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Targeting host-microbial interactions to develop otitis media therapies

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Abstract. Otitis media (OM; middle ear infection) is the most common reason for pre-school children to visit a doctor, be prescribed antimicrobials, or undergo surgery. Recent Cochrane reviews of clinical trials have identified that antibiotics and grommet surgery are only moderately effective in treating OM, with recurrent or persistent infection observed in one-third of children. Research efforts are focusing on developing improved therapies to treat OM and prevent disease recurrence. The recurrent nature of OM is mostly due to the persistence of bacterial pathogens within established biofilm in the middle ear. Promising novel therapies are harnessing host-microbe interactions to disrupt middle ear biofilm and permit antibiotics to work more effectively. New approaches are also being developed to prevent OM, including new vaccines and mining the host respiratory microbiome to develop novel bacterial therapies. This review describes how our improved knowledge of human and microbial interactions is driving development of OM therapies to improve health outcomes for children in Australia and worldwide.

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Globally, there are ~709 million cases of acute otitis media (OM), ~31 million cases of chronic OM and 21 000 deaths from OM complications each year[1]. In Australia, almost every child will experience an episode of OM by their second birthday. One quarter of Australian children will suffer from recurrent or persistent OM and hearing loss, for which grommet surgery is recommended to improve hearing and reduce the risk of infection. Approximately 35 000 surgeries for OM are conducted each year in Australia[2].

Acute OM (AOM) involves inflammation of the eardrum, which is often painful and associated with fever. Children that suffer from recurrent AOM (three episodes in 6 months or ≥4 in 12 months) are usually referred for grommet surgery (Figure 1*a*). In some children bacteria are never fully cleared and mucous (generated by the child's immune response to presence of bacteria) persists behind the eardrum; this is known as OM with effusion (OME) or 'glue ear'. Figure 1*b* (and Video S1, available as Supplementary material to this paper) shows aspiration of the sticky glue from the middle ear by an ENT surgeon. OME can occur without a preceding AOM episode. Persistent 'glue' in a child's ear results in conductive hearing loss and if left untreated can have a devastating impact on a child's learning, and social and emotional well-being[2,3]. This complication disproportionately affects Aboriginal and Torres Strait Islander children, who suffer the highest reported rates of OM and associated hearing loss in the world – more than double the incidence in non-Aboriginal Australian children[2].

Potential OM pathogens (otopathogens) reside in the nasopharynx and adenoids, usually as asymptomatic colonisers. Transition from colonisation of the upper respiratory tract to middle ear infection often involves a preceding respiratory virus infection, which aids otopathogen ascension to the middle ear through promotion of bacterial proliferation and increased mucous production[4]. Nontypeable *Haemophilus influenzae* (NTHi), *Streptococcus pneumoniae* and *Moraxella catarrhalis* are the leading otopathogens[5]. We have shown that these otopathogens survive in the 'glue' (biofilm) in the middle ear[6,7] (also shown in Figure 2), where they are up to 1000 times more resistant to antimicrobials and can evade host immune defences[8]. Recently, we have demonstrated that children with bacterial otopathogens detected in their middle ear at the time of grommet surgery are three times more likely to require repeat OM surgery[9]. Thus, ensuring that otopathogens are cleared from the middle ear at the time of first grommet surgery could be a strategy for preventing disease recurrence. Preventing the first episode of OM would be even better.

Current treatment strategies for OM

Antimicrobials

Cochrane reviews of randomised clinical trials (RCTs) have indicated that treating AOM and OME with antimicrobials only has a modest benefit on the symptoms of OM[10]. Long-term low-dose antibiotic treatment has been shown to prevent occurrence of AOM in high-risk children[11], but this must be balanced against the risk of adverse effects such as diarrhoea. Antibiotic use, particularly prolonged low-dose use, can also contribute to the growing threat of antimicrobial resistance.

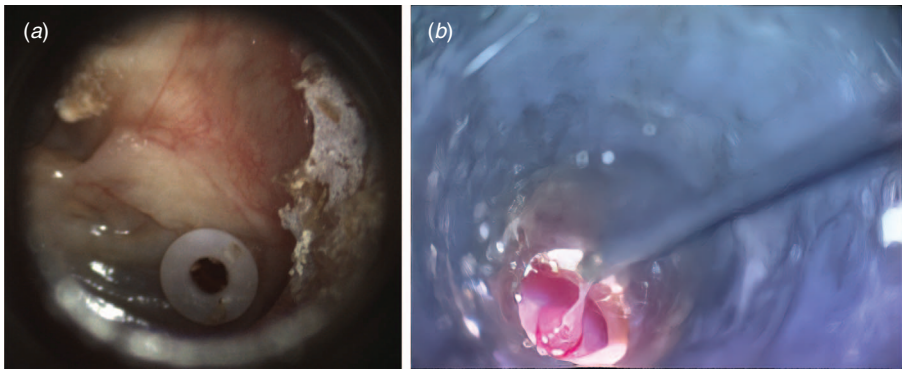

Figure 1. Grommet insertion and removal of effusion from the middle ear. (*a*) Otoscopy image of a grommet (small plastic tube) surgically inserted into the tympanic membrane for treatment of acute otitis media or otitis media with effusion (OME) (image courtesy of Clinical A/Professor Jafri Kuthubutheen). (*b*) Aspiration of middle ear fluid 'glue' from a child with OME, via an incision in the tympanic membrane and prior to grommet insertion (image courtesy of Professor Harvey Coates AO).

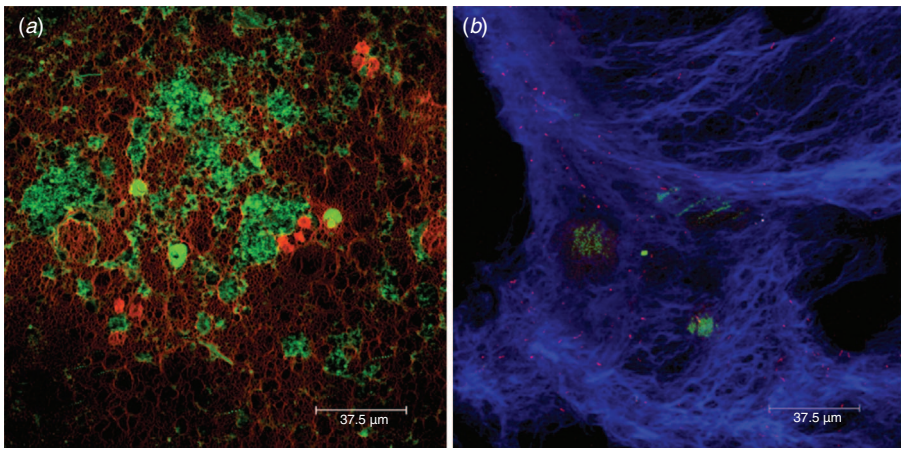

Figure 2. Maximum projection Confocal Laser Scanning microscopy images demonstrating presence of biofilm in middle ear effusion from a child undergoing grommet surgery for recurrent acute otitis media. (*a*) Live/dead staining of middle ear effusion demonstrating presence of live bacteria (green) surrounded by extracellular host DNA (red) within mature bacterial biofilms. (*b*) Fluorescence *in situ* hybridisation on the same middle ear effusion demonstrating multi-species bacterial biofilms using the following 16s rRNA probes: universal bacterial probe (red), *S. pneumoniae* (green), and *H. influenzae* (grey) plus Hoechst 33342 staining for all DNA (blue). *S. pneumoniae* and other unidentified bacteria were observed within biofilms throughout the host DNA in the middle ear fluid. Scale bar = 37.5 µm.

Surgery

Evidence on the effectiveness of OM surgery for OM is poor; a Cochrane review of five RCTs revealed only low-quality evidence for the benefits of grommet surgery[12]. While grommets do improve hearing in the short-term, they can also block with pus or lead to persistent otorrhoea (runny ears)[13]. Furthermore, disease often recurs in children, with >30% of patients returning for repeat grommets[13]. While private surgery waitlists for grommet surgery are only 4 to 6 weeks, the current wait time for grommet surgery at public Australian hospitals is unacceptably long at two years. This is an exceptionally long time for a child to suffer from reduced hearing, which can have major impacts on speech development, education outcomes and social and emotional wellbeing[2,3].

Current clinical preventative strategies for OM

Vaccines

Vaccination remains the gold standard for preventing infections. However, because NTHi has high strain diversity and no polysaccharide capsule, and *S. pneumoniae* has high serotype diversity, it is challenging to develop effective OM vaccines. Currently no OM-targeted vaccines are licensed[14].

Probiotics

Both orally and nasally delivered Lactobacillus and α-Streptococci species can have a moderate untargeted impact on recurrent OM[15]. However, there is no evidence that probiotics protect against initial episodes of OM (Cochrane review of 16 RCTs)[15].

Development of novel therapies to treat OM

Thermoresponsive ototopical gels

Hydrogels that are liquid at room temperature and gel-like at 37°C can be used for controlled delivery of OM therapies. Otiprio® is a new licenced therapy that delivers the antibiotic ciprofloxacin over 10–14 days in a thermoresponsive gel[16]. This gel can be applied into the middle ear at the time of grommet surgery, removing the need for parental application of antibiotic drops and thereby enhancing

compliance. However, antibiotics, even when delivered in a slow-release gel, will have limited effect on established biofilms.

Anti-biofilm treatments

Since the discovery of biofilm in the middle ear of OM patients[17], it is now widely accepted that persistence of infection and recalcitrance to treatment is predominantly driven by biofilm. Biofilms in the ears of children with OM are composed of a combination of host and microbial factors that protect the otopathogens[6]. Researchers are targeting biofilms to enhance treatment for recurrent AOM and chronic OME.

(1) *Therapeutic anti-biofilm vaccine*: antibodies to NTHi proteins (PilA and OmpP5) have been shown to disrupt established NTHi biofilms in the chinchilla model of OM[18]. The biofilm destabilisation is antibody mediated and occurs by targeting the type IV pilus (PilA) responsible for twitching motility, and also the tightly co-regulated quorum signalling molecule (LuxS), both of which are essential for biofilm formation and dispersal[18]. Otopathogens released from the destabilised biofilm are highly susceptible to antibiotics[19]. The PilA protein is included in a trivalent sub-unit NTHi vaccine that was tested in clinical trials in adults with chronic lung disease[20]. While PilA vaccination was safe and induced high antibody titres in the trial, the ability of these antibodies to destabilise OM biofilms in humans has not been assessed. Future trials with antibiofilm therapeutic vaccines in children with chronic NTHi OME are warranted.

(2) *Antibody therapy*: Bacterial extracellular DNA (eDNA) is abundant within bacterial biofilms. This eDNA is arranged in lattices and the critical protein that maintains the biofilm structure is integration host factor (IHF). IgG or Fab-fragments targeting protective epitopes within the DNA-binding tip domains of IHF have been shown to disrupt established biofilms *in vitro* and to mediate resolution of disease in the chinchilla OM NTHi biofilm model[21].

(3) *Anti-neutrophil extracellular trap (NET) therapy*: Dornase alfa (Pulmozyme®) is a DNAse-based therapy routinely used to breakdown biofilm in the lungs of cystic fibrosis (CF) patients. We have shown that Pulmozyme® breaks down NET-derived DNA in middle ear biofilms from OM patients to allow antibiotics to effectively kill the remaining otopathogens *in vitro*[6]. Our Phase I trial (CTN#2011/0635) in 60 children demonstrated that Pulmozyme® application into the middle ear at time of grommet surgery was safe, with no adverse events (manuscript in preparation). Our current Phase II randomised control trial is assessing safety and effectiveness of five daily applications of Pulmozyme® post-surgery (ACTRN12619001306101). Study end-points include safety and tolerability of an extended dosing regimen, recurrence of OM, and need for repeat surgery within 2 years of treatment.

Development of novel therapies to prevent OM

Vaccines

Progress on vaccine development for OM prevention was reviewed following the 2019 international OM meeting[14]. In brief, multi-species vaccines are required to prevent OM but their development is challenging. However, vaccines against NTHi and *M. catarrhalis*, and pneumococcal vaccines with broader serotype coverage, are all in current clinical trials. Vaccines against respiratory viruses are also useful in preventing OM, as demonstrated for influenza[14], and must be tested for new vaccines where possible, i.e. respiratory syncytial virus vaccines. Development of anti-biofilm prophylactic vaccines hold great promise with pre-clinical models demonstrating protection from biofilm formation in the middle ear[22].

Microbiome-derived probiotic therapies

The human respiratory microbiome has been described as 'the gatekeeper to respiratory health'[23] and a potential source of novel therapies. The use of respiratory commensal bacteria as probiotics, rather than gut commensals, for OM prevention is under investigation, with evidence of effectiveness in some but not all studies[24]. We demonstrated that the human respiratory commensal *Haemophilus haemolyticus* can be used to inhibit NTHi colonisation and infection of human respiratory epithelium *in vitro*[25]. In addition, intranasal administration of a closely related murine commensal, *Muribacter muris*, prevented NTHi colonisation and development of NTHi OM in mice[26]. Inflammatory responses to NTHi were curbed in mice receiving *M. muris*[26], which is important given that inflammation plays a major role in OM pathogenesis including neutrophil recruitment and NET formation. We are now undertaking a first-in-human study on the safety and tolerability of intranasally delivered *H. haemolyticus* to healthy adults prior to clinical trials in children to assess impact on OM prevention.

Conclusions

Fundamental research into human-microbial interactions involved in OM has led to significant advances in developing novel approaches to treat and prevent OM. Engaging stakeholder recognition in the value of OM prevention is essential to ensure further investment in development of these new OM therapies. Better treatment and prevention of OM will improve antimicrobial stewardship and conserve healthcare resources, and more importantly help bring equity to hearing health and educational outcomes: when kids can hear, they can learn.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of funding

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Biographies

Dr Lea-Ann S Kirkham is Co-Director of the Wesfarmers Centre of Vaccines and Infectious Diseases and Co-Head of the Bacterial Respiratory Infectious Disease Group at the Telethon Kids Institute. She is an infectious disease microbiologist with a major interest in developing therapies to prevent respiratory infections. In 2021 she was awarded an NHMRC investigator grant to translate her pre-clinical findings with a novel immunotherapy for ear and lung infections into human trials.

Dr Ruth B Thornton is a Senior Research Fellow in the Centre for Child Health Research at the University of Western Australia and an Honorary Research Fellow at the Telethon Kids Institute. Together with Dr Kirkham, she leads the Bacterial Respiratory Infectious Disease Group. Dr Thornton is an immunologist with considerable experience in host-pathogen interactions. She currently leads the ATOMIC ears clinical trial at Perth Children's Hospital, investigating the safety and effectiveness of using an anti-biofilm agent to treat recurrent and chronic otitis media.