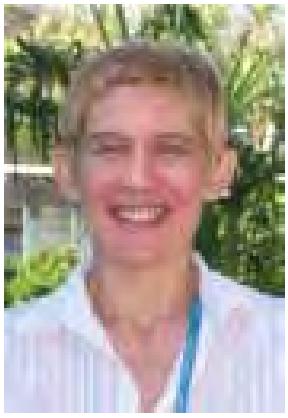
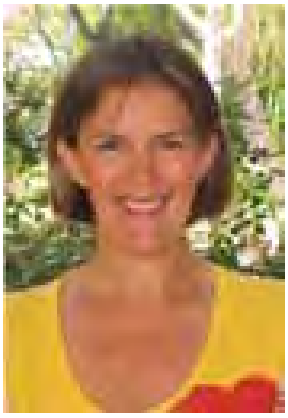
Otitis media: an ongoing microbial challenge





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Young Australian Indigenous children in remote Northern Territory communities suffer excessively high rates of otitis media (OM) and remain at high risk of suppurative complications with poor audiological and educational sequelae. Efforts to effectively treat this condition are confounded by the frequent failure of standard antibiotic treatment regimens, despite good therapeutic compliance and susceptibility of the major pathogens. OM has a complex, polymicrobial aetiology. *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* are the recognised major pathogens; however, bacteria from at least 15 other genera have been identified in middle ear effusions. It is often unclear whether detection of many of these bacteria is circumstantial, or if they are contributing to the infection either directly, or through competitive or cooperative processes. While culture remains the gold standard for detecting the major pathogens, molecular methods are changing the ways we explore the complex polymicrobial dynamics of OM.

OM, or middle ear infection, is a complex and dynamic continuum, which is generally believed to commence with bacterial colonisation of the nasopharynx. OM pathogens ascend the Eustachian tube to the middle ear space, initiating an inflammatory response and accumulation of middle ear fluid, causing bulging and possible redness of the tympanic membrane, pain and fever. Acute episodes (AOM) may resolve to non-acute middle ear effusion (OME) and hearing loss may persist. Alternatively, acute otitis media (AOM) may lead to perforation of the tympanic membrane, which can progress to chronic suppurative OM (CSOM) if untreated.

While OM is common in all populations, the frequency of the different manifestations varies. In Australia, non-Indigenous children frequently experience OME and occasionally suffer AOM. Perforation of the tympanic membrane and suppurative complications are uncommon [1]. In contrast, Indigenous children in remote communities experience high rates of AOM and CSOM in addition to recurrent episodes and persistent OME [2]. The most recent study from the Northern Territory found that among 709 Indigenous children examined from 29 remote communities, 24% had perforated tympanic membranes (15% CSOM, 2% dry perforation, 7% AOM with perforation). A further 26% had AOM without perforation and only 8% had bilaterally normal middle ears [3]. In another study, CSOM was identified as the most common health problem in Aboriginal children in Far North Queensland [4]. Effective therapeutic intervention is critical to minimising the risk of Indigenous children progressing to CSOM and the prevention of permanent audiological sequelae.

What we understand about OM bacteriology from culture-based studies

Two large studies in Israel and Finland have reported culture data from middle ear fluid obtained by tympanocentesis during AOM [5, 6]. Both studies reported *Streptococcus pneumoniae,* non-capsular *Haemophilus influenzae* (NCHi) and *Moraxella catarrhalis* as the most common causes of AOM. For Australian Indigenous children, culture of ear discharge in cases of AOM with perforation (tympanocentesis has not been possible), found NCHi in 55 to 60% specimens, *S. pneumoniae* in 30 to 40% and *M. catarrhalis* in less than 10% of ear discharge specimens [7].

An acute episode may resolve clinically within 24 to 48 hours, leaving OME that may persist for several weeks or months [8]. The conventional OM pathogens are cultured from middle

ear effusions of OME patients; however, culture rates are low. Bacteria from at least 15 genera have been reported in middle ear effusions [9-11], many with documented resistance to recommended OM treatments [9].

Recurrent AOM with perforation may lead to CSOM. The bacteriology of CSOM transitions to one dominated by opportunistic pathogens, particularly *Pseudomonas aeruginosa*, which is associated with approximately 20% to 50% CSOM in Indigenous [12] and non-Indigenous children [13-16]. Approximately one in five diagnoses of CSOM in young Indigenous children are associated with NCHi (22%), whereas *S. pneumoniae* is rarely cultured (3%) [12].

Why is OM so prevalent in Indigenous communities?

An extension of Cole's vicious circle hypothesis [17] is proposed to explain the high rates of OM and respiratory infection in Indigenous children (Figure 1) [18, 19]. Respiratory tract infections commence very early in life for these children and involve a high density of multiple bacterial species and strains that predict recurrent episodes of AOM and progression to CSOM [20, 21]. The density of these pathogens, their dominance among the nasopharyngeal flora and the multiplicity of strains colonising simultaneously, escalate during the first weeks of life [22] and are all significantly associated with the presence and severity of current ear disease (either no OM, OME or suppurative OM) (Figure 2) [21].

High rates of transmission result in an accumulation of strains at a rate greater than can be cleared by the infant immune response or by damaged mucosae and the vicious circle is repeated. These factors contribute to chronic ear and respiratory disease throughout childhood and often into adult life.

Molecular investigation of OM microbial dynamics

Despite years of culture-based research, we still lack a comprehensive understanding of the microbiology underlying OM. A better understanding of the polymicrobial pathogenesis is needed if more effective OM therapies are to be achieved. A range of culture-independent, largely nucleic acid–based, technologies have emerged, which allow deeper exploration of OM pathogenesis.

Polymerase chain reaction (PCR), the mainstay of culture-independent methods, is increasingly used to test OM samples, because it provides greater sensitivity and can allow identification of bacteria in culture-negative samples. Middle ear effusions are largely reported as 'sterile' by culture (>50% of the specimens [11]); however, molecular techniques detect bacteria in the same culture-negative effusions [23]. In some cases, questions remain regarding the viability and pathogenic nature of organisms that are only detected by PCR [24] and this area requires further investigation. PCR is also useful in the investigation of atypical OM pathogens such as *Alloiococcus otitidis,* a controversial and difficult to culture proposed middle ear pathogen, which is commonly only detected by PCR [25, 26].

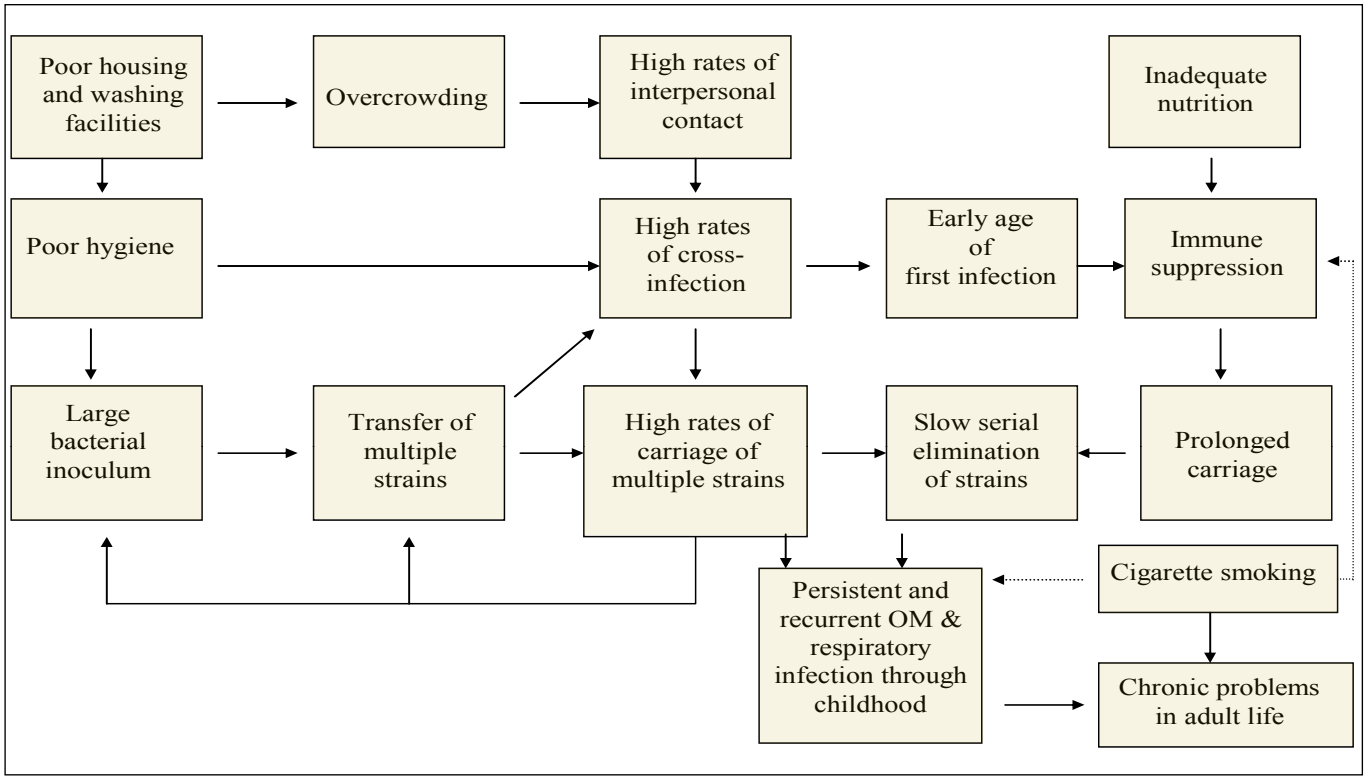

Figure 1: Factors influencing endemicity and disease associated with respiratory bacteria.

Fluorescence *in-situ* hybridisation (FISH) is a nucleic acid–based method that is being used to understand the role of biofilm in OM pathogenesis. In one FISH-based study, biofilm was detected in 92% of middle ear mucosal biopsies from 26 children with OME or recurrent OM [27]. Such findings suggest an explanation for the false-negative culture of middle ear samples, as biofilms are inherently difficult to culture. In some cases, it may also explain the persistence and recalcitrance of OM, as bacteria growing in biofilm are more resistant to antibiotics than planktonic cells [28].

Bacterial strain typing is also largely by molecular methods. Even classical phenotypic methods, such as pneumococcal serotyping, can be done directly from specimens using multiplex PCR [29-31], or microarray [32]. International multi-locus sequence typing (MLST) databases (25 to date) provide a powerful tool for epidemiologic studies [33]. Less expensive spin-offs from MLST, such as SNP typing [34], using real-time PCR technology, are becoming more popular. Like MLST, these techniques provide portable data, but central data repositories are not currently available.

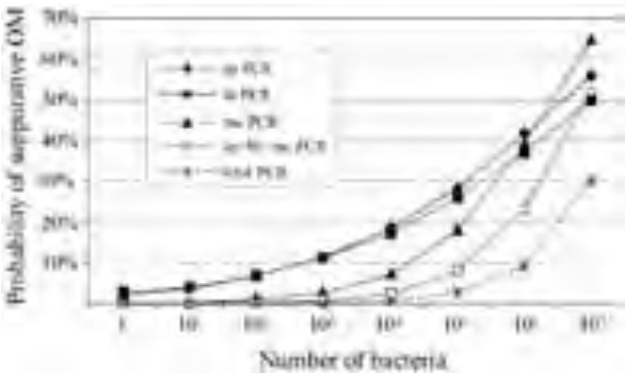

Figure 2: Predicted probability of suppurative OM with bacterial load based on the logistic regression model of data acquired by real-time quantitative PCR (from [21]). These data demonstrate the rise in predicted probability of suppurative OM (AOM with or without perforation) as bacterial load increases. sp, *S. pneumoniae;* hi, *H. influenzae;* mc, *M. catarrhalis;* total bacterial load.

Culture-independent methods are also increasingly recognised as important tools for understanding the complex microbial environments on human mucosal surfaces [35]. Perhaps the most exciting use of culture-independent methods is the applications made possible by Next-Generation sequencing. Current sequencing platforms, such as Roche 454 GS-FLX™ and Illumina sequencing technologies can provide millions of sequences in just a few hours. This facilitates not just identification of targeted species, but identification of all bacteria and, if desired, all genes present in an environment. If applied to OM, such studies may not only allow better understanding of factors controlling the composition of mucosal flora, but also understanding of the interactions between bacteria and human cells (especially the immune system), respiratory viruses and other respiratory bacterial flora. The technologies may also allow us to measure the effects of interventions, such as pneumococcal and *H. influenzae* vaccines and antimicrobial therapy, on bacterial populations associated with the respiratory mucosa.

Conclusion

Whilst there are some general indications that Indigenous child health has improved over the last 30 years, such as increased birth weight and lower infant mortality, there is evidence to suggest that morbidity associated with infections such as pneumonia and OM has not changed [36]. For Indigenous children and others at increased risk of early-onset OM, better understanding of the infectious process is needed if effective therapeutic interventions are to be realised. Culture-based studies can tell us much about

individual pathogens, but deeper exploration of the complex underlying bacteriology can only be achieved through the application of culture-independent methods. We believe that future investigations using molecular technologies will lay the foundation for the design of more effective OM interventions.

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