PLOS | ONE

RESEARCH ARTICLE

Predominant Bacteria Detected from the Middle Ear Fluid of Children Experiencing Otitis Media: A Systematic Review

Chinh C. Ngo[1,2], Helen M. Massa[1,2]*, Ruth B. Thornton[3,4], Allan W. Cripps[1,2]

1 School of Medical Science, Griffith University, Gold Coast, Queensland, Australia, **2** Molecular Basis of Disease, Menzies Health Institute Queensland, Griffith University, Gold Coast, Queensland, Australia, **3** School of Paediatrics and Child Health, University of Western Australia, Perth, Western Australia, Australia, **4** Telethon Kids Institute, University of Western Australia, Perth, Western Australia, Australia

* h.massa@griffith.edu.au

CrossMark
click for updates

OPEN ACCESS

Citation: Ngo CC, Massa HM, Thornton RB, Cripps AW (2016) Predominant Bacteria Detected from the Middle Ear Fluid of Children Experiencing Otitis Media: A Systematic Review. PLoS ONE 11(3): e0150949. doi:10.1371/journal.pone.0150949

Editor: Sean Reid, Ross University School of Medicine, DOMINICA

Received: August 9, 2015

Accepted: February 22, 2016

Published: March 8, 2016

Copyright: © 2016 Ngo et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper, its references and its Supporting Information files.

Funding: The authors received no specific funding for this work. CNN acknowledges the Vietnam International Education Development, The Ministry of Education and Training (VIED-MOET) for a doctoral scholarship. RBT acknowledges the BrightSpark Foundation, Perth, Western Australia for a post-doctoral fellowship. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background

Otitis media (OM) is amongst the most common childhood diseases and is associated with multiple microbial pathogens within the middle ear. Global and temporal monitoring of predominant bacterial pathogens is important to inform new treatment strategies, vaccine development and to monitor the impact of vaccine implementation to improve progress toward global OM prevention.

Methods

A systematic review of published reports of microbiology of acute otitis media (AOM) and otitis media with effusion (OME) from January, 1970 to August 2014, was performed using PubMed databases.

Results

This review confirmed that *Streptococcus pneumoniae* and *Haemophilus influenzae*, remain the predominant bacterial pathogens, with *S*. *pneumoniae* the predominant bacterium in the majority reports from AOM patients. In contrast, *H*. *influenzae* was the predominant bacterium for patients experiencing chronic OME, recurrent AOM and AOM with treatment failure. This result was consistent, even where improved detection sensitivity from the use of polymerase chain reaction (PCR) rather than bacterial culture was conducted. On average, PCR analyses increased the frequency of detection of *S*. *pneumoniae* and *H*. *influenzae* 3.2 fold compared to culture, whilst *Moraxella catarrhalis* was 4.5 times more frequently identified by PCR. Molecular methods can also improve monitoring of regional changes in the serotypes and identification frequency of *S*. *pneumoniae* and *H*. *influenzae* over time or after vaccine implementation, such as after introduction of the 7-valent pneumococcal conjugate vaccine.

Competing Interests: AWC received a research grant from Danisco A/S on the use of probiotics for respiratory illness and a research grant and consultancy from Probiotec Pty Ltd on the use if nutritional supplements for immune health. The others authors declare that they have no competing interests. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Conclusions

Globally, *S. pneumoniae and H. influenzae* remain the predominant otopathogens associated with OM as identified through bacterial culture; however, molecular methods continue to improve the frequency and accuracy of detection of individual serotypes. Ongoing monitoring with appropriate detection methods for OM pathogens can support development of improved vaccines to provide protection from the complex combination of otopathogens within the middle ear, ultimately aiming to reduce the risk of chronic and recurrent OM in vulnerable populations.

Introduction

Otitis media, may be simply defined as inflammation of the middle ear, and is the most common reason a child under the age of 5 will visit their general practitioner and be prescribed antibiotics in socioeconomically developed countries [1]. OM has a range of clinical presentations including AOM, which is characterised by the rapid onset of local and systemic symptoms, including otalgia, fever, vomiting and accumulation of fluid in the middle ear cavity and OME, where the child experiences MEF accumulation without the systemic symptoms. Both of these presentations may occur recurrently or chronically [2]. Globally, more than 700 million cases of AOM are diagnosed each year, with 50% of affected children being under five years of age [3]. Recurrent AOM (RAOM) occurs where a patient has 3 diagnosed AOM episodes within six months or more than 4 episodes in 12 months [4] and is commonly observed in up to 65% of children by 5 years of age[4] OME typically resolves spontaneously within 3 months [5] however, 30–40% of children experience persistent or chronic fluid in the middle ear for more than 3 months (chronic OME, COME) and may require surgical intervention to aid resolution [5].

Irrespective of clinical presentation, OM is a multi-factorial disease, with many associated risk factors, including environmental, immunological deficiency, gender, age and microbial exposure [4, 6]. Despite this complexity, bacterial and viral pathogens, individually and together, are strongly associated with OM development, for example, only 4% children diagnosed with AOM had no bacterial or viral pathogen detected using culture and PCR [7].

The three bacteria most frequently identified in association with OM are: *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* [6], whilst the viruses most commonly associated with OM are respiratory syncytial virus, adenovirus, rhinovirus and coronavirus [6].

Monitoring both the identification and frequency of detection of common otopathogens in OM is central to evaluation of the effects of treatment and impact of vaccination programs. For example, pneumococcal carriage was reduced in association with the introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) in the US and has also increased prevention of early AOM infections [8].

Implementation of PCV7 into national immunisation programs (NIPs) has also resulted in shifts to non-vaccine pneumococcal serotypes isolated from invasive pneumococcal disease and OM [9–12]. Importantly, PCV7 implementation has resulted in *non-typeable (non-encapsulated) H. influenzae* (NTHi) detection frequency surpassing *S. pneumoniae* detection in AOM patients within a number of countries including the US [12], Spain [13] and France [14]. The emergence of non-vaccine serotypes and their potential role as pathogens in OM is an area of ongoing research interest.

This systematic review examines the frequency of detection of bacteria within MEF from children experiencing OM, globally. Ongoing surveillance of bacteria predominant in OM patients, improved detection methods such as PCR and monitoring the impact of immunisations will continue to inform treatment decisions and ultimately vaccine development aimed to prevent OM development in young children.

Methods

Data sources and searches

The design and construction of this systematic review was informed and in compliance with PRISMA (2009) [15] (see S1 PRISMA Checklist). Published literature, including conference abstracts, was searched via PubMed database and only original articles with an English abstract were retrieved and reviewed. Publication dates were restricted from January 1st, 1970 to August 30th, 2014, with five different search strategies used, as detailed below.

PubMed search strategy

The following terms were used in five literature searches:

- Search 1—Asian region: (otitis media (Asia (aetiology, otopathogens, pathogens, microbiology, bacteriology, bacteria))) or (otitis media (Bangladesh, Brunei, Cambodia, China, India, Indonesia, Iran, Israel, Japan, Korea, Laos, Lebanon, Malaysia, Pakistan, Philippines, Saudi Arabia, Singapore, Taiwan, Thailand, Turkey, Vietnam)) (S1 Fig)
- Search 2—American region: (otitis media (America (aetiology, otopathogens, pathogens, microbiology, bacteriology, bacteria))) or (otitis media (Argentina, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominica Republic, Ecuador, Honduras, Mexico, Panama, Paraguay, Uruguay, Venezuela, Canada, The US)) (S2 Fig)
- Search 3—African region: (otitis media (Africa (aetiology, otopathogens, pathogens, microbiology, bacteriology, bacteria))) or (otitis media (Algeria, Cameroon, Cote D'lvoire, Egypt, Mozambique, Namibia, Nigeria, South Africa, Zambia, Zimbabwe)) (S3 Fig)
- Search 4—European region: (otitis media (Europe (aetiology, otopathogens, pathogens, microbiology, bacteriology, bacteria))) or (otitis media (Britain, Denmark, England, France, Finland, Germany, Greece, Ireland, Italy, Netherlands, Norway, Poland, Portugal, Romania, Russia, Scotland, Spain, Sweden, Ukraine, Wales, The UK)) (S4 Fig)
- Search 5—Oceanian region: (otitis media (Oceania (aetiology, otopathogens, pathogens, microbiology, bacteriology, bacteria))) or (otitis media (Australia, Cook Islands, New Zealand, Papua New Guinea, Solomon Islands)) (S5 Fig)

Selection criteria

All articles in English were assessed for relevance by review of abstracts when available, prior to full review of each article. Pre-determined inclusion selection criteria were used: (i) only human studies (ii) age range one month– 18 years (majority of reported study populations below 8 years old) (iii) otopathogenic bacteria were identified from MEF samples of children experiencing a range of presentations of OM, including AOM/RAOM/AOMTF or OME/COME but not chronic suppurative OM (CSOM) (iv) MEF samples were collected through clinical perforation of the tympanic membrane (myringotomy or tympanocentesis) only, rather than spontaneous perforation.

Study selection and data extraction

All articles meeting the keyword criteria, identified in the primary database search, were screened independently for relevancy by two of the authors (C.C.N. and H.M.M) who read all article titles and abstracts in a standardised manner. Abstracts and articles were reviewed and compared to the inclusion criteria above and key data evaluating the reported details of the study population (age), types of OM, sample size, sample collection methods, microbiological method and years of study were recorded for included studies. Furthermore, where the detail of the sample collection method or clinical criteria for diagnosis of type of OM was inconclusive, the full paper was retrieved and reviewed to determine inclusion. Discussion between reviewers resolved any disagreement regarding inclusion. Additional information was sought from the authors of included reviews, via emailed request seeking additional unpublished information regarding vaccination status and/or serotyping data, if available for the study populations, or to clarify information, such as sampling methods used in their published study. All bibliographies of the included studies were manually searched for additional relevant references.

Data items, synthesis and analysis

Data illustrating the frequency of detection of predominant bacteria from each report were collated and recorded. The antibiotic sensitivity of these bacteria and the vaccination status of the participants were recorded, where available. Data tables within this narrative report were compiled from the primary results from each included report. Meta-analysis was not performed due to variation of the designs of the included studies and study population characteristics within the published reports.

Evaluation of study quality

In this study, included studies were previously published in peer reviewed journals. The variety of individual study designs prevented use of a validated instrument to assess the quality of included studies. The assessment of risk of bias of individual studies was performed at the study level by the use of inclusion criteria. For example, clinical criteria describing the varied presentations of OM may have varied over the thirty years of published literature reviewed. Reviewing authors ensured that all published studies clearly differentiated acute AOM by the presence of otalgia and inflamed and/or bulging tympanic membrane which remain consistent characteristics within current definitions. Where vaccination status of the study population is unknown, comparison of the study dates versus the implementation of the NIPs was used to inform potential vaccination status. Only studies that sampled the MEF after clinical perforation of the tympanic membrane were included in this report. This criterion is likely to have generated selection bias on the basis of MEF collection being performed primarily for research purposes, rather than clinical or treatment reasons. The likely outcome would result in skewing representation to include more severe cases undergoing surgical treatment. In addition, there is a relative paucity of reports from some world regions.

Results

The search "otitis media and children" identified 9,617 articles via PubMed database search over the period January 1st, 1970 to August 30th, 2014. After performing the key word screening for each of the 5 regions, as illustrated in Fig 1, 10,483 articles were identified, screened for eligibility and 857 abstracts and 110 full publications were reviewed. Sixty-six articles met all the previously described criteria. The number of articles varied for each of the 5 regions, studies

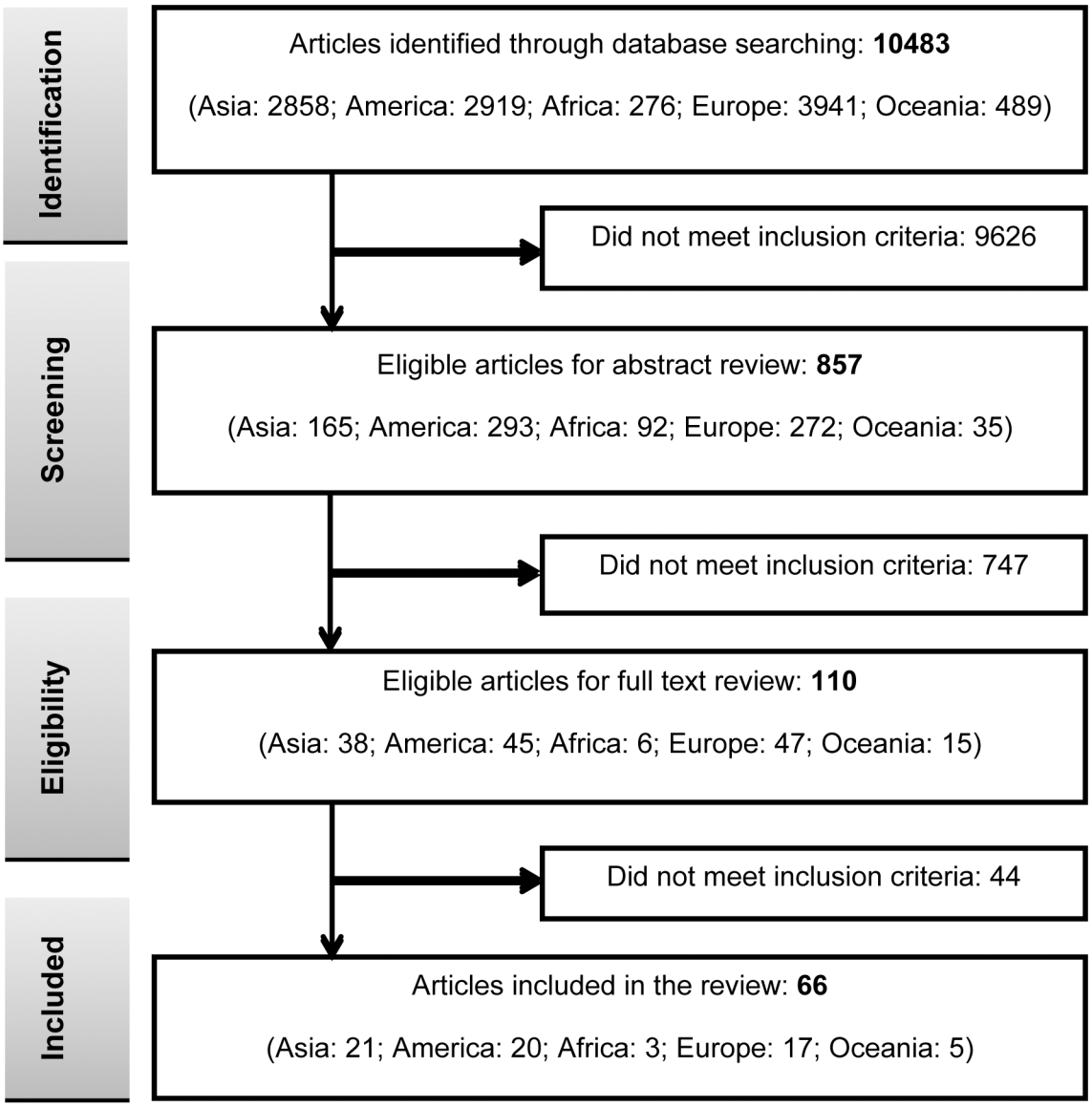

Fig 1. Literature search and selection.

doi:10.1371/journal.pone.0150949.g001

reporting from the Americas (n = 20), Europe (n = 17), Asia (n = 21), Africa (n = 3) and Oceania (n = 5) (Fig 1). Within the Americas, European and Asian regions, the reports analysed described 8 different countries within each region. In contrast, within the African and Oceania regions, reports were restricted to only two countries each; South Africa and Egypt and Australia and New Zealand, respectively.

Clinical variants of OM presentation included in this review are AOM (n = 38 studies; 58%), OME/COME (n = 24; 36%), RAOM/AOMTF (n = 9; 14%) and RAOM/OME (n = 1, 2%) within the included studies (n = 66). This percentage is greater than 100% due to 4 studies, in which, more than one type of OM was investigated [16–19]. All studies included in this review, reported microbiological findings from MEF collected via clinical perforation of the tympanic membrane.

Microbiology of AOM, RAOM/AOMTF and COME

Bacterial isolation overview. This systematic review includes 66 reports of bacterial isolation, using culture techniques, from MEF samples of patients experiencing AOM (n = 38, mean percentage of samples positive for bacteria 62%; range 25%–95%), RAOM/AOMTF (n = 12; mean percentage of samples positive for bacteria 45%, range 27%- 68%) and OME/COME (n = 24, mean percentage of samples positive for bacteria 36%; range 14%-73%) from across the world. Eight studies reported bacterial identification for more than one clinical presentation of OM. Bacterial isolation and identification were performed using PCR and bacterial culture in 17 reports, with only 2 reports from AOM patients [18, 20] rather than RAOM, OME or COME patients.

The wide variety of bacteria isolated in each of the reviewed studies, from patients with AOM, RAOM/AOMTF and OME/COME are shown in S1–S3 Tables, respectively. The frequency of detection of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* in these reports, identify them as the three predominant bacterial pathogens. Other bacteria isolated from the MEF included: *Streptococcus pyogenes* or Group A Streptococcus, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Chlamydia trachomatis*, *Alloiococcus otitidis*, *Klebsiella pneumoniae*, *Escherichia coli*. The variety of microbiota isolated and identified in these reports clearly varied with the experimental design, aims and methodology of each study and thus precludes direct comparisons of the frequency of individual pathogen detection. Thus, this review is focussed on the relative frequency of the predominant bacterial pathogens.

Previous reports for the different clinical presentations were collated and examined regionally. Identification of the three predominant bacteria within the MEF for children experiencing AOM, RAOM/AOMTF and OME/COME are presented in S1–S3 Tables, respectively. These tables summarise the published reports of bacteria isolated and identified from MEF using bacterial culture (S1–S3 Tables).

AOM. The bacterium most frequently identified from the MEF from children diagnosed with AOM, from previous reports across the world, was *S. pneumoniae* closely followed by *H. influenzae* (see Table 1). On average, *S. pneumoniae* detection was significantly higher than *H. influenzae* when all previous studies were pooled ($P<0.036$ 2-way ANOVA without replication) and these reports included samples collected between 1979 and 2010.

Regionally, in MEF from AOM patients, the average frequency of *S. pneumoniae* was higher than NTHi detection for all regions, although there was no significant difference between average detection frequencies for these bacteria in the US (27.8% NTHi versus 28.6% *S. pneumoniae*). In contrast, NTHi was the predominant bacteria, comparing average frequencies of bacterial detection for all 3 regions that reported RAOM/AOMTF; South America, Europe and Oceania. Two studies conducted in Costa Rica showed that *S. pneumoniae* was predominant in RAOM.

Within each world region, the trend toward more frequent detection of *S. pneumoniae*, that is, the average frequency of *S. pneumoniae* was higher than NTHi detection for all regions. Indeed, where multiple reports were available for the same country within a region, reports from a number of countries including Finland, Colombia, USA and Japan showed changes in the predominant bacteria identified. Multiple studies from the USA provide evidence of temporal trends in the detection frequency changing from *S. pneumoniae* in studies recruiting between 1989–1998 [21–23] to *H. influenzae* for similar studies recruiting between 2005–2009 [24, 25], then back to *S. pneumoniae* 2006–2010 [26] (Table 1).

For children experiencing AOM (Table 1), 29/38 of studies reported *S. pneumoniae* as the predominant bacteria compared to *H. influenzae* (primarily non-typeable) being clearly predominant bacteria in 6/38 studies (equivalent detection in 3 reports). The overall average

Table 1. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* detected from MEF of patients with AOM.

Countries/regions	OM	Age[a]	Size	Bacteria[b]			Ref.
				Hi	Spn	Mcat	
South America							
Argentina (1990–1992)	AOM	1m—11y	161	13.7%	20.5%	1.2%	[27]
Argentina (1996–1997)	AOM	15d—24m	367	24.5%	27.8%	0.5%	[28]
Brazil (1990–1995)	AOM	2m—5y	300	7.0%	16.0%	5.0%	[29]
Chile (1998–1999)	AOM	3m—9y	170	24.1%	37.1%	1.2%	[30]
Chile (1998–2002)	AOM	3m—9y	543	28.7%	39.8%	4.2%	[31]
Costa Rica (1992–1997)	AOM	4m—12y	398	14.1%	29.6%	1.5%	[32]
Costa Rica (1999–2001)	AOM	4m—12y	102	13.7%	42.2%	2.9%	[16]
Costa Rica (2002–2007)	AOM	2m—8y	880	23.0%	27.7%	8.8%	[17]
Colombia (1979–1985)	AOM	18d—11y	111	35.1%	23.4%	0.9%	[33]
Colombia (2008–2009)	AOM	3m—5y	83	28.9%	30.1%	ND[c]	[34]
Mexico (2008–2009)	AOM	3m—5y	99	31.3%	31.3%	2.0%	[35]
Venezuela (2008–2009)	AOM	3m—5y	82	41.5%	23.2%	1.2%	[36]
Average				**23.8%**	**29.1%**	**2.7%**	
Max				41.5%	42.2%	8.8%	
Min				7.0%	16.0%	0.5%	
North America							
The US (1989–1993)	AOM	2m—7y	815	23.3%	24.9%	15.0%	[21]
The US (1993–1995)	AOM	≤ 6y (86%)	159	28.3%	38.4%	25.8%	[22]
The US (1989–1998)	AOM	2m—7y	982	23.3%	25.8%	13.1%	[23]
The US (2005–2009)	AOM	2m—3y	184	31.5%	30.4%	7.6%	[24]
The US (2006–2008)	AOM	6m—3y	170	31.8%	20.6%	7.6%	[25]
The US (2008–2010)	AOM	4m—3y	208	28.4%	31.7%	13.9%	[26]
Average				**27.8%**	**28.6%**	**13.8%**	
Max				31.8%	38.4%	25.8%	
Min				23.3%	20.6%	7.6%	
Europe							
Spain (1989–1995)	AOM	1m—14y	104	28.8%	34.6%	1.0%	[37]
Finland (1980–1985)	AOM	≤ 3m	85	11.8%	18.8%	3.5%	[38]
Finland (1990–1992)	AOM	3m—8y	118	18.6%	22.0%	10.2%	[20]
Finland (1994–1995)	AOM	2m—2y	772	22.5%	26.0%	22.9%	[39]
Finland (1998–1999)	AOM	7m—6y	79	29.1%	49.4%	27.8%	[40]
Germany (2008–2010)	AOM	3m—5y	24	29.1%	ND	4.2%	[41]
Average				**23.3%**	**30.2%**	**11.6%**	
Max				29.1%	49.4%	27.8%	
Min				11.8%	18.8%	1.0%	
Asia							
Israel (1995–1996)	AOM	3m—3y	249	45.4%	37.8%	4.8%	[42]
Israel (1995–1999)	AOM	<2m	137	29.9%	40.9%	2.2%	[43]
Israel (1996–2003)	AOM	3m—3y	771	54.6%	35.9%	1.2%	[44]
Japan (1979–1980)	AOM	≤ 16y	406	32.0%	49.9%	ND	[45]
Japan (2001–2002)	AOM	1m—9y	81	7.4%	9.9%	7.4%	[46]
Japan (2003)	AOM	Children	138	21.7%	21.0%	3.6%	[47]
Japan (2002–2004)	AOM	≤ 10y	1092	20.8%	22.3%	4.4%	[48]
Japan (2006)	AOM	9m—8y	40	5.0%	12.5%	ND	[18]
Taiwan (2004)	AOM	4m—13y	96	13.5%	16.7%	ND	[49]

(*Continued*)

Table 1. (*Continued*)

Countries/regions	OM	Age[a]	Size	Bacteria[b]			Ref.
				Hi	Spn	Mcat	
Thailand (2008–2009)	AOM	3m—5y	107	17.8%	24.3%	6.5%	[50]
Turkey (1998–2000)	AOM	6m—10y	78	14.1%	23.1%	5.1%	[51]
Turkey (2002–2004)	AOM	6m—12y	180	13.3%	25.6%	10.0%	[52]
Turkey (2003–2004)	AOM	6m—12y	120	13.3%	23.3%	8.3%	[53]
Average				**22.2%**	**26.4%**	**5.4%**	
Max				54.6%	49.9%	10.0%	
Min				5.0%	9.9%	1.2%	
Africa							
South Africa (1999)	AOM	2m—7y	173	5.2%	20.20%	1.20%	[54]
Average (all regions)				**23.1%**	**27.8%**	**7.0%**	
Max (all regions)				54.6%	49.9%	27.8%	
Min (all regions)				5.0%	9.9%	0.5%	

[a]: d = day; m = month; y = year;

[b]: Hi: *H. influenzae*; Spn: *S. pneumoniae*; Mcat: *M. catarrhalis;*

[c] ND: Not detected

doi:10.1371/journal.pone.0150949.t001

Table 2. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* detected from MEF of patients with RAOM/AOMTF.

Countries	OM	Age[a]	Size	Bacteria[b]			Ref.
				Hi	Spn	Mcat	
South America							
Costa Rica (1999–2001)	RAOM	4m—11y	98	12.2%	32.7%	ND[c]	[16]
Costa Rica (1999–2001)	AOMTF	4m—11y	76	19.7%	17.1%	ND	[16]
Costa Rica (2002–2007)	RAOM	2m—8y	138	26.1%	29.0%	3.6%	[17]
Costa Rica (2002–2007)	AOMTF	2m—8y	90	24.4%	21.1%	4.4%	[17]
Europe							
France (2007–2009)	RAOM/ AOMTF	3m—3y	143[d]	31.5%	31.5%	1.4%	[55]
Netherlands (2008–2009)	RAOM	7m—6y	25	24.0%	ND	4.0%	[19]
Spain (2008–2010)	RAOM/ AOMTF	3m—3y	77	41.6%	16.9%	1.3%	[13]
Oceania							
Australia (2007–2009)	RAOM	7m—3y	143	13.3%	5.6%	6.3%	[56]
Australia (2007–2009)	RAOM	9m—3y	38	15.8%	7.9%	ND	[57]
New Zealand (2011)	RAOM/OME	3m—3y	325[e]	19.4%	8.0%	8.0%	[58]
Average (all regions)				22.8%	18.9%	4.1%	
Max (all regions)				41.6%	32.7%	8.0%	
Min (all regions)				12.2%	5.6%	1.3%	

[a] d = day; m = month; y = year;

[b] Hi: *H. influenzae*; Spn: *S. pneumoniae*; Mcat: *M. catarrhalis;*

[c] ND: Not detected;

[d] 18% from spontaneous otorrhoea;

[e] More than 60% of patients had RAOM

doi:10.1371/journal.pone.0150949.t002

frequency of detection for all studies was 27.8% (range 9.9%-49.9%) for *S. pneumoniae* compared to 23.1% (range 5.0%-54.6%) for *H. influenzae*, *M. catarrhalis* was detected in 34/38 studies at an average frequency of detection of 7.0% (range 0.5%–27.8%). Although detected less frequently overall, *M. catarrhalis* frequency, does not demonstrate a consistent temporal trend toward increasing detection in reports of AOM. Individual countries such as Finland, Costa Rica and Chile exhibit a temporal trend toward increasing rates of identification. In contrast, reports from the USA, Japan and Turkey show no consistent trend in identifications whilst reports from Israel do not support any temporal change in *M. catarrhalis* detection over time. Previous reports investigated chronic or longer term OM presentations such as RAOM/AOMTF (Table 2) or OME/COME (Table 3).

Examination of the RAOM/AOMTF reports (n = 7 and n = 3, respectively) did not demonstrate a predominant bacteria overall (Table 2) although the average bacterial detection frequency for *H. influenzae* was demonstrated as 22.8% (range 12.2%-41.6%) in comparison to 18.6%; (range 5.6%-32.7%) for *S. pneumoniae*. *M. catarrhalis* detection was very low (not detected in 3 of 10 reports) and average detection frequency from bacterial culture was 4.1% (range 1.3%-8%). There were no significant regional or temporal trends observed, due to the paucity of studies and the challenge arising from recruitment of overlapping clinical presentations (RAOM/AOMTF) within different study designs.

OME/COME. Overall, MEF samples from patients diagnosed with OME/COME were less likely to be culture positive for the 3 predominant bacteria in comparison to MEF samples from patients diagnosed with AOM. This is evidenced by the average frequency of detection for *H. influenzae* and *S. pneumoniae* from OME/COME patients being approximately half that reported from AOM patients (11.6% vs 23% and 6.5% vs 27%) for these bacteria respectively.

Globally, *H. influenzae* was the predominant bacteria identified within the MEF of patients experiencing OME/COME ($P<0.001$ 2-way ANOVA without replication) with the average detection frequency was 11.6% (range 3.2%-21.6%) compared to *S. pneumoniae* detection 6.5% (range 1.3%-16.2%).

Regionally, *H. influenzae* was identified most frequently from patients with OME/COME in South and North America, Europe, Asia, Africa and Oceania however in the Asian and European regions, four of the nine (44%) and two of nine (22%) of studies reported that *S. pneumoniae* was predominant, respectively.

Within this review, the bacterial pathogens associated with OM in the MEF were examined regionally and evidence of development of antibiotic resistance recorded. Where resistance was reported, it was included in the summary for each region provided below.

Regional variation of *H. influenzae* strains and Antibiotic sensitivity

Overall, there is a general paucity of reports in which *H. influenzae* strains and antibiotic sensitivity have been reported from the MEF of OM patients. In general, the available reports highlight the high proportion of NTHi isolates compared to other *H. influenzae* strains identified from AOM patients across almost all world regions; the Americas, Europe, Asia and Oceania, with no evidence available from Africa. The proportion of *H. influenzae* isolates from AOM patients that were β-lactamase positive ranged less than 20% for most regions to over 83% in a report from Mexico [35] with no consistent patterns recognisable in regions where multiple reports were available. *H. influenzae* isolates from COME patients generally reported a proportion of β-lactamase positive strains. The majority of reports from across the world identified very high proportions of *M. catarrhalis* strains as β-lactamase positive.

In South America, AOM-associated *H. influenzae* strains isolated within South America are predominantly NTHi, ranging from 63% [36] to 100% [17, 35] and *H. influenzae* types a, b, c,

Table 3. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* detected from MEF of patients with OME/COME.

Countries/regions	OM	Age	Size	Bacteria*			Ref.
				Hi	Spn	Mcat	
Americas							
Brazil (2001–2002)	OME/COME	11m—10y	128	10.2%	6.2%	3.9%	[59]
The US (1995)	COME	9m—15y	97	21.6%	5.2%	5.2%	[60]
Average				**15.9%**	**5.7%**	**4.6%**	
Max				21.6%	6.2%	5.2%	
Min				10.2%	5.2%	3.9%	
Europe							
Finland (1981)	COME	5m—15y	110	3.6%	2.7%	0.9%	[61]
Wales (1986–1987)	COME	≤ 7y (67%)	259	12.4%	2.7%	0.4%	[62]
England (1989)	COME	< 10y (Mostly)	102	16.7%	3.9%	1.0%	[63]
Finland (1993–1994)	OME	5m—12y	165	8.5%	10.3%	6.7%	[64]
Finland (1993–1994)	OME/COME	< 12y	123	14.6%	11.4%	6.5%	[65]
Finland (1996–1997)	OME	1y—9y	67	9.0%	3.0%	9.0%	[66]
Spain (2007)	OME/COME	1y—12y	40	12.5%	2.5%	ND**	[67]
Netherlands (2008–2009)	COME	< 6y	94	19.1%	5.3%	9.6%	[19]
UK (2012)	COME	≤ 18y (83%)	62	3.2%	6.50%	4.8%	[68]
Average				**11.1%**	**5.4%**	**4.9%**	
Max				19.1%	11.4%	9.6%	
Min				3.2%	2.5%	0.4%	
Asia							
Turkey (1999)	COME	2y—14y	37	10.8%	16.2%	2.7%	[69]
Korea (2004–2008)	COME	2y—7y	289	ND	1.7%	ND	[70]
Korea (2000–2002)	COME	1y—11y	278	7.9%	1.4%	ND	[71]
Japan (2006)	COME	9m—8y	76	5.3%	1.3%	1.3%	[18]
Japan (1988)	OME	≤ 15y (73%)	613	5.7%	3.3%	0.5%	[72]
Iran (2007–2008)	COME	2y—13y	63	9.5%	15.9%	9.5%	[73]
Iran (2009–2010)	COME	1y—12y	63	4.8%	9.5%	9.5%	[74]
Lebanon (1996–1997)	OME	2y—10y	47	19.1%	ND	4.3%	[75]
Lebanon (2009–2010)	COME	< 13y	107	19.6%	8.4%	3.7%	[76]
Average				**10.3%**	**7.2%**	**4.5%**	
Max				19.6%	16.2%	9.5%	
Min				4.8%	1.3%	0.5%	
Africa							
Egypt (1993)	COME	15m—8y	104	18.3%	5.8%	8.7%	[77]
Egypt (2003–2008)	OME/COME	3y—10y	50	ND	12.0%	14.0%	[78]
Average				**18.3%**	**8.9%**	**11.4%**	
Max				18.3%	12.0%	14.0%	
Min				18.3%	5.8%	8.7%	
Oceania							
Australia (1995–2000)	COME	11m—10y	45	6.7%	ND	4.4%	[79]
New Zealand (1994)	COME	11m—8y	105	16.2%	8.6%	12.4%	[80]
Average				**11.5%**	**8.6%**	**8.4%**	
Max				16.2%	8.6%	12.4%	
Min				6.7%	8.6%	4.4%	
Average (all regions)				**11.60%**	**6.5%**	**5.7%**	

(*Continued*)

Table 3. (*Continued*)

Countries/regions	OM	Age	Size	Bacteria*			Ref.
				Hi	Spn	Mcat	
Max (all regions)				21.6%	16.2%	14.0%	
Min (all regions)				3.2%	1.3%	0.4%	

[a] d = day; m = month; y = year;

[b] Hi: *H. influenzae*; Spn: *S. pneumoniae*; Mcat: *M. catarrhalis;*

[c] ND: Not detected

doi:10.1371/journal.pone.0150949.t003

d and f which were identified in AOM patients in Chile [30] and Venezuela [36]. Less than 20% of all AOM-associated *H. influenzae* strains were β-lactamase positive [17, 28–30, 34, 36]. In contrast, a recent study from Mexico reported that 83% of AOM-associated *H. influenzae* strains were β-lactamase positive [35], whilst other reports demonstrated that 100% of AOM-associated *M. catarrhalis* strains were β-lactamase positive [17, 29].

In North America, all AOM-related *H. influenzae* strains reported were non-typeable [81], with 20%-50% of these strains being β-lactamase positive [24, 81]. Furthermore, 100% of *M. catarrhalis* strains isolated from AOM patients were β-lactamase positive [81, 82]. Recently, two studies have reported that *H. influenzae* was the bacteria detected most often in MEF from children with AOM since the introduction of pneumococcal vaccine into the NIP [24, 25].

In Europe, a recent study from Germany, reported 86% of AOM-related *H. influenzae* strains were non-typeable, with *H. influenzae* type b and f also being identified in low frequencies (<10%) [41]. In France, more than 15% of AOM-associated *H. influenzae* strains isolated were β-lactamase positive [14, 55] and 95% of AOM-related *M. catarrhalis* strains were β-lactamase positive [14].

β-lactamase positive strains of *H. influenzae* were reported for 12% and 40% of strains isolated from OME/COME patients in England [63] and Spain [67], respectively.

In Asia, more than 80% of *H. influenzae* strains associated with AOM in this region were non-typeable and *H. influenzae* type a and b were identified in low frequencies (<20%) [52, 53, 83, 84], except two studies in Thailand [50] and Taiwan [49] where type b was reported in 63% and 100% of *H. influenzae* strains. Less than 20% of isolated *H. influenzae* strains were β-lactamase positive [43, 52, 53, 83, 85]. In contrast, all OM-associated *M. catarrhalis* strains isolated in Israel [43, 83, 85] and over 50% of strains isolated in Turkey [52, 53] were β-lactamase positive. Furthermore, more than 50% of *H. influenzae* isolates from children with COME in Lebanon were β-lactamase-negative ampicillin-resistant strains [76], however these strains were not typed.

In Africa, there are no reports of *H. influenzae* type from AOM patients in the African region, however a single report from Egypt confirmed that more than 80% and 60% of COME-associated *H. influenzae* and *M. catarrhalis* strains isolated were β-lactamase positive, respectively [77].

In Oceania, In Oceania, *H. influenzae* isolates from RAOM patients in Australia were all non-typeable strains with 17% of these strains were β-lactamase positive [56]. Similarly, in New Zealand, 95% of OM-related *H. influenzae* strains were non-typeable [58] and a single report of COME-associated *H. influenzae* strains in New Zealand reported that 6% (1/17) were type b and 94% (16/17) were not type b [80].

Changing microbial pattern pre- and post-PCV7 introduction

PCV7 has been introduced into NIPs since the year 2000. For a number of regions, including South America, Asia and Africa, this vaccine has either not yet been introduced to the NIP or only been recently introduced. For example, PCV7 was introduced into Israel's NIP and South Africa's NIP in 2009 [86, 87] or was recommended for high risk groups in Mexico in 2006 and subsequently introduced in to Mexico's NIP in 2008 [35]. Furthermore, the introduction of PCV7 into NIP or high risk groups within individual countries in a region may also impact the aetiology of OM within that region.

The demonstrated impacts of PCV7 immunisation on the aetiology of OM, since its introduction in 2000 includes: reduction of PCV7 serotype detection in OM, replacement of PCV7 serotypes in AOM by non-vaccine serotypes, particularly serotypes 19A and 3 and changes in dominant otopathogens between *H. influenzae* and *S. pneumoniae*.

In South America, PCV7 was introduced partially in Costa Rica and Venezuela in 2004 [17, 36, 88] and Mexico in 2006 [35]. The vaccine was implemented in NIP of Costa Rica in 2009 [89] and Mexico in 2008 [35, 89].

The overall rates of pneumococcal OM were reduced in the South American region after PCV7 introduction, as did the ratio of *S. pneumoniae* and *H. influenzae* isolated from patients with OM. For example, in Costa Rica, the frequency of *S. pneumoniae* isolated from patients with OM decreased from 42% (1999–2001) to 28% (2002–2007) after PCV7 introduction in 2004, while the percentage of isolated *H. influenzae* increased from 14% (1999–2001) to 23% (2002–2007) [16, 17]. Similarly, in Mexico, the proportion of *S. pneumoniae* isolated from patients with OM dropped from 52% (2002–2003) to 31% (2008–2009) [35, 90].

Persistence of PCV7 serotypes was demonstrated in early follow up studies in Costa Rica and Mexico, within 3–4 years of PCV7 introduction, in which PCV7 serotypes were identified in 60% and 40% of pneumococcal isolates from patients with OM respectively in these countries [35, 91].

Emergence of non-vaccine serotypes was observed in both countries, with serotype 3 accounting for 10% of identified pneumococcal isolates in Costa Rica [88, 92] and serotypes 19A, 15B and 28A identified in 23%, 10% and 7% of serotypes from OM patients in Mexico, after PCV introduction [35]. In the longer term, 4 years after PCV7 introduction in Venezuela, the predominant pneumococcal serotype isolated from children with OM was serotype 19A (41% of identified serotypes), followed by other non-PCV7 serotypes such as 6A (9%), 11 (9%) and 15 (9%) [36]. Overall, the emergence of non-PCV7 serotypes was concurrent with an overall reduction in the frequency of pneumococcal isolation from children with OM.

In North America, PCV7 was first introduced in the United States to prevent invasive pneumococcal disease in 2000 and 93.6% of children aged from 19 to 35 months had received at least 3 doses of PCV7 [93].

After PCV7 introduction, the predominant pathogen identified in OM in the US changed from *S. pneumoniae* to *H. influenzae*. Prior to PCV7 introduction, *S. pneumoniae* was the most common bacteria isolated from patients with AOM [22, 23, 82] but 1–3 years following PCV implementation, *H. influenzae* emerged as the most common isolates from patients with OM [12, 24].

Changes in the *S. pneumoniae* serotypes causing AOM had been observed after PCV7 introduction in the United States. Before PCV introduction, vaccine serotypes accounted for more than 70% of pneumococcal strains isolated from middle ear samples of children with AOM [94]. One to two years post-vaccine introduction, these serotypes accounted for about 50% of OM-associated pneumococcal strains isolated from middle ear samples [94] and six to eight years later they were not observed/reported amongst pneumococcal strains isolated from MEF

samples of children with OM [12]. Serotype 19A was the predominant serotype and accounted for 35% of OM-related *S. pneumoniae* strains isolated MEF samples [12]. Other non-PCV7 serotypes of *S. pneumoniae*, such as 3, 6A, 6C, 15, 23B and 11, were identified with lower frequencies in MEF samples of OM children [12, 94]. In recent years, although the pneumococcal serotypes are non-PCV7, frequencies of *S. pneumoniae* and *H. influenzae* isolates are equivalent [12].

In Europe, a 2008–2009 survey of 32 countries reported that only 17 countries recommended universal PCV7 immunization in children. The remaining countries had only used the vaccine for groups at high risk of infection or not used the vaccine universally [95]. Impacts of the vaccine on OM aetiology and pneumococcal serotypes identified from OM patients were observed in countries where the vaccine was introduced.

Following PCV7 introduction, the dominant otopathogen changed from *S. pneumoniae* to *H. influenzae* in France and Greece. In France, prior to the introduction of PCV7, *S. pneumoniae* was the otopathogen most commonly identified accounting for 54% of isolates and followed by *H. influenzae* (34%), however 1–2 years following PCV7 introduction, *H. influenzae* became the dominant bacteria, identified within approximately 46% of isolates and followed by *S. pneumoniae* (45%) [14]. This change in pattern was evident until 3–4 years post PCV7 implementation, where *S. pneumoniae* was again the most commonly isolated specie with mostly non-PCV7 serotypes [14]. Similarly, in Greece, *S. pneumoniae* isolation from the middle ear samples of children with AOM decreased from 42% to 31% post PCV introduction, while *H. influenzae* increased slightly from 34% to 37% [96].

Introduction of the PCV7 vaccine resulted in changes in the pneumococcal serotypes isolated from patients with OM. In France, pre-PCV7 vaccine introduction (this period, prior to 2004, is identified by the criterion that the rate of complete vaccination in infants under 2 years of age was below 50%), PCV7 serotypes accounted for more than 50% of *S. pneumoniae* associated with OM but 1–4 years post-PCV7 introduction, this decreased to less than 30% [14]. Similarly, in Spain, the percentage of PCV7 serotypes dropped from more than 60% to below 10% of pneumococcal strains isolated from patients with OM [97–99]. Following PCV7 introduction, the frequency of the non-PCV7 serotype 19A, increased from 20% to 50% in patients with OM in France [14] and from 8% to 35% in Spain [98, 99].

In Asia, Singapore and Israel introduced PCV7 into their NIPs in 2009 [86, 100–102]. Studies from Israel reported that PCV7 serotypes accounted for approximately 50% of *S. pneumoniae* strains associated with OM prior to the vaccine introduction [102, 103]. No studies have been identified in this review, which report rates following PCV7 introduction on microbial aetiology of OM or from other countries in this region.

In Africa, PCV7 was introduced into the NIP of South Africa in 2009 [87] and Gambia and Rwanda in 2010 [104]. A single study from South Africa examined *S. pneumoniae* serotypes pre- PCV7 and showed that PCV7 serotypes accounted for more than 90% of OM-associated *S. pneumoniae* [54].

In Nigeria, a shift in the predominant bacteria amongst the three main otopathogens detected from MEF samples of patients with AOM was observed, although information on PCV7 introduction has not been reported. In the early 2000s, *S. pneumoniae* was reported as the predominant otopathogen, followed by *H. influenzae* and *M. catarrhalis* [105], however more recently, *H. influenzae* was reported as the predominant otopathogen, followed by *M catarrhalis* and *S. pneumoniae* [106].

In Oceania, PCV7 was licenced and recommended for Australian Aboriginal children under 2 years of age and other children at high risk of pneumococcal infection in 2001. This recommendation was extended to all children in Australia in 2005 [107]. Pneumococcal serotypes recovered from middle ear samples showed progressive change after PCV7

immunisation. In 2000–2003, pre-PCV7 introduction, vaccine serotypes accounted for approximately 70% of the pneumococci isolated from ear discharges (ear swabs) of Australian children below 15 years of age [108]. In 2007–2009, 2–4 years post-PCV7 inclusion in NIP, non-PCV7 serotypes replaced the vaccine serotypes being isolated from middle ear samples from non-Aboriginal children with OM, accounting for more than 80% of identified serotype, of these serotype 19A made up 40% [56].

In contrast to other regions, *H. influenzae* has always been reported as the most common otopathogen isolated from both Aboriginal and non-Aboriginal children with OM in Australia [56, 79, 109–111]. Thus, there has not been a shift between *H. influenzae* and *S. pneumoniae* as the predominant otopathogen observed pre- and post- PCV7 introduction in the country. Similar observations are reported for New Zealand, with *H. influenzae* identified as the most common bacteria isolated from children with OM (RAOM or OME/COME) before and after pneumococcal vaccine introduction (2008)[58, 80, 112].

Improvement of detection of existing and new otopathogens by PCR

The frequency of bacterial detection within MEF samples from children with OM have been compared using both culture and PCR. Across the world, 17 studies have reported bacterial identification, the majority (n = 15) examined MEF from patients with COME or RAOM, with 2 reports from AOM patients.

Overall, PCR detection methods improved the detection frequency of bacteria although the magnitude of the improvement varied between individual reports and ranged from no difference to 9-fold increase in bacterial detection. On average, the improvement in the frequency of detection for bacteria identified within the MEF of children with OM is 3.2 times higher using PCR compared to bacterial culture, for both *S. pneumoniae* and *H. influenzae*. The sensitivity of PCR techniques also improved *M. catarrhalis* detection by 4.5- fold, on average across all studies (Table 4).

The enhanced sensitivity of detection observed using PCR has resulted in detection of *M. catarrhalis* and *S. pneumoniae* in culture negative samples [18, 19, 71, 113].

PCR confirmation of the same predominant bacterium being identified within the same sample was observed in 10/17 reports however, in the 6 reports showing a different predominant bacteria, 4/6 changed to *M. catarrhalis* and 2/6 changed to *H. influenzae*. A predominant bacterium was not identified in one study. Where PCR identified a different predominant bacterium, there was no recognisable pattern compared to the culture result, with 3 changes from *H. influenzae* and 3 from *S. pneumoniae* as the predominant bacterium from the culture.

Globally, and consistent with the results from bacterial culture, PCR results confirmed overall that *H. influenzae* was the most frequently detected bacterium in the MEF of children experiencing COME/RAOM (11/15 studies). *M. catarrhalis* was predominant in 3/4 studies and all three bacteria were equally frequent in one study from Iran. The two studies that examined AOM patients MEF, identified *M. catarrhalis* most frequently, although *Alloiococcus otitidis* (*A. otitidis*) was equally or more frequently identified in these reports.

Regionally, from PCR results, *H. influenzae* was detected most frequently in the Americas, Asia and Oceania. In Europe, *H. influenzae* and *M. catarrhalis* were equally frequent (2/4 studies each).

PCR detection for *A. otitidis* from the MEF of OM patients, has been reported from a range of countries, including Turkey [114], Japan [18], Iran [74], Finland [20, 65, 66], Sweden [115], the United Kingdom [116] and the United States [25, 117]. Collectively, *A. otitidis* was detected in 20% to 60% of MEF samples from OM patients by PCR, but was largely undetected using culture (Table 4). Furthermore, *A. otitidis* was more frequently identified in patients with OME

Table 4. Comparison of otopathogen detection in MEF using culture and PCR.

Countries	OM	Culture					PCR					Ref.
		Bacteria	Spn	Hi	Mcat	Aot[a]	Bacteria	Spn	Hi	Mcat	Aot	
Brazil (2001–2002)	COME/ RAOM	20%	6%	10%	4%	-	62%	13%	39%	10%	-	[59]
US (1995)	COME	32%	5%	22%	5%	-	131%	30%	55%	46%	-	[60]
Finland (1990–1992)	AOM	51%	22%	19%	10%	ND[b]	58%	20%	11%	27%	25%	[20]
Finland (1993–1994)	COME	33%	11%	15%	7%	ND	131%	35%	33%	63%	20%	[65]
Finland (1996–1997)	OME	21%	3%	9%	9%	ND	76%	21%	18%	37%	46%	[66]
Netherlands (2008–2009)	RAOM	28%	ND	24%	4%	-	84%	13%	42%	29%	-	[19]
Netherlands (2008–2009)	COME	34%	5%	19%	10%	-	63%	7%	32%	24%	-	[19]
Iran (2007–2008)	COME	36%	16%	10%	10%	-	151%	19%	95%	37%	-	[73]
Iran (2009–2010)	COME	25%	10%	5%	10%	24%	32%	11%	11%	10%	40%	[74]
Korea (2000–2002)	COME	9%	1%	8%	ND	-	45%	5%	29%	11%	-	[71]
Turkey (1999)	COME	30%	16%	11%	3%	-	143%	57%	70%	16%	-	[69]
Japan (2005)	COME	10%	ND	5%	5%	-	48%	12%	23%	13%	-	[113]
Japan (2006)	OME	7%	1%	5%	1%	ND	27%	8%	12%	7%	61%	[18]
Japan (2006)	AOM	18%	13%	5%	ND	ND	41%	13%	8%	20%	50%	[18]
Australia (2007–2009)	RAOM	25%	6%	13%	6%	-	66%	6%	47%	13%	-	[56]
Egypt (2003–2008)	OME/ COME	26%	12%	-	14%	-	92%	48%	-	56%	-	[78]
New Zealand (2011)	RAOM/ OME	19%	8%	19%	8%	-	65%	23%	43%	39%	-	[58]
PCR vs culture average		**3.3**										
Hi (PCR vs culture) average		**3.2**										
Spn (PCR vs culture) average		**3.2**										
Mcat (PCR vs culture) average		**4.5**										

[a] Aot: *A. otitidis;*

[b] ND: Not detected

doi:10.1371/journal.pone.0150949.t004

compared to patients with AOM [18, 117] and, was the most commonly detected bacteria in the middle ear of patients with OME/COME, detected using PCR [18, 74, 117, 118]. The use of PCR methodologies has improved detection rates for organisms that are difficult to culture, for example, detection of *A. otitidis* within MEF samples from patients with OM requires more than 3 days in culture, thus PCR analysis of MEF samples has improved detection of this organism and has resulted in consideration of *A. otitidis* having a role in OM pathogenesis [58, 67, 74, 119].

Discussion

The frequency of bacterial pathogens of OM, identified within the middle ear fluid using primarily microbial culture, but also PCR, were reviewed over a range of clinical presentations, using reports published since 1970. This review focussed on pathogens actually identified within the MEF rather than extrapolation of upper respiratory tract pathogens coincident in the nasopharynx. Improved understanding of the polymicrobial nature of OM and its varied clinical presentations is informed by the identification of specific bacteria (and viruses) within the middle ear.

Overall, the frequency of bacterial isolation from MEF samples of patients with AOM was generally higher than that from other presentations including RAOM/AOMTF or OME/ COME. This finding is consistent with recognition of AOM as an acute, frequently bacterial caused infection, whilst OME and COME are considered as a consequence of a previous AOM

episode, or inflammation resulting from the presence of bacterial biofilm or intracellular infection within the middle ear epithelial cell [57]. COME and OME may also result from inflammatory and allergic conditions such as rhino-sinusitis [120].

This review confirms OM as a polymicrobial disease, with the same predominant microbes identified in MEF samples from patients with AOM, RAOM, AOMTF, OME and COME globally. Consistent with previous reports, the three bacteria most frequently identified in the MEF from culture and considered causal for OM were *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. In Latin American countries, *Streptococcus pyogenes (S. pyogenes)* was also frequently identified as a predominant bacterium, pathogenic for OM. Increasingly, A. otitidis has been identified within the MEF using PCR techniques, although this bacterium's role in OM pathogenesis is still under investigation [121, 122]. Culture based detection of *A. otitidis* within MEF samples from patients with OM requires more than 3 days in culture and thus this organism may not have been successfully identified and reported in previous studies [58, 67, 74, 119].

S. pneumoniae remains the most frequently detected bacteria from patients with AOM in Asia, Africa, America and Europe. No significant differences between the clinical manifestations of AOM, resulting from the different pneumococcal serotypes, were identified but in general, PCV7 serotypes are reported to cause more otalgia or ear ache than non-PCV7-serotypes [123]. Additionally, children with AOM caused by *S. pneumoniae* are reported to experience higher fever and more redness of tympanic membrane than those with AOM caused by *H. influenzae* or *M. catarrhalis* [124], thus increasing the likelihood of presentation for medical treatment. AOM associated with *S. pneumoniae* is less likely to resolve without treatment when compared to that caused by *M. catarrhalis* [125].

Overall, use of PCV7 has initially reduced the incidence of AOM caused by *S. pneumoniae* and altered the serotypes causal for AOM. Replacement of PCV7 serotypes by non-vaccine serotypes has been observed in MEF samples of children with OM in all countries where the vaccine has been used, with serotype 19A, a predominant serotype recovered from vaccinated children with OM. A new genotype of this serotype, isolated from children with AOM was resistant to all USA Food and Drug Administration approved antimicrobial drugs for AOM treatment in children [126]. Therefore, 13-valent pneumococcal conjugate vaccine (PCV13), which includes serotype 19A, may help to reduce the incidence of OM. Since 2010, PCV13 has been licensed and recommended for children in a number of developed countries such as the United States, the United Kingdom, Singapore and Australia [101, 127–129].

In contrast to AOM, *H. influenzae* is most frequently detected bacteria in the MEF of patients with RAOM or OME/COME globally and, with the exception of Australia, is the second most common pathogen in patients with AOM across the world. In Australia, *H. influenzae* is the predominant bacterium for AOM and OME for indigenous and non-indigenous children [110, 111, 130]. Bilateral AOM, eye symptoms, previous treatment with antibiotics, protracted and recurrent disease, are more likely for AOM *caused by H. influenzae* when compared with caused by other pathogens [131, 132].

In most studies included in this review, less than 50% of *H. influenzae* strains recovered from patients with OM were β-lactamase producers. β-lactamase is responsible for bacterial resistance to the β-lactam antibiotics, commonly used as the first line of treatment for OM, such as aminopenicillins, cephalosporins, cephamycins and carbapenems [133, 134]. Widespread use of these antibiotics for the treatment of OM has contributed to the development of antimicrobial resistance. β-lactamase-negative ampicillin-resistant (BLNAR) *H. influenzae* strains, have been isolated from the MEF of patients with OM in Mexico [35], Lebanon [76] and Japan [135]. The BLNAR strains identified from MEF in the latter two reports [76, 135] have been isolated elsewhere in the upper respiratory tract and reflect the most frequently detected mechanism of ampicillin resistance for *H. influenzae* across the world [136]. Although

not based on MEF samples, a recent report has described the changing frequency of identification of BLNAR *H. influenzae* isolates, both globally and regionally, and confirmed reductions in detection in Europe and Africa between 2004–2008 and 2009–2012 whilst the proportion of detections rose in other regions including Latin America and the Middle East [137].

In this review, NTHi strains were most frequently reported and these strains are unaffected by *H. influenzae* b conjugate vaccination alone (Hib) [138]. Increasingly, inclusion of *H. influenzae* antigens to improve the opportunity for vaccine development is under development [139].

PCV7 introduction has been associated increased identification of *H. influenzae* in patients with OM when compared with *S. pneumoniae*. *H. influenzae* has gradually replaced *S. pneumoniae* as the predominant otopathogen in children with AOM for 1–5 years following PCV7 introduction and, in the absence of an approved vaccine for NTHi prevention, this shift could create another challenge for treatment and prevention of OM.

Until recently, most NTHi vaccine research interest was focussed on the 10-valent pneumococcal NTHi protein D conjugate vaccine (PHiD-CV10), which contains 10 pneumococcal serotypes and protein D of *H. influenzae*. This vaccine is immunogenic against protein D and well tolerated in studies conducted in America, Europe, Asia, Africa [140–145] and Australia [129]. Originally considered a potential candidate to replace PCV7, a recent study from Australia demonstrated that 19% of NTHi strains isolated from the nasal cavity, nasopharynx and bronchoalveolar lavage fluid from asymptomatic carriage or children with bronchiectasis were missing the *hpd* genes encoding protein D of NTHi [146]. Further research is needed to trial a number of already identified NTHi vaccine candidates, with respect to the safety, immunogenicity and efficacy against OM [1]. *M. catarrhalis* has been recognised as an otopathogen since 1920s with significant increased detection occurring since the 1980's [147]. Clinically, *M. catarrhalis* alone appears to be the least virulent pathogen [148] and is often associated with the first episode of disease, a younger age of onset, lower rates of spontaneous tympanic membrane perforation [149] and increased likelihood of spontaneous resolution [125]. *M. catarrhalis* however, most frequently occurs as a polymicrobial infection [149]. In this review, where culture findings of predominant bacteria were identified as different to that identified by PCR methods, *M. catarrhalis* was the new predominant bacterium.Most studies report that more than 90% of *M. catarrhalis* strains isolated from patients with OM were β-lactamase producers, although lower rates of 40%-50% have been reported from Turkey and Nigeria [52, 53, 106]. Whilst several vaccine candidates have been identified for *M. catarrhalis*, development of a vaccine strategy for *M. catarrhalis* is at an early stage [150–152].

Otitis media is associated with other bacteria including *S. pyogenes* (Group A Streptococcus), the fourth ranked otopathogen [82] identified in this review. This bacterium is otopathogenic and more often identified in older children who experience low rates of fever and respiratory symptoms but, increased rates of spontaneous perforation and mastoiditis, compared with AOM due to other otopathogens [153]. It is speculated that the role of *S. pyogenes* in OM aetiology maybe under-recognised in some regions.

Increasingly, new organisms such as *A. otitidis*, *Turicella otitidis*, *Pseudomonas otitidis* and *Corynebacterium mucifaciens* have been detected in patients with OM, but their significance in OM pathogenesis remains unclear [154]. Of these organisms, *A. otitidis* is the most commonly identified in the MEF from patients with OME/COME [18, 117]. *A. otitidis* may be associated with a protracted clinical course, development of the mucoid MEF characteristic of OME [65] and was recently reported to induce inflammation within the middle ear cavity [122, 155] thus potentially contributing as a secondary pathogen in AOM with perforation [156].

In this review, we have reported on the global detection of bacterial otopathogens in the MEF of children experiencing OM. Although three bacteria, *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* dominate, geographical differences are emerging. These differences relate to

PCV use, antimicrobial treatment and potentially other local regional factors. This highlights the need for ongoing surveillance and reporting of the microbiology of OM globally, in order to better understand the pathogenesis of this disease and to guide development of appropriate intervention strategies.

Conclusions

Despite a paucity of comprehensive prospective studies examining the microbial aetiology of AOM/RAOM and OME/COME locally within the middle ear of children throughout the world, we conclude that *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*, have remained remarkably consistent over the past 40 years, as the predominant bacteria causal for OM. Depending on the region, and the detection method, *S. pneumoniae* and *H. influenzae* vie for predominance as the bacteria most frequently isolated from the middle ear fluid from children with OM. A significant environmental change, the introduction of PCV7 and ongoing monitoring of serotype replacement throughout many regions of the world, continues to provide evidence of the predominance of these two bacteria in children with OM. Importantly, to identify and address the evolving development of bacterial resistance, such as β-lactamase production and potential pathogenicity of prevalent microbes such as *S. pyogenes* and *A. otitidis* ongoing monitoring is essential.

Future research is essential, whether development of vaccines that include multiple otopathogens [157], bacterial and viral, or trialling the use of probiotic supplementation to the upper respiratory tract [158] for children at high risk of OM. Investigators should continue to aim to minimise the incidence or indeed prevent OM pathogenesis and its long term sequelae for vulnerable children across the world.

Supporting Information

S1 Fig. Strategies for searching studies on pathogens of OM in Asia.
(DOCX)

S2 Fig. Strategies for searching studies on pathogens of OM in the Americas.
(DOCX)

S3 Fig. Strategies for searching studies on pathogens of OM in Africa.
(DOCX)

S4 Fig. Strategies for searching studies on pathogens of OM in Europe.
(DOCX)

S5 Fig. Strategies for searching studies on pathogens of OM in Oceania.
(DOCX)

S1 PRISMA Checklist. PRISMA Checklist.
(DOCX)

S1 Table. Proportion of bacteria detected from MEF samples of patients with AOM.
(DOCX)

S2 Table. Proportion of bacteria detected from MEF samples of patients with RAOM/AOMTF.
(DOCX)

S3 Table. Proportion of bacteria detected from MEF samples of patients with OME/COME.
(DOCX)

Acknowledgments

Authors, who, upon email contact, assisted this review by providing clarification or additional information about their research.

Author Contributions

Conceived and designed the experiments: AWC HMM. Performed the experiments: CCN HMM. Analyzed the data: CCN HMM RBT AWC. Contributed reagents/materials/analysis tools: CCN HMM. Wrote the paper: CCN HMM RBT AWC.

References

1. Cripps AW, Kyd J. Bacterial otitis media: current vaccine development strategies. Immunol Cell Biol. 2003; 81(1):46–51. PMID: 12534945
2. Kong K, Coates HLC. Natural history, definitions, risk factors and burden of otitis media. Med J Aust. 2009; 191(9):s39–s43. PMID: 19883355
3. Monasta L, Ronfani L, Marchetti F, Montico M, Brumatti LV, Bavcar A, et al. Burden of Disease Caused by Otitis Media: Systematic Review and Global Estimates. PLos One. 2012; 7(4):1–12.
4. Rovers MM. The burden of otitis media. Vaccine. 2008; 26(suppl 7):G2–G4. doi: 10.1016/j.vaccine.2008.11.005 PMID: 19094933
5. Physicians AAoF. Otitis media with effusion. Pediatrics. 2004; 113:1412–29. PMID: 15121966
6. Massa HM, Cripps AW, Lehmann D. Otitis media: viruses, bacteria, biofilms and vaccines. Medical journal of Australia. 2009; 191(9):s44–s9. PMID: 19883356
7. Chonmaitree T, Ruohola A, Hendley JO. Presence of Viral Nucleic Acids in the Middle Ear Acute Otitis Media Pathogen or Bystander? Pediatr Infect Dis J. 2012; 31(4):325–30. doi: 10.1097/INF.0b013e318241afe4 PMID: 22173136
8. Weil-Olivier C, van der Linden M, de Schutter I, Dagan R, Mantovani L. Prevention of pneumococcal diseases in the post-seven valent vaccine era: a European perspective. BMC Infect Dis. 2012; 12 (207):1–12.
9. Mokaddas E, Albert MJ. Impact of pneumococcal conjugate vaccines on burden of invasive pneumococcal disease and serotype distribution of Streptococcus pneumoniae isolates: an overview from Kuwait. Vaccine. 2012; 30:G27–G40.
10. Isaacman DJ, McIntosh ED, Reinert RR. Burden of invasive pneumococcal disease and serotype distribution among Streptococcus pneumoniae isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. Int J Infect Dis. 2010; 14(3):197–209.
11. Hsu KK, Shea KM, Stevenson AE, Pelton SI. Changing serotypes causing childhood invasive pneumococcal disease: Massachusetts, 2001–2007. Pediatr Infect Dis J. 2010; 29(4):289–93. doi: 10.1097/INF.0b013e3181c15471 PMID: 19935447
12. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. The Pediatric Infectious Disease Journal. 2010; 29(4):304–9. doi: 10.1097/INF.0b013e3181c1bc48 PMID: 19935445
13. Pumarola F, Marès J, Losada I, Minguella I, Moraga F, Tarragó D, et al. Microbiology of bacteria causing recurrent acute otitis media (AOM) and AOM treatment failure in young children in Spain: shifting pathogens in the post-pneumococcal conjugate vaccination era. Int J Pediatr Otorhinolaryngol 2013; 77(8):1231–6. doi: 10.1016/j.ijporl.2013.04.002 PMID: 23746414
14. Dupont D, Mahjuob-Messai F, Francois M, Doit C, Mariani-Kurkdjian P, Bidet P, et al. Evolving microbiology of complicated acute otitis media before and after introduction of the pneumococcal conjugate vaccine in France. Diagnostic Microbiology and Infectious Disease. 2010; 68:89–92. doi: 10.1016/j.diagmicrobio.2010.04.012 PMID: 20727478
15. Moher D, Liberati A, Tetzlaff J, Altman D, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009; 6(7).
16. Arguedas A, Dagan R, Soley C, Loaiza C, Knudsen K, Porat N, et al. Microbiology of otitis media in Costa Rican children, 1999 through 2001. Pediatr Infect Dis J. 2003; 22(12):1063–8. PMID: 14688566
17. Aguilar L, Alvarado O, Soley C, Abdelnour A, Dagan R, Arguedas A. Microbiology of the middle ear fluid in Costa Rican children between 2002 and 2007. Int J Pediatr Otorhinolaryngol 2009; 73:1407–11. doi: 10.1016/j.ijporl.2009.07.005 PMID: 19683349

18. Harimaya A, Takada R, Hendolin PH, Fujii N, Ylikoski J, Himi T. High incidence of *Alloiococcus otitidis* in children with otitis media, despite treatment with antibiotics. J Clin Microbiol. 2006; 44(3):946–9. PMID: 16517881

19. Stol K, Verhaegh SJ, Graamans K, Engel JA, Sturm PD, Melchers WJ, et al. Microbial profiling does not differentiate between childhood recurrent acute otitis media and chronic otitis media with effusion. Int J Pediatr Otorhinolaryngol 2013; 77(4):488–93. doi: 10.1016/j.ijporl.2012.12.016 PMID: 23369612

20. Leskinen K, Hendolinb P, Virolainen-Julkunen A, Ylikoski J, Jeroa J. Alloiococcus otitidis in acute otitis media. International Journal of Pediatric Otorhinolaryngology. 2004; 68:51–6. PMID: 14687687

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

22. Rodriguez W, Schwartz R, Thorne M. Increasing incidence of penicillin- and ampicillin-resistant middle ear pathogens. Pediatr Infect Dis J. 1995; 14(12):1075–8. PMID: 8745021

23. Patel JA, Nguyen DT, Revai K, Chonmaitree T. Role of respiratory syncytial virus in acute otitis media: implications for vaccine development. Vaccine. 2007; 25(9):1683–9. PMID: 17156899

24. Grubb MS, Spaugh DC. Microbiology of acute otitis media, Puget Sound region, 2005–2009. Clinical Pediatrics. 2010; 49(8):727–30. doi: 10.1177/0009922810361365 PMID: 20185479

25. Kaur R, Adlowitz DG, Casey JR, Zeng M, Pichichero ME. Simultaneous assay for four bacterial species including Alloiococcus otitidis using multiplex-PCR in children with culture negative acute otitis media. Pediatr Infect Dis J. 2010; 29(8):741–5. doi: 10.1097/INF.0b013e3181d9e639 PMID: 20335823

26. Casey JR, Kaur R, Friedel VC, Pichichero ME. Acute otitis media otopathogens during 2008 to 2010 in Rochester, New York. Pediatr Infect Dis J 2013; 32(8):805–9. doi: 10.1097/INF.0b013e31828d9acc PMID: 23860479

27. MacLoughlin GJF, Barreto DG, Torre Cdl, Pinetta EA, Castillo Fd, Palma L. Cefpodoxime proxetil suspension compared with cefaclor suspension for treatment of acute otitis media in paediatric patients. Journal of Antimicrobial Chemotherapy. 1996; 37:565–73. PMID: 9182113

28. Commisso R, Romero-Orellano F, Montanaro PB, Romero-Moroni F, Romreo-Diaz R. Acute otitis media: bacteriology and bacterial resistance in 205 pediatric patients. International Journal of Pediatric Otorhinolaryngology. 2000; 56:23–31. PMID: 11074112

29. Sih TM. Acute otitis media in Brazilian children: analysis of microbiology and antimicrobial susceptibility. The Annals of Otology, Rhinology & Laryngology. 2001; 110(7):662–6.

30. Rosenblut A, Santolaya ME, Gonzalez P, Corbalan V, Avendano LF, Martinez MA, et al. Bacterial and viral etiology of acute otitis media in Chilean children. The Pediatric Infectious Disease Journal. 2001; 20(5):501–7. PMID: 11368107

31. Rosenblut A, Santolaya ME, Gonzalez P, Borel C, Cofre J. Penicillin resistance is not extrapolable to amoxicillin resistance in Streptococcus pneumoniae isolated from middle ear fluid in children with acute otitis media. The Annals of Otology, Rhinology & Laryngology. 2006; 115(3):186–90.

32. Arguedas A, Loaiza C, Perez A, Vargas F, Herrera M, Rodriguez G, et al. Microbiology of acute otitis media in Costa Rican children. Pediatr Infect Dis J. 1998; 17(8):680–9. PMID: 9726340

33. Trujillo H, Callejas R, Mejia GI, Castrillon L. Bacteriology of middle ear fluid specimens obtained by tympanocentesis from 111 Colombian children with acute otitis media. Pediatr Infect Dis J. 1989; 8(6):361–3. PMID: 2787494

34. Sierra A, Lopes P, Zapata MA, Vanegas B, Castrejon MM, DeAntonio R, et al. Non-typeable Haemophilus influenzae and Streptococcus pneumonia as primary causes of acute otitis media in colombian children: a prospective study. BMC Infectious Diseases. 2011; 11(4):1–11.

35. Parra MM, Aguilar GM, Echaniz-Aviles G, Rionda RG, Estrada MdLA, Cervantes Y, et al. Bacterial etiology and serotypes of acute otitis media in Mexican children. Vaccine. 2011; 29:5544–9. doi: 10.1016/j.vaccine.2011.04.128 PMID: 21596081

36. Naranjo L, Suarez JA, DeAntonio R, Sanchez F, Calvo A, Spadola E, et al. Non-capsulated and capsulated Haemophilus influenzae in children with acute otitis media in Venezuela: a prospective epidemiological study. BMC Infectious Diseases. 2012; 12(40):1–10.

37. del Castillo F, Garcia-Perea A, Baquero-Artigao F. Bacteriology of acute otitis media in Spain: a prospective study based on tympanocentesis. Pediatr Infect Dis J. 1996; 15(6):541–3.

38. Karma PH, Pukander JS, Sipilä MM, Vesikari TH, Grönroos PW. Middle ear fluid bacteriology of acute otitis media in neonates and very young infants. Int J Pediatr Otorhinolaryngol. 1987; 14(2–3):141–50. PMID: 3125118

39. Kilpi T, Herva E, Kaijalainen T, Syrjanen R, Takala AK. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. Pediatr Infect Dis J. 2001; 20:654–62. PMID: 11465836

40. Ruohola A, Meurman O, Nikkari S, Skottman T, Salmi A, Waris M, et al. Microbiology of acute otitis media in children with tympanostomy tubes: prevalences of bacteria and viruses. Clin Infect Dis 2006; 43:1417–22. PMID: 17083014

41. Grevers G, Wiedemann S, Bohn J, Blasius R, Harder T, Kroeniger W, et al. Identification and characterization of the bacterial etiology of clinically problematic acute otitis media after tympanocentesis or spontaneous otorrhea in German children. BMC Infect Dis. 2012; 12(1):312.

42. Leibovitz E, Piglansky L, Raiz S, Greenberg D, Yagupsky P, Press J, et al. Bacteriologic efficacy of a three-day intramuscular ceftriaxone regimen in nonresponsive acute otitis media. Pediatr Infect Dis J. 1998; 17(12):1126–31. PMID: 9877360

43. Turner D, Leibovitz E, Aran A, Piglansky L, Raiz S, Leiberman A, et al. Acute otitis media in infants younger than two months of age: microbiology, clinical presentation and therapeutic approach. Pediatr Infect Dis J. 2002; 21:669–74. PMID: 12237601

44. Polachek A, Greenberg D, Lavi-Givon N, Broides A, Leiberman A, Dagan R, et al. Relationship among peripheral leukocyte counts, etiologic agents and clinical manifestations in acute otitis media. Pediatr Infect Dis J. 2004; 23(5):406–13. PMID: 15131462

45. Sugita R, Kawamura S, Ichikawa G, Goto S, Fujimaki Y. Studies of anaerobic bacteria in chronic otitis media. Laryngoscope. 1981; 91(5):816–21. PMID: 7231031

46. Sagai S, Suetake M, Yano H, Yoshida M, Ohyama K, Endo H, et al. Relationship between respiratory syncytial virus infection and acute otitis media in children. Auris Nasus Larynx 2004; 31(4):341–5. PMID: 15571905

47. Suzuki A, Watanabe O, Okamoto M, Endo H, Yano H, Suetake M, et al. Detection of Human Metapneumovirus From Children With Acute Otitis Media. The Pediatric Infectious Disease Journal. 2005; 24(7):665–7.

48. Yano H, Okitsu N, Hori T, Watanabe O, Kisu T, Hatagishi E, et al. Detection of respiratory viruses in nasopharyngeal secretions and middle ear fluid from children with acute otitis media. Acta Oto-Laryngologica. 2009; 129:19–24. doi: 10.1080/00016480802032777 PMID: 18607974

49. Tseng T-C, Chen L-C, Wang P-C, Huang C-H, Chen Y-H. Clinical report on the bacteriology of acute otitis media in childhood. FJJM. 2007; 5(1):1–8.

50. Intakorn P, Sonsuwan N, Noknu S, Moungthong G, Pircon J-Y, Dyke MKV, et al. Haemophilus influenzae type b as an important cause of culture-positive acute otitis media in young children in Thailand: a tympanocentesis-based, multi-center, cross-sectional study. BMC Pediatrics. 2014; 14(157):1–9.

51. Oğuz F, Unüvar E, Süoğlu Y, Erdamar B, Dündar G, Katircioğlu S, et al. Etiology of acute otitis media in childhood and evaluation of two different protocols of antibiotic therapy: 10 days cefaclor vs. 3 days azitromycin. Int J Pediatr Otorhinolaryngol. 2003; 67(1):43–51. PMID: 12560149

52. Guven M, Bulut Y, Sezer T, Aladag I, Eyibilen A, Etikan I. Bacterial etiology of acute otitis media and clinical efficacy of amoxicillin—clavulanate versus azithromycin. International Journal of Pediatric Otorhinolaryngology. 2006; 70(70):915–23.

53. Bulut Y, Guven M, Otlu B, Yenisehirli G, Aladag I, Eyibilen A, et al. Acute otitis media and respiratory viruses. Eur J Pediatr. 2007; 166:223–8. PMID: 16967296

54. Huebner RE, Wasas AD, Hockman M, Klugman KP. Bacterial aetiology of non-resolving otitis media in South African children. The journal of Laryngology & Otology. 2003; 117:169–72.

55. Couloigner V, Levy C, Francois M, Bidet P, Hausdorff WP, Pascal T, et al. Pathogens implicated in acute otitis media failures after 7-valent pneumococcal conjugate vaccine implementation in France: distribution, serotypes, and resistance levels. Pediatr Infect Dis J. 2012; 31:154–8. doi: 10.1097/INF.0b013e3182357c8d PMID: 21983212

56. Wiertsema SP, Kirkham LS, Corscadden KJ, Mowe EN, Bowman JM, Jacoby P, et al. Predominance of nontypeable *Haemophilus influenzae* in children with otitis media following introduction of a 3 + 0 pneumococcal conjugate vaccine schedule. Vaccine. 2011; 29:5163–70. doi: 10.1016/j.vaccine.2011.05.035 PMID: 21621576

57. Thornton RB, Wiertsema SP, Kirkham LS, Rigby PJ, Vijayasekaran S, Coates HL, et al. Neutrophil extracellular traps and bacterial biofilms in middle ear effusion of children with recurrent acute otitis media—a potential treatment target. PLoS One. 2013; 8:e53837. doi: 10.1371/journal.pone.0053837 PMID: 23393551

58. Mills N, Best E, Murdoch D, Souter M, Neeff M, Anderson T, et al. What is behind the ear drum? The microbiology of otitis media and the nasopharyngeal flora in children in the era of pneumococcal vaccination. J Paediatr Child Health. 2015; 51(3):300–6. doi: 10.1111/jpc.12710 PMID: 25175818

59. Pereira MBR, Pereira MR, Cantarelli V, Costa SS. Prevalence of bacteria in children with otitis media with effusion. Jornal de Pediatria. 2004; 80(1):41–8. PMID: 14978548

60. Post JC, Preston RA, Aul JJ, Larkins-Pettigrew M, Rydquist-White J, Anderson KW, et al. Molecular analysis of bacterial pathogens in otitis media with effusion. JAMA. 1995; 273(20):1598–604. PMID: 7745773

61. Sipilä P, Jokipii AM, Jokipii L, Karma P. Bacteria in the middle ear and ear canal of patients with secretory otitis media and with non-inflamed ears. Acta Otolaryngol. 1981; 92(1–2):123–30. PMID: 7315245

62. Sriwardhana KB, Howard AJ, Dunkin KT. Bacteriology of otitis media with effusion. J Laryngol Otol 1989; 103(3):253–6. PMID: 2495334

63. Diamond C, Sisson PR, Kearns AM, Ingham HR. Bacteriology of chronic otitis media with effusion. J Laryngol Otol. 1989; 103(4):369–71. PMID: 2497219

64. Jero J, Karma P. Bacteriological findings and persistence of middle ear effusion in otitis media with effusion. Acta Otolaryngol Suppl. 1997; 529:22–6. PMID: 9288259

65. Leskinen K, Hendolin P, Virolainen-Julkunen A, Ylikoski J, Jero J. The clinical role of Alloiococcus otitidis in otitis media with effusion. International Journal of Pediatric Otorhinolaryngology. 2002; 66:41–8. PMID: 12363421

66. Hendolin PH, Kärkkäinen U, Himi T, Markkanen A, Ylikoski J. High incidence of Alloiococcus otitis in otitis media with effusion. Pediatr Infect Dis J 1999; 18(10):860–5. PMID: 10530580

67. Martínez IM, Ramos MA, Masgoret PE. Bacterial implication in otitis media with effusion in the childhood. Acta Otorrinolaringol Esp. 2007; 58(9):408–12. PMID: 17999905

68. Daniel M, Imtiaz-Umer S, Fergie N, Birchall JP, Bayston R. Bacterial involvement in otitis media with effusion. Int J Pediatr Otorhinolaryngol. 2012; 76(10):1416–22. doi: 10.1016/j.ijporl.2012.06.013 PMID: 22819485

69. Gok U, Bulut Y, Keles E, Yalcin S, Doymaz MZ. Bacteriological and PCR analysis of clinical material aspirated from otitis media with effusions. Int J Pediatr Otorhinolaryngol. 2001; 60(1):49–54. PMID: 11434953

70. Jung H, Lee SK, Cha SH, Byun JY, Park MS, Yeo SG. Current bacteriology of chronic otitis media with effusion: high rate of nosocomial infection and decreased antibiotic sensitivity. J Infect 2009; 59 (5):308–16. doi: 10.1016/j.jinf.2009.08.013 PMID: 19715725

71. Park C-W, Han J-H, Jeong J-H, Cho S-H, Kang M-J, Tea K, et al. Detection rates of bacteria in chronic otitis media with effusion in children. J Korean Med Sci. 2004; 19:735–8. PMID: 15483353

72. Kurono Y, Tomonaga K, Mogi G. Staphylococcus epidermidis and Staphylococcus aureus in otitis media with effusion. Arch Otolaryngol Head Neck Surg 1988; 114(11):1262–5. PMID: 3262358

73. Shishegar M, Faramarzi A, Kazemi T, Bayat A, Motamedifar M. Polymerase chain reaction, bacteriologic detection and antibiogram of bacteria isolated from otitis media with effusion in children, shiraz, iran. Iran J Med Sci. 2011; 36(4):273–80. PMID: 23115412

74. Khoramrooz SS, Mirsalehian A, Emaneini M, Jabalameli F, Aligholi M, Saedi B, et al. Frequency of Alloicoccus otitidis, Streptococcus pneumoniae, Moraxella catarrhalis and Haemophilus influenzae in children with otitis media with effusion (OME) in Iranian patients. Auris Nasus Larynx. 2012; 39:369–73. doi: 10.1016/j.anl.2011.07.002 PMID: 21868180

75. Matar GM, Sidani N, Fayad M, Hadi U. Two-step PCR-based assay for identification of bacterial etiology of otitis media with effusion in infected Lebanese children. J Clin Microbiol. 1998; 36(5):1185–8. PMID: 9574673

76. Nasser SC, Moukarzel N, Nehme A, Haidar H, Kabbara B, Haddad A. Otitis media with effusion in lebanese children: prevalence and pathogen susceptibility. The journal of Laryngology & Otology. 2011; 125:928–33.

77. el-Shamy HA. Bacteriology of chronic secretory otitis media in children. J Egypt Public Health Assoc. 1993; 68:495–505. PMID: 7775877

78. Aly BH, Hamad MS, Mohey M, Amen S. Polymerase Chain Reaction (PCR) Versus Bacterial Culture in Detection of Organisms in Otitis Media with Effusion (OME) in Children. Indian J Otolaryngol Head Neck Surg. 2012; 64(1):51–5. doi: 10.1007/s12070-011-0161-6 PMID: 23449820

79. Stuart J, Butt H, Walker P. The microbiology of glue ear in Australian Aboriginal children. J Paediatr Child Health. 2003; 39(9):665–7. PMID: 14629496

80. Watson P, Voss L, Barber C, Aickin R, Bremner D, Lennon D. The microbiology of chronic otitis media with effusion in a group of Auckland children. N Z Med J. 1996; 109(1022):182–4. PMID: 8657383

81. Block SL, Hedrick J, Harrison CJ, Tyler R, Smith A, Findlay R, et al. Pneumococcal serotypes from acute otitis media in rural Kentucky. Pediatr Infect Dis J. 2002; 21:859–65. PMID: 12352810

82. Bluestone CD, Stephenson JS, Martin LM. Ten-year review of otitis media pathogens. Pediatr Infect Dis J. 1992; 11(8):s7–s11. PMID: 1513611

83. Dagan R, Johnson CE, McLinn S, Abughali N, Feris J, Leibovitz E, et al. Bacteriologic and clinical efficacy of amoxicillin/clavulanate vs. azithromycin in acute otitis media. Pediatr Infect Dis J. 2000; 19 (2):95–104. PMID: 10693993

84. Chiu NC, Lin HY, Hsu CH, Huang FY, Lee KS, Chi H. Epidemiological and microbiological characteristics of culture-proven acute otitis media in Taiwanese children. J Formos Med Assoc. 2012; 111 (10):536–41. doi: 10.1016/j.jfma.2011.07.015 PMID: 23089688

85. Leibovitz E, Piglansky L, Raiz S, Greenberg D, Hamed KA, Ledeine JM, et al. Bacteriologic and clinical efficacy of oral gatifloxacin for the treatment of recurrent/nonresponsive acute otitis media: an open label, noncomparative, double tympanocentesis study. Pediatr Infect Dis J 2003; 22(11):943–9. PMID: 14614364

86. Ben-Shimol S, Greenberg D, Givon-Lavi N, Elias N, Glikman D, Rubinstein U, et al. Rapid reduction in invasive pneumococcal disease after introduction of PCV7 into the National Immunization Plan in Israel. Vaccine. 2012; 30(46):6600–7. doi: 10.1016/j.vaccine.2012.08.012 PMID: 22939907

87. Jones SA, Groome M, Koen A, Van NN, Sewraj P, Kuwanda L, et al. Immunogenicity of seven-valent pneumococcal conjugate vaccine administered at 6, 14 and 40 weeks of age in South african infants. PLoS One. 2013; 8(8):e72794. doi: 10.1371/journal.pone.0072794 PMID: 24015277

88. Abdelnour A, Soley C, Guevara S, Porat N, Dagan R, Arguedas A. Streptococcus pneumoniae Serotype 3 among Costa Rican Children with Otitis Media: clinical, epidemiological characteristics and antimicrobial resistance patterns. BMC Pediatrics. 2009; 9(52):1–6.

89. Lee LH, Gu X, Nahm MH. Towards New Broader Spectrum Pneumococcal Vaccines: The Future of Pneumococcal Disease Prevention. Vaccines. 2014; 2:112–28. doi: 10.3390/vaccines2010112 PMID: 26344470

90. Gomez-Barreto D, Monteros LEEdl, Lopez-Enriquez C, Suarez RR, Torre Cdl. Streptococcus pneumoniae serotypes isolated from middle ear of Mexican children diagnosed with acute otitis media. salud pública de méxico. 2011; 53(3):207–11. PMID: 21829885

91. Guevara S, Abdelnour A, Soley C, Porat N, Dagan R, Arguedas A. Streptococcus pneumoniae serotypes isolated from the middle ear fluid of Costa Rican children following introduction of the heptavalent pneumococcal conjugate vaccine into a limited population. Vaccine. 2012; 30(26):3857–61. doi: 10.1016/j.vaccine.2012.04.010 PMID: 22521846

92. Arguedas A, Dagan R, Guevara S, Porat N, Soley C, Perez A, et al. Middle ear fluid Streptococcus pneumoniae serotype distribution in Costa Rican children with otitis media. The Pediatric Infectious Disease Journal. 2005; 24(7):631–4. PMID: 15999006

93. Centers for Disease Control and Prevention. Preventing Pneumococcal Disease Among Infants and Young Children. 2000.

94. McEllistrem MC, Adams JM, Patel K, Mendelsohn AB, Kaplan SL, Bradley JS, et al. Acute otitis media due to penicillin-nonsusceptible Streptococcus pneumoniae before and after the introduction of the pneumococcal conjugate vaccine. Clin Infect Dis. 2005; 40(12):1738–44. PMID: 15909260

95. Gomes HDC, Muscat M, Monnet DL, Giesecke J, Lopalco PL. Use of seven-valent pneumococcal conjugate vaccine (PCV7) in Europe, 2001–2007. EUROSURVEILLANCE. 2009; 14(12):1–6.

96. Stamboulidis K, Chatzaki D, Poulakou G, Ioannidou S, Lebessi E, Katsarolis I, et al. The impact of the heptavalent pneumococcal conjugate vaccine on the epidemiology of acute otitis media complicated by otorrhea. Pediatr Infect Dis J. 2011; 30:551–5. doi: 10.1097/INF.0b013e31821038d9 PMID: 21297521

97. Gene A, Garcia-Garcia JJ, Domingo A, Palacin PWyE. Etiology of acute otitis media in a children's hospital and antibiotic sensitivity of the bacteria involved. Enferm Infecc Microbiol Clin. 2004; 22 (7):377–80. PMID: 15355766

98. Fenoll A, Aguilar L, Vicioso M-D, Robledo O, Granizo J-J. Increase in serotype 19A prevalence and amoxicillin non-susceptibility among paediatric Streptococcus pneumoniae isolates from middle ear fluid in a passive laboratory-based surveillance in Spain, 1997–2009. BMC Infectious Diseases. 2011; 11(239):1–8.

99. Alonso M, Marimon JM, Ercibengoa M, Pérez-Yarza EG, Pérez-Trallero E. Dynamics of Streptococcus pneumoniae serotypes causing acute otitis media isolated from children with spontaneous middle-ear drainage over a 12-year period (1999–2010) in a region of northern Spain. PLoS One. 2013; 8 (1):e54333. doi: 10.1371/journal.pone.0054333 PMID: 23349853

100. Lu C-Y, Santosham M, Members A. Survey of national immunization programmes and vaccine coverage rates in Asia Pacific countries. Vaccine. 2012; 30:2250–5. doi: 10.1016/j.vaccine.2011.10.070 PMID: 22075085

101. Tyo KR, Rosen MM, Zeng W, Yap M, Pwee KH, Ang LW, et al. Cost-effectiveness of conjugate pneumococcal vaccination in Singapore: comparing estimates for 7-valent, 10-valent, and 13-valent vaccines. Vaccine. 2011; 29:6686–94. doi: 10.1016/j.vaccine.2011.06.091 PMID: 21745516

102. Porat N, Amit U, Givon-Lavi N, Leibovitz E, Dagan R. Increasing Importance of Multidrug-Resistant Serotype 6A Streptococcus pneumoniae Clones in Acute Otitis Media in Southern Israel. The Pediatric Infectious Disease Journal. 2010; 29(2):126–30. doi: 10.1097/INF.0b013e3181b78e6e PMID: 19927039

103. Somech I, Dagan R, Givon-Lavi N, Porat N, Raiz S, Leiberman A, et al. Distribution, dynamics and antibiotic resistance patterns of Streptococcus pneumoniae serotypes causing acute otitis media in children in southern Israel during the 10 year-period before the introduction of the 7-valent pneumococcal conjugate vaccine. Vaccine. 2011; 29(25):4202–9. doi: 10.1016/j.vaccine.2011.03.103 PMID: 21497634

104. Kim SY, Lee G, Goldie SJ. Economic evaluation of pneumococcal conjugate vaccination in The Gambia. BMC Infect Dis. 2010; 10(260):1–18.

105. Ako-Nai AK, Oluga FA, Onipede AO, Adejuyigbe EA, Amusa YB. The characterization of bacterial isolates from acute otitis media in Ile-Ife, southwestern Nigeria. Journal of Tropical Pediatrics. 2002; 48:15–23. PMID: 11866331

106. Akinjogunla OJ, Eghafona NO, Enabulele IO. Aetiologic Agents of Acute Otitis Media (AOM): Prevalence, Antibiotic Susceptibility, β-Lactamase (βL) and Extended Spectrum β-Lactamase (ESBL) Production. Journal of Microbiology, Biotechnology and Food Sciences. 2011; 1(3):333–53.

107. Giele C, Moore H, Bayley K, Harrison C, Murphy D, Rooney K, et al. Has the seven-valent pneumococcal conjugate vaccine had an impact on invasive pneumococcal disease in Western Australia? Vaccine. 2007; 25(13):2379–84. PMID: 17064825

108. Watson M, Brett M, Brown M, Stewart MG, Warren S, Network ftNSWP. Pneumococci responsible for invasive disease and discharging ears in children in Sydney, Australia. journal of medical microbiology. 2007; 56:819–23. PMID: 17510269

109. Dawson VM, Coelen RJ, Murphy S, Graham D, Dyer H, Sunderman J. Microbiology of chronic otitis media with effusion among Australian Aboriginal children: role of *Chlamydia trachomatis*. Aust J Exp Biol Med Sci. 1985; 63:99–107. PMID: 4040361

110. Gibney KB, Morris PS, Carapetis JR, Skull SA, Smith-Vaughan HS, Stubbs E, et al. The clinical course of acute otitis media in high-risk Australian Aboriginal children: a longitudinal study. BMC Pediatr. 2005; 5(16):1–8.

111. Leach AJ, Morris PS. The burden and outcome of respiratory tract infection in Australian and Aboriginal children. Pediatr Infect Dis J. 2007; 26(10):S4–S7. PMID: 18049380

112. Walls T, Best E, Murdoch D, Mills N. Vaccination to prevent otitis media in New Zealand. N Z Med J. 2011; 124(1340):6–9. PMID: 21952379

113. Hamamoto Y, Gotoh Y, Nakajo Y, Shimoya S, Kayama C, Hasegawa S, et al. Impact of antibiotics on pathogens associated with otitis media with effusion. J Laryngol Otol. 2005; 119(11):862–5. PMID: 16354337

114. Kalcioglu MT, Oncel S, Durmaz R, Otlu B, Miman MC, Ozturan O. Bacterial etiology of otitis media with effusion; focusing on the high positivity of Alloiococcus otitidis. New Microbiol. 2002; 25(1):31–5. PMID: 11837388

115. Neumark T, Ekblom M, Brudin L, Groth A, Eliasson I, Molstad S, et al. Spontaneously draining acute otitis media in children: An observational study of clinical findings, microbiology and clinical course. Scandinavian Journal of Infectious Diseases. 2011; 43:891–8. doi: 10.3109/00365548.2011.591820 PMID: 21736512

116. Beswick AJ, Lawley B, Fraise AP, Pahor AL, Brown NL. Detection of Alloiococcus otitis in mixed bacterial populations from middle-ear effusions of patients with otitis media. Lancet. 1999; 354 (9176):386–9. PMID: 10437868

117. Holder RC, Kirse DJ, Evans AK, Peters TR, Poehling KA, Swords WE, et al. One third of middle ear effusions from children undergoing tympanostomy tube placement had multiple bacterial pathogens. BMC Pediatrics. 2012; 12(87):1–7.

118. Güvenç MG, Midilli K, Inci E, Kuşkucu M, Tahamiler R, Ozergil E, et al. Lack of Chlamydophila pneumoniae and predominance of Alloiococcus otitidis in middle ear fluids of children with otitis media with effusion. Auris Nasus Larynx. 2010; 37(3):269–73. doi: 10.1016/j.anl.2009.09.002 PMID: 19879704

119. Ashhurst-Smith C, Hall ST, Walker P, Stuart J, Hansbro PM, Blackwell CC. Isolation of Alloiococcus otitidis from Indigenous and non-Indigenous Australian children with chronic otitis media with effusion. FEMS immunology and medical microbiology. 2007; 51(1):163–70. PMID: 17666076

120. Morris PS, Leach AJ. acute and chronic otitis media. Pediatr Clin N Am. 2009; 56:1383–99.

121. Ashhurst-Smith C, Hall S, Burns C, Stuart J, Blackwell C. Induction of inflammatory responses from THP-1 cells by cell-free filtrates from clinical isolates of Alloiococcus otitidis. Innate Immun. 2014; 20 (3).

122. Ashhurst-Smith C, Hall ST, Burns CJ, Stuart J, Blackwell CC. In vitro inflammatory responses elicited by isolates of Alloiococcus otitidis obtained from children with otitis media with effusion. Innate Immun. 2014; 3:Abstract.

123. Palmu AA, Jokinen JT, Kaijalainen T, Leinonen M, Karma P, Kilpi TM. Association of clinical signs and symptoms with pneumococcal acute otitis media by serotype—implications for vaccine effect. Clin Infect Dis. 2005; 40(1):52–7. PMID: 15614692

124. Rodriguez WJ, Schwartz RH. Streptococcus pneumoniae causes otitis media with higher fever and more redness of tympanic membranes than Haemophilus influenzae or Moraxella catarrhalis. Pediatr Infect Dis J. 1999; 18(10):942–4. PMID: 10530598

125. Courter JD, Baker WL, Nowak KS, Smogowicz LA, Desjardins LL, Coleman CI, et al. Increased clinical failures when treating acute otitis media with macrolides: a meta-analysis. Ann Pharmacother 2010; 44:471–8. doi: 10.1345/aph.1M344 PMID: 20150506

126. Pichichero ME, Casey JR. Emergence of a Multiresistant Serotype 19A Pneumococcal Strain Not Included in the 7-Valent Conjugate Vaccine as an Otopathogen in Children. JAMA. 2007; 298 (15):1772–8. PMID: 17940232

127. Nuorti JP, Whitney CG. Prevention of Pneumococcal Disease Among Infants and Children—Use of 13-Valent Pneumococcal Conjugate Vaccine and 23-Valent Pneumococcal Polysaccharide Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report (CDC). 2010; 59:1–18.

128. Jefferies JMC, Macdonald E, Faust SN, Clarke SC. 13-valent pneumococcal conjugate vaccine (PCV13). Human Vaccines. 2011; 7(10):1012–8. doi: 10.4161/hv.7.10.16794 PMID: 21941097

129. Newall AT, Creighton P, Philp DJ, Wood JG, MacIntyre CR. The potential cost-effectiveness of infant pneumococcal vaccines in Australia. Vaccine. 2011; 29:8077–85. doi: 10.1016/j.vaccine.2011.08.050 PMID: 21864617

130. Smith-Vaughan HC, Binks MJ, Marsh RL, Kaestli M, Ward L, Hare KM, et al. Dominance of Haemophilus influenzae in ear discharge from Indigenous Australian children with acute otitis media with tympanic membrane perforation. BMC Ear Nose Throat Disord. 2013; 13(1):1–9.

131. Palmu AA, Herva E, Savolainen H, Karma P, Mäkelä PH, Kilpi TM. Association of clinical signs and symptoms with bacterial findings in acute otitis media. Clin Infect Dis. 2004; 38(2):234–42. PMID: 14699456

132. Barkai G, Leibovitz E, Givon-Lavi N, Dagan R. Potential contribution by nontypable Haemophilus influenzae in protracted and recurrent acute otitis media. Pediatr Infect Dis J. 2009; 28:466–71. PMID: 19504729

133. Livermore DM. b-Lactamases in Laboratory and Clinical Resistance. CLINICAL MICROBIOLOGY REVIEWS. 1995; 8(4):557–84. PMID: 8665470

134. Wilke MS, Lovering AL, Strynadka NCJ. b-Lactam antibiotic resistance: a current structural perspective. Current Opinion in Microbiology. 2005; 8:525–33. PMID: 16129657

135. Yano H, Yamazaki Y, Qin L, Okitsu N, Yahara K, Irimada M, et al. Improvement rate of acute otitis media caused by Haemophilus influenzae at 1 week is significantly associated with time to recovery. J Clin Microbiol. 2013; 51(11):3542–6. doi: 10.1128/JCM.01108-13 PMID: 23966504

136. Tristram S, Jacobs M, Appelbaum P. Antimicrobial resistance in Haemophilus influenzae. Clin Microbiol Rev. 2007; 20(2):368–89. PMID: 17428889

137. Tomic V, Dowzicky M. Regional and global antimicrobial susceptibility among isolates of Streptococcus pneumoniae and Haemophilus influenzae collected as part of the Tigecycline Evaluation and Surveillance Trial (T.E.S.T.) from 2009 to 2012 and comparison with previous years of T.E.S.T. (2004–2008). Ann Clin Microbiol Antimicrob 2014; 13(52):1–8.

138. Agrawal A, Murphy TF. Haemophilus influenzae Infections in the H. influenzae Type b Conjugate Vaccine Era. JOURNAL OF CLINICAL MICROBIOLOGY. 2011; 49(11):3728–32. doi: 10.1128/JCM.05476-11 PMID: 21900515

139. Murphy TF. Vaccine development for non-typeable Haemophilus influenzae and Moraxella catarrhalis: progress and challenges. Expert Rev Vaccines. 2005; 4(6):843–53. PMID: 16372880

140. Ruiz-Palacios GM, Guerrero ML, Hernández-Delgado L, Lavalle-Villalobos A, Casas-Muñoz A, Cervantes-Apolinar Y, et al. Immunogenicity, reactogenicity and safety of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in Mexican infants. Hum Vaccin. 2011; 7(11):1137–45. doi: 10.4161/hv.7.11.17984 PMID: 22048109

141. Lagos RE, Muñoz AE, Levine MM, Lepetic A, François N, Yarzabal JP, et al. Safety and immunogenicity of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in Chilean children. Hum Vaccin. 2011; 7(5):511–22. PMID: 21441782

142. Dicko A, Santara G, Mahamar A, Sidibe Y, Barry A, Dicko Y, et al. Safety, reactogenicity and immunogenicity of a booster dose of the 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) in Malian children. Hum Vaccin Immunother. 2013; 9(2):382–8. PMID: 23291945

143. van den Bergh MR, Spijkerman J, François N, Swinnen K, Borys D, Schuerman L, et al. Immunogenicity, safety, and reactogenicity of the 10-valent pneumococcal nontypeable Haemophilus influenzae protein D conjugate vaccine and DTPa-IPV-Hib when coadministered as a 3-dose primary vaccination schedule in The Netherlands: a randomized controlled trial. Pediatr Infect Dis J 2011; 30(9):e179–e8.

144. Kim CH, Kim JS, Cha SH, Kim KN, Kim JD, Lee KY, et al. Response to primary and booster vaccination with 10-valent pneumococcal nontypeable Haemophilus influenzae protein D conjugate vaccine in Korean infants. Pediatr Infect Dis J. 2011; 30(12):e235–e43. doi: 10.1097/INF.0b013e31822a8541 PMID: 21817957

145. Lim FS, Koh MT, Tan KK, Chan PC, Chong CY, Shung YYW, et al. A randomised trial to evaluate the immunogenicity, reactogenicity, and safety of the 10-valent pneumococcal non-typeable Haemophilus influenzae protein D conjugate vaccine (PHiD-CV) co-administered with routine childhood vaccines in Singapore and Malaysia. BMC Infect Dis. 2014; 14(530):1–13.

146. Smith-Vaughan HC, Chang AB, Sarovich DS, Marsh RL, Grimwood K, Leach AJ, et al. Absence of an important vaccine and diagnostic target in carriage- and disease-related nontypeable Haemophilus influenzae. Clin Vaccine Immunol. 2014; 21(2):250–2. doi: 10.1128/CVI.00632-13 PMID: 24285816

147. Verduin CM, Hol C, Fleer A, Dijk Hv, van Belkum A. Moraxella catarrhalis: from Emerging to Established Pathogen. CLINICAL MICROBIOLOGY REVIEWS. 2002; 15(1):125–44. PMID: 11781271

148. Coffey JDJ, Martin AD, Booth HN. Neisseria catarrhalis in exudate otitis media. Arch Otolaryngol. 1967; 86(4):403–6. PMID: 6041112

149. Broides A, Dagan R, Greenberg D, Givon-Lavi N, Leibovitz E. Acute otitis media caused by Moraxella catarrhalis: epidemiologic and clinical characteristics. Clin Infect Dis 2009; 49:1641–7. doi: 10.1086/647933 PMID: 19886799

150. Tan TT, Riesbeck K. Current progress of adhesins as vaccine candidates for Moraxella catarrhalis. Expert Rev Vaccines. 2007; 6(6):949–56. doi: 10.1586/14760584.6.6.949 PMID: 18377357

151. Mawas F, Ho MM, Corbel MJ. Current progress with Moraxella catarrhalis antigens as vaccine candidates. Expert Rev Vaccines. 2009; 8(1):77–90. doi: 10.1586/14760584.8.1.77 PMID: 19093775

152. Smidt M, Bättig P, Verhaegh SJ, Niebisch A, Hanner M, Selak S, et al. Comprehensive antigen screening identifies Moraxella catarrhalis proteins that induce protection in a mouse pulmonary clearance model. PLoS One. 2013; 8(5):1–15.

153. Segal N, Givon-Lavi N, Leibovitz E, Yagupsky P, Leiberman A, Dagan R. Acute Otitis Media Caused by Streptococcus pyogenes in Children. Clinical Infectious Diseases. 2005; 41:35–41. PMID: 15937760

154. Roland PS. controversial etiopathologic agents in otitis media. Ear, Nose & Throat Journal. 2007; 86 (11):2–4.

155. Harimaya A, Fujii N, Himi T. Preliminary study of proinflammatory cytokines and chemokines in the middle ear of acute otitis media due to Alloiococcus otitidis. Int J Pediatr Otorhinolaryngol. 2009; 73 (5):677–80. doi: 10.1016/j.ijporl.2008.12.033 PMID: 19185927

156. Marsh RL, Binks MJ, Beissbarth J, Christensen P, Morris PS, Leach AJ, et al. Quantitative PCR of ear discharge from indigenous Australian children with acute otitis media with perforation supports a role for Alloiococcus otitidis as a secondary pathogen. BMC Ear, Nose and Throat Disorders. 2012; 12 (11).

157. Cripps AW, Otczyk DC. Prospects for a vaccine against otitis media. Expert Rev Vaccines. 2006; 5 (4):517–34. PMID: 16989632

158. Niittynen L, Pitkäranta A, Korpela R. Probiotics and otitis media in children. Int J Pediatr Otorhinolaryngol. 2012; 76(4):465–70. doi: 10.1016/j.ijporl.2012.01.011 PMID: 22305688