interventies zouden routinematig microbiologische monsters moeten verzamelen om de off-target impact verder te onderzoeken en zodoende aan te tonen welke antibiotische behandeling de verstoring van de diversiteit van de microbiota en de verspreiding van antibiotica resistentiegenen minimaliseert.

In **hoofdstuk 6** bespreek ik de belangrijkste bevindingen van mijn proefschrift in de context van de huidige literatuur, inclusief de uitdagingen rondom het includeren van deelnemers in de trial en reflecteer ik op de implicaties voor toekomstig onderzoek binnen de eerste lijn. In de loop van de trial kwamen we verschillende operationele uitdagingen tegen die de voortgang van onze trial beïnvloedden, waaronder leveringsproblemen van onze onderzoeksmedicatie en de impact van de COVID-19-pandemie. Uitdagingen bij het includeren van voldoende deelnemers in klinische studies binnen de gestelde tijd is een wereldwijd probleem. Factoren die een negatieve invloed kunnen hebben op de werving van studiedeelnemers zijn onder andere een lager dan verwachte incidentie, interfereren van studiehandelingen met de alledaagse praktijk, extra tijd die de clinici moeten investeren, voorkeur voor bepaalde behandeling van én de belasting van studiedeelname voor de patiënt. Voortbouwend op onze eerdere ervaringen met succesvolle onderzoeken in de eerstelijnszorg, hebben we verschillende strategieën geïmplementeerd om de inclusiesnelheid te verhogen. Desondanks zijn we er niet in geslaagd om ons beoogde aantal deelnemers te behalen. Vanaf nu zou een pilot studie, inclusief een beslissing of, en zo ja hoe, er doorgegaan moet worden, dienen worden overwogen om de kans te vergroten dat het benodigde aantal deelnemers zal worden bereikt. Bovendien is het onontbeerlijk om landelijk samen te werken om grote (effectiviteit)studies in de eerste lijn succesvol af te ronden. Bovendien is het belangrijk dat onderzoekers nieuwe methodieken ter bevordering van de inclusie blijven exploreren, zoals het integreren van onderzoek in de reguliere zorg. Ten slotte kan worden afgevraagd of traditionele gerandomiseerde studies de eerste keus methodiek dient te blijven om effectiviteit van interventies in de eerstelijnszorg te evalueren. Het gebruik van observationele studies met real-world data en big data zijn veelbelovend. Deze transitie brengt echter belangrijke methodologische uitdagingen met zich mee, waaronder het minimaliseren van het risico op bias en het controleren voor confounding. Zoals benadrukt in recente literatuur ligt het echter het meest voor de hand dat observationele studies met real-world data in de toekomst de bevindingen uit gerandomiseerde studies zullen aanvullen in plaats van deze volledig te vervangen.

## List of publications

### Publications based on studies presented in this thesis

**Hullegie S**, Damoiseaux RAMJ, Hay AD, Zuithoff NPA, van Dongen TMA, Little P, Schilder AGM, Venekamp RP. Topical or oral antibiotics in childhood acute otitis media and ear discharge: a randomized controlled non-inferiority trial. *Fam Pract*. 2024 Jun 24;cmæ034. doi: 10.1093/fampra/cmæ034

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## Dankwoord/Acknowledgements

In 2017 ben ik gestart met mijn gecombineerde promotie en opleidingstraject en onderweg hebben vele mensen mijn pad gekruist. Graag wil ik hier degenen bedanken die mij hebben ondersteund en die hebben bijgedragen aan het onderzoek, want promoveren doe je zeker niet alleen.

Allereerst wil ik mijn dank uitspreken aan alle deelnemers van de PLOTS-studie en aan de huisartsen die de kinderen hebben aangedragen voor deelname.

Uiteraard wil ik ook mijn promotieteam, prof. dr. Roger Damoiseaux, prof. dr. Anne Schilder, dr. Roderick Venekamp en dr. Thijs van Dongen, hartelijk bedanken voor de fijne begeleiding en inspiratie.

Beste Roger, dank voor je positieve en betrokken houding en begeleiding. Jouw relativeringsvermogen, overstijgende blik en focus op de klinische relevantie hebben mij geïnspireerd en gestimuleerd. Daarnaast heb ik genoten van je humor en de fijne sfeer tijdens de promotie overleggen.

Beste Anne, dank voor je scherpzinnigheid en voor jouw nuttige feedback. Jouw uitgebreide ervaring, brede kennis en blik vanuit het KNO-veld hebben elk manuscript tot een hoger niveau getild.

Beste Roderick, dank voor je tomeloze betrokkenheid en fijne begeleiding. Jouw gedrevenheid, punctualiteit en razendsnelle feedback hebben ervoor gezorgd dat ik dit promotietraject succesvol heb kunnen afronden. Elk overleg met jou zorgde voor een motivatie boost. Ik waardeer het zeer dat je ondanks je overvolle agenda in de laatste fase ook zo betrokken bent geweest. Heel veel dank hiervoor.

Beste Thijs, met name in het begin van mijn promotietraject ben jij nauw betrokken geweest. Dank voor je fijne en laagdrempelige manier van begeleiden. Leuk en nuttig dat PLOTS heeft kunnen voortbouwen op jouw LOT-expertise.

De leden van de beoordelingscommissie, Prof. dr. M.H. Blanker, Prof. dr. L.J. Bont, Prof. dr. R.J. Stokroos, Prof. dr. J.H.H.M. van de Wijgert en Prof. dr. D.L.M. Zwart wil ik hartelijk bedanken voor het lezen en beoordelen van mijn proefschrift.

Verder wil ik graag alle co-auteurs bedanken voor hun waardevolle input en expertise. Ik ben dankbaar dat ik met jullie heb mogen samenwerken.

Lidian Izeboud en Marjon van der Meer jullie waren als studieverpleegkundigen voor de PLOTS-studie onmisbaar voor de praktische onderzoek gerelateerde zaken en de huisbezoeken. Dank voor jullie pragmatische en flexibele houding. Het was plezierig om met jullie samen te werken.

Doordat mijn traject van 2017 tot 2024 heeft gelopen, heb ik vele kamergenoten, collega's en AIOTO's zien komen en gaan. Bedankt allemaal voor de gezelligheid. Valentijn en Kevin jullie zijn het langst mijn kamergenoten geweest, dank voor de gezelligheid en uiteraard ook jullie statistische/methodologische kennis. Het is fijn om te zien dat er na COVID-19 een leuke nieuwe actieve HAG promovendi groep is ontstaan. De gezelligheid, koffiepauzes, lunches, borrels en sportieve activiteiten zorgde voor veel werkplezier. In het bijzonder wil ik Rick, Joline en Merijn bedanken voor het waarnemen in tijden van afwezigheid. Dankzij jullie kon de PLOTS-studie altijd doorgaan, heel fijn dat ik op jullie kon rekenen.

De HAG-infectie groep wil ik bedanken voor de inspirerende maandelijkse bijeenkomsten.

Afdeling datamanagement van het Julius Centrum, in het bijzonder Jildou Zwerver, dank voor de goede samenwerking. Beste Peter Zuithoff, dank voor je hulp bij de analyses van de PLOTS-studie. Jouw enthousiasme en kennis over statistiek is inspirerend.

Beste opleiders van de huisartsopleiding, Huub Lamers, Hanneke Schlaman en Karin Pouls. Bedankt voor jullie interesse en steun bij het combineren van de huisartsopleiding met mijn promotieonderzoek.

Het schrijven van een proefschrift kent zijn ups en downs, en dan is het fijn om familie en vrienden om je heen te hebben die je steunen. Ik ben dankbaar voor de geweldige vriendinnen en vrienden die in de loop der jaren mijn leven hebben verrijkt. Enschede vriendinnen, Geneeskunde chickies, huisgenoten, hockey-, fiets-, en roeimaten, vrienden van Q, dank allen voor de mooie gezellige en sportieve momenten de afgelopen jaren. Het afronden van mijn proefschrift betekent meer tijd voor nieuwe avonturen, ik kijk er naar uit om nog vele mooie momenten met jullie te delen.

Lieve Muriël en Ilse, wat is het fijn dat jullie mijn paranimfen willen zijn. Vanuit de tennisbaan in Enschede is onze vriendschap alleen maar sterker geworden sinds jullie in de buurt wonen. Muur, jouw creativiteit, levenslust en tomeloze energie zijn bewonderingswaardig en zorgen voor geweldige uitjes. Ils, jij bent de stabiliteit en attentie zelve, wat is het fijn om zo'n fijne en warme vriendin te hebben.

Lieve schoonfamilie, dank voor jullie liefde en de Franse verrijking in mijn leven.



Lieve familie, wat ben ik blij met jullie en wat heb ik een geluk dat ik in dit warme nest ben opgegroeid. Lieve Karin, Ellen en Marieke, wat is het fijn om jullie als zussen te hebben en wat ben ik trots op jullie alle drie. Lieve Kaat, ik heb jou als oudste zus gevolgd en ben ook geneeskunde gaan studeren. Dank voor jouw zorgzaamheid, liefde en het geduld tijdens het aanhoren van al mijn werkperikelen. Lieve El, jouw doorzettingsvermogen, optimisme en energie zijn echt uitzonderlijk. Lieve Jack, Morris, Joan en Miles, wat is het fijn dat jullie bij onze familie zijn aangesloten. Lieve Miek, het is zo bijzonder en fijn dat onze band ondanks de afstand onveranderd blijft, aan een half woord hebben wij genoeg, wat een geluk dat jij mijn lieve attente twinnie bent.

Lieve pap en mam, ik heb zoveel aan jullie te danken. De stabiele basis en het vertrouwen dat jullie mij hebben gegeven, hebben ervoor gezorgd dat ik mijn eigen weg kon volgen en sta waar ik nu sta. Lieve pap, jouw interesse voor filosofie en wetenschap hebben mij geïnspireerd om ook de onderzoekswereld in te gaan. Dank je voor het sparren en het motiveren. Lieve mam, ik ken geen onbaatzuchtiger persoon op deze hele wereld. Jij straalt zoveel liefde en betrokkenheid uit en staat altijd voor ons klaar. Bedankt voor jullie liefde en geduld!

Lieve Querian, wat een geluk dat onze paden in 2017 elkaar hebben gekruist. Vanaf het begin heb jij mij zoveel rust en vertrouwen gegeven, en het blijft mij verbazen dat het alleen maar leuker wordt. Jouw zorgzaamheid, humor en sterke fietsbenen brengen mij zoveel vreugde. Onze gedeelde passie voor sport, de bergen, avontuur en kamperen maakt het leven zoveel mooier. Ik kan mij geen betere vader voor Tibo indenken en kijk er enorm naar uit om samen met onze kleine alle toekomstige ervaringen en avonturen te delen en te beleven.



## About the author

Saskia Hullegie was born on August 23<sup>rd</sup> 1991 in Enschede, the Netherlands. In 2009 she started her study medicine at the University Medical Centre Utrecht. During her research internships at the Julius Center for Health Sciences and Primary Care, Utrecht and at the Oxford University's Nuffield Department of Primary Care Health Sciences her interest in scientific research grew. After obtaining her medical degree in 2016, Saskia worked as an internal resident at Gelre Hospital in Apeldoorn. In 2017, she started her PhD trajectory about the management of acute otitis media and ear discharge in children under supervision of prof. dr. R.A.M.J. Damoiseaux, prof. dr. A.G.M. Schilder, dr. R.P. Venekamp and dr. T.M.A. van Dongen. She combined her PhD research project with the General Practice Training in Utrecht and the Postgraduate Master of Epidemiology at the Utrecht University. She obtained her Master of Epidemiology degree in 2020. From 2019 until 2022 she was a member of the Scientific Workgroup of the Dutch National Organisation of General Practitioners in Training (Landelijke Organisatie Van Aspirant Huisartsen, LOVAH). Furthermore, she acquired her University Teaching Qualification (BKO) in 2022. Currently, she is completing her General Practice Training. In the future she aims to combine working as a General Practitioner with research and education.

