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The microbiome of otitis media with effusion in Indigenous Australian children

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ABSTRACT

Introduction: Indigenous Australian children have a high prevalence of otitis media with effusion (OME) and associated conductive hearing loss. Only three microbiological studies of middle ear fluid (MEF) from Indigenous Australian children with OME have been reported. All of these were reliant on culture or species-specific PCR assays. The aim of this study was to characterise the middle ear fluid (MEF), adenoid and nasopharyngeal (NP) microbiomes of Indigenous Australian children, using culture-independent 16S rRNA gene sequencing.

Methods: MEF, NP swabs and adenoid specimens were collected from 11 children in the Alice Springs region of Central Australia. Bacterial communities in these specimens were characterised using 16S rRNA gene sequencing.

Results: The microbiota in MEF samples were dominated (>50% relative abundance) by operational taxonomic units (OTUs) consistent with *Alloiococcus otitidis* (6/11), *Haemophilus influenzae* (3/11) or *Streptococcus* sp. (specifically, Mitis group streptococci which includes *Streptococcus pneumoniae*) (1/11). Anatomical site selectivity was indicated by the presence of a single conserved *Haemophilus* OTU in 7/11 MEF samples. In comparison, there were ten distinct *Haemophilus* OTUs observed across the NP and adenoid samples. Despite significant differences between the MEF and NP/adenoid microbiomes, *Streptococcus* sp., *H. influenzae* and *Moraxella catarrhalis* OTUs were common to all sample types. Co-occurrence of classical otopathogens in paired MEF and NP/Adenoid samples is consistent with earlier culture-based studies.

Conclusion: These data highlight the need to further assess *H. influenzae* traits important in otitis media and to understand the role of canal flora, especially *A. otitidis*, in populations with a high prevalence of tympanic membrane perforation.

1. Introduction

Otitis media affects up to 90% of children in remote Indigenous communities in Australia [1]. Around 40% of these children have otitis media with effusion (OME; the presence of middle ear fluid behind an intact tympanic membrane, without signs or symptoms of infection) [1]. The conductive hearing loss that results from OME may impact speech development and educational outcomes in children, compounding the significant social, financial and educational disadvantage seen in Australian Indigenous populations [2].

Understanding the complex microbiology associated with OME is critical to achieving effective therapy. The bacteriology of OME

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has been extensively researched in non-Indigenous populations, primarily using culture and PCR-based methods [3,4]. Culture-based studies of middle ear fluid (MEF) collected by tympanocentesis or myringotomy from non-Indigenous children with OME have identified *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* as the most commonly detected species [4]. Only three studies of MEF from Indigenous children with OME have been reported [5–7] all reliant on culture or species-specific PCR assays. In a study by Dawson et al. [5], 3 of 29 MEFs from 18 Indigenous children with OME were culture-positive, isolating *Proteus* sp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Stuart et al. [6] cultured 45 MEFs from 27 Indigenous children with OME, identifying *S. aureus*, *Corynebacterium* sp. and *H. influenzae* as the most commonly detected species. Ashhurst-Smith et al. [7] cultured 22 MEFs from 22 Indigenous children with OME, reporting *Alloiococcus otitidis* and *Corynebacterium* sp. as the most frequently isolated species. Detection of species such as *A. otitidis* and *Corynebacterium* sp. in MEF from children with OME remains controversial as these species are ear canal commensals [8], and thus, may indicate a degree of MEF contamination during sample collection. The significance of these species in MEF from populations with high prevalence of spontaneous tympanic membrane perforation is less clear, as their detection may also indicate secondary middle ear infection following an earlier perforation [9].

Since NP colonisation is an important antecedent of otitis media, analysis of nasopharyngeal (NP) specimens has also been used to investigate OME microbiology [10]. Culture-based studies have shown a high correlation between isolation of otopathogens in the NP and MEF of children with otitis media [11,12]. While studies of NP microbiology are important in OME, detailed analysis of MEF collected by tympanocentesis or myringotomy is required to improve understanding of the underlying OME microbiology.

It is important that studies of OME microbiology use DNA-based methods as the bacteria present in MEF may be refractory to standard culture [13] and may not be targeted by commonly deployed PCR assays [14]. The limitations inherent to culture and targeted PCR methods can be overcome through the use of 16S rRNA gene sequencing approaches. To our knowledge, this approach has been limited to one OME study, where analysis of a MEF, an adenoid biopsy and a tonsilar biopsy from one non-Indigenous child was performed [15].

The aim of this study was to characterise the microbiome of MEF collected from Indigenous children with OME using aseptic surgical techniques. Additionally, we sought to compare the middle ear and NP microbiomes of these children, and describe the relationship between these sites.

2. Material and methods

2.1.1. Sample collection

This study of OME microbiota was performed using baseline specimens collected from children randomised to the surgical arms of a randomised controlled trial (RCT) of three OME treatments; medical management, adenoidectomy and grommet insertion or adenoidectomy and myringotomy (Australia and New Zealand Clinical Trials Registration 12611001073998). Inclusion criteria for the RCT were: (1) Indigenous children aged 3–10 years living in Central Australia, and (2) a diagnosis of bilateral OME present for ≥6 months. The clinical criteria for the diagnosis of OME were the presence of an immobile tympanic membrane on pneumatic otoscopy, supported by an air-bone gap on audiometry (conductive hearing loss >20 dB) and a Type B tympanogram. Middle ear fluid, an adenoid biopsy and a NP swab were collected at the time of surgery. Results from children enrolled in the RCT during May and June 2014 are included in this report.

2.1.2. Ethical approval

Ethical approval for the study was obtained from the Central Australian Human Research Ethics Committee (HOMER 12–16) and the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (2011–1686).

2.1.3. Surgical procedures and sample collection

All clinical specimens were collected under general anaesthesia at Alice Springs Hospital during May and June 2014. Intraoperative sample collection and processing was supervised by the principal author to minimise the potential for contamination of samples undergoing 16S rRNA gene analyses. Prior to myringotomy, wax and other debris were removed from the ear canal using suction and a Jobson Horne Probe (Adept Medical Ltd, Auckland, NZ). Following myringotomy, a suction catheter was used to collect MEF into an Argyle Specimen Trap (Covidien, Massachusetts, USA) by aspiration of 2 mL of sterile saline through the suction catheter. NP swabs were collected by passing a sterile Pediatric FLOQ Swab (Copan, California, USA) along the floor of the nasal cavity into the nasopharynx, then keeping it *in situ* for 5 s while rotating 180 degrees. The swab was removed and placed into 1 mL skim milk tryptone glucose glycerol broth (STGGB). Adenoidectomy was then undertaken using a curette technique. All specimens were stored on ice until the end of the surgical procedure, and then transferred to a −80 °C freezer within 2 h of collection.

2.1.4. DNA extraction and qPCR for bacterial load estimation

All specimens were thawed on ice. Swabs of the external surface of the thawed adenoid biopsies were collected and processed for DNA extraction to provide an adenoid sample with reduced amounts of human DNA (henceforth referred to as the adenoid swab). Total DNA was extracted from all clinical samples and two DNA extraction negative controls containing extraction reagents only. The DNA was extracted using a QIAamp kit (Qiagen, Victoria, Australia) with enzymatic and bead-beating pretreatment (described in detail in the Supplementary materials). The bacterial load was estimated by qPCR as described previously [9] using a dedicated aliquot of extracted DNA (see Supplementary materials).

2.1.5. 16S rRNA gene sequencing

16S rRNA gene sequencing was performed using the Illumina MiSeq platform (Illumina, Victoria, Australia). Libraries were generated by amplifying the V1–V3 hypervariable region of the 16S rRNA gene using fusion degenerate primers 27F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGRGTTTGATCMTGGCTCAG-3′) and 519R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTNTTACNGCGGCKGCTG-3′) with ligated overhang Illumina adapter consensus sequences (underlined). Library generation was performed according to the Illumina 16S Metagenomic Sequencing Library Preparation Guide [16] with minor modification (see Supplementary materials). A MiSeq V3 reagent kit (Illumina, Victoria, Australia) was used for library sequencing. Reads from Illumina sequencing were used as raw data for bioinformatic analyses. Data are deposited in the SRA database under study accession number SRP051439.