Are Biofilms behind Adenotonsillar Disease?

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Introduction

Adenotonsillar Disease describes the group of diseases including Adenotonsillar Hypertrophy (ATH) and Recurrent Tonsillitis (RT). ATH is a disorder of enlarged tonsils and adenoids, which is highly prevalent in children. The enlarged tonsils and adenoids often block the airways of children during sleep, leading to Obstructive Sleep Apnoea (OSA) with long-term reduction in cognitive development and increased cardiovascular morbidity [1]. The pathogenesis of ATH has been linked to recurrent infection as ATH children frequently experience concurrent RT infections. Children with RT do not necessarily develop ATH [2]. Previous studies have shown evidence of bacterial infection from biopsies of children with ATH, including *S. pneumoniae, S. aureus*, *M. Catarrhalis*, *Group A streptococcus* (GAS), and *nontypeable haemophilus influenzae* (NTHi). Several of these species have also been found in tonsils and adenoids of children with RT. The aim of this study is to identify and characterise the respiratory pathogens present in biofilms from children with RT and ATH.

Methods

Genetic analysis of biofilms was performed using qPCR from biofilms of patients with RT (n=22) and ATH (n=56). Determination of bacterial species was confirmed using Fluorescent *in-situ* hybridisation (FISH) from adenoids and tonsil tissue of 10 participants (RT=5 and ATH=5). FISH staining was achieved using 16s probes specific for bacterial species (*S. pneumoniae, S. aureus*, *M. Catarrhalis*, *Group A streptococcus* (GAS), *nontypeable haemophilus influenzae* (NTHi)) with directly conjugated fluorophores to enable unique identification. A universal bacteria probe and nuclear stain was included as counterstains. Confocal laser microscopy (CLM) was used to capture images (Nikon A1Si confocal, 60x 1.4NA objective, Nikon, Tokyo, Japan), and images analysed using ImageJ.

Results

This method was originally designed and optimised to show biofilms in middle ear effusions from children with chronic/recurrent Otitis Media [3]. This method has since been used on Broncho-alveolar lavages from children with chronic lung diseases to demonstrate the presence of Biofilms. Preliminary data indicates that this method will also be applicable to the adenoid and tonsil tissue from children with ATH and RT (Figure 1). There was no significant difference found in the bacterial profiles between children with ATH compared to RT (unpublished data

Discussion

The presence of biofilm would help further support the microbiome data and providing more evidence that ATH is a result of persistent infection and not allergy.

References

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2. Roberts, A.L., et al., *Detection of group A Streptococcus in tonsils from pediatric patients reveals high rate of asymptomatic streptococcal carriage.* BMC Pediatrics, 2012.
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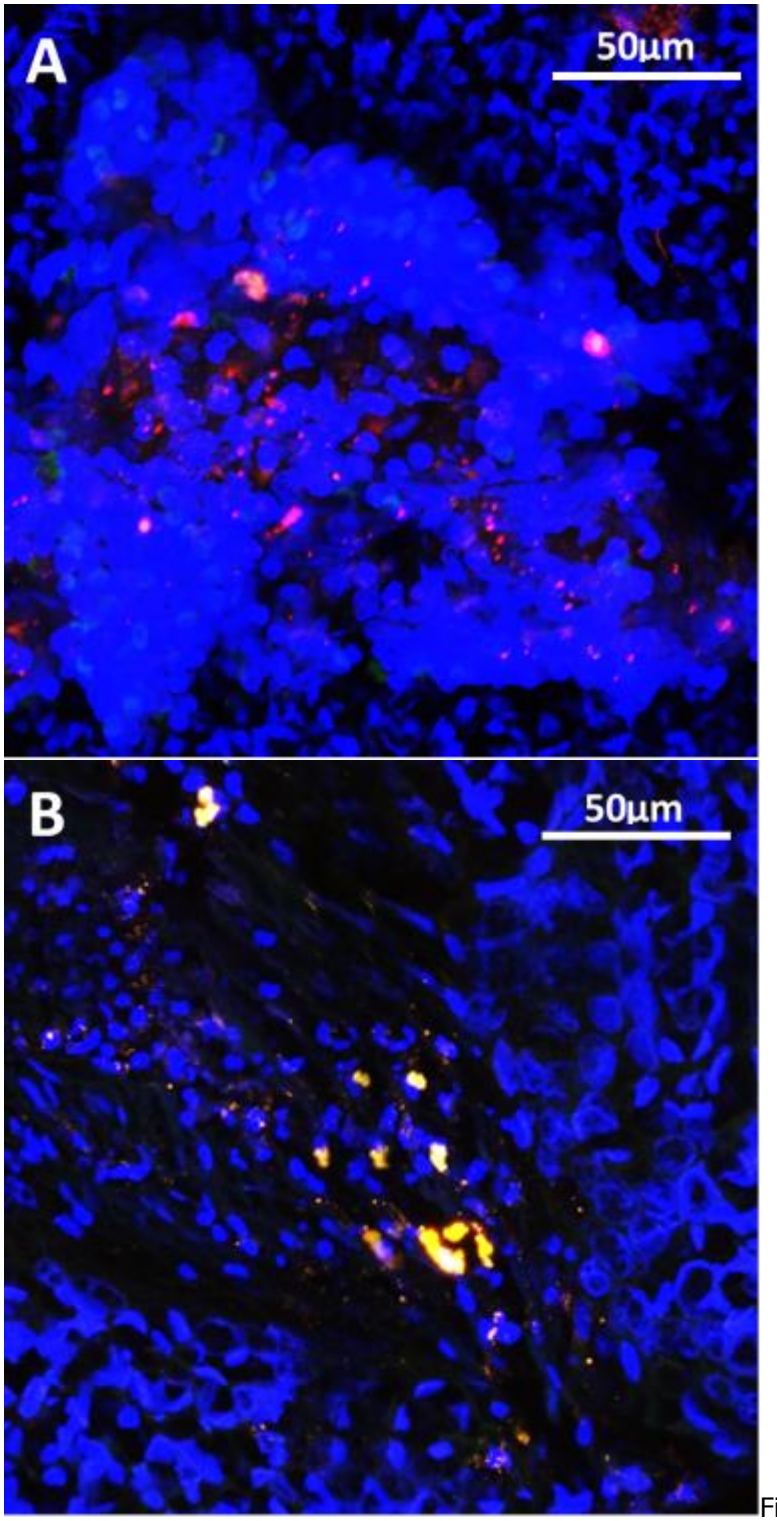

Figure 1: Preliminary images From FISH staining on tonsil tissue from a child with RT for; A) *nontypeable haemophilus influenzae* (NTHi) and *S. pneumoniae* B) *S. aureus* and, *Group A streptococcus* (GAS).