Non-typeable Haemophilus influenzae (NTHi) is one of the common causes of OM in all forms. There is currently no vaccine against NTHi. The adhesins HMW (isoforms HMW1 and HMW2) and Hia are expressed by NTHi and facilitate attachment to the human airway epithelium. Strains express either HMW1 and/or 2 (~75% of strains) or Hia (~25% of strains). Expression of both HMW and Hia is phase-variable: changes in the length of simple-sequence repeats located in the encoding genes promoter regions results in changes in expression levels of these adhesins. HMW1 has been previously demonstrated to bind a 2-3 sialyl-lactosamine, located predominantly in the upper respiratory tract, and HMW2 binds a 2-6 sialyl-lactosamine, which is found in both the upper and lower human airway, and is a receptor for other human pathogens, such as Influenza A virus. However, the target for Hia is currently unknown. We hypothesized that host glycans also act as a receptor for Hia-mediated adherence to host cells. We examined the glycan-binding ability of Hia using glycan arrays and Surface Plasmon Resonance (SPR). Glycan array and SPR results show that Hia binds preferentially to sialylated glycans, with highest binding of both proteins to a 2-6 sialyl-lactosamine, the same receptor as HMW2. Our analysis of HMW1, HMW2, and Hia, shows that both HMW2 and Hia, but not HMW1, preferentially bind to the human form of sialic acid, N-acetylneuraminic acid (Neu5Ac; the only form expressed in humans) over N-glycolylneuraminic acid (Neu5Gc). HMW1 shows no preference for Neu5Ac over Neu5Gc. Humans are one of the few mammals unable to produce Neu5Gc; a preference for structures containing Neu5Ac over Neu5Gc for both HMW2 and Hia demonstrates that unrelated NTHi adhesins have evolved to bind human specific glycan structures with highest avidity. Both HMW1/2 and Hia are currently being investigated as vaccine candidates; a combined vaccine approach would provide 100% strain coverage. Blocking host colonization by NTHi may result in lowered carriage rates, and decrease the incidence of NTHi disease. Knowledge of the host structures bound by these key adhesins will aid in the development of a vaccine against NTHi.
Presenting Author: Erin Baschal

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Title: Biological Networks Underlying Genetic Susceptibility to Upper and Lower Airway Conditions

Abstract/Summary: Introduction: A unified airway theory has been proposed, by which the upper and lower respiratory tracts act as a single functional unit. This theory is typically applied to allergic disease, in part due to the observation that patients with allergic rhinitis are more likely to have asthma, and vice versa. Additional support for this theory is that the middle ear mucosa, upper airway, and lower airway are all lined with ciliated epithelium, which is involved in the active transport of mucus and particulate matter. It stands to reason that these tissues may respond similarly to environmental insult. In addition, data from the Human Microbiome Project indicates that the microbiome of the upper and lower airway show remarkable similarities. We believe that this unified airway theory can also be applied to infections, with the host genetic background contributing to susceptibility to both upper and lower airway infections. Objective: The goal of this study is to investigate the unified airway theory with respect to infectious and non-allergic disease, through both literature search and analysis of our own RNA- Sequencing data. Methods: Literature searches were completed in PubMed for chronic rhinosinusitis with and without nasal polyps, otitis media, chronic bronchitis, pneumonia, and idiopathic pulmonary fibrosis. Genome-wide studies were included in these analyses (linkage, genome-wide association studies (GWAS), and whole exome sequencing). Candidate gene studies were not included. Linkage results were included if reported with LOD≥3.3. GWAS results were included using two thresholds, first with a standard threshold for genome-wide significance (p<5x10^-8), and also with a more lenient threshold (p<2.5x10^-6). Genes identified by exome sequencing were included if p<2.5x10^-6. NetworkAnalyzer software was used to perform GO-term enrichment analyses on the combined gene list (upper and lower airways) and for each condition separately. Results: Initial analyses suggest that there may be shared genetic susceptibility to both upper and lower airway infections. As expected, genes related to the immune response, including antigen processing and presentation, are enriched in both upper and lower airway infections. Other enrichments include extracellular matrix, cytoskeleton, cilia, and calcium ion binding. Enrichments specific to otitis media are actin cytoskeleton, carbohydrate metabolism, and sensory perception of sound. Conclusion: Further analyses will be completed to fully investigate the common and condition-specific pathways and networks. In addition, we are in the process of examining the intersection of RNA-Seq datasets from our samples, for infections of the ear, nose, and lung. Eventually, these results may provide insight into the pathogenesis of both upper and lower airway infections, including the role of the host genetic susceptibility. This information may also allow for preventative measures and improved treatments for these infections.
**Presenting Author:** Jemima Beissbarth  
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**Title:** The impact of pneumococcal vaccine schedules on nasopharyngeal microbiology of Indigenous infants aged 12 months  
**Abstract/Summary:**  
**Introduction:** Indigenous infants living in the remote north of Australia are at high risk of otitis media (OM). Participants were randomised (1:1:1) at 28-38 days of age to either Synflorix (PHiD-CV10) or Prevenar13 (PCV13) at 2, 4 and 6 months of age or an early start combination schedule of PHiD-CV10 at 1, 2 and 4 months followed by PCV13 at 6 months.  
**Objective:** To determine whether the combination schedule provides superior protection against vaccine type Streptococcus pneumoniae (Spn) compared to PHiD-CV10 alone, and superior protection against non-typeable Haemophilus influenzae (NTHi) compared to PCV13 alone, post primary schedule at 12 months of age. Method: Nasopharyngeal (Np) swabs, and Ear discharge (ED) swabs (if present), were collected at 12 months of age into STGGB, then transported, stored and cultured using standard methods. Antimicrobial resistance profiles for Spn, NTHi, Moraxella catarrhalis (Mc) and Staphylococcus aureus (Sa) were determined, and Spn isolates were serotyped using the Quellung method. Results: 259 Np swabs from 261 enrolled infants were collected. From 212 swabs processed to date, 74%, 58%, 81% and 5% were positive for Spn, NTHi, Mc and Sa respectively. Of the cultured Spn, 11% were PCV13 vaccine types (VT), predominantly 19A (4%) and 19F(3%). 37 ED swabs were collected from 28 infants in the cohort. Of the 32 ED swabs cultured to date, 16%, 52%, 3% and 19% were culture-positive for Spn, NTHi, Mc and Sa respectively. One child had a VT-Spn-positive ED. There were no statistically significant differences in VT-Spn or NTHi in the NP or ED by vaccine group, when comparing PCV13 with the combination group for NTHi (Np: 36/71 (50%) v 43/68 (74.1%), p=0.171; ED: 6/12 (50%) v 1/6 (16.7%), p=0.173) and comparing PHiD-CV10 with the combination group for VT-Spn (Np: 10/73 (13.7%) v 10/68 (14.7%), p=1.000; ED: 0/11 (0%) v 1/9 (11.1%), p=0.450) Conclusion: These preliminary data suggest that the use of a combined primary series of vaccination with PHiD-CV10 at 1, 2, and 4 months followed by PCV13 at 6 months of age compared to a primary series with either PHiD-CV10 or PCV13 did not result in reduced NP carriage with NTHi or Spn. These results should be cautiously interpreted due to the small number of swabs available for analysis.
Presenting Author: Tori Bootpetch Roberts

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Title: Identification of Rare, Missense PLG Variants in Families with Otitis Media

Abstract/Summary: Introduction: The PLG gene encodes plasminogen which, when activated by proteolysis, is converted to plasmin and angiostatin. Plasmin degrades blood plasma proteins, including fibrin in clots, while angiostatin inhibits angiogenesis. Deficiency in plasminogen results in a build-up of fibrin-rich lesions on the mucous membranes of the body. Plasminogen deficiency is most frequently associated with ligneous conjunctivitis, in which the wood-like pseudomembranous lesions develop on the mucous membranes in the eyes. However, similar lesions have also been observed in the middle ear, gingiva, respiratory tract, and female reproductive tract. Previous studies that show association between PLG variants and plasminogen deficiency consider homozygotes to be affected and heterozygotes to be “healthy”. Interestingly plasminogen-deficient Plg-/- mice spontaneously develop chronic otitis media (OM). Previously a rare PLG variant c.112A>G (p.(Lys38Glu)) was identified as a genome-wide significant risk factor for OM. Objective: We aim to identify rare, coding PLG variants in families with OM. Method: DNA samples from affected individuals from 28 Minnesota and 214 Finnish families were submitted for exome sequencing. Coding variants within PLG were Sanger-sequenced in additional family members. PLG variants were checked for minor allele frequency (MAF) within the Finnish population in the genome Aggregation Database (gnomAD) and prediction of functional effect on protein based on multiple bioinformatics tools, namely CADD, MutationTaster, PolyPhen-2 and PROVEAN/SIFT. Results: From the families with exome data, three Minnesota and five Finnish families were positive for rare, damaging PLG variants. Four PLG variants were identified as heterozygous, namely: c.112A>G (p.(Lys38Glu)) in one Finnish and two Minnesota families; c.782G>A (p.(Arg261His)) in three Finnish families; c.1481C>T (p.(Ala494Val)) in one Finnish proband; and c.2045T>A (p.(Ile682Asn)) in one Minnesota family. Three out of four multi-affected families showed incomplete co-segregation of PLG variants with OM in an autosomal dominant pattern, suggesting intra-familial heterogeneity or the occurrence of phenocopies. Two of the identified variants, c.112A>G (p.(Lys38Glu)) and c.782G>A (p.(Arg261His)), were previously associated with autosomal recessive type I plasminogen deficiency, however no second coding PLG variant was identified in the same families. All four variants were predicted to be deleterious by majority of bioinformatics tools used, with CADD scores of 19.0-27.6. The four variants were rare (MAF<0.005) in all gnomAD populations, except for c.1481C>T (p.(Ala494Val)) which had a MAF=0.056 in the Ashkenazi Jewish population. Majority of our families with PLG variants had recurrent acute OM. Conclusion: We identified four PLG variants in eight families with autosomal dominant OM, suggesting a novel association between heterozygous PLG variants and familial OM.
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Title: Topical and Systemic Interventions for Chronic Suppurative Otitis Media: A Suite of Cochrane Reviews
Abstract/Summary: Introduction: Chronic suppurative otitis media (CSOM) is the leading cause of preventable, permanent hearing loss globally. Objective: To systematically review the effectiveness of the most common topical and systemic treatments for CSOM using seven key comparisons identified in a scoping review and global consultation process including patients, clinicians and researchers: i) topical antibiotics, ii) topical antibiotics with steroids, iii) systemic antibiotics, iv) topical versus systemic antibiotics, v) antiseptics, vi) topical antibiotics versus topical antiseptics and vii) aural toileting (ear cleaning). Method: The Cochrane ENT Information Specialist searched the Cochrane ENT Register; Central Register of Controlled Trials; Ovid Medline; Ovid Embase; CINAHL; Web of Science; Clinicaltrials.gov; ICTRP and additional sources for published and unpublished trials. The date of the search was 12 March 2018. The reviews included randomised controlled trials with at least a one-week follow-up with patients (adults and children) who had chronic ear discharge of unknown cause; or CSOM, where the ear discharge had continued for more than two weeks. We used the standard Cochrane methodological procedures. Our primary outcomes were: resolution of ear discharge or ‘dry ear’ (whether otoscopically confirmed or not), measured at between one week and up to two weeks; two weeks to up to four weeks; and after four weeks; health-related quality of life using a validated instrument; ear pain (otalgia) or discomfort or local irritation. Secondary outcomes included hearing; serious complications; suspected ototoxicity including sensorineural hearing loss; balance problems/dizziness/vertigo; tinnitus. The same methods and outcome measures are shared across the seven reviews allowing evidence for all treatment options to be compared for relative effectiveness and quality of evidence. Results: The searches retrieved a total of 2990 unique references. After title and abstract screening we assessed 212 full texts. 49 studies met the inclusion criteria (with some studies addressing multiple comparisons): 26 studies examined topical antibiotics +/- steroids, 16 studies examined systemic antibiotics, 5 studies examined topical antiseptics, 6 studies examined topical versus systemic antibiotics and 8 studies examined antibiotics versus antiseptics and 3 studies examined aural toilet for CSOM. Conclusion: The findings from these reviews will help inform global policy and practice in CSOM treatment and direct future research efforts in trials examining the effectiveness of topical and systemic treatments for CSOM. This suite of Cochrane reviews has highlighted a lack of high-quality clinical trials for CSOM treatments, with poor reporting of adverse effects, limited duration of follow-up and few studies reported the impact of treatment on hearing outcomes.
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Title: Creating an Otitis Media Research Network in Aboriginal Medical Services in Australia
Abstract/Summary: Introduction: Australian Aboriginal and Torres Strait Islander peoples have one of the highest rates of complicated otitis media in the world. Best practice research and knowledge translation requires that research is done in close partnership with Aboriginal communities. Objective: To describe key factors in the successful establishment of the Aboriginal Medical Service otitis media research network. Methods: Two clinical trials examining management of otitis media in Aboriginal and Torres Strait Islander children are currently being run in 6 Aboriginal Medical Services (AMSs) nationally, commencing with the WATCH (Watchful waiting for Aboriginal and Torres Strait Islander Children) trial in 2014 and the addition of the INFLATE (autoinflation for otitis media with effusion) trial in 2017. Five AMSs have left the research network over this time due to inability to recruit and organisational changes. Process evaluation has been undertaken to understand and enhance the running of the trials and the network. This comprises thematic analysis of committee meeting minutes and interviews with site-based research officers, carers of participants, healthcare providers at sites and community reference group members. Results: We will present the findings of our process evaluation which relate to the enablers and challenges of establishing an otitis media research network in this context. Challenges to establishing a network include balancing research with clinical imperatives, organisational changes in AMSs and lower than expected numbers of children presenting to services with otitis media. Key enabling factors relate to relationship building, research staffing, funding models, flexible governance, clinical research which is aligned with community priorities, and two-way capacity building. Conclusion: An otitis media research network in Aboriginal Medical Services has long been needed. We have demonstrated that this is feasible and our ongoing successful recruitment demonstrates sustainability. Consideration of the specific enablers and challenges in this context is important to success.
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Title: Development of a Primary Pediatric Middle Ear Epithelial Cell Model for the Study of MUC5B Regulation in Otitis Media
Abstract/Summary: Objectives: Chronic Otitis Media (OM) is characterized by the production of fluid in the middle ear often mucoid, with accumulation of MUC5B mucin. Current middle ear (ME) human cell models employ immortalized cell lines which are limited in their ability secrete mucin proteins. Our laboratory has developed a novel in vitro model using fibroblast reconditioned media with patient middle ear epithelial cells enabling the study of MUC5B protein in chronic OM development. Methods: Primary middle ear epithelial cells (pMEECs) were recovered brushing the middle ear cavity of 2 pediatric patients during cochlear implant placement at Children's National Medical Center with consent of the legal guardians and in accordance with the Institutional Review Board. Cells were cultured in conditional medium containing calf bovine serum, irradiated 3T3 NIH fibroblast secretions and 10µM Y-27632 dihydrochloride (Rho kinase inhibitor) to induce proliferation. Cells were then plated in transwells and differentiated at air-liquid interface (ALI) with BEBM supplemented with epithelial specific singlequots (Lonza). Pictures were taken; proteins were analyzed by western blot and mass spectrometry, and mRNA by quantitative PCR after 1 to 4 weeks of culture. Results: pMEECs required a mean of 20 days to proliferate in conditional medium. Bright field microscope pictures showed pMEECs cultured at ALI formed a tight epithelium until week 2 to week 4 depending on the patient, and had a differentiated airway epithelial phenotype. Keratins 5, 14 and 15 were detected by immunofluorescence and western blot to confirm epithelial cell differentiation. Importantly, MUC5B mRNA was expressed at all-time points in pMEECs for both patients. MUC5B protein was detected in pMEEC lysates for both patients by western blot. Interestingly, MUC5B was secreted only by one patient sample (detected by mass spectrometry), showing an increase in MUC5B peptide count (PC) overtime between day 1 (0 PC) and week 4 of differentiation (69 PC = 1.05% total PC). Conclusion: We successfully created a primary pediatric middle ear epithelial cell model potent to produce mucins for the study of MUC5B regulation in OM.
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Title: Aboriginal Australian Otitis Prone children have reduced natural antibody titres to NTHi vaccine candidate antigens associated with attachment and biofilm formation
Abstract/Summary: Introduction: Nontypeable Haemophilus influenzae (NTHi) is the most common bacterial otopathogen associated with otitis media (OM), persisting in biofilms on the middle ear mucosa of otitis-prone (OP) children. Australian Aboriginal children acquire NTHi in early infancy and have exceptionally high rates of NTHi OM. Protein-based vaccines specifically targeting NTHi disease are under development. These include antigens associated with both infection and persistence (rsPilA, ChimV4 and Protein D). We have previously shown that Australian Aboriginal OP children have lower Protein D serum IgG titres than their non-Aboriginal counterparts. Objective: To assess the natural serum antibody titres to NTHi vaccine candidate antigens rsPilA, ChimV4, OMP26 (and confirm Protein D titres) in children; focusing on high-risk OP cohorts including Australian Aboriginal children. Method: Serum was collected from Aboriginal OP children (n=77), non-Aboriginal OP children (n=70) and healthy non-Aboriginal controls (n=36). Naturally acquired antigen-specific serum IgG was measured using an in-house multiplex fluorescent bead immunoassay. Antibody titres were adjusted for age, and Geometric Mean Concentrations (GMCs) compared between cohorts using a univariate analysis model. Results: Australian Aboriginal OP children had lower serum IgG titres to rsPilA, ChimV4 and Protein D (GMC: 88.94, 429.66 and 194.81 AU/mL respectively) compared to non-Aboriginal OP children (GMC: 240.57, 1210.76 and 493.24 AU/mL respectively), and Healthy Controls (227.69, 872.18 and 509.28 AU/mL respectively) p<0.05. No significant differences between non-Aboriginal OP children and healthy children were observed. Serum IgG titres to OMP26 were similar between groups (GMC: Australian Aboriginal OP children 1060 AU/mL, non-Aboriginal OP children 1054 AU/mL and healthy children 820.2 AU/mL), p>0.5. Conclusion: Australian Aboriginal OP children had lower antibody titres to most major NTHi vaccine candidate antigens, suggesting a failure to develop antibodies in response to NTHi exposure. Similar anti-OMP26 IgG titres show that this deficiency does not exist to all NTHi antigens. Our data further demonstrate that both population and antigen-specific differences occur in response to infection with NTHi, and this may contribute to the increased susceptibility of Australian Aboriginal children to OM. Importantly, this vulnerable population may benefit from active immunisation with antigens associated with biofilm/adhesion and should be a major consideration for development of future vaccines.

Introduction: Approximately 10% of remote Aboriginal and Torres Strait Islander (Indigenous) children younger than 3 years of age have healthy ears. Indigenous Health services across the country struggle to deliver diagnostic and management services and are challenged by high staff turnover. User-friendly mobile phone health care apps with evidence-based information that is suitable and accessible for all potential users are needed. Objective: An OMapp may assist in improving diagnosis and management of ear disease and conductive hearing loss in Indigenous children with a high disease burden. Methods: In 2010, The Recommendations for Clinical Care Guidelines on the Management of Otitis Media in Aboriginal and Torres Strait Islander Populations was published. In 2016, the National Health and Medical Research Council Centre of Research Excellence for Indigenous Children's Health Ears (CRE_ICHEAR) appointed a Technical Advisory Group to update this guideline following the GRADE approach. In 2018, the guideline was incorporated into an OMapp. Results: (a) Key Features of the OMapp: The OMapp is a multi-platform app available free of charge with downloadable content for off-line use. It has four basic windows: (i) Clinical (Diagnosis and Management): diagnostic, prevention and treatment algorithms for all types of OM; (ii) Communication tools: audio recordings in multiple Aboriginal languages to assist caregiver understanding of their child’s ear health and hearing needs; (iii) Educational resources: for professionals, families and children including videos of pneumatic otoscopy, audiograms and tympanograms, hearing loss simulations, and cartoons to explain the ear and hearing health service pathways; and (iv) Guidelines: evidence summaries for all strategies and recommendations for prevention and treatment with links to GRADEpro Summary of Findings tables, strength of recommendations, quality, effect size and a simple Population Intervention Comparison Outcome Time (PICOT) statement for each intervention and for multiple outcomes. (b) Pros and cons: OMapp is fully downloadable for access offline. The user-friendly windows and audio-visual features are intended to suit a diversity of users. Translation into Indigenous languages will enhance comprehension and adherence to recommended strategies for OM and hearing loss prevention and treatment. Updates necessarily demand internet connection, which is not universal across Australia.
Conclusion: The potential impact of the OMapp in reducing the burden of OM among Australian Indigenous children should be rigorously evaluated. Future capability for maintaining the OMapp evidence base in real-time will be an ongoing challenge.
Presenting Author: Malene Demant

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Title: Parental Perceptions and Management Strategies for Otitis Media In Greenland

Abstract/Summary: Introduction Otitis media (OM) in Greenland is a substantial problem and the country prevalence is among the highest in the world. However, little is known about how Greenlandic Inuit parents perceive and manage everyday life in families with children suffering from OM. We hypothesize that having a child with OM has dire consequences for the families that go beyond the advice and treatment offered in primary health care due to the nature of the disease.

Objective The aim of this study was to investigate the perception of and management strategies for everyday life among Greenlandic Inuit parents with children suffering from OM.

Methods We conducted a qualitative study based on semi-structured interviews and focus groups with parents to children with OM. The interviews took place in three different regions; the capital Nuuk (17,000 inhabitants), and two smaller towns (2,000-3,000 inhabitants) in West and East Greenland. Access to specialized health care, including Ear, Nose, and Throat specialists, differs among the regions, creating an underlying difference on the limitations of referral and thereby level of care. We conducted the data analysis using Systematic Text Condensation, a cross-case method.

Results In total, 29 parents participated in the study. Although most parents perceived OM as a result of genetic or environmental dispositions, individual perceptions and cultural beliefs of causal associations between parental behavior and the occurrence of OM co-existed with the general understanding of medical explanation models for OM. This created a sense of guilt among the parents. Parents felt either in control of managing the disease of the child and used medically well-established strategies such as systematic ear mopping and antibiotics as prescribed. Others felt frustrated and considered contact to the health clinics as futile, thereby managing the disease by ‘waiting it out’. Emerging themes were shame and stigma related to the symptoms of OM in the local communities, which had led to social isolation as a consequence for several of the interviewed families with children suffering from OM.

Conclusion Our results indicate that Greenlandic Inuit families are impacted by OM in a complex and severe manner. Guilt, shame and social isolation were predominant themes influencing the everyday life of the affected families. The parental perceptions of the disease and the management strategies go beyond the scope of the medical explanation and treatment models which poses a potential challenge for the parents’ experiences with the present treatment offer and indicate that the consequences of the disease reach beyond ear pain and fever. Due to the high prevalence of OM in Greenland the results of our study underline the need to develop a more holistic approach to prevention and treatment targeted children with OM and their families - both at the clinical level as well as a part of public health promotion at the community level.
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Title: Prevention of non-typeable Haemophilus influenzae colonisation and otitis media in mice by microbial interference with a closely-related commensal species

Abstract/Summary: Introduction: Nontypeable Haemophilus influenzae (NTHi) is a major otitis media (OM) pathogen, and colonisation of the nasopharynx with NTHi is a prerequisite to development of OM. Therapies that prevent NTHi colonisation may therefore prevent OM and reduce the global OM burden. Microbial interference describes the use of commensal bacterial species to compete with pathogens for binding sites, nutrients and space in order to beneficially alter the host microflora. This strategy has been shown to prevent pneumococcal OM in children (using a nasal α-Streptococci probiotic spray), to prevent meningococcal carriage in humans and experimental meningococcal meningitis in mice (using intranasal delivery of the closely related commensal Neisseria lactamica). We have previously shown that pretreatment of human respiratory epithelium with Haemophilus haemolyticus, a closely related commensal of NTHi, can prevent NTHi colonisation and infection of the epithelial cells. We have now assessed whether the murine equivalent of H. haemolyticus (Muribacter muris) can prevent NTHi colonisation and OM using a murine NTHi OM ascension model.

Objective: To determine whether intranasal pre-treatment of mice with M. muris can prevent NTHi colonisation and development of NTHi OM. Method: BALB/c mice were intranasally treated with either 5x10^7 colony-forming units (CFU) of M. muris (n=12) or saline (n=15) on Day 0. On Day 1, mice were challenged with 1x10^4.5 plaque-forming units of Influenza A virus (strain MEM, H1N3) followed by a Day 3 intranasal challenge with 5x10^7 CFU of NTHi (spectinomycin resistant strain R2866Specr). Mice were monitored daily and scored clinically. Nasal washes and middle ear bullae were collected on Day 6. Homogenised ear tissue and nasal washes were plated onto selective media for viable bacterial counts. Results: Pre-treatment of mice with M. muris reduced NTHi nasopharyngeal colonisation density from a median 4.32 Log10CFU/mL in controls to 2.20 Log10CFU/mL (p=0.0002). Twenty five percent of mice pre-treated with M. muris developed NTHi OM (3/12) compared with 53% of mice with no pre-treatment (8/15); p=0.24. Of the mice that had NTHi detected in their middle ear, the density of NTHi was lower in those pretreated with M. muris compared with controls (median 2.20 Log10CFU/mL versus 4.34 Log10CFU/mL; p=0.133. Mice that were pre-treated with M. muris also had fewer symptoms of disease compared with controls, with less weight loss (10.4% versus 13.8%; p=0.0006) and lower clinical scores (5.54 vs 7.22; p<0.00001) compared with controls. Conclusion: We have demonstrated that microbial interference can reduce NTHi colonisation and development of OM. Pre-treatment with the commensal also protected mice from the clinical symptoms of disease, potentially lessening OM severity. Human colonisation studies on microbial interference of NTHi are warranted in attempt to provide an effective therapy to reduce the burden of OM.
Presenting Author: Lena Hafrén
Authors: L. Hafrén1, D. N. Frank2, A. P. Giese3, T. B. Roberts2, T. Yarza4, G. Abes4, P. S. Mattila1, M. Sale9, Z. Ahmed3, T. Chonmaitree5, C. Chiong4, R. L. Santos-Cortez2 1University Hospital Of Helsinki, Department Of ENT, H&N Surgery, Helsingfors, , Finland 2University Of Colorado, School Of Medicine, Aurora, CO, USA 3University Of Maryland School Of Medicine, Department Of Otorhinolaryngology, Baltimore, MA, USA 4University Of Philippines, Manila, , Philippines 5University Of Texas Medical Branch, Galveston, TX, USA 9University Of Virginia, Center For Public Health Genomics, School Of Medicine, Department Of Biochemistry And Molecular Genetics, And Department Of Public Health Sciences, Charlottesville, VA, USA
Institutions: 1 University Hospital Of Helsinki, 2 University Of Colorado, 3 University Of Maryland School Of Medicine, 4 University Of Philippines, 5 University Of Texas Medical Branch, 9 University Of Virginia,
Title: SPINK5 Variants Confer Susceptibility to Autosomal Dominant Non-syndromic Otitis Media
Abstract/Summary: Introduction: Previously population-specific rare variants within genes A2ML1 and FUT2 were observed to confer risk for otitis media. These studies were performed by exome and Sanger sequencing, linkage and association analyses, microbiome sequencing and analyses, and middle-ear specific protein localization in wild-type mouse. Objective: To assess how SPINK5, which encodes an epithelial serine protease inhibitor, increase susceptibility to autosomal dominant non-syndromic otitis media. Methods: Exome sequencing on DNA from Filipino, European-American, and Finnish cohorts with otitis media. Microbiome analyses on oral, nasopharyngeal and middle ear samples. Expression studies on murine tympanic membrane and middle ear mucosa. Results: Genome-wide-significant linkage (LOD=4.5) for the rare SPINK5 variant c.1682A>G (p.Glu561Gly) in a genetically heterogeneous, intermarried, indigenous Filipino population. A second rare, missense SPINK5 variant c.802C>T (p.Arg268Cys) co-segregates with otitis media in a European-American family and was also observed in four additional European-American or Finnish probands with otitis media. Seven additional SPINK5 missense, stop or UTR variants were identified in multi-ethnic probands, including one that is absent in the gnomAD database. SPINK5 is expressed in tympanic membrane and middle ear mucosa. Carriage of a SPINK5 variant is associated with overall shifts in the microbiota of the oral cavity and nasopharynx. Additionally in SPINK5 variant carriers compared to wild-type individuals we identified relatively abundant bacterial taxa in the middle ears that are more similar to taxa identified in the outer ear or nasopharynx. Conclusion: These findings suggest that changes in the head and neck microbiota due to SPINK5 variants also play a role in otitis media susceptibility.
Presenting Author: Alistair Harrison
Authors: A. Harrison1, R. L. Hardison1, A. R. Fullen1, R. M. Wallace1, S. S. Justice1,2, K. M. Mason1,2  1Nationwide Children's Hospital, The Center For Microbial Pathogenesis, Columbus, OH, USA 2Ohio State University, The Ohio State College Of Medicine, Columbus, OH, USA
Institutions: 1 Nationwide Children's Hospital, 2 Ohio State University,
Title: Sequential Episodes of Experimental Otitis Media Promote Pathogenic Lifestyles Through Microevolution of Nontypeable Haemophilus influenzae
Abstract/Summary: Introduction: Nontypeable Haemophilus influenzae (NTHI) is a causative agent of both chronic and recurrent otitis media (OM). During chronic infection, bacterial adaptation promotes fitness and facilitates long term survival within the host. Objective: We used a preclinical chinchilla model of OM to determine the potential role for microevolution in the adaptation of NTHI during sequential episodes of the disease. Methods: OM was introduced into three chinchillas. Seven days following inoculation, NTHI were recovered from middle ear effusions and mucosal-associated biofilm. These two bacterial populations were used to infect each of three naive chinchillas. After a 7-day infection, bacteria were again recovered and used to infect a third and a subsequent fourth cohort. Results: Between the first and the last cohorts we observed a significant increase in biofilm development and in the bacteria recovered from biofilms during the first week of infection, suggesting in vivo strain adaptation by passage through prior animals. We also observed a significant increase in the number of intracellular bacterial communities (IBCs) of NTHI within middle ear mucosae, as well as increased fibrosis and inflammation. The genomes of the bacteria collected from the fourth cohort were sequenced and, compared to the parent strain, mutations were identified in 14 genes. These mutations were categorized into six classes based on mutation type and the locus where the mutation occurred: transcriptional phase variation, translational phase variation, intragenic single nucleotide polymorphisms (SNPs), intragenic SNPs, promoter region SNPs, or deletions. Moreover, in three genes multiple separate mutations were observed in a single locus. Confirmatory sequencing showed a subset of these mutations arose within the first 7 days of infection. Conclusions: These data provide the first demonstration of microevolution during experimental OM, with mutations arising along with increased bacterial growth, more severe markers of disease and greater biofilm and IBC formation over time. A deeper understanding of microevolution of NTHI during disease will give insight into how IBCs develop and the bacteria survive for protracted periods of time within the host. Understanding how strains of NTHI enter this chronic lifestyle will ultimately reveal targets for therapeutic modalities in the treatment of OM.
Abstract/Summary: Introduction:
The reported incidence of allergy being related to Eustachian Tube Dysfunction (ETD) and/or Otitis Media with Effusion (OME) as determined by allergy testing ranges from 15% to 93% in children up to 18 years, and up to 35% among adults.\[1\] Most epidemiologic studies have shown that patients with OME have an increased prevalence of atopic conditions when compared with non-OME controls.\[2\] Related symptoms of allergic rhinitis (AR) and asthma are associated with OME among 80% of children and 30% among adults respectively.\[3\] Meta-analysis of clinical evidence suggests a strong correlation of AR and OME among children.\[4\] Current guidelines from otologists and allergists support the role of allergy in the development of OME.\[5\] Yet, recent International Consensus states “there is no convincing evidence that treating allergy has any effect on OME”,\[6\] ignoring published data demonstrating that up to 89% of OME patients partially or completely resolve ETD symptoms after immunotherapy\[7\] or that 86% of children allergic to foods resolved their OME on food elimination diets.\[8\] The lack of agreement on this relationship has lead many physicians to discount the role that allergy might play in OME, even though it has been shown that allergy is associated with a 2- to 4.5-fold increased incidence of OME.\[9\]

We sought to discover if the disparity of the reported relationship of allergy to ETD might be due to the allergy testing method employed: skin prick tests (SPT) vs intradermal dilution testing (IDT).

Methods: There is no absolute way to evaluate allergy-testing techniques other than symptom resolution with allergy-directed therapy. This prospective study evaluated symptom improvement using symptom severity questionnaires completed before and after allergy immunotherapy (AIT) by 110 patients with chronic OME, asthma and chronic allergic rhinitis. Patients were tested for twelve antigens using IDT and treated for all positives.

Results: Positive SPT correlated with positive IDT at dilutions equal to or weaker than 1:12,500 weight/volume (w/v), but not at 1:2,500 or 1:500. These stronger dilutions identified 63 (57%) patients, with an additional 435 positive allergens, who would not otherwise have been diagnosed. Even among SPT+/IDT+ patients, IDT detected 3.26 times more allergens per patient than did SPT (336 vs 103; p
Disparity of Allergy Testing Results Among Patients with Allergic Rhinitis and Eustachian Tube Dysfunction

D. Hurst, MD, PhD  oto72hurst@gmail
Bruce Gordon, MD  Alan McDaniel, MD

AOM and OME are 2 distinct diseases

Patients with OME have an increased prevalence of atopic conditions when compared with non-OME controls.¹

86% of children allergic to foods resolved their OME on food elimination diets.²

Related symptoms of allergic rhinitis (AR) and asthma are associated with OME among 80% of children and 30% among adults respectively.³

Up to 89% of OME patients partially or completely resolve ETD symptoms after immunotherapy⁴

Meta-analysis of clinical evidence suggests a strong correlation of AR and OME among children.⁵

Current guidelines from otologists and allergists support the role of allergy in the development of OME.⁶⁻⁸

Among 2.4 Billion children - allergy is associated with a 2- to 4.5-fold increased incidence of OME.⁹

110 consecutive patients with symptoms of Eustachian Tube Dysfunction

<table>
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<tr>
<th>Symptom</th>
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<th>Female</th>
<th>%</th>
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<table>
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<th>Symptom</th>
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<th>After</th>
<th>Improvement</th>
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<td>100</td>
<td>48</td>
<td>43</td>
<td>81</td>
<td>74</td>
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</tbody>
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Purpose of this Study:

1) Determine if the sensitivity of testing methods for allergy may account for the failure of many otologists to associate allergy with ETD.

2) Determine the incidence of positive IDT in the face of a negative SPT in OME patients.

3) Determine if + IDT represents true allergic disease or are IDT’s false positives when SPT is negative?

It is the host’s immunologic status that holds the key to understanding the underlying mucosal response that is responsible for his/her sinusitis, asthma or middle ear disease.

The middle ear, like the sinuses, are bony cavities lined by Pseudostratified, ciliated, columnar epithelium. Allergy causes edema, influx and degranulation of eosinophils and mast cells leading to ETD and mucous production.

Conclusion

ETD and OME are the result of Allergic Disease

A diagnosis of allergy based entirely on skin prick tests will miss as many as 57% of patients and 88% of the possibly significant allergens.

Treatment of allergens detectable ONLY by IDT among 110 patients with ETD has shown to result in

> ALL patients experiencing 64% subjective improvement

> 94% experiencing objective improvement in their OME.

Contrary to current guidelines, IDT does offer increased sensitivity in the diagnosis of atopic disease and provides significant, clinically relevant information.

Failure to diagnose allergy in OME patients may account for the misconception that allergy is unrelated to OME and thus may account for why so many otologists fail to associate the two and miss the diagnosis!

Additional References


Presenting Author: Ramon Gordon Jensen

Authors:

Institutions: Head and Neck Surgery & Audiology, Rigshospitalet University Hospital

Title: Epidemiological Data on Hearing Impairment among Greenlandic Adolescents: Item Development and Findings from the Health Behaviour in School-aged Children Study 2018

Abstract/Summary: Objectives – Ear-infections is the leading cause of hearing impairment among children worldwide and a major public health problem in many indigenous populations. Studies have shown that early onset of impaired hearing compromise communication skills, academic performance, psychosocial behavior and emotional development, but studies are based on clinical examinations and not from representative studies of populations as studies on self-reported hearing impairment are scarce. The purpose of the present study was therefore two-sided; first to follow modern psychometric methods to develop an item bank for collection of data on hearing impairment among Greenlandic adolescents, and secondly to report data on the child-reports on hearing impairment from a national survey.

Methods – Data was part of the 2018 survey including 2,273 students corresponding to 47.6% of all Greenlandic schoolchildren in the age range from 10 to 17 years. The analyses performed aimed to describe the data characteristics and the frequency of self-reported hearing impairment among Greenlandic adolescents. Descriptive statistics are presented for hearing impairment, and binary logistic regression illustrate the strengths of association of school-related (risk) factors and poor self-rated health on hearing impairment.

Results – An average of 4% reported to have pain in the ear almost every day, and almost 10% reported ear pain at least weekly. 5% reported to have such impaired hearing that they were not at all able to follow what happened in school. 56% reported that their hearing impairment had lasted for less than 3 months, and 30% reported to have had hearing impairment for more than 1 year. 13% reported to have had them for more than 10 years. Logistic regression showed that girls had significantly higher odds of low self-rated health and poor school environment when experiencing impaired hearing. For boys tendencies were different and no associations were statistically significant except experiencing an academic achievement below average.

Conclusion – The study confirms clinical knowledge and case stories that there are large proportions of Greenlandic adolescents, who have impaired hearing. The updated development of items collecting data among children and adolescents for practical use in epidemiological studies are now available in Danish, Greenlandic and English. Longitudinal studies will examine causal associations between hearing impairment and other risk factors as well as social and health outcomes, to prevent this widespread detrimental impairment. The developed items on perceived hearing impairment will support future studies in finding unrecognized hearing impairment among children and adolescents. Furthermore, we hope these surveys will be beneficial to children with hearing impairment attending school.
Presenting Author: Lea-Ann Kirkham
Authors: Telethon Kids Institute
Title: Prevention of non-typeable Haemophilus influenzae colonisation and otitis media in mice by microbial interference with a closely-related commensal species
Abstract/Summary: Introduction: Nontypeable Haemophilus influenzae (NTHi) is a major otitis media (OM) pathogen, and colonisation of the nasopharynx with NTHi is a prerequisite to development of OM. Therapies that prevent NTHi colonisation may therefore prevent OM and reduce the global OM burden. Microbial interference describes the use of commensal bacterial species to compete with pathogens for binding sites, nutrients and space in order to beneficially alter the host microflora. This strategy has been shown to prevent pneumococcal OM in children (using a nasal α-Streptococci probiotic spray), to prevent meningococcal carriage in humans and experimental meningococcal meningitis in mice (using intranasal delivery of the closely related commensal Neisseria lactamica). We have previously shown that pre-treatment of human respiratory epithelium with Haemophilus haemolyticus, a closely related commensal of NTHi, can prevent NTHi colonisation and infection of the epithelial cells. We have now assessed whether the murine equivalent of H. haemolyticus (Muribacter muris) can prevent NTHi colonisation and OM using a murine NTHi OM ascension model.
Objective: To determine whether intranasal pre-treatment of mice with M. muris can prevent NTHi colonisation and development of NTHi OM.
Methods: BALB/c mice were intranasally treated with either 5x10^7 colony-forming units (CFU) of M. muris (n=12) or saline (n=15) on Day 0. On Day 1, mice were challenged with 1x10^4.5 plaque-forming units of Influenza A virus (strain MEM, H1N3) followed by a Day 3 intranasal challenge with 5x10^7 CFU of NTHi (spectinomycin resistant strain R2866Spe). Mice were monitored daily and scored clinically. Nasal washes and middle ear bullae were collected on Day 6. Homogenised ear tissue and nasal washes were plated onto selective media for viable bacterial counts.
Results: Pre-treatment of mice with M. muris reduced NTHi nasopharyngeal colonisation density from a median 4.32 Log_{10}CFU/mL in controls to 2.20 Log_{10}CFU/mL (p=0.0002). Twenty five percent of mice pre-treated with M. muris (3/12) compared with 53% of mice with no pre-treatment (8/15); p=0.24. Of the mice that had NTHi detected in their middle ear, the density of NTHi was lower in those pretreated with M. muris compared with controls (median 2.20 Log_{10}CFU/mL versus 4.34 Log_{10}CFU/mL; p=0.133. Mice that were pre-treated with M. muris also had fewer symptoms of disease compared with controls, with less weight loss (10.4% versus 13.8%; p=0.0006) and lower clinical scores (5.54 vs 7.22; p
Presenting Author: Oded Kraus
Authors: 
Institutions: Assuta Ashdod University hospital 
Title: Next Generation Middle Ear Evaluation
Abstract/Summary: In this session we will cover a number of evolving techniques some of which are already in practice to more accurately diagnose different middle ear states
Presenting Author: Chuan-Ming Li
Authors: C. Li1, H. J. Hoffman1, L. Chen1, C. L. Themann2, G. A. Flamme3, M. L. Rice4 1National Institute On Deafness And Other Communication Disorders (NIDCD), National Institutes Of Health (NIH), Epidemiology And Statistics Program, Bethesda, MD, USA 2National Institute For Occupational Safety And Health (NIOSH), Centers For Disease Control And Prevention (CDC), Hearing Loss Prevention Team, Cincinnati, OH, USA 3Stephenson And Stephenson Research And Consulting, LLC–West, Forest Grove, OR, USA 4University Of Kansas, Child Language Doctoral Program, Lawrence, KS, USA
Institutions: 1 National Institute On Deafness And Other Communication Disorders (NIDCD), National Institutes Of Health (NIH), 2 National Institute For Occupational Safety And Health (NIOSH), Centers For Disease Control And Prevention (CDC), 3 Stephenson And Stephenson Research And Consulting, LLC–West, 4 University Of Kansas,
Abstract/Summary: Objective: To describe associations between parental report of ear infections (otitis media history since birth) and difficulty hearing with measured hearing loss and reading achievement scores in early primary school. Method: ECLS–K:2011 children (n=18,170) were drawn from a national sample of public and private schools in 2010–11. Information on children's health, including medically-diagnosed ear infections (EIs) and hearing trouble (HT), was reported by parents; additional information was provided by teachers, schools, and daycare providers. Children’s cognitive, socio-emotional, and physical development were directly assessed. Pure-tone audiometry was performed on a subsample of over 3500. Reading scale scores based on the full set of assessment items were calculated using item response theory (IRT) procedures. The IRT scale scores represent estimates of the number answered correctly if children had received all of the questions in a given content domain. Using national sampling weights, logistic regression models were statistically-adjusted for covariates. Results: Prevalence of EIs was reported for specific time intervals: birth to age 2 (45% of children), age 2 to kindergarten entry (72%), kindergarten year (30%), first grade (20%), second grade (17%) and third grade (15%) for participants without missing data (n=6,580). Prevalence estimates for each time interval using the full sample of children were similar. By third grade, 80% of children had at least one EI and 30% had one or more time periods with recurrent EIs (3 or more). Overall, 2.8% of children had reported HT (80% “a little” HT; 20% “moderate/worse” HT). Among those who had EIs, HT was reported for 6.2% in kindergarten, 7.4% in first grade, 7.2% in second grade, and 7.0% in third grade. Measured hearing thresholds were 5 to 7 dB higher (worse) on average at frequencies of 2, 4, and 8 kHz for children with reported HT. After controlling for children’s age, sex, race/ethnicity, birth weight, complications at birth, recurrent EIs (3 or more) in any time interval before kindergarten entry was significantly associated with HT in kindergarten (odds ratio [OR]=3.6; 95% CI: 2.0-6.6), first (OR=4.9; 95% CI: 2.5-9.4), second (OR=5.4; 95% CI: 3.0-9.6), and third grade (OR=5.1; 95% CI: 2.7-9.8). Children with HT had lower reading scores in kindergarten (HT vs no HT, 0.26 vs 0.59), first grade (1.34 vs 1.74), second grade (1.97 vs 2.32), and third grade (2.38 vs 2.74), p<0.0001 for each grade. Conclusion: EIs are associated with reported HT and HT is associated with children’s academic achievement; hence, EIs indirectly impact children’s academic (reading) achievement test scores in early primary school.
Presenting Author: Paola MARCHISIO
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Institutions: 1 Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, 2 Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico, 3 Foundation IRCCS Cà Granda Ospedale Maggiore Policlinico,
Title: Nasopharyngeal Microbiome Analysis in Healthy and Otitis-Prone Children: Focus on History of Spontaneous Tympanic Membrane Perforation
Abstract/Summary: Introduction: The etiology of acute otitis media is ascribable to bacteria that colonize the upper respiratory tract and become more virulent under favorable circumstances. Recurrent acute otitis media has relevant effects, in terms of direct/indirect costs and antibiotic misuse. The most common complication of acute otitis media is otorrhea, whose epidemiology has been proved different from not-complicated acute otitis media and whose clinical management is often more challenging as soon as it becomes recurrent. Recently several trials have focused on nasal probiotic therapies, in order to prevent colonization by the most common otopathogens, but unfortunately results are still poorly clear. Objective: To compare nasopharyngeal microbiome of children with recurrent acute otitis media (otitis-prone children) to the one detected in healthy controls; to compare nasopharyngeal microbiome of otitis-prone children without otorrhea to otitis-prone children with recurrent otorrhea. Method: During winter 2016-17 nasopharyngeal swabs were collected from 112 children (mean age 3.5 years, SD ±1.5) including 36 healthy children, 39 otitis-prone children without otorrhea and 37 otitis-prone children with recurrent otorrhea. DNA was subsequently extracted and 16S rRNA gene V3-V4 regions were PCR amplified and sequenced using Illumina Mi Seq technology. Results: A higher relative abundance of Dolosigranulum and Corynebacterium genera was detected in the nasopharynx of healthy children (15.1% and 8%) in comparison with otitis-prone group without otorrhea (8.8% and 4.7%) and otitis-prone group with otorrhea (5.3% and 3.7%). On the other hand, Staphylococcus, Alloiococcus and Bifidobacterium were detected more often in otitis-prone children (2.4%,1.9% and 1.3%) than in the healthy group (0.7%, 0% and 0.4%). In all groups, the most abundant genera were Moraxella, Streptococcus and Haemophilus, followed by Dolosigranulum and Corynebacterium. Dolosigranulum and Corynebacterium showed a co-occurrence pattern with positive correlation in all groups, with negative correlation with Streptococcus and Haemophilus. Conclusions: To our knowledge, this is the first study comparing nasopharyngeal microbiota in not-complicated otitis-prone children to the one detected in otitis-prone children with recurrent otorrhea. Moreover, our study provides a characterization of the upper respiratory tract microbiome in children who experienced recurrent otorrhea. Although our data did not achieve statistical significance, we believe that this topic is worthy of further in depth-analysis, as it could potentially explain the clinical and epidemiological differences between complicated or not complicated otitis-prone children. In line with previous studies, our study identifies Dolosigranulum and Corynebacterium to be more abundant in the healthy group, strengthening the hypothesis that these two genera could be fundamental elements of the healthy respiratory tract microbiome.
Abstract/Summary: Acute otitis media (AOM) prevention represents a primary goal of the pediatric practice. This is mostly true for recurrent forms, but it can also be applied to the first episode of the disease, in otherwise healthy children. Considering that AOM onset is favored by a wide range of predisposing factors, and that it usually follows a viral infection of the upper respiratory tract, AOM prevention attempts mostly rely on the reduction of risk factor contribution, of the viral respiratory infection and of the bacterial colonization of the upper respiratory mucosa. In synthesis:
  • the removal of risk factors is undoubtedly useful, but the precise quantification of benefits in the prevention of recurrent AOM is often lacking
  • the use of influenza vaccine appears to be effective in the prevention of AOM.
  • pneumococcal vaccines are beneficial in reducing recurrent AOM in infants not yet suffering from recurrences and are to be definitively recommended in order to prevent the burden of AOM in the first year of life.
  • systemic and topic probiotics seem a promising method in the prevention of AOM and upper respiratory tract infections, but, because of the contrasting results of the available studies, further clinical evaluation is needed in order to assess their true potential.
  • as regards CAM, despite the large use in children with recurrent respiratory diseases and AOM, robust data on the efficacy of CAM in preventing AOM are lacking.
  • the use of antibiotic prophylaxis should be considered only in children for whom the burden of disease of recurrent acute otitis media outweighs the cost of this kind of therapy, in terms of both possible side effects and contribution to the development of bacterial resistance.
**Abstract/Summary:** BACKGROUND: Acute otitis media (AOM) pathophysiology presumes bacterial super-infection complicating an antecedent viral nasopharyngeal infection. Studies of nasopharyngeal secretions serve as reliable surrogate to evaluate the involvement of viruses to AOM and upper/lower respiratory tract infections (URIs/LRIs).

METHODS: We identified children aged 0-6 years admitted to our Pediatrics department in a University-affiliated, secondary hospital with uncomplicated AOM and concurrent URI/LRI during October-April between 2012-2017, when viral studies are performed. Studies were performed either using antigen detection tests, for respiratory syncytial virus (RSV) and influenza A/B (2012-2016) and for a variety of common respiratory viruses, utilizing multiplex polymerase chain reaction assays (2017).

RESULTS: 249 children with a median age of 15 months (IQR 7-22) were included. There were 155 (62%) males. In 88 (35%) cases, viral studies were positive, most of them in children
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Authors: E. M. Mokrzan1, L. A. Novotny1, K. L. Brockman1, L. O. Bakaletz1,2 1Nationwide Children's Hospital, Columbus, OH, USA 2The Ohio State University, College Of Medicine, Columbus, OH, USA
Institutions: 1 Nationwide Children's Hospital, 2 The Ohio State University,
Title: NTHI and M. catarrhalis Released from a Polymicrobial Biofilm by Antibodies against NTHI Type IV Pili are Hypersensitive to Antibiotic Mediated Killing
Abstract/Summary: Objective: Otitis media (OM) is often polymicrobial, with nontypeable Haemophilus influenzae (NTHI) and Moraxella catarrhalis frequently co-cultured from clinical specimens. The chronic and recurrent nature of OM is due to bacterial biofilms in the middle ear; therefore, strategies to eradicate these biofilms are needed. We have focused our vaccine development efforts on PilA, the majority subunit of the NTHI Type IV pilus, which is important for adherence, colonization, motility and biofilm formation. Antibodies against a recombinant, soluble form of PilA (rsPilA) disrupt and prevent the formation of NTHI biofilms in vitro. Moreover, immunization with rsPilA both prevents and resolves experimental NTHI-induced OM. Epidemiological studies show that M. catarrhalis is rarely found as a sole OM pathogen and instead often co-infects with NTHI, which suggests a symbiotic relationship that facilitates polymicrobial OM. Thus we hypothesized that vaccine strategies that are effective against NTHI-induced OM might also mediate a collateral, albeit indirect, benefit in terms of resolution of polymicrobial OM. Here we explored the effects of anti-rsPilA exposure on polymicrobial biofilms formed by these two predominant OM pathogens. Method: We established biofilms formed by NTHI + M. catarrhalis at temperatures typical of the nasopharynx (34°C) or middle ear (37°C), exposed them to anti-rsPilA, then quantitated how many of each species was disrupted from the biofilm and now present in the supernatant. Results: Notably, anti-rsPilA dispersed both NTHI and M. catarrhalis from biofilms at 34°C and at 37°C. With the knowledge that bacteria newly-released from biofilms often display greatly enhanced antibiotic sensitivities, we then examined the relative sensitivity of newly-released NTHI and M. catarrhalis to first-line antibiotics used to treat OM. When newly-released NTHI were exposed to trimethoprim-sulfamethoxazole, we observed significantly more killing at 34°C and at 37°C than for NTHI in broth suspension (p<0.05). Similarly, we observed significantly more killing of newly-released M. catarrhalis exposed to clarithromycin than M. catarrhalis in broth at both temperatures (p<0.01). These data demonstrated that anti-rsPilA-mediated dispersal of bacteria from residence in a biofilm resulted in significantly increased antibiotic sensitivity in both the newly-released NTHI and M. catarrhalis. Conclusion: The results of this study revealed that immunization with rsPilA could induce antibodies that disrupt a dual-species biofilm despite specifically targeting only a single pathogen. Moreover, when needed, immunization with rsPilA could offer a new and potentially powerful defense against the growing threat of antibiotic resistance by combining the power of active immunization with the killing activity of traditional antibiotics but now effective at a markedly reduced dose. Funded by NIH-R01-DC003915
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Title: The Transcriptional Landscape of the In Vitro Murine Middle Ear Epithelium.
Abstract/Summary: Introduction: Current knowledge suggests that otitis media (OM) is caused by an unrestrained response of the middle ear epithelium to an exogenous trigger, often a pathogen. The mechanisms underpinning epithelial remodelling in OM remain unclear but they result in the development of an abnormal mucociliary epithelium that contributes to the development of characteristic exudates. Research into the pathogenesis of OM is limited because of difficulties in accessing appropriate samples but murine models are often employed. To complement these studies we recently described a novel in vitro model of mouse middle ear epithelial cells (mMEECs) cultured at an air liquid interface (ALI). In this model cells isolated from dissected bullae, undergo mucociliary differentiation into the varied epithelial cell populations (ciliated and secretory) seen in the native middle ear cavity. The model offers the possibility to understand the processes underpinning the development of OM in a genetically tractable manner. Objective: To investigate genome wide gene expression profiles of mMEECs during differentiation to better understand the biology of this in vitro model of the murine middle ear. Methods: We used gene expression array analysis on triplicate samples of mMEECs cells cultured at the ALI. mMEECs isolated from dissected bullea were placed into culture and when confluent were differentiated at an ALI. We compared the gene expression profiles of original (uncultured) middle ear cells, confluent cultures of undifferentiated cells (day 0 of ALI) and cells that had been differentiated for 7 days at an ALI. Microscopy and PCR was used to validate findings in mMEECs and in mouse tissues. Results. Multidimensional scaling analysis showed that samples from each set grouped together. >5000 genes were differentially expressed among the three groups of cells. Approximately 4000 genes were differentially expressed between the original cells and day 0 of ALI culture. The original cell population was shown to contain a mix of cell types, including contaminating inflammatory cells and reticulocytes that were lost on culture. Approximately 500 genes were upregulated during differentiation. These included some secretory genes (Lyz1, Lyz2 – the most induced, Muc5b) and some enzymes (Aldh1a1, Cyp2a5) but most were associated with the process of ciliogenesis. Conclusion. Our in vitro model of differentiated murine middle ear epithelium exhibits a transcriptional profile consistent with the mucociliary epithelium seen within the middle ear. Knowledge of the transcriptional landscape of this epithelium will provide a basis for understanding the phenotypic changes seen in murine models of OM.
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Title: Immunization with a Bandaid Protects Against Experimental Otitis Media Due to nontypeable Haemophilus influenzae  

Abstract/Summary: Objective: We’ve developed a strategy for immunization that involves placement of vaccine formulations on to a bandaid which is then affixed to the skin. This simple regimen, called transcutaneous immunization (TCI), has potential to be equally effective as injectable vaccination, yet may be less expensive, encourage greater compliance and promote widespread distribution to regions without ready access to healthcare. Herein, we fully characterized TCI to resolve active experimental otitis media (OM) due to nontypeable Haemophilus influenzae (NTHI), reveal the molecular mechanisms that underlie the significant efficacy achieved and further, demonstrate boostability and durability against an additional episode of OM. Methods: TCI was performed by placement of band aids behind the outer ears of chinchillas. Formulations consisted of an immunogen called ‘chimV4’ which targets two critical adhesins expressed by NTHI: the Type IV pilus (involved in NTHI adherence, motility and biofilm formation) and outer membrane protein P5 (important for NTHI adherence), mixed with a potent adjuvant called ‘dmLT’ or dmLT alone. Band aids were placed on days 0 and 7. We characterized the maturation of B-cells within the nasal-associated lymphoid tissue (NALT) as an immune inductive site after completion of a primary immunization series and upon receipt of a boosting bandaid 60 days later. In a second study, chinchillas were immunized by band aid as before, then challenged on day 14 by direct inoculation of the middle ear with NTHI to induce active OM. To demonstrate prevention of a subsequent episode of OM, middle ears were inoculated with NTHI a second time 60 days later and resolution of active disease determined by video otoscopy and quantitation of bacterial burden. Results: TCI by bandaid induced a significant increase in long-lived memory B-cells and chimV4-specific antibody-secreting cells in the NALT (P≤ 0.05), compared to cells collected from animals administered dmLT, an outcome that was further augmented upon receipt of a boosting bandaid 60 days later (P≤ 0.05). Moreover, the immune response established after a TCI was durable, as upon a second challenge with NTHI animals administered chimV4+dmLT eradicated NTHI from the middle ear and resolved signs of inflammation within 3 days, compared to animals administered dmLT only (P≤ 0.001). Conclusions: Our collective data prove that TCI by bandaid to administer a dual NTHI adhesive protein immunogen is a highly effective therapeutic and also preventative strategy against NTHI-induced OM. The immune response shaped by TCI can be recalled and expanded, and a single immunization series was satisfactory to protect against an additional episode of OM, all characteristics that are essential to a successful vaccine and delivery strategy. Importantly, the simplicity of TCI with a bandaid has tremendous potential to expand the reach of vaccines against OM to underserved regions.  

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Title: Immunologic dysfunction in the otitis prone child
Abstract/Summary: Objective. Acute Otitis Media (AOM) is a multifactorial disease influenced by the immunological response occurring mostly in young children who are immunologically naïve to AOM pathogens. We sought to identify deficiencies in fundamental immune defense mechanisms in otitis prone children during a 10-year prospective study.
Methods. Nasopharyngeal and blood samples were prospectively collected children at 6-36 months of age (6, 9, 12, 15, 18, 24 and 30-36 months old) and at every AOM episode. The diagnosis of AOM was made by validated otoscopyists and confirmed by tympanocentesis. Stringently-defined otitis prone (sOP) classification was made if a child had 3 AOMs within 6 months or 4 AOMs within a year. Cytokines/chemokines were quantitated with molecular methods. B cells, T cells and antigen presenting cells (APCs) and immunology signaling pathways were identified, characterized and quantitated by flow cytometry, Elispot and molecular methods.
Results. We identified dysfunction in innate responses that cause an immunopathological impact in the nasopharynx resulting in pathogenesis. These include inadequate expression of TLRs, proinflammatory cytokine secretion, epithelial cell repair and defects in professional APCs. Adaptive immunity defects in B cell function and immunologic memory were identified resulting in low levels of antibody to major otopathogen-specific protein antigens. CD4+ and CD8+ T cell function and memory defects were identified. We sought a mechanistic explanation in B cell dysfunction by examination of TNF family receptors (TNFRs) TACI, BCMA, and BAFFR receptor expression and found significantly lower BAFFR and TACI expression; significantly lower proliferation of B-cells stimulated with exogenous BAFF; and diminished expression of co-stimulatory receptors B7–1 and B7–2 among sOP children. We sought a solution for T cell dysfunction and found addition of exogenous Th17-promoting cytokines restored Th17 function in cells from sOP children. We found that sOP children are unusually vulnerable to other respiratory infections: viral URI, sinusitis, lobar pneumonia and influenza suggesting their broad immunologic defects are associated with susceptibility to other infections.
Conclusions. In the first years of life when the diagnosis of otitis proneness is stringently applied by requiring microbiologic confirmation of authentic repeated AOMs, broad immunologic deficits in innate and adaptive immunity can be identified and present a new opportunity for therapeutic intervention.
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Title: The Yield of Respiratory Viruses Detection Testing is Age-Dependent in Children with Uncomplicated Acute Otitis Media
Abstract/Summary: Introduction: Acute otitis media (AOM) pathophysiology presumes bacterial super-infection complicating an antecedent nasopharyngeal viral infection that ascends to the middle ear. Studies of nasopharyngeal secretions serve as reliable surrogate to evaluate the contribution of viruses to AOM and upper/lower respiratory tract infections (URIs/LRIs). Objectives: We sought to study the results of NP respiratory viral panel tests obtained from children with uncomplicated AOM hospitalized during the URI/LRI seasons in 6 consecutive calendrical years which had seen a shift from traditional antigen detection assays to a PCR-based multiplex assay. A secondary objective was to study the yield of these tests in relation to children's age, in order to identify which age group would benefit the most at the greatest cost effectiveness Methods: We identified children aged 0-6 years admitted to our Pediatrics department in a university-affiliated, secondary hospital with uncomplicated AOM and concurrent URI/LRI during October-April between 2012-2017, when viral studies are performed. Viral studies were performed either using antigen detection tests, for respiratory syncytial virus (RSV) and influenza A/B (2012-2016) and for various common respiratory viruses, utilizing multiplex polymerase chain reaction assays (2017). Results: 249 children with a median age of 15 months (IQR 7-22) were included. There were 155 (62%) males. In 88 (35%) cases, viral studies were positive, most of them in children <24 months (78, 89%). RSV was positive in 52 (59%) cases, followed by influenza A, 11 (13%) and B, 5 (6%). RSV co-infection was documented in 10 (11%) cases. Children ≤12 months were the age group with most positive test results (43, 49%) (p=0.004), and had statistically significant more positive RSV results (31, 60%) when compared with older children (p=0.038). There were no statistically significant differences in the positive viral results between those who were treated with antibiotics before admission and those who were not (p=0.318). Conclusion: The yield of nasopharyngeal viral studies in uncomplicated AOM is limited to children <24 months, with the greatest benefit in infants aged ≤12 months.
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Title: Diverse Paths to Serum Resistance in Nontypeable Haemophilus influenzae Isolated from Children with Otitis Media

Abstract/Summary: Introduction: Nontypeable Haemophilus influenzae (NTHi) is a human-restricted opportunistic pathogen that causes considerable morbidity, particularly in otitis media (OM) infections. Like many bacteria, NTHi is highly diverse, including variation among isolates’ serum resistance (SR) levels. Although several SR mechanisms have been identified in model NTHi strains (including phase-variable genes), how these mechanisms are distributed across NTHi and what role SR plays in OM infections has not been rigorously assessed, despite the known presence of complement in the middle ear. Objective: Using a well-curated collection of 212 clinical NTHi strains isolated longitudinally from healthy or otitis media prone-children, we set out to: 1) survey SR across the collection to test for correlations with NTHi clonal type and subject health status; 2) identify loci and alleles responsible for variability in SR; and 3) measure the response to selection for SR among diverse serum sensitive-strains. Methods: 1) We implemented a novel high-throughput assay to quantify SR across our collection and applied multivariate regression analysis to test any associations with clinical metadata. 2) We collected whole genome assemblies for each strain for the performance of genome-wide associations studies to identify SR genes within and among NTHi lineages. 3) We subjected phylogenetically diverse sensitive-strains to serial serum selection, and used genomic sequencing to identify differences between sensitive parental strains and newly evolved SR strains. Results: As expected, we found broad phenotypic variation among strains collected from both cohorts of children. By using selectively compromised serum, we discovered that diverse sensitive-strains were differentially susceptible to the distinct branches of complement. Testing for associations between NTHi SR, clinical provenance, and bacterial genomic variation are ongoing; preliminary results suggest rapid switches in the SR phenotype, even within closely related strains infecting the same subject and only sometimes explainable by switching of known phase-variable genes. Remarkably, after serial serum exposure, diverse sensitive strains (n=18) rapidly increased in SR by ~400-fold on average, despite their phylogenetic diversity and distinct pathway sensitivity profiles. Genomic analyses to identify the causative mutations is ongoing. Conclusion: These results underscore the rapid and dynamic changes within NTHi strains during carriage and disease; they indicate fluctuating selective pressures acting on the SR trait. This indicates unknown fitness costs associated with SR-associated surface structures (particularly lipooligosaccharide moieties). Our new bacterial genomic and phenotypic resource will elucidate the ‘genetic architecture’ of the highly variable SR phenotype across a large diverse collection of clinical strains, which may inform translational endeavors, particularly in NTHi vaccine development.
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Title: Epidemiological Data on Hearing Impairment among Greenlandic Adolescents: Item Development and Findings from the Health Behaviour in School-aged Children Study 2018
Abstract/Summary: Objectives – Ear-infections is the leading cause of hearing impairment among children worldwide and a major public health problem in many indigenous populations. Studies have shown that early onset of impaired hearing compromise communication skills, academic performance, psychosocial behavior and emotional development, but studies are based on clinical examinations and not from representative studies of populations as studies on self-reported hearing impairment are scarce. The purpose of the present study was therefore two-sided; first to follow modern psychometric methods to develop an item bank for collection of data on hearing impairment among Greenlandic adolescents, and secondly to report data on the child-reports on hearing impairment from a national survey. Methods – Data was part of the 2018 survey including 2.273 students corresponding to 47.6% of all Greenlandic schoolchildren in the age range from 10 to 17 years. The analyses performed aimed to describe the data characteristics and the frequency of self-reported hearing impairment among Greenlandic adolescents. Descriptive statistics are presented for hearing impairment, and binary logistic regression illustrate the strengths of association of school-related (risk) factors and poor self-rated health on hearing impairment. Results – An average of 4% reported to have pain in the ear almost every day, and almost 10% reported ear pain at least weekly. 3% reported to have inflammation from the ear at least weekly. 5% reported to have such impaired hearing that they were not at all able to follow what happened in school. 56% reported that their hearing impairment had lasted for less than 3 months, and 30% reported to have had hearing impairment for more than 1 year. 13% reported to have had them for more than 10 years. Logistic regression showed that girls had significantly higher odds of low self-rated health and poor school environment when experiencing impaired hearing. For boys tendencies were different and no associations were statistically significant except experiencing an academic achievement below average. Conclusion – The study confirms clinical knowledge and case stories that there are large proportions of Greenlandic adolescents, who have impaired hearing. The updated development of items collecting data among children and adolescents for practical use in epidemiological studies are now available in Danish, Greenlandic and English. Longitudinal studies will examine causal associations between hearing impairment and other risk factors as well as social and health outcomes, to prevent this widespread detrimental impairment. The developed items on perceived hearing impairment will support future studies in finding unrecognized hearing impairment among children and adolescents. Furthermore, we hope these surveys will be beneficial to children with hearing impairment attending school.
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**Title:** Bacterial Reservoirs in Otitis-prone Children are Associated with Repeat Surgery Outcomes: A Cohort Study  
**Abstract/Summary:** Introduction: Recurrent acute otitis media (rAOM) remains a common and challenging paediatric disease. Bacteria associated with rAOM, predominantly non-typeable Haemophilus influenzae (NTHi), are highly immune-evasive and refractory to antibiotics. Children with rAOM (otitis-prone) usually require ventilation tube insertion (VTI) surgery and/or other otorhinolaryngology surgery; and repeat operations are common. Identifying children that may require repeat surgery is important for clinical management. Objective: To identify risk factors associated with follow up otorhinolaryngology surgery and repeat VTI in children undergoing VTI surgery for rAOM. Method: For this cohort study, 186 children with a history of rAOM undergoing VTI surgery were recruited between November 2007 and May 2009. Middle ear effusion (MEE) samples were collected from 148 children during VTI surgery. Follow up clinical information was collected retrospectively in 2015 from 142 children. Demographic risk factors (age at the time of VTI surgery, gender, recent antibiotic usage, median number of AOM episodes, and day-care attendance) were assessed against follow up surgery requirements. Microbiological risk factors (viral and bacterial detection by PCR) were calculated against repeat surgery for the subset of followed-up children where MEE samples were available (116/142). Results: Children were grouped according to requirements for follow up otorhinolaryngology surgery (no repeat surgery; surgery -ve: n=52, and repeat surgery; surgery +ve: n=90). Age, gender, antibiotic usage, day-care attendance and number of AOM episodes were similar between groups (p≥0.073). Presence of a common respiratory virus was associated with follow up otorhinolaryngology surgery (76% in surgery+ve versus 58.5% in surgery-ve; p=0.05). PCR detection of bacterial otopathogen in MEE at the time of surgery was associated with follow up otorhinolaryngology surgery (64.4% in surgery +ve versus 39% in surgery -ve; p=0.009), and specifically detection of NTHi (52.1% in surgery +ve versus 29.3% in surgery -ve; p=0.019). Of the 90 children who had follow up otorhinolaryngology surgery, 72.2% had repeat VTI. PCR detection of otopathogen in the MEE at the time of VTI was more common children who went on to have repeat VTI than those who did not (67.3% in VTI +ve versus 44.1% in VTI -ve; p=0.005). More specifically, NTHi detection in MEE at the time of VTI was associated with repeat VTI (52.7% in VTI +ve versus 35.6% in VTI -ve; p=0.044). Conclusion: Detection of bacterial otopathogens in the middle ear at the time of VTI is a strong indicator of children at-risk of repeat surgery. NTHi was the dominant otopathogen detected in the middle ear, indicating that NTHi-targeted treatment strategies are likely to reduce repeat otorhinolaryngology surgery in children with rAOM.
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Title: Cationic Nanoparticle Enhanced trans-Tympanic Membrane Drug Delivery for Noninvasive Treatment of Otitis Media

Abstract/Summary: Introduction: Otitis media (OM) is typically treated with systemic oral antimicrobial agents, which often cause therapeutic resistance/side-effects and many children with chronic OM undergo surgery, as no alternative non-invasive therapies exist. The impermeability of TM, driven by tight junctions on the epithelial layer has limited the topical delivery efficacy of therapeutics to the ME cavity. Increasing the therapeutic flux through TM is critical for success of topical treatment. We report the potential of cationic nanoparticle (CNP)-based drug carriers for local non-invasive trans-TM drug delivery of drugs. Objective: We hypothesize that CNPs applied topically in outer-ear can effectively and rapidly translocate therapeutics into the ME cavity through the TM via enhanced diffusion. We synthesized and characterized antibiotic and steroid loaded CNP formulations for acute and chronic OM and tested the delivery efficacy enhancements of these formulations through the TM using an ex vivo chinchilla model. Method: To examine the role of NP charge and size in enhancing Fick’s diffusion across TM, we synthesized Ag2S quantum dots (QD) (~8nm, charge -26mV) and Gd2O3 NPs doped with 1% Ne (~100nm, charge +42mV). These NPs luminesce at 1000–1300nm with 808nm excitation, thus allowing imaging based detection. 200ul NPs aqueous suspension was applied to the external auditory canal (EAC) of excised chinchilla auditory bullae maintained with PBS buffer in ME and was sampled at varying time-intervals to detect diffusion of NPs. The diffusion of NPs was quantitatively verified by ICP-MS for Gd and Ag. The ability of ~100nm CNPs to diffuse through intact TM and deliver drugs was tested with biocompatible drug delivery systems based on cationic DOTAP(1,2-dioleoyl-3-trimethylammonium-propane) liposomes, with both hydrophobic (Dexamethasone, 2mg/ml) and hydrophilic (Ceftriaxone (CFX), 6.5mg/ml) cargoes and quantified by HPLC and LC-MS assays. The antibacterial efficacy of antibiotic loaded NPs was also tested with standard bacterial killing assays. Results: The TM maintained a tight barrier with 0.05% of -ve-charge Ag2S QD diffusing to ME cavity, whereas intriguingly ~5% or 100X higher delivery of much larger but +ve charged Gd2O3 particles was detectable by imaging within 15min of application and confirmed by ICP-MS. For both the steroid and antibiotic cargoes, transport of drugs to ME was detected within 15min of application compared to minimal or undetected levels in ME for equivalent free drug application. CFX-Liposomes delivered 32.5ug/ml in ME in 15min far exceeding the MIC (~1ug/ml) for SP and NTHi strains. The CFX loaded liposomes maintained antibacterial activity equivalent to free drug, when tested on NTHi bacterial cultures. Conclusion: Local non-invasive trans-TM delivery of antibiotics and steroids is enhanced by CNPs formulations and non-invasive delivery of therapeutic levels of antimicrobials is feasible with a topical ear-drop type application.
OBJECTIVE: Chronic and recurrent otitis media (OM) is difficult to treat with antibiotics, in part because OM pathogens form biofilms in the middle ear. These biofilms are 1000 times more resistant to antibiotics than free-living bacteria. In addition to biofilm formation, nontypeable Haemophilus influenzae (NTHi) has evolved a powerful mechanism to adapt to and persist within its' human host termed the ‘phasevarion’ (phase variable regulon). NTHi uses the phasevarion to rapidly and reversibly regulate the expression of multiple genes. Phasevarion switching results in two genetically identical but phenotypically unique subpopulations (e.g., ‘ON’ and ‘OFF’) of a single NTHi strain. Subpopulation diversity increases the adaptability and survivability of NTHi in its’ human host. When exposed to conditions similar to chronic OM, temperatures ≥37°C and alkaline pH, the phasevarion has an influence on biofilm formation by NTHi. Due to the unique biofilms formed by ‘ON’ and ‘OFF’ subpopulations, we hypothesized that there were also likely differences in the antibiotic susceptibility of the biofilm resident NTHi. This study investigates the role of the phasevarion in the relative susceptibility of NTHi to antibiotics in any of three unique lifestyles: planktonic, biofilm-resident, and those newly released from a biofilm due to the action of any of several known cues/agents. METHODS: To study the phasevarion, genetically modified variants, termed ‘locked ON’ or ‘locked OFF,’ were assayed. These locked variants cannot phase-vary which allows us to separate the two phenotypic subpopulations and assess how NTHi uses the phasevarion to respond to biological cues without the confounding issue of ongoing phase-variation. These variants were tested for sensitivity to commonly prescribed antibiotics for OM, ampicillin, cefdinir, or trimethoprim/sulfamethoxazole. Antibiotic susceptibility for planktonically grown bacteria was tested via use of the published MIC90 of the antibiotic. Biofilms were grown in conditions similar to a healthy middle ear (37°C, pH 7) or those common during chronic OM (37°C, pH 9). Due to the resistant nature of biofilms, to see differences in susceptibility between locked ON vs. OFF variants, biofilms were treated with 1000X MIC90, then architectural differences (via confocal microscopy) and biofilm resident bacterial viability were determined. The antibiotic susceptibility of bacteria newly released from a biofilm via exposure to antibodies that target the type IV pilus adhesin, PilA, or the DNA-binding protein, integration host factor, each of which is known to disrupt an NTHi biofilm, was also tested. RESULTS: There was no difference in the antibiotic sensitivity between locked ON or locked OFF variants when grown planktonically. Regardless of pH, biofilm-resident bacteria of the locked OFF variant were twice as sensitive to ampicillin than the locked ON counterpart. However, antibiotic susceptibility of ON and OFF variants newly released from a biofilm is likely to provide data of greatest interest. CONCLUSIONS: To date, our data demonstrate that in conditions similar to chronic OM, NTHi utilizes the phasevarion to modify both biofilm formation and resistance to antibiotics. A complete understanding of the NTHi phasevarion is crucial to develop efficient therapies and possibly prevent infections by this organism and as such, our work in this area continues. Support NIH/NIDCD R01DC015688
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**Title:** In vitro Biofilm Formation Distinguishes Strains of Streptococcus pneumoniae that Persist in the Nasopharynx from Non-persistent Strains in Children with Acute Otitis Media  
**Abstract/Summary:** Objective: Persistent nasopharyngeal (NP) carriage of Streptococcus pneumoniae is associated with recurrence of acute otitis media (AOM) and prolonged middle ear effusion. Biofilm is found frequently in the middle ear in those with chronic otitis media, but the role of biofilm formation in persistent carriage remains uncertain. Our aim was to study whether in vitro biofilm formation distinguishes between pneumococcal strains that persist in the NP compared to those that do not. We also studied the genomic differences between persistent and non-persistent strains. Method: Clinical isolates were obtained from the NP of children (6-35 months) with AOM enrolled in a study of antimicrobial treatment vs. placebo. Children were followed for at least 2 months, and NP cultures were taken at follow-up visits. Persistent carriage was defined as detection of the same serotype of S. pneumoniae from the NP for ≥45 days. Biofilm formation was assessed by measuring optical density (OD) values in microtiter plates after crystal violet staining. The whole genome sequencing data was generated using the Illumina HiSeq platform. We performed comparative genomic analysis of 63 persistent and 16 non-persistent strains using SEED, BLAST, and BRIG. Results: Persistent carriage was detected in 18% (31/177) of children. In vitro biofilm formation was significantly greater among persistent strains of S. pneumoniae compared with serotype matched, non-persistent strains [mean OD 0.367 (SD 0.109) vs. 0.292 (SD 0.066); P=0.01]. Overall bacterial growth did not differ between persistent and non-persistent strains [mean growth 0.656 (SD 0.163) vs. 0.609 (SD 0.157); P=0.38]. Repeat isolates from the same child demonstrated comparable in vitro results, suggesting that persistent strains were already better biofilm producers at the time of initial colonization. Comparative genomic analysis indicated a great homogeneity among the subsequent strains from the same child. Genes coding for cell wall surface anchor family proteins, phage replication proteins, diaminobutyrate-pyruvate transaminase, and histidinol-phosphate aminotransferase appeared to be more common among persistent strains as compared with non-persistent strains, but the results were not consistent between different serotypes. Conclusion: In vitro biofilm formation distinguishes persistent strains of S. pneumoniae from those that are cleared from the NP. Further studies are needed to identify genes that contribute to the persistence of S. pneumoniae in the NP.
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Title: Baseline and end of therapy hearing in Aboriginal children with chronic suppurative otitis media receiving standard topical treatment plus adjunct therapies: a factorial randomised control trial
Abstract/Summary: Introduction: Hearing is significantly affected by chronic suppurative otitis media (CSOM). This remains high among Australia Aboriginal children in remote communities, with impacts on learning and education outcomes. A factorial design randomised controlled trial was conducted to evaluate adjunct treatment with twice daily antiseptic ear washes (betadine) or no wash, and oral cotrimoxazole ([4mg/kg trimethoprim] BD) or placebo equivalent. The intervention period was 16 weeks. All children also received current standard treatment (twice daily ear cleaning and ciprofloxacin drops). Twenty-eight urban and remote communities in the Northern Territory participated between 2015-2018. Objective: To determine if adjunct betadine ear washes or oral cotrimoxazole improve hearing outcomes at the 12 month follow up. Method: Children with CSOM aged 6 months to 17 years of age were eligible. CSOM was defined as discharge through a perforation for >6 weeks in the worst ear. Routine audiological assessments were included if undertaken within a 2-year period before randomisation (pre-intervention) or 6 to 18 months (post-intervention). Hearing level was defined as the pure tone 4 frequency average hearing (dB) at 500, 1000, 2000 and 4000 Hz in the better hearing and worse hearing ears. Grades of hearing loss were defined for the worse ear as normal (<16dB), mild (16 to 30dB), moderate (31 to 60dB) and severe (61 to 80dB). Mean hearing level differences were estimated using analysis of variance models Results: Of the 280 children with CSOM, an audiogram was performed at baseline in 165 children and at ~12 months after randomisation in 129 children; 89 children had both baseline and 12-month audiograms. The mean hearing level in the worse hearing ear was around 33dB both before and after the intervention period. 3% of children had normal hearing in their worse ear before the intervention period. Comparisons of post-intervention hearing levels in the worse ear were: i) betadine 32.3dB (SD 10.28, n=65) versus no betadine 33.31dB (SD 9.75, n=64); mean difference -1 dB (95% Confidence Interval (CI) -4.5 to 2.5); and ii) cotrimoxazole 32.4dB (SD 9.51, n=61) versus placebo 33.16dB (SD 10.46, n=68); mean difference of -0.76 (95%CI -4.3 to 2.7). There were no differences between the unadjusted and adjusted estimates. Conclusion: Our analysis of children at ~12 months follow-up shows the very high level of persistent hearing loss in children at baseline. There was no difference in hearing levels between adjunct betadine washes versus no wash, or between adjunct oral cotrimoxazole versus placebo. The average hearing loss was above 30dB which WHO states is disabling hearing loss in children. Further studies are needed to determine which medical treatments optimise the hearing in children with CSOM. Strategies for prevention are also needed. In this high risk population, the environmental causes of persistent and severe OM must be addressed.
Presenting Author: Ruth Thornton
Authors: Telethon Kids Institute
Title: Bacterial reservoirs in the middle ear of otitis-prone children predict clinical outcomes
Abstract/Summary: Introduction: Children with recurrent acute otitis media (rAOM) are managed through ventilation tube insertion (VTI), and repeat operations are common. Identifying the contribution of bacterial otopathogen persistence in the middle ear, and increased likelihood of repeat VTI reveals a therapeutic target that could improve clinical management for children with rAOM.
Objective: To investigate if the presence of bacterial otopathogen in middle ear effusion (MEE) from children aged 6 to 36 months undergoing VTI for rAOM is associated with repeat VTI over the following 8 years.
Method: 186 children aged 6 to 36 months and undergoing VTI surgery were recruited into this study between 2007 and 2009. Most (90%) were undergoing first time VTI for rAOM, while the other 10% had previous otorhinolaryngology surgery. When present, MEE samples were collected during VTI (from 148/186 children). Follow up clinical information was collected retrospectively from medical notes for 142 children in 2015. Of these 142 children, MEE PCR data for bacterial otopathogen detection and respiratory viruses was available for 114 children.
Results: Children were grouped according to PCR detection of bacterial otopathogens in their MEE (PCR-ve; n=51 and PCR+ve; n=63). Age, gender, antibiotic usage, day-care attendance and number of AOM episodes were similar between groups (p≥0.095). Nontypeable Haemophilus influenzae (NTHi) was the most frequently detected otopathogen in MEE (79% of all PCR+ve MEE). Of the children who had PCR+ve MEE, 58.7% required repeat VTI surgery during the follow up period compared to 31.4% of children with no bacterial otopathogen detected in their MEE (Odds Ratio, OR = 3.1 [95% CI 1.4 - 6.8]; p = 0.004). Similar proportions of children in each group underwent otorhinolaryngology procedures that did not include repeat VTI during the follow up period (17.6% versus 15.9%; OR 0.9; 95% CI [0.3, 2.4], p = 0.800). Children with no otopathogen detected in their MEE were less likely to require follow up otorhinolaryngology procedures (OR = 0.3 [95% CI 0.1 - 0.7]; p = 0.005). Detection of any of the 11 common respiratory viruses in MEE was not associated with repeat VTI.
Conclusion: Presence of bacterial otopathogen, and specifically NTHi, in the middle ear at the time of VTI surgery is a strong indicator of children at-risk of repeat VTI. Detection of bacterial otopathogen in the MEE at first VTI would identify patients most likely to benefit from targeted treatment strategies such as anti-NTHi therapies. This could improve management of children with rAOM.
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Title: Micro RNAs Implication in the Progression of Middle Ear Infection  
Abstract/Summary: Objective: Otitis Media (OM) affects millions of children every year and costs over $2 billion/year to the US healthcare system. OM is an infection of the middle ear mostly due to Non-typeable Haemophilus influenza (NTHi), and characterized by fluid build-up. During chronic OM, this fluid becomes mucoid as a result of the remodeling of the middle ear epithelium. Currently, there is no medication beneficial to chronic OM, leading to surgery to clear the middle ear. Micro RNAs (miRNAs) are small RNA sequences (<22 nucleotides) that can be carried in small vesicles (exosomes) for cell to cell communication, regulating gene expression. In this study we aimed at investigating the role of miRNAs in the progression of OM. Methods: Middle ear fluids (MEF) were collected during tympanostomy tube placement at Children's National with consent. A human middle ear cell line (HMEEC) was grown and exposed to NTHi lysates to simulate acute stages of OM. Exosomes from both MEF and cell secretions were isolated with Exoquick-TC, miRNAs purified with SeraMir kit and analyzed by Nanostring. HMEEC were transfected with miR-378 mimic or negative control and gene expression for MUC5B, MUC5AC and IL-8 was assayed by PCR, the transcriptome was analyzed by mRNA-seq technique performed by Children's National Genomics Core. miR-378 transfection efficiency was evaluated by PCR and flow cytometry to detect its presence inside of HMEEC. Results: miR-378 was the most upregulated miRNA in HMEEC secretions in response to NTHi lysates and one of the most abundant in MEF. Notably, miR-378 was previously shown to increase mucin expression in airway epithelial cells. Given these findings, we proceeded to test its effect on HMEEC. Cells were transfected with miR-378 using lipofectamine and the presence of miR-378 in cells was confirmed by PCR and flow cytometry. miR-378 exposure resulted in mRNA induction after 24hrs: mucins MUC5B 3.7-fold, MUC5AC 20-fold and IL-8 2-fold compared to negative control miRNA (p<0.05). As a potential mechanism of action, the preliminary mRNA-seq results showed that miR-378 induces Wnt-1 pathway (3-fold, p<0.0001) and the downregulation of HIF1-α inhibitor (1.9-fold, p<0.00001) after 6hrs of incubation, pathways previously implicated in mucin regulation. Conclusion: This study shows the mucogenic and inflammatory effect of miR-378 on the middle ear epithelium. This work will provide the groundwork to find treatment strategies limiting the progression of OM targeting miRNAs.
Presenting Author: Rick T Van Uum

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Title: Effectiveness of a Multifaceted Intervention to Educate General Practitioners about Pain Management in Children with Acute Otitis Media: a Cluster Randomized Controlled Trial

Abstract/Summary: Introduction. Ear pain is the predominant symptom of childhood acute otitis media (AOM) and analgesics are the cornerstone of AOM management. Nevertheless, current evidence suggest that symptomatic management with analgesic management is suboptimal in daily practice, which in turn may lead to unnecessary discomfort, doctor consultation and antibiotic prescribing. Objective. To assess the effectiveness of a multifaceted intervention aimed at educating general practitioners (GPs) about pain management in children with acute otitis media (AOM). Methods. Pragmatic cluster randomized controlled trial with the GP practice as unit of randomization. 83 GPs and 11 GP trainees in 37 practices (intervention n=19; control n=18) across the Netherlands recruited 224 children with GP-confirmed AOM and ear pain (intervention n=94; control n=130) between February 2015 and May 2018. GPs in practices allocated to the intervention group received a blended GP educational program (online and face-to-face training). They were trained to explicitly discuss pain management with parents using an information leaflet, and prompted to prescribe analgesics (paracetamol, and ibuprofen in case of insufficient pain relief with paracetamol alone) in weight-appropriate dosage. GPs in the practices allocated to the control group provided usual care. The primary outcome was parent-reported mean ear pain score (scale 0-10) over the first three days. Results. Primary outcome data was available for 209 children (93.3%). Mean ear pain scores over the first three days were similar between groups (4.66 versus 4.36; adjusted mean difference -0.05, 95% confidence (CI) -0.93 to 0.83), whereas on these days analgesic use, and in particular ibuprofen, was higher in the intervention group than in the control group. Although children in the intervention group received fewer antibiotic prescriptions at the index consultation than those in the control group (mean rate 0.23 versus 0.39; adjusted rate ratio 0.67, 95% CI 0.43 to 1.04), the total number of antibiotic prescriptions during the 28-day follow-up was similar: mean rate 0.43 versus 0.47, respectively (adjusted rate ratio 0.98, 95% CI 0.69 to 1.39). Children in the intervention group consulted their GP more often for AOM-related complaints during follow-up: mean rate 0.70 versus 0.41 (adjusted rate ratio 1.73, 95% CI 1.15 to 2.62). Other secondary outcomes were similar in both groups. Conclusion. Our intervention aimed at educating GPs about pain management in children with AOM led to an increase in analgesic use, in particular ibuprofen, but this did not result in lower parent-reported ear pain scores. We therefore suggest not to routinely use ibuprofen in children with AOM.
Presenting Author: Rebecca Walker

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Title: Nasal microbiota profiles and chronic otitis media with effusion

Abstract/Summary: Objective: To determine the relationship between nasal microbial composition and risk of chronic otitis media with effusion (COME) in preschool children. Method: A case-control study of 178 preschool children aged 3 and 4 years was conducted. The cases were undergoing placement of tympanostomy tubes for COME. The controls were healthy children from primary care practices. Nasal swabs were collected and a structured questionnaire was administered. The microbial community of the anterior nares was assessed by amplifying the V1-3 region of the 16S rRNA gene and sequenced on the Illumina MiSeq platform. Results: Children with COME had a lower Shannon diversity index than healthy controls (1.62 [.80] versus 1.88 [.84], respectively; P=.046). The nasal microbiota of cases and controls differed in composition using Bray-Curtis dissimilarity (p=.002). Children with COME had a higher abundance of otopathogens and lower abundance of commensals including alpha haemolytic Streptococci and Lactococcus. However, the proportion of controls in which any reads of otopathogens were detected did not differ from the proportion of cases (P≥.05). Cluster analysis revealed 4 distinct nasal microbial profiles. Profiles that were Corynebacterium-dominated (aOR 4.18 [95%CI, 1.68-10.39], Streptococcus-dominated (aOR 3.12 [95%CI, 1.08-9.06], or Moraxella-dominated (aOR 4.70 [95%CI, 1.73-12.80] were associated with COME, compared to a more mixed microbial profile when controlling for age, ethnicity, and recent antibiotics use. Conclusion: Children with COME have a less diverse nasal microbial composition with a higher abundance of pathogens, compared to healthy children who have a more mixed bacterial profile with a higher abundance of commensals. The presence of otopathogens in the nasal passages of controls may indicate that they are respiratory pathobionts, remaining asymptomatic until activated into overgrowth and dispersion by stimuli such as viral upper respiratory infection. Further research is required to determine how nasal microbial composition may be involved in the pathogenesis or maintenance of COME, and whether modification of the nasal microbiota is an effective prophylactic or treatment for children at risk of COME.
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Title: Hearing Outcomes at 1 to 3 Years of Age for Aboriginal Children Living in Remote Communities Allocated to Combination Schedules of Pneumococcal Conjugate Vaccines
Abstract/Summary: Aboriginal children living in remote communities of the Northern Territory in are at high risk of early and persistent otitis media, which causes disabling hearing loss and social disadvantage. Objective: To measure conductive hearing loss (CHL) in young Aboriginal children at high risk of otitis media. Methods: Aboriginal infants 28 to 38 days of age were allocated (1:1:1) to either Prevenar13™ (P, PCV13) at 2-4-6 months of age (_PPP), Synflorix™ (S, PHID-CV10) at 2-4-6 months (_SSS), or an investigational schedule of Synflorix at 1-2-4 months plus Prevenar13 at 6 months (SSSP). At 12 months consented infants were randomised to S or P. Outcomes were measured at 1, 2, 4, 6, 7, 12, 18, 24, 30, 36 months of age. Hearing (dB, 3 frequency average) was assessed by an audiologist in a sound-proof booth at 12, 18, 24, 30 and 36 months. Results: 425 infants were randomised to _PPP(143), _SSS(141), or SSSP(141). 261 were randomised to S or P at 12 months of age. 167 hearing assessments are included in this analysis. Investigators remain blinded. Overall, 17% showed no detectable CHL(<16dB), 64% mild CHL (16 to 30 dB), and 19% moderate CHL (31 to 45 dB). Of 61 assessments in children < 30 months of age, these figures were 0%, 77%, and 21%. Of 106 assessments in children > 30 months, these figures were 26%, 56% and 18%. Of 122 cases of any OM and where a hearing assessment was also made, 82 (67%) had mild CHL, 29 (24%) moderate CHL and 11 (9%) had no detectable CHL. Conclusion: Among Aboriginal infants in remote communities, almost all children (90%) with OM have mild or moderate CHL. Differences in infant vaccine schedules may not impact on this outcome. No child less than 30 months of age had normal hearing. Any child with a clinical diagnosis of any OM requires appropriate medical treatment, hearing assistance, and strategies for language stimulation and support with communication. As AOM is asymptomatic, primary health care services must ensure that every ear of every child is assessed at every opportunity. Prevention remains a huge challenge. Social determinants such as household crowding are major predictors of early onset OM and a childhood of chronic conductive hearing loss.
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Title: Non-Invasive, Cross-Sectional Optical Middle Ear Imaging Compared with Acoustic Measurements during Otitis Media

Abstract/Summary: Objective: Non-invasively characterizing middle ear conditions during otitis media (OM) is often intrinsically challenging due to the anatomical location of middle ear. Although wideband acoustic immittance (WAI) assesses middle ear function by measuring the sound conduction over a range of frequencies, its lack of structural information has made it challenging to determine the effects of various middle ear conditions on WAI during OM in vivo. Furthermore, standard otoscopy only allows for surface visualization of the tympanic membrane without any quantitative characteristics of the middle ear or middle ear effusions (MEEs). The purpose of this study is to: (1) quantify physical characteristics (presence, relative turbidity, and amount) of MEEs in vivo using optical coherence tomography (OCT), a non-invasive optical imaging technique, and (2) correlate WAI measurements with quantitative characteristics determined from cross-sectional OCT images. Method: A total of 21 subjects (average age of 7 ± 4 years) visiting an outpatient pediatric clinic were recruited under an approved IRB protocol. A total of 29 ears (normal: 18, OM with effusion: 8, and acute OM: 3 ears, based on pediatrician’s diagnosis) were included in the study. Standard otoscopy, OCT, tympanometry, and WAI measurements were collected in a clinical setting. OCT images were analyzed to assess the presence of a MEE and/or middle ear biofilm, type of MEE (relative turbidity based on the amount of scattering), and amount of MEE fluid (relative fluid level). These OCT metrics were then utilized to categorize subject ears into: no MEE (control), biofilm without a MEE, serous-scant, serous-severe, mucoid-scant, and mucoid-severe MEE groups. The power absorbance of each group was correlated to evaluate statistical significance at \(\alpha=0.05\). Results: The presence of a MEE decreased the power absorbance. The power absorbance from 2-5 kHz showed large variance across the groups, suggesting the dependence on both the type and amount of MEEs. The mucoid MEE group showed significantly less power absorbance at 1.65 kHz (\(p=0.04\)) and 2.65 kHz (\(p=0.01\)), when compared with the serous MEE group. The greater amounts of MEE fluid significantly decreased the power absorbance, especially at higher (>2 kHz) frequencies. The lower amounts of MEE fluid did not significantly affect the power absorbance, as expected. Conclusion: A portable, handheld OCT-otoscope can non-invasively determine physical characteristics of the middle ear and MEEs during OM. Quantitative cross-sectional OCT images can not only provide additional structural information about the middle ear space, but can also be utilized to better understand abnormal WAI measurements from the early- to late/severe-stages of OM. Further investigations to correlate acoustic measurements with other physical characteristics of middle ear conditions in vivo are needed.
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Title: Acute otitis media in infants younger than two months of age: epidemiologic and microbiologic characteristics in the era of pneumococcal conjugate vaccines
Abstract/Summary: Objectives: To evaluate the epidemiology, microbiology, Streptococcus pneumoniae (SP) serotypes distribution and serious bacterial infections (SBIs) occurrence in infants <2 months of age with tympanocentesis-documented acute otitis media (AOM), before and after the introduction of pneumococcal conjugate vaccines (PCVs). Methods: The medical records of all hospitalized infants with AOM who underwent tympanocentesis during 2005-2014 were reviewed. Results: Of the 182 infants with AOM, 92 were diagnosed during 2005-2009 (prevaccine period) and 90 during 2010-2014 (postvaccine period). No changes were recorded in the number of AOM cases requiring tympanocentesis during 2005-2014 vs. 2010-2014 (92/12,619 patients, 0.007% vs. 90/13,563, 0.006%, P=0.57; the number of cases requiring tympanocentesis reached their lowest during 2013-2014. SP and nontypeable H. influenzae (NTHI) were isolated in 46/92 (50%) and 37/92 (40.2%) patients during 2005-2009 and decreased to 27/90 (30%) and 21/90 (23.3%), respectively, during 2010-2014 (P=0.006 and P=0.001). The number of culture-negative patients increased from 18/92 (19.6%) during 2005-2009 to 32/90 (35.6%) during 2010-2014 (P=0.02). There were only 6 (3.3%) patients <2 weeks of age. The most common SP vaccine serotypes isolated during 2005-2009 were 5, 3, 1, 19F and 14 (15.2%, 13.0%, 10.9%, 6.5%, and 4.3%, respectively) and 3, 5, 1, 14 and 19A (22.2%, 11.1%, 7.4%, 7.4%, and 7.4%, respectively) during 2010-2014. No changes were recorded in the percentages of vaccine serotypes (58.7% vs. 63%, P=0.7) and non-vaccine serotypes (41.3% vs. 37%, P=0.7) between 2005-2009 and 2010-2014. SBIs were recorded in 23/182 (12.64%) patients and urinary tract infections represented 19/23 (82.61%) of them (Escherichia coli isolated in 12, 63.2%). Conclusions: The overall number of AOM cases and of SP and NTHI-AOM decreased while culture-negative-AOM increased following the introduction of PCVs. SBIs associated with AOM were frequent and were represented mostly by urinary tract infections caused by pathogens unrelated to the etiologic agents of AOM.