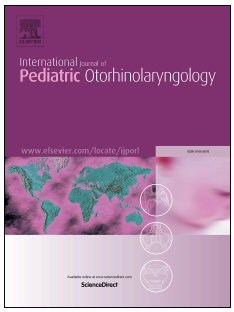

Panel 1: Biotechnology, biomedical engineering and new models of otitis media

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PANEL 1: BIOTECHNOLOGY, BIOMEDICAL ENGINEERING AND NEW MODELS OF OTITIS MEDIA

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ABSTRACT

OBJECTIVE: To summarize recently published key articles on the topics of biomedical engineering, biotechnology and new models in relation to otitis media (OM).

DATA SOURCES: Electronic databases: PubMed, Ovid Medline, Cochrane Library and Clinical Evidence (BMJ Publishing).

REVIEW METHODS: Articles on biomedical engineering, biotechnology, material science, mechanical and animal models in OM published between May 2015 and May 2019 were identified and subjected to review. A total of 132 articles were ultimately included.

RESULTS: New imaging technologies for the tympanic membrane (TM) and the middle ear cavity are being developed to assess TM thickness, identify biofilms and differentiate types of middle ear effusions. Artificial intelligence (AI) has been applied to train software programs to diagnose OM with a high degree of certainty. Genetically modified mice models for OM have further investigated what predisposes some individuals to OM and consequent hearing loss. New vaccine candidates protecting against major otopathogens are being explored and developed, especially combined vaccines, targeting more than one pathogen. Transcutaneous vaccination against non-typeable *Haemophilus influenzae* has been successfully tried in a chinchilla model. In terms of treatment, novel technologies for trans-tympanic drug delivery are entering the clinical domain. Various growth factors and grafting materials aimed at improving healing of TM perforations show promising results in animal models.

CONCLUSION: New technologies and AI applications to improve the diagnosis of OM have shown promise in pre-clinical models and are gradually entering the clinical domain. So are novel vaccines and drug delivery approaches that may allow local treatment of OM.

IMPLICATIONS FOR PRACTICE: New diagnostic methods, potential vaccine candidates and the novel trans-tympanic drug delivery show promising results, but are not yet adapted to clinical use.

KEYWORDS: otitis media, animal model, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, diagnostics, vaccines, treatment

INTRODUCTION

Otitis media (OM) is a major cause of health care visits, antibiotic prescriptions and surgery, especially in the pediatric population (1, 2). Its complications and sequelae are important causes of preventable hearing loss as well as serious infections, particularly in developing countries.

This report from our 'Biotechnology, Biomedical Engineering and New Models of Otitis Media' panel was drafted by 12 clinicians and scientists who convened during the 20th International Symposium on Recent Advances in Otitis Media, Hollywood, CA, USA in June 2019. We focused on articles on the above topics which were published since the last report in 2015 (3).

METHODS

Panel members were assigned to review the following topics: imaging of the tympanic membrane (TM) and the middle ear, bioengineering, mechanical models, animal models (advances in genetics, microbiology and vaccines), innovative treatment approaches, material science and tissue engineering.

Each panel member designed a topic-specific key-word search strategy for the various electronic databases [PubMed, Ovid Medline, the Cochrane Library and Clinical Evidence (BMJ Publishing)]. Databases were searched for publications with an English language abstract published from 5/31/2015 to 5/31/2019. Additional relevant articles identified during the meeting were added. In total, 132 articles were included in this manuscript.

RESULTS

1. Imaging Technologies

Visible light (pneumatic) otomicroscopy is considered the best diagnostic tool for OM currently available. Specific features of the TM, such as bulging and redness, are vital for making a correct diagnosis (4). However, otomicroscopes are not available everywhere, and require training. The second-best option is pneumatic otoscopy; however, it has multiple and well documented limitations regarding diagnostic certainty (5-8). To overcome current diagnostic challenges in otoscopy, several new technologies are being developed, aimed at mapping the TM and assessing and quantifying the presence of middle ear effusion (MEE). **Table 1** summarizes these recent advances in imaging technologies.

1.1 Tympanic Membrane

Optical coherence tomography (OCT) has been proposed to evaluate structural changes of the TM. OCT is an optical imaging technique analogous to ultrasound, enabling a cross-sectional view of the TM and the identification of thickness changes and biofilm attachment to the TM (9-12). OCT coupled with pneumatic otoscopy enables quantification of TM mobility (compliance) and middle ear pressure (13, 14).

Optical methods to delineate the three-dimensional (3D) contour of the TM have also been explored. One modality utilizes structured light to form a 3D image (15). Another 3D imaging method is light field, where a plenoptic camera records numerous measurements, corresponding to rays passing through different locations in the aperture. Plenoptic data can be demultiplexed to a set of multi-view images forming a 4-dimensional structure called the light field. From this, a 3D picture can be computed (16).

Another technology is terahertz otoscopy, based on the high affinity of terahertz waves for water. This technique has been explored *ex vivo*, but there is no clinical data to this date (17).

1.2 Middle Ear

Evaluation and diagnosis of the middle ear status using conventional oto(micro)scopy and tympanometry have remained dependent on the physician's experience and interpretation. Superior resolution and assessment of the middle ear cavity and its content can potentially reduce over-prescription of antibiotics for AOM, ventilating tube (VT) insertion if prolonged MEE is detected, and diagnostic exploratory surgery in challenging otologic pathology.

High frequency ultrasound (HFUS) has been successfully utilized *ex vivo* and *in vitro* to visualize middle ear pathologies, like ossicular pathologies, with high resolution (18-20). Spectral gradient acoustic reflectometry (SGAR) has been tested to predict the presence of MEE, with high sensitivity following short training, but specificity is relatively poor compared to tympanometry (21). Transmastoid ultrasound has been used to detect MEE with high accuracy, yet improved design of the probe for clinical use is needed (22).

Visual light techniques include multicolor reflectance imaging and narrow band imaging (NBI). Multicolor reflectance imaging produces high definition images with demarcation of morphological structures to detect MEE (23). The technique provides superior imagery of middle ear pathology compared to current methods, but patient movement and image distortion remain substantial challenges. NBI has been used to investigate middle ear anatomy using specific blue and green light wavelengths that interact with hemoglobin to enhance illustration of TM vascularity (24).

Near infrared light techniques include anti-confocal middle ear assessment and OCT. Anti-confocal spectroscopic measurements to assess middle ear inflammation by analyzing blood content have been successful *in vitro*, but *in vivo* assessment has yet to be conducted (25). OCT enables non-invasive characterization of middle ear pathology *in vivo*, in addition to TM imaging (**Figure 1**). OCT has the largest number of publications over the specified time period and, most notably, has been shown to facilitate non-invasive differentiation of the type of MEE (9, 10, 20, 26-34).

An otoscope sensitive to shortwave infrared (SWIR) wavelengths of light provides two primary advantages over conventional visible light-based pneumatic otoscopy: it can help identify MEE based on the strong light absorption by fluid in the SWIR spectral region, and can penetrate deeper through tissue, enabling a view of middle ear anatomy behind the TM (35, 36) (**Figure 2**).

Raman spectroscopy is able to distinguish serous from mucoid MEEs *ex vivo* (37). Scanning laser doppler vibrometry can detect changes in chinchilla TM motion evoked by sound during OM, with reduced amplitudes and a shift towards lower frequencies (38).

Synchrotron radiation phase-contrast imaging has shown improved contrast and thereby visualization and finite-element modeling of middle ear structures (39).

2. Bioengineering

2.1 Computerized Software

Artificial intelligence has been used to train computer software to diagnose OM from otoscopic images of the TM with a diagnostic accuracy of over 90% (40-42). A computer vision system was able to automatically detect VTs in otoscopic images of high as well as low quality. The offline training process constructed a 3-layer cascaded classifier, with each layer reflecting specific characteristics of the VT. When trained using 215 images, it could achieve a 90% accuracy in terms of classifying otoscopic images with and without VTs (43).

2.2 3D Models

3D reconstructions of cadaver ears from patients with chronic OM or cholesteatoma has shown reduced volumes of the bony portion of the Eustachian tube (ET) compared to normal controls (44). Computerized tomography (CT) scans have been used to measure ET diameter in healthy adults and young children <10 years old who exhibit reduced ET opening

efficiency, and thus were considered to be OM prone. A smaller diameter was detected in the latter (45). CT scans can reliably measure the length of the ET cartilaginous portion, but this measurement seems to be of limited prognostic value for surgical treatment, i.e. successful ET opening dilatation (46). Multi-scaled modeling has been employed to study if the ET is more affected by mucosal adhesion in children than in adults, potentially contributing to their increased susceptibility to OM (47). A 3D model of the chinchilla ear, based on X-ray micro-CT images, has helped characterizing middle ear functions (48). A conventional 2D monocular endoscope coupled with a computer-based 3D imaging system has been tested during otologic surgery for chronic otitis media (COM), cholesteatoma, otosclerosis and cochlear implant, thereby giving 3D vision endoscopic procedures (49). The presence of MEE has also been explored by a machine-learning software algorithm able to assess TM mobility using speakers and microphones within a normal smartphone (50).

3. Animal Models

Several animal models of OM have been used in recent years. The mouse has become one of the favored models because of the mouse life span, its easy breeding and the well-established methods for introducing genetic modifications. The similarities in auditory structure between mouse and human and the similarity of the genomes make the mouse a valuable model to study the genetics of hearing.

3.1 Genetics

In the past four years, new genetic models to study the predisposition to OME have been developed:

(1) FLI1 and ETS1 are transcription factors known to be homozygous in Jacobsen syndrome. They play a role in the development of the nose, middle ear cavity and ossicles.

The Fli1+/− and Ets1+/−Fli1+/− mice exhibit hearing impairment associated with chronic OM, a small middle ear cavity, and fusions deformities of the ossicles (51).

(2) Ages-with-stiffened-joints mutant mice, which have a point mutation in the Enpp1 gene develop conductive hearing loss, MEE and ME deformities at an early age (52).

(3) Mutations in the EDA, EDAR and EDARADD genes are associated with the development of OM, rhinitis and nasopharyngitis (53).

(4) A point mutation in the Nischarin (NISCH) gene results in the development of conductive hearing loss due to chronic OM. Homozygous mice spontaneously develop chronic OM at three weeks of age, and sometimes an inflamed TM (54).

(5) BPIFA1, a bacterial permeability-increasing fold innate defense protein, is one of the most abundant secretory proteins in the upper respiratory tract. Bpifa1 knock out mice do not develop spontaneous OM up to six months, although BPIFA1 is highly expressed in the middle ear epithelium. However, deletion of Bpifa1 in Junbo mice resulted in significant exacerbation of the phenotype including thickening of the middle ear mucosa and increased collagen deposition. This finding indicates a role of BPIFA1 in mucosal protection (55).

3.2 New Animal Models

A novel *in vitro* model of mouse middle ear epithelial cells, incorporating an air-liquid interface (ALI) has been developed (56) and recapitulated the characteristics of the native murine middle ear epithelium. After bacterial infection, it mimicked features of the epithelium in OM.

3.3 Microbiology and New Vaccines

Animal models and cell cultures have been used in vaccine research, including studies of basic immunologic mechanisms and those aimed at finding vaccine candidate molecules and protein carriers (57).

Streptococcus pneumoniae (Spn), non-typeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis* (Mcat) are the major AOM bacteria. With viral-bacterial interactions playing an important role in the pathophysiology of AOM, vaccines against respiratory syncytial virus (RSV) and influenza virus are being developed (58, 59).

3.3.1 Streptococcus pneumoniae (Spn)

Vaccines against a larger number of pneumococcal serotypes than in the current 10- and 13-valent pneumococcal conjugated vaccines are being developed, but no new trials have been conducted with them (60). The main findings on research about pneumococcal OM are summarized in **Table 2.** Most research has focused on the identification of virulence peptides in both encapsulated and non-encapsulated strains and the investigation of quorum sensing peptides in bacterial persistence (61).

Recent studies have shown that a secreted metabolite byproduct of the LuxS/AI-2 quorum sensing system enables Spn to utilize galactose as a carbon source and increases the production of capsular polysaccharide and the development of a hypervirulent OM phenotype in a mouse model (62). Single nucleotide polymorphisms in the raffinose uptake and utilization genes *rafR* or *rafK* dictate the nature of pneumococcal disease (63) and genes involved in the metabolism of sugars influence the virulence of bacteria during OM in chinchillas (64).

New vaccines: The pneumococcal polysaccharide capsule has been extensively studied because of its role in AOM development (65). Attempts at developing peptide

vaccines have involved chitosan nanoparticles, used as vectors for pneumococcal surface adhesin protein A (PsaA) (66) and the development of a conjugate recombinant PsaA vaccine with the *Haemophilus influenzae* type b (Hib) polysaccharide (67).

Non-encapsulated Spn strains lack genes involved in capsule synthesis. Loss of these genes have been shown to dampen the OM phenotype in mouse and chinchilla models (68-70) (**Figure 3**). Mice vaccinated with trivalent vaccine including some of these genes have shown reduced inflammatory responses in experimentally induced Spn-AOM (69). Hib-PsaA conjugate vaccine can induce both anti-Hib and anti-PsaA immune responses in young mice and elicits effective protection against Spn-induced AOM (67).

These studies emphasize the importance of developing combined vaccines that can target multiple virulence factors.

3.3.2 Non-typeable Haemophilus influenzae (NTHi)

The major findings of studies on the pathophysiology of NTHi-induced OM have been summarized in **Table 3**.

Several studies suggest that pre-existing effusion in the middle ear of mice that spontaneously develop chronic OM promotes infection and persistence of NTHi in the middle ear (55, 71). A direct correlation between fluid viscosity and bacterial load has been observed in Junbo mice (72). This is supported by a study on children with OME, which identified NTHi as the predominant pathogen in MEEs with a higher content of mucins (73). Other studies have focused on understanding the factors involved in the persistence of NTHi during OM (74, 75). A chinchilla study has revealed that early disease is accompanied by host immunosuppression and actin morphogenesis, along with bacterial aerobic respiration (76).

Phase variable alleles in NTHi are involved in epigenetic regulation of several virulence genes, and the impact of one such allele being turned 'ON' or 'OFF' has been evaluated. Chinchillas infected with bacteria where the allele had been turned OFF at inoculation but shifted ON in the middle ear has shown a more severe disease compared to those where it was either inoculated OFF and remained OFF or inoculated ON and remained ON (77, 78). An *in vitro* model of middle ear epithelial cells cultured at the air-liquid interface has been developed and used to study the effect of NTHi infection on middle ear epithelium (79, 80).

Given the genetic diversity of NTHi, there was an increasing interest in "microbiome-sparing", i.e. developing approaches targeted towards genes specific to the disease-causing strains of NTHi, such identifying surface-exposed proteins (SEPs) in different strains of NTHi which can act as receptors, secretory systems and sensors, and function to establish host-pathogen interactions (81-85). Another strategy has been to develop new approaches to target bacterial biofilms (86).

New vaccines: Several outer membrane proteins including outer membrane protein 1, 2, 4 and 6, Tbp1, Tbp2, protein D, Haemophilus adhesin protein, high-molecular-weight protein 1 and 2, and *H. influenzae* adhesin (Hia) have been reported as possible vaccine candidates (87, 88).

3.3.3 Moraxella catarrhalis (MCat)

Key observations from studies on MCat studies have been summarized in **Table 4**.

New vaccines: MCat obtained from patients with OM has been examined with genome mining, showing that AfeA, a substrate binding protein, is an excellent candidate vaccine antigen. It was present in all examined strains, it is highly conserved among clinical

isolates, it expresses abundant epitopes on the bacterial surface, it is highly immunogenic and induces protective immune responses in the mouse following aerosol challenge with MCat. It is expressed during human infection (89, 90). Substrate binding protein 2 and sulfate binding protein are other promising vaccine antigens candidates (91, 92).

MCat components involved in adherence to host tissue have been studied (93). Lactoferrin binding protein A and oligopeptide permease A were identified as potential candidates for vaccine development (94, 95).

3.4 Polymicrobial Infections

Table 5 summarizes recent findings on polymicrobial infections in OM.

Neuraminidase NanA works synergistically with influenza A neuraminidase to exacerbate colonization by Spn (58). Intranasal inoculation with live attenuated influenza virus before or after intranasal pneumococcal inoculation has been shown to increase the transfer of bacteria into the middle ear cavity in mice (96).

There has been increasing interest in methicillin-resistant *Staphylococcus aureus* (MRSA) as a cause of OM in combination with other otopathogens in pre-clinical and clinical studies (97-100).

3.5 Viral Infections

Apart from influenza virus, there are currently no available vaccines against respiratory viruses; no significant advances have been reported in the last 4 years. Yet, it has been reported that the administration of RSV fusion protein can induce potent neutralizing antibody responses against RSV, which may facilitate the development of an effective vaccine (101-103).

4. Innovative Treatment Approaches

4.1 Trans-tympanic Drug Delivery

Trans-tympanic drug delivery approaches allow antimicrobials to enter the middle ear without systemic side effects. A drug delivery system that forms a strong hydrogel on the TM surface has been developed, thus allowing the antibiotic to flux into the middle ear (104). All chinchillas (n=10) treated with this 1% hydrogel-ciprofloxacin combination cleared NTHi-induced OM, in contrast to 63% of animals who received 1% ciprofloxacin ear drops alone. In another chinchilla study, trans-tympanic delivery of ciprofloxacin also cleared Spn-induced OM (105). Trans-tympanic delivery minimized systemic side effects.

Peptides have also been studied for trans-tympanic drug delivery: peptides that can actively cross an intact TM into the middle ear were identified in rats with OM (106, 107) (**Figure 4**).The addition of six specific amino acids further enhanced the transport capacity of the peptides (108). In an *in vitro* study done on human TM fragments discarded during otologic surgery, human trans-tympanic transport capacity was found to be as effective as that of rats, rabbits or guinea pigs (109) (**Figure 5**). Trans-tympanic delivery of analgesics has also been achieved in chinchillas with experimentally-induced AOM (110).

4.2 Transcutaneous Immunization

Transcutaneous immunization against NTHi using band-aids placed post-auricularly has been tested in chinchillas. The use of antibodies against the major subunit of type IV Pili resulted in eradication of NTHi, disruption of mucosal biofilms and rapid resolution of AOM (111). One band-aid vaccine resolved experimental NTHi-induced AOM in chinchillas significantly faster than saline alone (112). Monoclonal antibodies directed against specific epitopes of bacterial DNA binding proteins common in biofilm matrixes have been shown to be highly effective in disrupting biofilms in vitro and resolve experimental OM in chinchilla and murine models (86).

4.3 Other Treatment Options

The effect of caffeic acid phenethyl ester and thymoquinone on OME treatment has been studied in a rat model. Submucosal neutrophil leukocyte count was significantly lower among rats receiving the intra-peritoneal treatment (10 mg/kg), as compared to rats receiving thymoquinone, methylprednisolone, or saline (113).

The use of Hypericum perforatum (St. John's Wort) on prevention of myringosclerosis after myringotomy has also been evaluated in rats (114). Oral or topical administration of this extract suppressed inflammation and fibroblastic activity, thus reducing the severity of myringosclerosis. In another study, clarithromycin showed similar effects in an animal model (115).

Mucosal biofilms play a significant role in OME and many strategies to remove biofilms have been investigated. Middle ear irrigation with saline or 1% baby shampoo was effective in reducing biofilm formation in chinchilla middle ears. Irrigation treatment did not affect hearing, vestibular or facial nerve functions (116).

5. Materials Science

It has been shown in rats that human adipose-derived stromal cells could regenerate temporal bone defects following mastoidectomy; rats that received human adipose cells had significantly higher bone formation compared to controls (117). Tissue-engineered autologous middle ear cell sheets have been examined to regenerate middle ear mucosa lost during surgery in a rabbit model. While granulation tissue formation and bone hyperplasia were inhibited, increased mucosal regeneration was observed in the cell sheet-grafted group compared to controls (118). Ossicular prostheses in which silver nanoparticles have been embedded have been tested *in vitro* and demonstrated that the released silver had an antimicrobial effect (119).

Several studies have been promising for improved TM regeneration. Heparin binding epidermal growth factor-like growth factor enhances regeneration of TM perforations in mice (120). In another study in rats, epidermal growth factor-releasing nanofibrous patches showed improved TM healing (121). Similarly, improved TM regeneration has been achieved using chitosan patch scaffolds incorporated with insulin-like growth factor-binding protein 2 in a rat model (122). Bioprinted acellular grafts have been developed to treat TM perforations with the use of endoscopic imaging to create customized grafts, showing improved healing in chinchillas (123). In humans with small to moderately sized perforations, bacterial cellulose graft myringoplasty as an alternative to fat graft/temporal fascia myringoplasty have shown similar post-operative healing and hearing results (124). The use of gelatin sponge soaked with fibroblast growth factor during myringoplasty may also improve TM regeneration (125, 126).

A novel VT made from Nitinol with titanium dioxide coating has been shown to inhibit biofilm formation *in vitro* when inoculated with a carbenicillin-resistant *Pseudomonas aeruginosa* strain (127).

ET balloon dilation has been introduced to overcome ET dysfunction and its sequala, chronic OM, yet evidence is only available from case series showing short-term improvement of symptoms (128, 129). To prolong the effect of ET dilation, the application of a stent has been proposed. A cobalt-chrome coronary stent introduced from the nasopharynx into the ET has been shown to enhance middle ear ventilation in sheep (130). Whether this intervention has an effect on the disease course of OM remains to be seen.

DISCUSSION

New ways of preventing, diagnosing and treating OM have continued to be investigated, though most new findings have not yet been translated to testing in humans.

Prevention with polymicrobial vaccines and the targeting of multiple antigens is promising in the laboratory, but it remains to be seen to what extent OM could be prevented in a clinical setting, and whether the effect would be lasting.

Improving the diagnostic accuracy of OM to avoid unnecessary antibiotic overuse without compromising the outcome for those actually suffering from the disease is still of high priority. Easy-to-learn, reliable diagnostic methods could improve the management, especially of pediatric patients, with OM, but the techniques have to be widely used and taught, and affordable for doctors in primary care if they are to make a difference.

Trans-tympanic drug delivery has the potential to reduce not only systemic side effects for the individual, but also the selective pressure of antibiotics, thus giving a scope for a slow-down of antimicrobial resistance development.

IMPLICATIONS FOR CLINICAL PRACTICE AND FUTURE RESEARCH GOALS

Further development of new techniques may provide better ways of diagnosing, preventing and treating OM and its sequelae. The use of experimental models to further elucidate the basic properties of disease mechanisms shows promising results and warrants further exploring. Further development of these novel modalities may provide an enhanced ability to diagnose middle ear disease in conjunction with, or as replacement of, current technologies in the future. They might also make it easier to evaluate new means of prevention, pharmaceutical delivery or surgical intervention studied clinically or in animal models. The use of experimental models to further elucidate the basic properties of the disease mechanisms to be able to target new treatment models shows promising results and new fields to explore further.

AUTHOR CONTRIBUTIONS

Authors MG, AGMS, AH and TM concepted the study rationale, reviewed and integrated input from all other co-authors; authors PAT and AFR reviewed new treatment approaches; authors AM, SK and SB reviewed animal models; authors TAV and RMN reviewed imaging technologies and bioengineering, and author GvI reviewed material science.

All authors actively participated in drafting the manuscript, approved the final version and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DISCLOSURES

Ryan M. Nolan, is the Co-Founder and VP of Clinical Operations, PhotoniCare, Inc. Tal Marom and Anne G.M. Schilder are on the Scientific Advisory Board of Novus Therapeutics, Inc. Allen F. Ryan is a Co-founder of and stockholder in Otonomy, Inc. The other authors declare no conflicts of interest, and there has been no financial support received for this work. Funding for the generation and publication of this panel report was made possible in part by 1 R13 DC017389-01 from the National Institute on Deafness and Other Communication Disorders.

REFERENCES

1. Disease GBD, Injury I, Prevalence C. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392(10159):1789-858.
2. Guys N, Ramsey T, Kandinov A, Svider PF, Jyung RW, Hong R, et al. Interplay Between Socioeconomic Status and Otitis Media Disease Burden in Resource-rich and Resource-poor Nations. Otol Neurotol. 2018;39(9):e817-e24.
3. Preciado D, Granath A, Lin J, Val S, Kurabi A, Johnston N, et al. Panel 8: Report on Recent Advances in Molecular and Cellular Biochemistry. Otolaryngol Head Neck Surg. 2017;156(4_suppl):S106-S13.
4. Ovnat Tamir S, Shemesh S, Oron Y, Marom T. Acute otitis media guidelines in selected developed and developing countries: uniformity and diversity. Arch Dis Child. 2017;102(5):450-7.
5. Rosenfeld RM, Shin JJ, Schwartz SR, Coggins R, Gagnon L, Hackell JM, et al. Clinical Practice Guideline: Otitis Media with Effusion (Update). Otolaryngol Head Neck Surg. 2016;154(1 Suppl):S1-S41.
6. Takata GS, Chan LS, Morphew T, Mangione-Smith R, Morton SC, Shekelle P. Evidence assessment of the accuracy of methods of diagnosing middle ear effusion in children with otitis media with effusion. Pediatrics. 2003;112(6 Pt 1):1379-87.
7. Pichichero ME, Poole MD. Assessing diagnostic accuracy and tympanocentesis skills in the management of otitis media. Arch Pediatr Adolesc Med. 2001;155(10):1137-42.
8. Rosenfeld RM. Diagnostic certainty for acute otitis media. Int J Pediatr Otorhinolaryngol. 2002;64(2):89-95.
9. Dsouza R, Won J, Monroy GL, Hill MC, Porter RG, Novak MA, et al. In vivo detection of nanometer-scale structural changes of the human tympanic membrane in otitis media. Sci Rep. 2018;8(1):8777.
10. Monroy GL, Shelton RL, Nolan RM, Nguyen CT, Novak MA, Hill MC, et al. Noninvasive depth-resolved optical measurements of the tympanic membrane and middle ear for differentiating otitis media. Laryngoscope. 2015;125(8):E276-82.
11. Hubler Z, Shemonski ND, Shelton RL, Monroy GL, Nolan RM, Boppart SA. Real-time automated thickness measurement of the in vivo human tympanic membrane using optical coherence tomography. Quant Imaging Med Surg. 2015;5(1):69-77.
12. Pande P, Shelton RL, Monroy GL, Nolan RM, Boppart SA. A Mosaicking Approach for In Vivo Thickness Mapping of the Human Tympanic Membrane Using Low Coherence Interferometry. J Assoc Res Otolaryngol. 2016;17(5):403-16.
13. Won J, Monroy GL, Huang PC, Dsouza R, Hill MC, Novak MA, et al. Pneumatic low-coherence interferometry otoscope to quantify tympanic membrane mobility and middle ear pressure. Biomed Opt Express. 2018;9(2):397-409.
14. Shelton RL, Nolan RM, Monroy GL, Pande P, Novak MA, Porter RG, et al. Quantitative Pneumatic Otoscopy Using a Light-Based Ranging Technique. J Assoc Res Otolaryngol. 2017;18(4):555-68.
15. Das AJ EJ, Ge Z, Dolcetti S, Chen D, Raskar R. A compact structured light based otoscope for three dimensional imaging of the tympanic membrane. Photonic Therapeutics and Diagnostics XI Vol 9303. (International Society for Optics and Photonics; 2015:93031F.).
16. Bedard N, Shope T, Hoberman A, Haralam MA, Shaikh N, Kovacevic J, et al. Light field otoscope design for 3D in vivo imaging of the middle ear. Biomed Opt Express. 2017;8(1):260-72.
17. Ji YB, Moon IS, Bark HS, Kim SH, Park DW, Noh SK, et al. Terahertz otoscope and potential for diagnosing otitis media. Biomed Opt Express. 2016;7(4):1201-9.
18. Rainsbury JW, Landry TG, Brown JA, Adamson RA, Bance M. High Frequency Ex Vivo Ultrasound Imaging of the Middle Ear to Show Simulated Ossicular Pathology. Otol Neurotol. 2016;37(5):586-92.

19. Landry TG, Rainsbury JW, Adamson RB, Bance ML, Brown JA. Real-time imaging of in-vitro human middle ear using high frequency ultrasound. Hear Res. 2015;326:1-7.
20. Bance M, Zarowski A, Adamson RA, Casselman JW. New Imaging Modalities in Otology. Adv Otorhinolaryngol. 2018;81:1-13.
21. Erkkola-Anttinen N, Laine MK, Tahtinen PA, Ruohola A. Can changes in parentally measured acoustic reflectometry levels predict the middle ear status? Int J Pediatr Otorhinolaryngol. 2017;95:72-4.
22. Chen CK, Fang J, Wan YL, Tsui PH. Ultrasound characterization of the mastoid for detecting middle ear effusion: A preliminary clinical validation. Sci Rep. 2016;6:27777.
23. Valdez TA, Spegazzini N, Pandey R, Longo K, Grindle C, Peterson D, et al. Multi-color reflectance imaging of middle ear pathology in vivo. Anal Bioanal Chem. 2015;407(12):3277-83.
24. Zhang H, Wong PY, Magos T, Thaj J, Kumar G. Use of narrow band imaging and 4K technology in otology and neuro-otology: preliminary experience and feasibility study. Eur Arch Otorhinolaryngol. 2018;275(1):301-5.
25. Jung DS, Crowe JA, Birchall JP, Somekh MG, See CW. Anti-confocal assessment of middle ear inflammation. Biomed Opt Express. 2017;8(1):230-42.
26. Dsouza R, Won J, Monroy GL, Spillman DR, Boppart SA. Economical and compact briefcase spectral-domain optical coherence tomography system for primary care and point-of-care applications. J Biomed Opt. 2018;23(9):1-11.
27. Monroy GL, Hong W, Khampang P, Porter RG, Novak MA, Spillman DR, et al. Direct Analysis of Pathogenic Structures Affixed to the Tympanic Membrane during Chronic Otitis Media. Otolaryngol Head Neck Surg. 2018:194599818766320.
28. Park K, Cho NH, Jeon M, Lee SH, Jang JH, Boppart SA, et al. Optical assessment of the in vivo tympanic membrane status using a handheld optical coherence tomography-based otoscope. Acta Otolaryngol. 2018;138(4):367-74.
29. Tan HEI, Santa Maria PL, Wijesinghe P, Francis Kennedy B, Allardyce BJ, Eikelboom RH, et al. Optical Coherence Tomography of the Tympanic Membrane and Middle Ear: A Review. Otolaryngol Head Neck Surg. 2018:194599818775711.
30. Monroy GL, Pande P, Nolan RM, Shelton RL, Porter RG, Novak MA, et al. Noninvasive in vivo optical coherence tomography tracking of chronic otitis media in pediatric subjects after surgical intervention. J Biomed Opt. 2017;22(12):1-11.
31. Monroy GL, Pande P, Shelton RL, Nolan RM, Spillman DR, Jr., Porter RG, et al. Non-invasive optical assessment of viscosity of middle ear effusions in otitis media. J Biophotonics. 2017;10(3):394-403.
32. Pande P, Shelton RL, Monroy GL, Nolan RM, Boppart SA. Low-cost hand-held probe for depth-resolved low-coherence interferometry. Biomed Opt Express. 2017;8(1):338-48.
33. Park K, Cho NH, Jang JH, Lee SH, Kim P, Jeon M, et al. In vivo 3D imaging of the human tympanic membrane using a wide-field diagonal-scanning optical coherence tomography probe. Appl Opt. 2017;56(9):D115-D9.
34. Pande P, Monroy GL, Nolan RM, Shelton RL, Boppart SA. Sensor-Based Technique for Manually Scanned Hand-Held Optical Coherence Tomography Imaging. J Sens. 2016;2016.
35. Valdez TA, Carr JA, Kavanagh KR, Schwartz M, Blake D, Bruns O, et al. Initial findings of shortwave infrared otoscopy in a pediatric population. Int J Pediatr Otorhinolaryngol. 2018;114:15-9.
36. Carr JA, Valdez TA, Bruns OT, Bawendi MG. Using the shortwave infrared to image middle ear pathologies. Proc Natl Acad Sci U S A. 2016;113(36):9989-94.
37. Pandey R, Zhang C, Kang JW, Desai PM, Dasari RR, Barman I, et al. Differential diagnosis of otitis media with effusion using label-free Raman spectroscopy: A pilot study. J Biophotonics. 2018;11(6):e201700259.
38. Wang X, Gan RZ. Surface Motion of Tympanic Membrane in a Chinchilla Model of Acute Otitis Media. J Assoc Res Otolaryngol. 2018;19(6):619-35.

39. Elfarnawany M, Rohani SA, Ghomashchi S, Allen DG, Zhu N, Agrawal SK, et al. Improved middle-ear soft-tissue visualization using synchrotron radiation phase-contrast imaging. Hear Res. 2017;354:1-8.
40. Tran TT, Fang TY, Pham VT, Lin C, Wang PC, Lo MT. Development of an Automatic Diagnostic Algorithm for Pediatric Otitis Media. Otol Neurotol. 2018;39(8):1060-5.
41. Chuen-Kai S, Chung-Hisang C, Chun-Nan C, Meng-Hsi W, Chang EY. Transfer representation learning for medical image analysis. Conf Proc IEEE Eng Med Biol Soc. 2015;2015:711-4.
42. Cha D, Pae C, Seong SB, Choi JY, Park HJ. Automated diagnosis of ear disease using ensemble deep learning with a big otoendoscopy image database. EBioMedicine. 2019;45:606-14.
43. Wang X, Valdez TA, Bi J. Detecting tympanostomy tubes from otoscopic images via offline and online training. Comput Biol Med. 2015;61:107-18.
44. Pauna HF, Monsanto RC, Schachern P, Paparella MM, Cureoglu S. A 3-D analysis of the protympanum in human temporal bones with chronic ear disease. Eur Arch Otorhinolaryngol. 2017;274(3):1357-64.
45. Paltura C, Can TS, Yilmaz BK, Dinc ME, Develioglu ON, Kulekci M. Eustachian tube diameter: Is it associated with chronic otitis media development? Am J Otolaryngol. 2017;38(4):414-6.
46. Falkenberg-Jensen B, Hopp E, Jablonski GE, Pripp AH, Silvola JT. The cartilaginous Eustachian tube: Reliable CT measurement and impact of the length. Am J Otolaryngol. 2018;39(4):436-40.
47. Malik J, Ghadiali SN. Multi-scale modeling of an upper respiratory airway: Effect of mucosal adhesion on Eustachian tube function in young children. Clin Biomech (Bristol, Avon). 2018.
48. Wang X, Gan RZ. 3D finite element model of the chinchilla ear for characterizing middle ear functions. Biomech Model Mechanobiol. 2016;15(5):1263-77.
49. Chen CK, Hsieh LC, Hsu TH. Novel three-dimensional image system for endoscopic ear surgery. Eur Arch Otorhinolaryngol. 2018;275(12):2933-9.
50. Chan J, Raju S, Nandakumar R, Bly R, Gollakota S. Detecting middle ear fluid using smartphones. Sci Transl Med. 2019;11(492).
51. Carpinelli MR, Kruse EA, Arhatari BD, Debrincat MA, Ogier JM, Bories JC, et al. Mice Haploinsufficient for Ets1 and Fli1 Display Middle Ear Abnormalities and Model Aspects of Jacobsen Syndrome. Am J Pathol. 2015;185(7):1867-76.
52. Tian C, Harris BS, Johnson KR. Ectopic Mineralization and Conductive Hearing Loss in Enpp1asj Mutant Mice, a New Model for Otitis Media and Tympanosclerosis. PLoS One. 2016;11(12):e0168159.
53. Azar A, Piccinelli C, Brown H, Headon D, Cheeseman M. Ectodysplasin signalling deficiency in mouse models of hypohidrotic ectodermal dysplasia leads to middle ear and nasal pathology. Hum Mol Genet. 2016;25(16):3564-77.
54. Crompton M, Purnell T, Tyrer HE, Parker A, Ball G, Hardisty-Hughes RE, et al. A mutation in Nischarin causes otitis media via LIMK1 and NF-kappaB pathways. PLoS Genet. 2017;13(8):e1006969.
55. Mulay A, Hood DW, Williams D, Russell C, Brown SDM, Bingle L, et al. Loss of the homeostatic protein BPIFA1, leads to exacerbation of otitis media severity in the Junbo mouse model. Sci Rep. 2018;8(1):3128.
56. Mulay A, Akram KM, Williams D, Armes H, Russell C, Hood D, et al. An in vitro model of murine middle ear epithelium. Dis Model Mech. 2016;9(11):1405-17.
57. Broker M, Berti F, Schneider J, Vojtek I. Polysaccharide conjugate vaccine protein carriers as a "neglected valency" - Potential and limitations. Vaccine. 2017;35(25):3286-94.
58. Wren JT, Blevins LK, Pang B, Basu Roy A, Oliver MB, Reimche JL, et al. Pneumococcal Neuraminidase A (NanA) Promotes Biofilm Formation and Synergizes with Influenza A Virus in Nasal Colonization and Middle Ear Infection. Infect Immun. 2017;85(4).
59. Nokso-Koivisto J, Marom T, Chonmaitree T. Importance of viruses in acute otitis media. Curr Opin Pediatr. 2015;27(1):110-5.

60. Marom T, Avraham E, Cinamon U, Tamir SO. The effect of immunization with pneumococcal conjugated vaccines on Streptococcus pneumoniae resistance patterns in acute otitis media. J Microbiol Immunol Infect. 2017;50(5):714-7.
61. Rowe HM, Mann B, Iverson A, Poole A, Tuomanen E, Rosch JW. A Cross-reactive Protein Vaccine Combined with PCV-13 Prevents Streptococcus pneumoniae- and Haemophilus influenzae-mediated Acute Otitis Media. Infect Immun. 2019.
62. Yadav MK, Vidal JE, Go YY, Kim SH, Chae SW, Song JJ. The LuxS/AI-2 Quorum-Sensing System of Streptococcus pneumoniae Is Required to Cause Disease, and to Regulate Virulence- and Metabolism-Related Genes in a Rat Model of Middle Ear Infection. Front Cell Infect Microbiol. 2018;8:138.
63. Minhas V, Harvey RM, McAllister LJ, Seemann T, Syme AE, Baines SL, et al. Capacity To Utilize Raffinose Dictates Pneumococcal Disease Phenotype. MBio. 2019;10(1).
64. Hu FZ, Krol JE, Tsai CHS, Eutsey RA, Hiller LN, Sen B, et al. Deletion of genes involved in the ketogluconate metabolism, Entner-Doudoroff pathway, and glucose dehydrogenase increase local and invasive virulence phenotypes in Streptococcus pneumoniae. PLoS One. 2019;14(1):e0209688.
65. Middleton DR, Paschall AV, Duke JA, Avci FY. Enzymatic Hydrolysis of Pneumococcal Capsular Polysaccharide Renders the Bacterium Vulnerable to Host Defense. Infect Immun. 2018.
66. Xu JH, Dai WJ, Chen B, Fan XY. Mucosal Immunization with PsaA Protein, Using Chitosan as a Delivery System, Increases Protection Against Acute Otitis Media and Invasive Infection by Streptococcus pneumoniae. Scandinavian Journal of Immunology. 2015;81(3):177-85.
67. Chen Z, Guo R, Xu J, Qiu C. Immunogenicity and protective immunity against otitis media caused by pneumococcus in mice of Hib conjugate vaccine with PsaA protein carrier. Front Med. 2016;10(4):490-8.
68. Keller LE, Bradshaw JL, Pipkins H, McDaniel LS. Surface Proteins and Pneumolysin of Encapsulated and Nonencapsulated Streptococcus pneumoniae Mediate Virulence in a Chinchilla Model of Otitis Media. Front Cell Infect Microbiol. 2016;6:55.
69. Jang AY, Seo HS, Lin S, Chung GH, Kim HW, Lim S, et al. Molecular characterization of pneumococcal surface protein K, a potential pneumococcal vaccine antigen. Virulence. 2017;8(6):875-90.
70. Bradshaw JL, Pipkins HR, Keller LE, Pendarvis JK, McDaniel LS. Mucosal Infections and Invasive Potential of Nonencapsulated Streptococcus pneumoniae Are Enhanced by Oligopeptide Binding Proteins AliC and AliD. MBio. 2018;9(1).
71. Hood D, Moxon R, Purnell T, Richter C, Williams D, Azar A, et al. A new model for non-typeable Haemophilus influenzae middle ear infection in the Junbo mutant mouse. Dis Model Mech. 2016;9(1):69-79.
72. Vikhe PP, Purnell T, Brown SDM, Hood DW. Cellular content plays a crucial role in Non-typeable Haemophilus influenzae infection of preinflamed Junbo mouse middle ear. Cell Microbiol. 2019;21(1):e12960.
73. Krueger A, Val S, Perez-Losada M, Panchapakesan K, Devaney J, Duah V, et al. Relationship of the Middle Ear Effusion Microbiome to Secretory Mucin Production in Pediatric Patients With Chronic Otitis Media. Pediatr Infect Dis J. 2017;36(7):635-40.
74. Hirano T, Kodama S, Kawano T, Suzuki M. Accumulation of Regulatory T Cells and Chronic Inflammation in the Middle Ear in a Mouse Model of Chronic Otitis Media with Effusion Induced by Combined Eustachian Tube Blockage and Nontypeable Haemophilus influenzae Infection. Infect Immun. 2016;84(1):356-64.
75. Hardison RL, Heimlich DR, Harrison A, Beatty WL, Rains S, Moseley MA, et al. Transient Nutrient Deprivation Promotes Macropinocytosis-Dependent Intracellular Bacterial Community Development. mSphere. 2018;3(5).
76. Harrison A, Dubois LG, St John-Williams L, Moseley MA, Hardison RL, Heimlich DR, et al. Comprehensive Proteomic and Metabolomic Signatures of Nontypeable Haemophilus influenzae-

Induced Acute Otitis Media Reveal Bacterial Aerobic Respiration in an Immunosuppressed Environment. Mol Cell Proteomics. 2016;15(3):1117-38.
77. Atack JM, Bakaletz LO, Jennings MP. High-Depth RNA-Seq Data Sets for Studying Gene Expression Changes Mediated by Phase-Variable DNA Methyltransferases in Nontypeable Haemophilus influenzae. Microbiol Resour Announc. 2019;8(2).
78. . !!! INVALID CITATION !!! {}.
79. Mulay A, Akram KM, Williams D, Armes H, Russell C, Hood D, et al. An in vitro model of murine middle ear epithelium. Disease Models & Mechanisms. 2016;9(11):1405-17.
80. Mulay A, Akram K, Bingle L, Bingle CD. Isolation and Culture of Primary Mouse Middle Ear Epithelial Cells. In: Bertoncello I, editor. Mouse Cell Culture Methods in Molecular Biology. 1940: Humana Press, New York, NY; 2019. p. 157-68.
81. Whitby PW, Seale TW, Morton DJ, Stull TL. Antisera Against Certain Conserved Surface-Exposed Peptides of Nontypeable Haemophilus influenzae Are Protective. PLoS One. 2015;10(9):e0136867.
82. Thofte O, Su YC, Brant M, Littorin N, Duell BL, Alvarado V, et al. EF-Tu From Non-typeable Haemophilus influenzae Is an Immunogenic Surface-Exposed Protein Targeted by Bactericidal Antibodies. Front Immunol. 2018;9:2910.
83. Su YC, Mukherjee O, Singh B, Hallgren O, Westergren-Thorsson G, Hood D, et al. Haemophilus influenzae P4 Interacts With Extracellular Matrix Proteins Promoting Adhesion and Serum Resistance. J Infect Dis. 2016;213(2):314-23.
84. Winter LE, Barenkamp SJ. Immunogenicity of Nontypeable Haemophilus influenzae Outer Membrane Vesicles and Protective Ability in the Chinchilla Model of Otitis Media. Clin Vaccine Immunol. 2017;24(10).
85. Kress-Bennett JM, Hiller NL, Eutsey RA, Powell E, Longwell MJ, Hillman T, et al. Identification and Characterization of msf, a Novel Virulence Factor in Haemophilus influenzae. PLoS One. 2016;11(3):e0149891.
86. Novotny LA, Jurcisek JA, Goodman SD, Bakaletz LO. Monoclonal antibodies against DNA-binding tips of DNABII proteins disrupt biofilms in vitro and induce bacterial clearance in vivo. EBioMedicine. 2016;10:33-44.
87. Murphy TF. Vaccines for Nontypeable Haemophilus influenzae: the Future Is Now. Clin Vaccine Immunol. 2015;22(5):459-66.
88. Behrouzi A, Vaziri F, Rahimi-Jamnani F, Afrough P, Rahbar M, Satarian F, et al. Vaccine Candidates against Nontypeable Haemophilus influenzae: a Review. Iran Biomed J. 2017;21(2):69-76.
89. Murphy TF, Brauer AL, Johnson A, Kirkham C. ATP-Binding Cassette (ABC) Transporters of the Human Respiratory Tract Pathogen, Moraxella catarrhalis: Role in Virulence. PLoS One. 2016;11(7):e0158689.
90. Murphy TF, Brauer AL, Johnson A, Wilding GE, Koszelak-Rosenblum M, Malkowski MG. A Cation-binding Surface Protein as a Vaccine Antigen to Prevent Moraxella catarrhalis Otitis Media and Infections in Chronic Obstructive Pulmonary Disease. Clin Vaccine Immunol. 2017.
91. Otsuka T, Kirkham C, Brauer A, Koszelak-Rosenblum M, Malkowski MG, Murphy TF. The Vaccine Candidate Substrate Binding Protein SBP2 Plays a Key Role in Arginine Uptake, Which Is Required for Growth of Moraxella catarrhalis. Infect Immun. 2016;84(2):432-8.
92. Murphy TF, Kirkham C, Johnson A, Brauer AL, Koszelak-Rosenblum M, Malkowski MG. Sulfate-binding protein, CysP, is a candidate vaccine antigen of Moraxella catarrhalis. Vaccine. 2016;34(33):3855-61.
93. Singh B, Alvarado-Kristensson M, Johansson M, Hallgren O, Westergren-Thorsson G, Morgelin M, et al. The Respiratory Pathogen Moraxella catarrhalis Targets Collagen for Maximal Adherence to Host Tissues. MBio. 2016;7(2):e00066.
94. Perez AC, Johnson A, Chen Z, Wilding GE, Malkowski MG, Murphy TF. Mapping Protective Regions on a Three-Dimensional Model of the Moraxella catarrhalis Vaccine Antigen Oligopeptide Permease A. Infect Immun. 2018;86(3).

95. Yassin GM, Amin MA, Attia AS. Immunoinformatics Identifies a Lactoferrin Binding Protein A Peptide as a Promising Vaccine With a Global Protective Prospective Against Moraxella catarrhalis. J Infect Dis. 2016;213(12):1938-45.
96. Mina MJ, Klugman KP, Rosch JW, McCullers JA. Live attenuated influenza virus increases pneumococcal translocation and persistence within the middle ear. J Infect Dis. 2015;212(2):195-201.
97. Sung CM, Kim HC, Cho YB, Shin SY, Jang CH. Evaluating the ototoxicity of an anti-MRSA peptide KR-12-a2. Braz J Otorhinolaryngol. 2018;84(4):441-7.
98. Yadav MK, Chae SW, Im GJ, Chung JW, Song JJ. Eugenol: a phyto-compound effective against methicillin-resistant and methicillin-sensitive Staphylococcus aureus clinical strain biofilms. PLoS One. 2015;10(3):e0119564.
99. Yadav MK, Chae SW, Go YY, Im GJ, Song JJ. In vitro Multi-Species Biofilms of Methicillin-Resistant Staphylococcus aureus and Pseudomonas aeruginosa and Their Host Interaction during In vivo Colonization of an Otitis Media Rat Model. Front Cell Infect Microbiol. 2017;7:125.
100. Yadav MK, Chae SW, Im GJ, Chung JW, Song JJ. Eugenol: A Phyto-Compound Effective against Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus Clinical Strain Biofilms. Plos One. 2015;10(3).
101. Graham BS. Vaccine development for respiratory syncytial virus. Curr Opin Virol. 2017;23:107-12.
102. Mazur NI, Higgins D, Nunes MC, Melero JA, Langedijk AC, Horsley N, et al. The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. Lancet Infect Dis. 2018;18(10):e295-e311.
103. Battles MB, McLellan JS. Respiratory syncytial virus entry and how to block it. Nat Rev Microbiol. 2019.
104. Yang R, Sabharwal V, Okonkwo OS, Shlykova N, Tong R, Lin LY, et al. Treatment of otitis media by transtympanic delivery of antibiotics. Science translational medicine. 2016;8(356):356ra120.
105. Yang R, Sabharwal V, Shlykova N, Okonkwo OS, Pelton SI, Kohane DS. Treatment of Streptococcus pneumoniae otitis media in a chinchilla model by transtympanic delivery of antibiotics. JCI insight. 2018;3(19).
106. Kurabi A, Beasley KA, Chang L, McCann J, Pak K, Ryan AF. Peptides actively transported across the tympanic membrane: Functional and structural properties. PLoS One. 2017;12(2):e0172158.
107. Kurabi A, Pak KK, Bernhardt M, Baird A, Ryan AF. Discovery of a Biological Mechanism of Active Transport through the Tympanic Membrane to the Middle Ear. Sci Rep. 2016;6:22663.
108. Kurabi A, Schaerer D, Chang L, Pak K, Ryan AF. Optimisation of peptides that actively cross the tympanic membrane by random amino acid extension: a phage display study. Journal of drug targeting. 2018;26(2):127-34.
109. Kurabi A, Schaerer D, Noack V, Bernhardt M, Pak K, Alexander T, et al. Active Transport of Peptides Across the Intact Human Tympanic Membrane. Sci Rep. 2018;8(1):11815.
110. Yang R, Saarinen R, Okonkwo OS, Hao Y, Mehta M, Kohane DS. Transtympanic Delivery of Local Anesthetics for Pain in Acute Otitis Media. Mol Pharm. 2019;16(4):1555-62.
111. Novotny LA, Jurcisek JA, Ward MO, Jr., Jordan ZB, Goodman SD, Bakaletz LO. Antibodies against the majority subunit of type IV Pili disperse nontypeable Haemophilus influenzae biofilms in a LuxS-dependent manner and confer therapeutic resolution of experimental otitis media. Mol Microbiol. 2015;96(2):276-92.
112. Novotny LA, Clements JD, Bakaletz LO. Therapeutic Transcutaneous Immunization with a Band-Aid Vaccine Resolves Experimental Otitis Media. Clinical and vaccine immunology : CVI. 2015;22(8):867-74.

113. Gulmez MI, Okuyucu S, Dokuyucu R, Gokce H. The effect of caffeic acid phenethyl ester and thymoquinone on otitis media with effusion in rats. International journal of pediatric otorhinolaryngology. 2017;96:94-9.
114. Egilmez OK, Kokten N, Ekici AI, Kalcioglu MT, Yesilada E, Tekin M. The effect of Hypericum perforatum L. (St. John's Wort) on prevention of myringosclerosis after myringotomy in a rat model. International journal of pediatric otorhinolaryngology. 2015;79(7):1128-34.
115. Genc G, Koyuncu M, Kutlar G, Guvenc T, Gacar A, Aksoy A, et al. Does systemic clarithromycin therapy have an inhibitory effect on tympanosclerosis? An experimental animal study. The Journal of laryngology and otology. 2015;129(2):136-41.
116. Abi Hachem R, Goncalves S, Walker T, Angeli S. Middle ear irrigation using a hydrodebrider decreases biofilm surface area in an animal model of otitis media. Laryngoscope investigative otolaryngology. 2018;3(3):231-7.
117. Kim YJ, Park SG, Shin B, Kim J, Kim SW, Choo OS, et al. Osteogenesis for postoperative temporal bone defects using human ear adipose-derived stromal cells and tissue engineering: An animal model study. J Biomed Mater Res A. 2017;105(12):3493-501.
118. Yaguchi Y, Murakami D, Yamato M, Hama T, Yamamoto K, Kojima H, et al. Middle ear mucosal regeneration with three-dimensionally tissue-engineered autologous middle ear cell sheets in rabbit model. J Tissue Eng Regen Med. 2016;10(3):E188-94.
119. Ziabka M, Dziadek M, Menaszek E, Banasiuk R, Krolicka A. Middle Ear Prosthesis with Bactericidal Efficacy-In Vitro Investigation. Molecules. 2017;22(10).
120. Santa Maria PL, Kim S, Varsak YK, Yang YP. Heparin binding-epidermal growth factor-like growth factor for the regeneration of chronic tympanic membrane perforations in mice. Tissue engineering Part A. 2015;21(9-10):1483-94.
121. Seonwoo H, Shin B, Jang KJ, Lee M, Choo OS, Park SB, et al. Epidermal Growth Factor-Releasing Radially Aligned Electrospun Nanofibrous Patches for the Regeneration of Chronic Tympanic Membrane Perforations. Adv Healthc Mater. 2019;8(2):e1801160.
122. Seonwoo H, Kim SW, Shin B, Jang KJ, Lee M, Choo OS, et al. Latent stem cell-stimulating therapy for regeneration of chronic tympanic membrane perforations using IGFBP2-releasing chitosan patch scaffolds. J Biomater Appl. 2019;34(2):198-207.
123. Kuo CY, Wilson E, Fuson A, Gandhi N, Monfaredi R, Jenkins A, et al. Repair of Tympanic Membrane Perforations with Customized Bioprinted Ear Grafts Using Chinchilla Models. Tissue Eng Part A. 2018;24(5-6):527-35.
124. Mandour YMH, Mohammed S, Menem MOA. Bacterial cellulose graft versus fat graft in closure of tympanic membrane perforation. Am J Otolaryngol. 2019;40(2):168-72.
125. Kanemaru SI, Kanai R, Yoshida M, Kitada Y, Omae K, Hirano S. Application of Regenerative Treatment for Tympanic Membrane Perforation With Cholesteatoma, Tumor, or Severe Calcification. Otol Neurotol. 2018;39(4):438-44.
126. Omae K, Kanemaru SI, Nakatani E, Kaneda H, Nishimura T, Tona R, et al. Regenerative treatment for tympanic membrane perforation using gelatin sponge with basic fibroblast growth factor. Auris Nasus Larynx. 2017;44(6):664-71.
127. Joe H, Seo YJ. A newly designed tympanostomy stent with TiO2 coating to reduce Pseudomonas aeruginosa biofilm formation. J Biomater Appl. 2018;33(4):599-605.
128. Alper CM, Teixeira MS, Richert BC, Douglas Swarts J. Presentation and eustachian tube function test results in children evaluated at a specialty clinic. Laryngoscope. 2018.
129. Huisman JML, Verdam FJ, Stegeman I, de Ru JA. Treatment of Eustachian tube dysfunction with balloon dilation: A systematic review. Laryngoscope. 2018;128(1):237-47.
130. Pohl F, Schuon RA, Miller F, Kampmann A, Bultmann E, Hartmann C, et al. Stenting the Eustachian tube to treat chronic otitis media - a feasibility study in sheep. Head Face Med. 2018;14(1):8.

131. Cuevas RA, Eutsey R, Kadam A, West-Roberts JA, Woolford CA, Mitchell AP, et al. A novel streptococcal cell-cell communication peptide promotes pneumococcal virulence and biofilm formation. Mol Microbiol. 2017;105(4):554-71.
132. Aggarwal SD, Eutsey R, West-Roberts J, Domenech A, Xu W, Abdullah IT, et al. Function of BriC peptide in the pneumococcal competence and virulence portfolio. PLoS Pathog. 2018;14(10):e1007328.
133. Brockman KL, Jurcisek JA, Atack JM, Srikhanta YN, Jennings MP, Bakaletz LO. ModA2 Phasevarion Switching in Nontypeable Haemophilus influenzae Increases the Severity of Experimental Otitis Media. Journal of Infectious Diseases. 2016;214(5):817-24.
134. Winter LE, Barenkamp SJ. Immunogenicity of Nontypeable Haemophilus influenzae Outer Membrane Vesicles and Protective Ability in the Chinchilla Model of Otitis Media. Clinical and Vaccine Immunology. 2017;24(10).
135. Devaraj A, Buzzo J, Rocco CJ, Bakaletz LO, Goodman SD. The DNABII family of proteins is comprised of the only nucleoid associated proteins required for nontypeable Haemophilus influenzae biofilm structure. Microbiologyopen. 2018;7(3).
136. Murrah KA, Turner RL, Pang B, Perez AC, Reimche JL, King LB, et al. Replication of type 5 adenovirus promotes middle ear infection by Streptococcus pneumoniae in the chinchilla model of otitis media. Pathog Dis. 2015;73(2):1-8.

FIGURES AND LEGENDS

Figure 1: Optical coherence tomography (OCT) images of the tympanic membrane (TM) and underlying middle ear space. Top: Both normal thickness and monomer (thinned) portions (arrow) of the TM can be assessed in OCT's cross-sectional views. Bottom: Middle ear effusion (MEE) and air-fluid boundary (arrow) can be directly visualized as well using OCT.

Figure 2: Otoscopy Images on the SWIR.

(A) With evidence of the promontory and incus seen through the tympanic membrane.

(B) Blackout effect in otitis media with effusion due to water absorption in the SWIR.

Figure 3: A novel *in vitro* model of the primary mouse middle ear epithelial cells.

Timeline for culture of mouse middle ear epithelial cells (mMECs). Bullae were dissected, treated with pronase for dissociation of the epithelial cells, and fibroblasts were excluded from culture by differential adherence to plastic (A). Epithelial cells were grown in submerged culture until confluence (B), before ALI was induced (C). Cultures were terminated at ALI day 14, at which point the cultures were composed of flat polygonal and compactly clustered pseudostratified cells with active cilia (D).

Scale bar: 200 μm.

This figure has been adapted with permission from Disease Models & Mechanisms 2016 9: 1405-1417; doi: 10.1242/dmm.026658

Figure 4: A phage display library was used to investigate trans-tympanic delivery.

In this method, bacteriophages are genetically engineered to express one of 1010 random peptides on surface filaments. Peptides were selected by application of successively selected libraries to the surface of the TM, with sampling from the middle ear, which resulted in

library collapse to several very rare sequences that could actively cross the membrane carrying phage as cargo.

Figure 5: (A) *In vitro* assay developed to evaluate transport of a phage bearing a trans-tympanic peptide across fragments of the TM, from the upper to the lower chamber. (B) Transport of a trans-tympanic peptide phage TM-3 across the tympanic membranes of various species, including the human membrane.

Table 1: Recent Advances in Middle Ear Imaging

Technique	Basic Principles	Purpose	Results	Benefits/Limitations	References
Acoustic Techniques					
High frequency ultrasound (HFUS)	HFUS waves are used to visualize behind the eardrum. High frequency waves allow for higher resolution at smaller depths.	Visualization of middle ear anatomy and pathology.	HFUS allows for visualization of the middle ear past an intact TM in real time.	HFUS enables visualization of middle ear and contents, however no *in vivo* studies have been done and HFUS may not translate to imaging through thicker soft tissue	(18-20)
Spectral gradient acoustic reflectometry (SGAR)	Inaudible sonar waves are reflected off the middle ear wall to assess the presence/absence of effusion.	MEE detection.	SGAR has high sensitivity, but suffers poor specificity.	SGAR has a high negative predictive value, and is relatively easy technology to use clinically, even on less than compliant patients. SGAR however cannot	(21, 50)

				detect effusion progression/clearance or differentiate from AOM. Also, SGAR is not superior to nor synergistic with tympanometry.	
Transmastoid ultrasound	Detection of effusion via the mastoid air system.	MEE detection.	Transmastoid ultrasound has a high accuracy rate (81%).	Transmastoid ultrasound has a high accuracy but overcoming states when probes cannot be inserted into the external ear canal is a drawback.	(22)
Visible Light Techniques					
Multicolor reflectance imaging	By incorporating RGB (multicolor) narrow-band reflectance imaging to a	Detection of AOM, MEE, and cholesteatoma	The high definition images depict middle ear mucosal structures	Multicolor reflectance imaging provides high quality imagery of middle ear pathology. However,	(23)

	standard video otoscope, differences in tissue properties can be assessed.		to a better extent, combined with better demarcation of morphological structures.	this technology is particularly susceptible to patient movement and image distortion.	
Narrow band imaging (NBI)	Narrow band light penetrates the tissue at different depths and indicate areas of hypervascularity.	Improved visualization of tissue based on varying degrees of vascularity.	NBI is a feasible technology for measuring the extent of a disease, and small residual disease is not ignored.	NBI enables differentiation of healthy and diseased tissue, and the extent of the disease.	(24)
Near Infrared Techniques					
Anti-confocal middle ear	Spectroscopic measurements to assess	MEE detection.	On phantom ear models, human MEE	While this in vitro study shows promising results for detection of	(25)

assessment	inflammatory states of middle ear, by analyzing blood content.		inserted was detected.	MEE, extrapolation into use in humans may prove less capable due to patient movement.	
Optical coherence tomography (OCT)	Low coherence (broadband) interferometry (light interference) penetrates the tissue to obtain depth visualization in high resolution. Scanning the near infrared beam laterally enables 2/3D imaging.	MEE detection and differentiating acute or chronic OM states.	Using the TM thickness combined with the presence of MEE enabled differentiation of fluid type. Additionally, OCT uniquely enables visualization of middle ear biofilm structure in vivo.	OCT enables structural and dynamic visualization of TM thickness, middle ear contents (as well as MEE differentiation), and biofilm structure, in high resolution. While OCT can be used to visualize the ossicles, it cannot image through them. Yet multiple clinical studies have been conducted using OCT, and this modality has the largest body	(26-34)

				of evidence of the imaging techniques discussed.	
OCT: Quantitative pneumatic otoscopy	Measure TM micro-displacement differences using a pneumatic OCT-otoscope. TM compliance and middle ear pressure are calculated via displacement amplitude ratio.	MEE detection and differentiating acute or chronic OM states.	Proof of technical capability shown, wherein decreased compliance in MEE cases, decreased amplitude ratio in AOM cases, due to positive middle ear pressure.	Pneumatic OCT-otoscopy enables objective quantification of both middle ear mobility (compliance) and middle ear pressure. However, this technique still suffers the requirement of an ear canal seal to collect data.	(14)
OCT: TM thickness mapping	TM thickness mapping using a 1-D OCT-otoscope and sampling	Visualization of middle ear anatomy and pathology.	Proof of concept shown, wherein TM thickness was seen to	While this new application for a low-cost OCT device shows promise, this approach struggles	(11, 38)

	TM thickness measurements at 500 different TM locations.		increase 100-200% when MEE and/or AOM are present.	with differentiation of MEE type and suffers a relatively long image processing time.	
Radiology Techniques					
Synchrotron radiation phase-contrast imaging (SR-PCI)	A phase-shifted beam interferes with the original beam to produce measurable fringes that correspond to the surfaces and structural boundaries of the sample.	Visualization of middle ear anatomy and pathology.	SR-PCI edge-enhancement provides clearer visualization of bone structure and brighter borders for higher density structures, as well as soft tissues like TM, ligaments, and joints.	SR-PCI provides improved contrast and detectability of soft tissue in intensity profile compared to absorption contrast micro-CT. Images provide a more accurate 3D reconstruction of the ossicles. However, this radiative imaging modality poses standard health risks.	(39)

Table 2: Recent Advances in Research on Spn induced OM

Field of research	Model	Key results	References
Identification of novel virulence peptides and quorum sensing pathways	Chinchilla middle ear epithelial cells	A novel virulence peptide 1 (vp1), under the Rgg family of transcription regulators, was characterized. Infection with mutant Vp1 pneumococcal strain produced biofilms with reduced biomass and thickness which was restored by addition exogenous synthetic VP1.	(131)
	Mouse model	briC (Biofilm regulating peptide induced by Competence) gene was identified as a novel peptide involved in pneumococcal competence and virulence and induced by the ComE, the master regulator of quorum sensing competence signaling pathway. Mice challenged with briC-deleted mutant of the D39Δ pneumococci show reduced bacterial counts in nasal lavages.	(132)
	Rat model	Transbullar infection of rats using Spn strain with mutation in the quorum sensing component, LuxS, led to no biofilm formation in the middle ears and resulted in lower bacterial titers in the middle ear effusions.	(62)

Targeting the pneumococcal polysaccharide capsule	Mouse model	Uncapsulated pneumococci were more susceptible to *in vitro* phagocytosis assays and exhibited reduced ability to colonize the nasal passages in an in vivo murine model in comparison to capsulated variants.	(65)
		Chitosan nanoparticles were used as vectors for pneumococcal surface adhesin protein A (PsaA), a lipoprotein common to all serotypes of pneumococcus. Intranasal inoculation of mice with these particles induced protection against pneumococcus (serotype 14) induced AOM.	(66)
		Mice immunized with a conjugate recombinant PSA vaccine with the Hib polysaccharide (Hib-PSA) improved elimination pneumococcus and reduced inflammation of the middle ear.	(67)
Targeting non-encapsulated strains (NESp) of pneumococci	Mouse and chinchilla models	NESp expressing AliC and AliD are were found to be more virulent compared to mutants and enhanced murine nasopharyngeal colonization The OM phenotype was significantly attenuated in absence of AliC and AliD in the chinchilla model.	(70)

Table 3: Recent Advances in Research on NTHi induced OM

Pathophysiology of NTHi induced OM			
Novel emerging concepts	Animal model utilized	Supporting observations	References
Pre-existing middle ear inflammation in genetic models of OM can provide a niche in which micro-aerophilic bacteria such as NTHi can successfully establish an infection and persist following transfer through the ET	Evi1Jbo/+ (Junbo mouse)- spontaneous chronic OM	Single intranasal dose of NTHi in a model of spontaneous OM development induced robust infection rates of up to 90%. Pre-existing middle ear fluid was a pre-requisite for successful NTHi infection.	(71)
	Bpifa1-/-Evi1Jbo/+- spontaneous chronic OM	Loss of the putative innate immunity protein BPIFA1 alone did not cause spontaneous or NTHi-induced OM development in young mice, but deletion of BPIFA1 in the pre-existing inflammatory Junbo middle ears led to significant exacerbation of OM severity.	(55)
Clinical features such as the	Evi1Jbo/+ (Junbo	NTHi was absent in the serous ear fluids, but almost 100% ears containing highly	(72)

type and viscosity of middle ear fluid pathology can influence bacterial load during OM development	mouse)-spontaneous chronic OM	viscous fluid were culture positive. Viscous fluids were accompanied by higher neutrophil infiltration and percentage of necrotic and apoptotic cells and reduced number of monocytes.	
Suppressor T-cells (Treg cells) confer infectious tolerance to NTHi in the middle ear during chronic OM, contributing to persistence of infection and inflammation	Trans-bullar NTHi inoculation of murine bullae followed by persistently blocking the ET for 2 months with the introduction of a gelatin plug post infection	Culture positive ears showed mucosal inflammation and elevated of Treg cells. Depletion of Treg cells caused a 99.9% reduction in NTHi bacterial counts in the middle ear fluids and in levels of pro-inflammatory cytokines.	(74)

Intracellular entry of NTHi contributes to bacterial persistence during OM	Chinchilla model *In vitro* NTHi infection of chinchilla middle ear epithelial cell line	Heme-iron restriction of NTHI led to bacterial entry into epithelial cells and formation of intracellular bacterial communities (IBCs). IBCs survive the hostile microenvironment by escaping the endolysosomal pathway for degradation. Prevention of macropinocytosis in cultured middle ear epithelial cells reduced the number of IBCs.	(75, 76)
NTHi variable regulons contribute to increased disease severity	*In vivo* chinchilla infection with NTHi ModA2 variants	NTHi variants in which ModA2 was OFF at inoculation but shifted ON in the middle ear showed a more severe disease phenotype than those with challenged with NTHi variants, that were either inoculated OFF and remained OFF or inoculated ON and remained ON.	(77, 133)
A novel model of the murine middle ear epithelium	NTHi infection of primary cultures of mouse middle ear	This new *in vitro* model recapitulates the differentiated in situ murine middle ear epithelium as indicated by expression of various epithelial markers at a transcriptional and proteomic level.	(79, 80)

	epithelial cells at the Air-liquid interface	The model demonstrated effectiveness for studying longitudinal NTHi infections.	
Development of Therapeutic Strategies			
Strategy	Animal model utilized	Key findings	References
Targeting intracellular bacterial persistence by blocking invasion of NTHi into host cells	Chinchilla model *In vitro* chinchilla middle ear epithelial cell line	Pharmacological blockade of the actin-remodeling complex, Arp2/3 which is involved in invasion of NTHi by the host cells prevented the formation of IBCs. Inhibition of macropinocytis and re-direction of internalized bacteria towards the endolysosomal pathway of degradation can serve as a therapeutic intervention.	(76)
Identification of surface-exposed proteins (SEPs)	Infant rat model of AOM	Antisera against five of the most highly SEPs offered protection to NTHi invasive infection.	(81)
	Rabbit	Identification of antibody accessible moieties on the intracellular elongation factor thermo-unstable (EF-Tu) protein that are capable of mounting a host	(82)

	immunization	immune response.	
	Evi1Jbo/+ (Junbo mouse)	*Haemophilus* outer member lipoprotein e (P4) acts as a receptor for host extracellular matrix proteins lamin, fibronectin and vitronectin. P4 deficient NTHi showed reduced binding to extracellular matrix and reduced middle ear infection.	(83)
	Chinchilla model	Antisera against NTHi outer membrane vesicles resulted in an opsonophagocytic killing of homologous and heterologous NTHi strains. Immunization with prototype OMVs provided protection to infection by homologous strains of NTHi.	(134)
	Chinchilla model	Macrophage survival factor (Msf) was identified as a new NTHi virulence gene family. Deletion of Msf1-4 displayed decreased in phagocytosis and survival in	(85)

		macrophages which was restored by a single copy of msfA1 gene.	
Broad spectrum approach for biofilm disruption	Chinchilla model	Monoclonal antibodies (MAbs) against DNA-binding tip regions of the alpha- and beta subunits of the DNAIIB protein, integration host factor (IHF) disrupted biofilm formation.	(135)
Combinatorial immunization	Chinchilla model – novel transcutaneous route	combining the majority subunit of Type IV pili (Tfp) of NTHI called rsPilA in a vaccine formulation with the DBAIIB protein, IHF significantly resolved planktonic and adherent populations of NTHi.	(86)

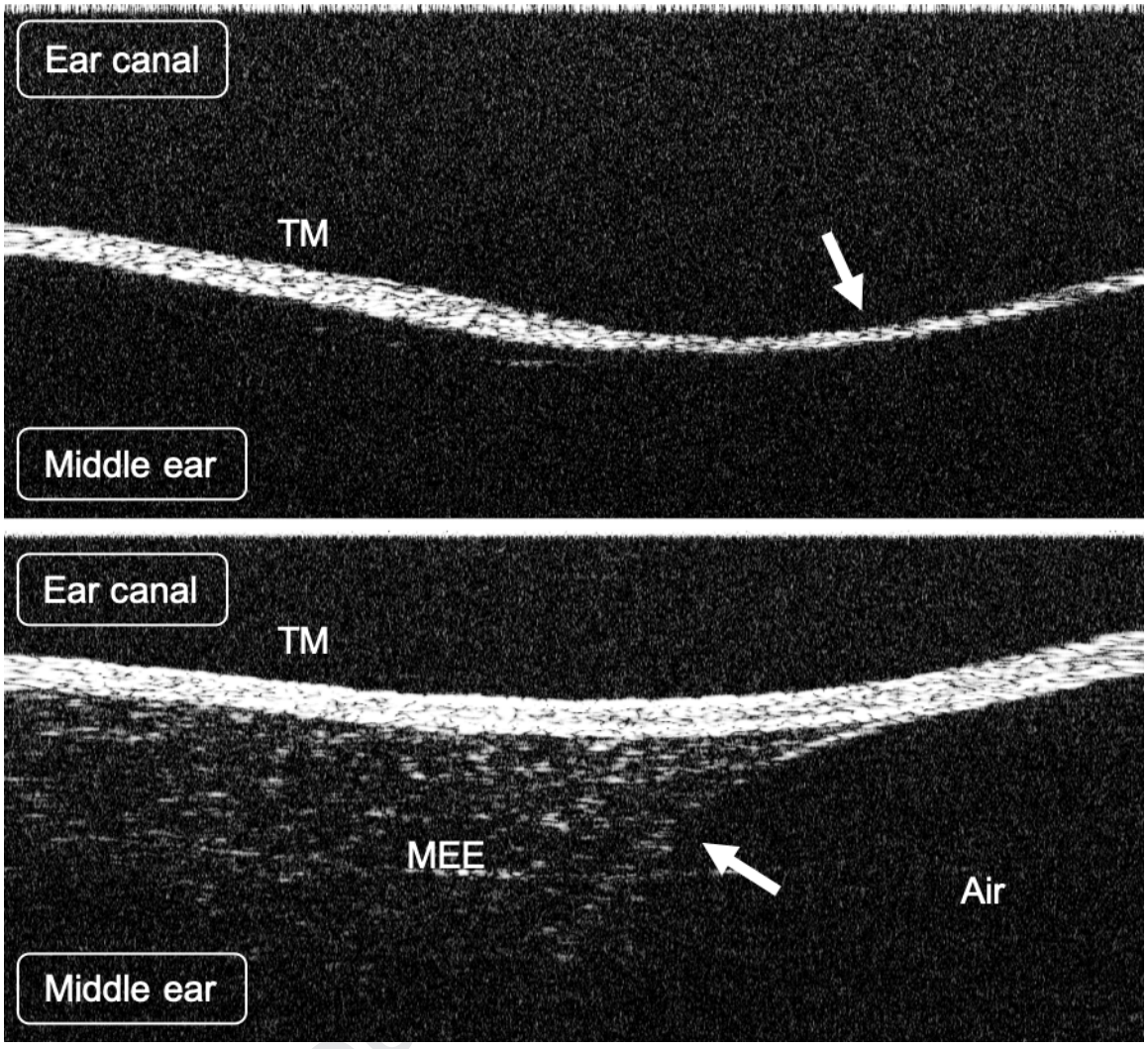
Table 4: Recent Advances in Research on MCat induced OM

Field of research	Model	Key results	References
Study of mechanisms by which MCat adheres to host tissue	Smoke-induced COPD mouse model	Clinical isolates of Mcat adhered to network-forming collagens IV and VI and fibrillar collagen types I, II, and III through the trimeric autotransporter adhesins ubiquitous surface protein A2 (UspA2) and UspA2H receptors. UspA2 and UspA2H deletion mutants showed reduced adherence to the respiratory tract in the COPD mouse model, compared to wild-type bacteria.	(93)

Table 5: Recent Advances in Research on Polymicrobial OM

Field of research	Model	Key results	References
Polymicrobial infection of viruses and Spn	Mouse and chinchilla models	Mice inoculated by pneumococci deficient in the primary neuraminidase, NanA exhibited reduced nasal colonization which was only partially restored upon co-infection with IAV, which also expresses neuraminidase. IAV potentiated middle ear colonization by NanA-deficient pneumococci to a lesser extent that the Wt strain. Intranasal vaccination of mice with live attenuated Influenza virus before or after pneumococcal increased the transfer of bacteria into the middle ear.	(58, 96)
		Precedent intranasal infection of chinchillas with Wt type 5 adenovirus increased the incidence of middle ear infection upon challenge with Spn. Inoculation with non-replicating mutant adenovirus led to less frequent middle ear infection.	(136)

Polymicrobial infection of MRSA with other otopathogens	Rat model	RNA-sequencing of total transcriptome after co-infection of rat middle ears by MRSA and *Pseudomonas aeruginosa* showed that exclusive differential induction of a number of host response genes that were not expressed with single species infection.	(97, 100)
	Rat and guinea pig models	Eugenol, a naturally occurring phytogen and the KR-12 peptide of human cathelicidin LL-37 were shown to have an antimicrobial effect against MRSA.	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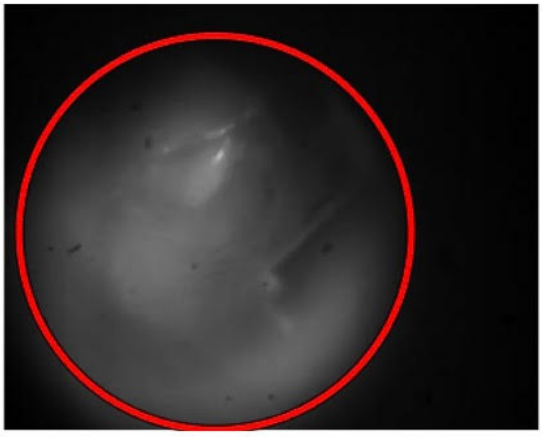
TM
Middle ear
Ear canal
TM
MEE
Air
Middle ear



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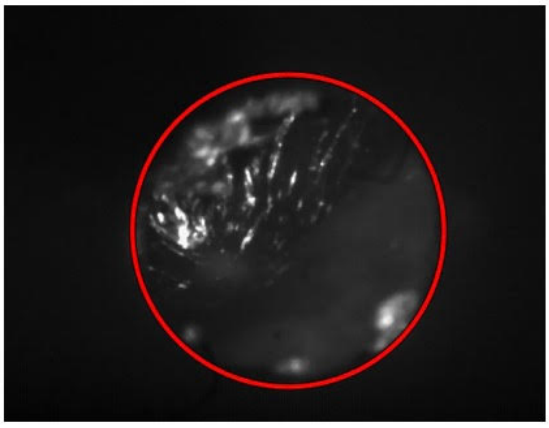
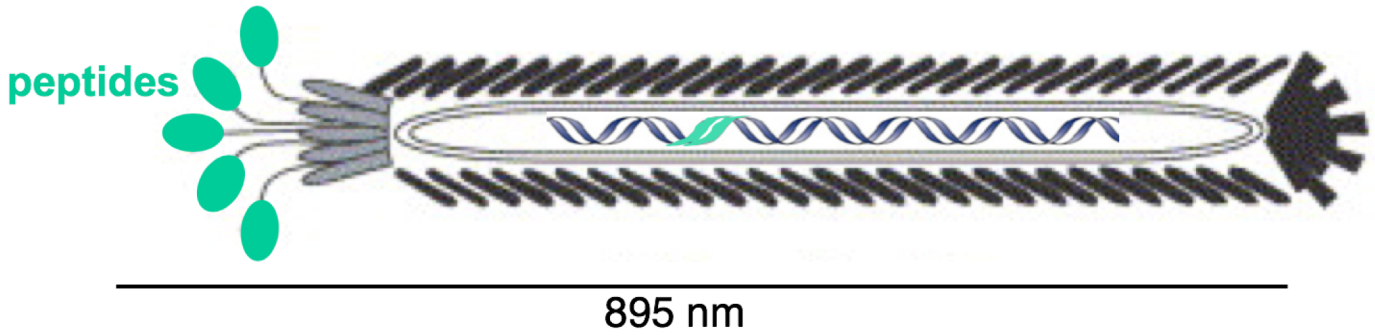


B

Figure 1



Phage library engineered to express 10^{12} random 12-mer peptides

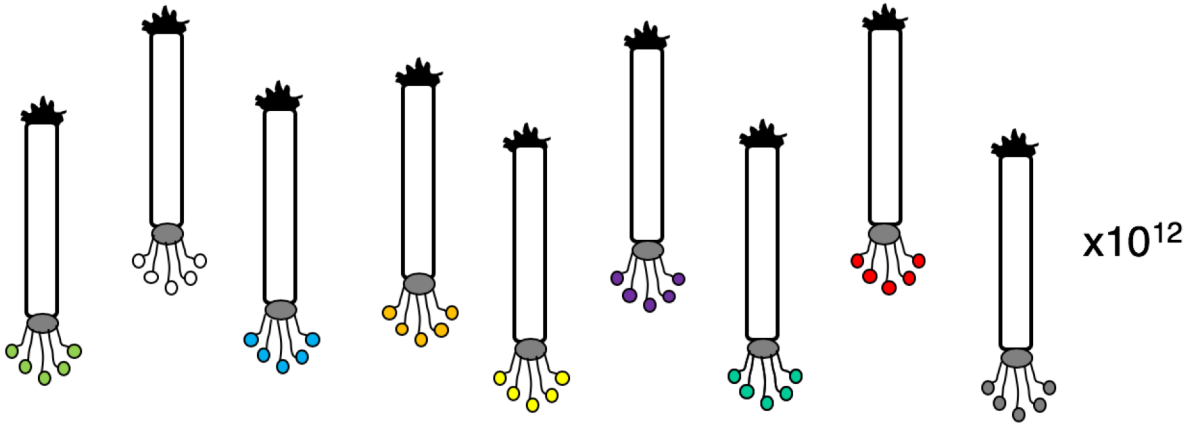
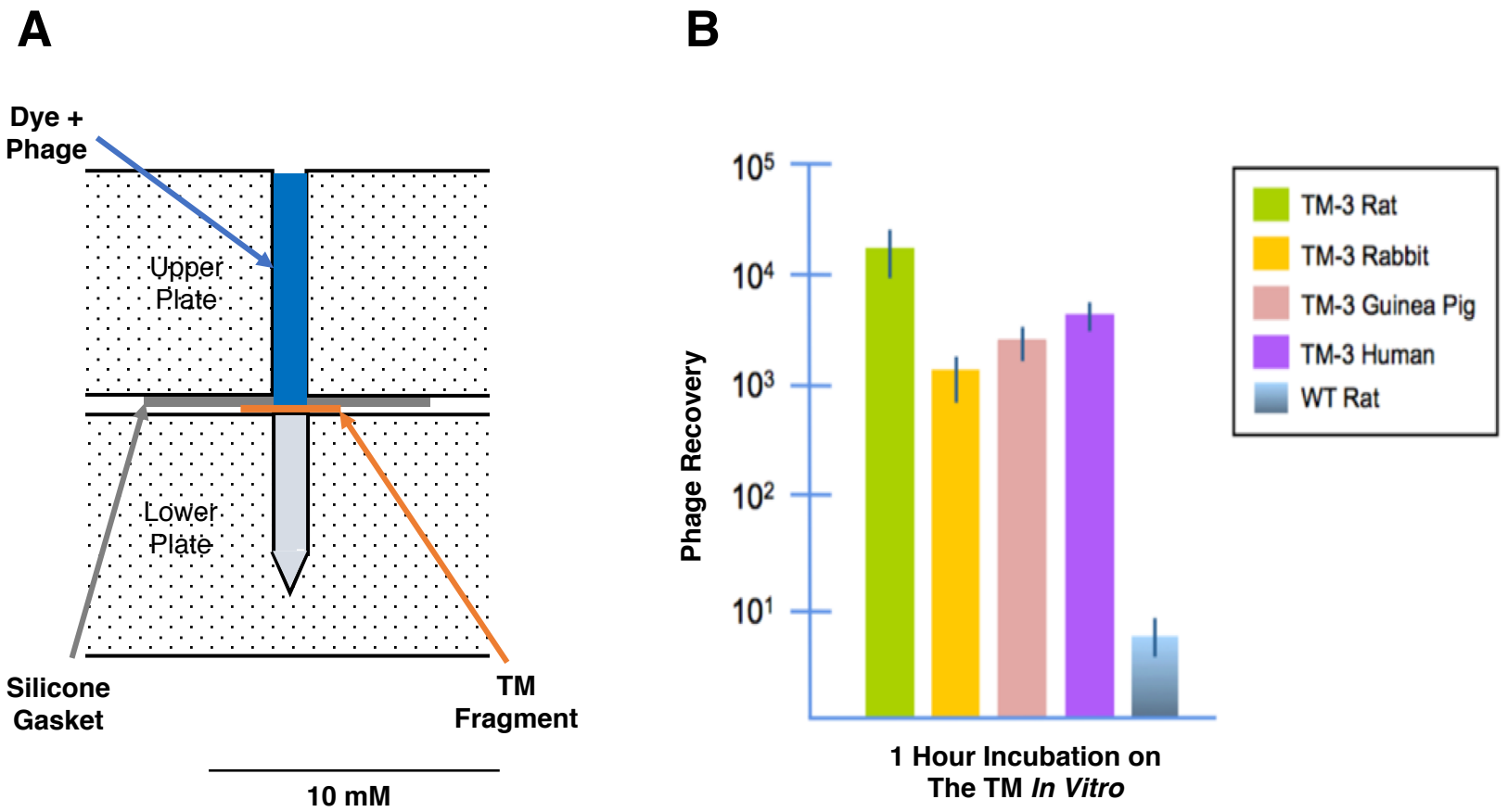


Figure 2


A
Dye +
Phage
Upper
Plate
Lower
Plate
Silicone
Gasket
TM
Fragment
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B
Phage Recovery
10^5
10^4
10^3
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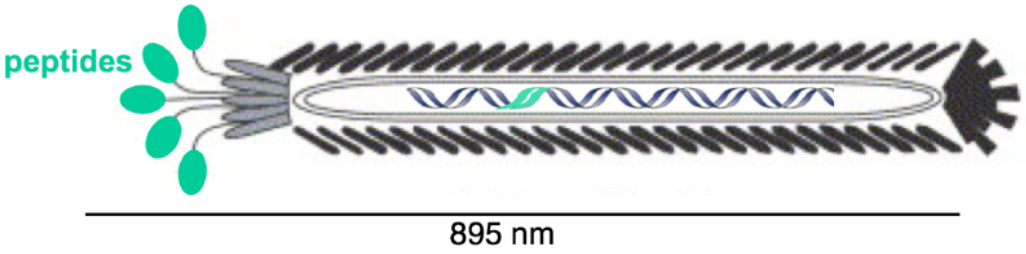
10^1
TM-3 Rat
TM-3 Rabbit
TM-3 Guinea Pig
TM-3 Human
WT Rat
1 Hour Incubation on
The TM *In Vitro*



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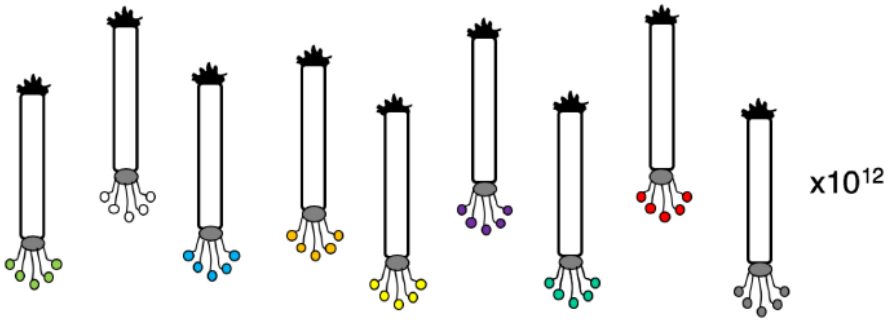

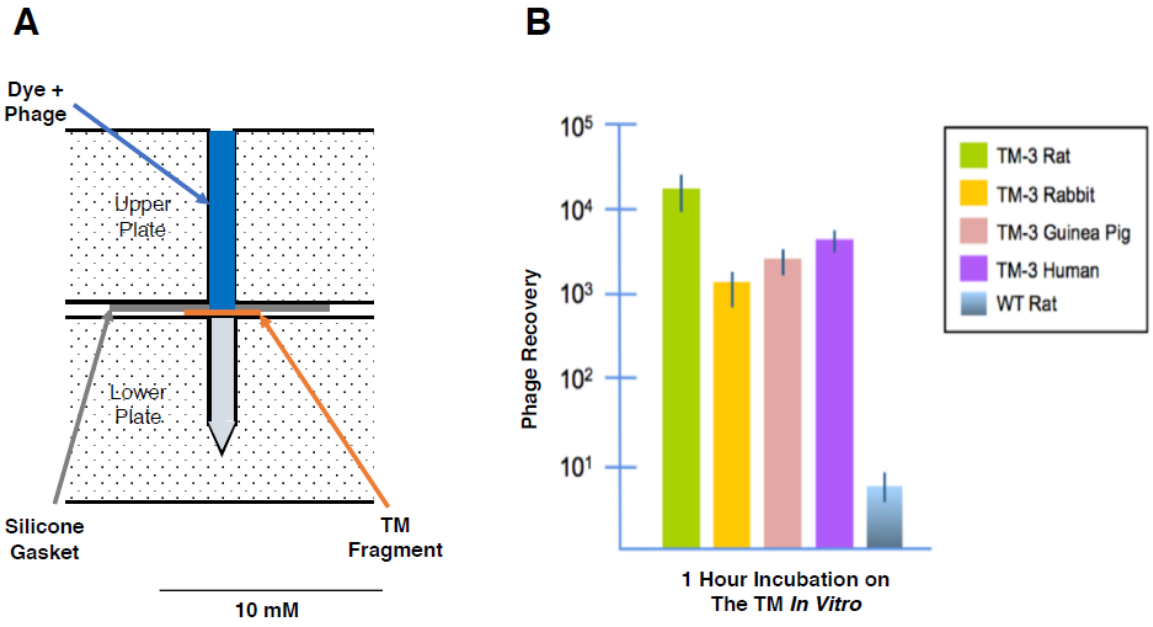
A
Dye +
Phage
Upper
Plate
Lower
Plate
Silicone
Gasket
TM
Fragment
10 mM
B
Phage Recovery
10^5
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10^3
10^2
10^1
TM-3 Rat
TM-3 Rabbit
TM-3 Guinea Pig
TM-3 Human
WT Rat
1 Hour Incubation on
The TM *In Vitro*