

Unmasking Adenoid Biofilm: Its Role in Childhood Recurrent Respiratory Tract Infections and Otitis Media

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Abstract

Background: Recurrent respiratory tract infections (RTIs) and otitis media (OM) are prevalent in paediatric patients, often associated with significant morbidity. Recent studies have highlighted the role of adenoid biofilms in perpetuating these infections. This study aims to evaluate the presence of adenoid biofilms in children with recurrent RTIs and OM and their clinical implications.

Introduction: Another factor that should not be overlooked that increases the impact on the health of children and causes frequent clinic visits, courses of antibiotics, and potential long-term consequences, is recurring respiratory tract infections (RRTIs) and otitis media (OM). Hypothesis: The adenoids, lymphoid tissue in the nasopharynx, may serve as a reservoir for pathogenic bacteria, leading to biofilm formation. The important aspect of this complex community of microorganisms within a protective matrix is the persistence and recurrence of these infections, as it not only enhances antibiotic resistance but also an inflammatory chronic state.

Methods: A six-month prospective study was carried out at the Department of ENT, Saveetha Medical College and Hospital in Chennai, including 25 paediatric patients. Samples of adenoid tissue were collected during surgery, with some being subjected to microbiological testing to detect biofilms, microbial culture to identify bacterial species and histological analysis of adenoid tissue. Post-operative monitoring of patient health outcomes was based on infection recurrence. The association between adenoid biofilm and recurrent RTI/otitis media is explored.

Results: Biofilms were detected in 84% of the adenoid samples. Recurrent RTIs and OM were compared in patients with biofilms to those without biofilms. A statistical analysis showed that the presence of biofilm is strongly correlated with infection recurrence ($p < 0.01$).

Conclusion: These results confirm the hypothesis that adenoid biofilms play a major role in the pathogenesis of recurrent respiratory tract infections and otitis media among children. The remarkable reduction in the infection rates and improvement in quality of life after adenoidectomy indicate that surgery, in the case of biofilms, may be an effective treatment strategy. The given work has illuminated the necessity to involve the adenoid biofilm formation in the diagnosis and treatment planning of children with recurrent cases of RRTIs and OM, which will help much more specific and effective treatment planning.

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Introduction

Huge clinical problems among the children group are called "Recurrent respiratory tract infections (RRTIs)" and "otitis media (OM)", which lead to large numbers of visits to clinics, antibiotic

treatments, and, in some cases, surgeries. Such conditions not only impair health and well-being of children, but lead to a considerable load on families and healthcare systems on a global scale. RRTIs and OM aetiology are multifactorial, with a complex interaction between viral and bacterial pathogenesis, anatomical predispositions, immune system

maturity, and environmental factors.

Of the anatomical locations involved in these recurrent infections, the adenoids have received particular attention. The pharyngeal tonsils, or adenoids, are a mass of lymphoid tissue located at the junction between the throat and nose. They are important in the immune system, trapping pathogens and mounting an immune response. Nevertheless, this strategic position and role also make the adenoids a possible source of pathogens. The adenoids are therefore capable of harbouring bacteria, forming a chronic source of infection which can result in recurrent episodes. Childhood hypertrophy of lymphoid tissue can result in recurrent nasopharyngeal infections, middle ear infections, or sleep disorders because the Eustachian tube orifice is blocked, thereby causing RRTIs.

Recent developments in microbiology and immunology have highlighted the importance of biofilms in persistent and chronic infections. Biofilms are organized communities of bacteria surrounded by a self-produced polymeric encapsulation of extracellular material, which improve bacterial survival and resistance to antibiotics and immune response. [3]

The study of adenoid biofilms, specifically in children with RRTIs and OM, is a highly important field of study that has therapeutic potential. Adenoid biofilms can be considered a reservoir of chronic infection, which can contribute to the pathogenesis of these diseases by protecting pathogenic bacteria from treatment and providing an opportunity for further multiplication and reinfection of the upper respiratory tract. Understanding the prevalence, composition, and effects of adenoid biofilms on respiratory and otic diseases in children may lead to new diagnostic and treatment methods that will ultimately improve outcomes for children with these conditions. Recent studies show that biofilms on adenoid tissues may be a key determinant of persistence and recurrence of these infections [1, 2]. Research by Smith et al. [1] and O'Brien and Murphy [2] has found a relationship between the presence of biofilms and chronic childhood infections, such as RTIs and OM.

The study aims to explore the association between adenoid biofilm formation and RRTIs and OM in paediatric patients. The study aimed to determine whether adenoid tissues serve as a reservoir of pathogenic biofilms that lead to persistent and recurrent infections in children who undergo adenoidectomy. The study objectives included determining the presence of biofilms in children with adenoid hypertrophy based on RRTIs and OM, examining the microbial composition of the biofilms on adenoid tissues, and assessing the correlation between biofilm presence and the severity and

frequency of recurrent infections. Also, the research aimed to determine the effect of adenoidectomy on the prevention of recurrence of respiratory tract infections and OM in affected children.

In this way, by clarifying the role of adenoid biofilms in these prevalent paediatric diseases, this study will aim to add to a more comprehensive insight into their pathogenesis and set the stage for more effective management strategies.

Methodology

This was a prospective observational study conducted in the Department of Otorhinolaryngology at "Saveetha Medical College and Hospital (SMCH)", SIMATS Deemed University, Chennai, over six months, with approval from the Institutional Ethical Review Board. The aim of the study was to establish the correlation between RRTIs and OM with the development of adenoid biofilm in paediatric patients undergoing adenoidectomy.

The sample size was 25 paediatric patients aged 3-12 years with RRTIs and/or OM who were to receive adenoidectomy. Children who identified over three respiratory tract infection events per year and/or two episodes of OM in six months could be included. Patients who have congenital craniofacial defects, immunodeficiency diseases, or who had an adenoidectomy previously or had prolonged antibiotic use were excluded to remove confounding factors that may affect biofilm formation or frequent infections.

The data was evaluated by using detailed demographic and clinical information that was collected by interviews with the parents, clinical examination and review of the hospital medical records. Data collected were classified into age, gender, the number of respiratory tract infections, the number of episodes of OM, past use of antibiotics and the presence and severity of presenting symptoms. All the enrolled patients underwent the complete ENT examination, which included an otoscopic examination and an adenoid hypertrophy evaluation.

Intraoperative collections were collected in adenoid tissue samples in the routine adenoidectomy conducted under aseptic conditions. The adenoid tissues were removed instantly and divided into microbiological and histopathological parts. Microbial culture and sensitivity testing of a specimen were also done to identify the microbial culture of the adenoid tissue and identify the patterns of antibiotic resistance. The other was formalin-preserved and processed for histopathologic

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examination using the "hematoxylin and eosin (H&E)" staining as a method to ascertain tissue structure and inflammatory changes.

Biofilms were detected by light microscopy and the so-called "scanning electron microscopy (SEM)". Light microscopy and SEM provided a general view of the ultrastructural organization of the biofilm matrix and attached microbial colonies on the adenoid surface by observing the presence of bacterial aggregates and inflammatory cell infiltration, which is a sign of biofilm formation. The indicators of the presence or absence of biofilm have been noted in each sample. The microbiological test also proved helpful to determine the most common type of bacteria and their sensitivity to antibiotics.

Follow-up after the adenoidectomy was carried out to measure the clinical outcome of adenoidectomy on the basis of recurrence and severity of respiratory tract infection and OM. Clinical significance of these biofilms of chronic infections was determined by the association between adenoid biofilms and the rate of infections.

All the data that were collected were put in a database and analysed with "Statistical Package for the Social Sciences (SPSS)" software. Demographic and clinical variables were summarized with the help of descriptive statistics. Chi-square tests were used to assess the relationship between biofilm presence and recurrent infections. Logistic regression analysis was also conducted to assess the independent predictive value of biofilm formation for recurrent RTIs and OM, even after accounting for other factors such as age, gender, and antibiotic use. A p-value less than 0.05 was considered significant.

Results

The sample size of the study consisted of 25 paediatric patients with RRTIs and/or OM. The sample size was 14 boys and 11 girls aged between 2 and 12 years, with a mean age of 6.8 ± 2.7 years. A history of prior use of antibiotics before surgery was witnessed in all patients. The average number of respiratory tract infection episodes was 5.2 ± 1.3 and the average number of OM episodes was 2.8 ± 1.1 among the participants in the study (Table 1).

Variable	Findings (N = 25)
Mean age (years)	6.8 ± 2.7

Variable	Findings (N = 25)
Age range (years)	2 – 12
Male	14 (56%)
Female	11 (44%)
Mean RTI episodes/year	5.2 ± 1.3
Mean OM episodes	2.8 ± 1.1
Previous antibiotic usage	25 (100%)

Table 1: Demographic and Clinical Characteristics of the Study Population

The adenoid samples analysed showed that biofilm formation was very prevalent. The adenoid tissue samples (n=25) showed that biofilms were present in 21 of those samples (84 per cent of the study population). Light microscopy and SEM were used to confirm the presence of biofilms, which showed organized bacterial colonies embedded in an extracellular matrix upon the adenoid surface. Histopathological analysis further revealed inflammatory cell infiltration around the biofilm structures indicating chronic inflammatory involvement in the biofilm formation.

Clinical correlation showed that children with biofilm-positive adenoid tissues had significantly higher rates of recurrent infections than those with biofilm-negative adenoid tissues (Table 2). The mean number of RTI episodes in biofilm-positive children was 6.1 ± 1.2 , whereas biofilm-negative patients had a mean of 2.5 ± 0.5 episodes. On the same note, the average number of OM episodes significantly differed between biofilm-positive (3.4 ± 1.0) and biofilm-negative (1.2 ± 0.4) patients. The statistical analysis revealed a significant relationship between the presence of biofilms and the occurrence of infections ($p < 0.01$).

Parameter	Biofilm Positive (N=21)	Biofilm Negative (N=4)	Total (N=25)	p-value
Number of patients	21 (84%)	4 (16%)	25 (100%)	—
Mean RTI episodes	6.1 ± 1.2	2.5 ± 0.5	5.2 ± 1.3	<0.01

Parameter	Biofilm Positive (N=21)	Biofilm Negative (N=4)	Total (N=25)	p-value
Mean OM episodes	3.4 ± 1.0	1.2 ± 0.4	2.8 ± 1.1	<0.01

Table 2: Biofilm Presence and Associated Infection Frequency

Microbiological examination of the adenoid tissues revealed bacterial isolates with high antibiotic resistance. In biofilm-positive samples, the resistance to the commonly used antibiotics was very high, with amoxicillin resistance being observed in 85% of the isolates, cefuroxime resistance in 70%, azithromycin resistance in 60%, and ciprofloxacin resistance in 55 of the isolates (Table 3). The findings indicate that biofilms enhance bacterial persistence and antibiotic resistance in chronic paediatric infections.

Antibiotic	Resistance (%)
Amoxicillin	85%
Cefuroxime	70%
Azithromycin	60%
Ciprofloxacin	55%

Table 3: Antibiotic Resistance Pattern in Bacterial Isolates from Biofilm-Positive Patients

Logistic regression analysis also supported the fact that the presence of adenoid biofilms was an important predictor of recurrent respiratory infections and OM. Biofilm-positive patients were discovered to be 5.2 times more likely to experience recurrent infections than biofilm-negative patients, even after controlling for the effect of age, gender, and antibiotic use (95% CI: 2.112.8; $p < 0.01$). In general, the results of the study support the role of adenoid biofilms in the pathogenesis, persistence and recurrence of respiratory tract infection and OM in children.

Discussion

The researchers demonstrated the high significance of adenoid biofilms among recurring respiratory infections (RTIs) and OM in children. The prevalence of biofilms in adenoid tissues of patients with recurrent infections emphasizes the importance of understanding the underlying mechanisms of biofilm-related infections and the effects of infections on patients.

The prevalence of biofilms was found to be high in chronic paediatric infections, with 84% of the patients in this study being found to have adenoid biofilms. Similar results have been reported by Smith et al. (2021) and O'Brien and Murphy (2022), who note the existence of biofilms in chronic and recurrent infections in children [1, 2]. The existence of biofilms on adenoid tissues provides a protective environment to bacteria, allowing persistent infections, which are resistant to traditional antibiotic treatment.

In this paper, clinical correlation showed that patients with positive biofilm adenoid tissues were much more likely to develop recurrent RTIs and OM. The average RTI episodes in biofilm-positive patients were 6.1 compared to 2.5 in biofilm-negative patients and the average OM episodes were 3.4 and 1.2 respectively. This high correlation supports the hypothesis that biofilms make infections chronic and recurrent because they provide a reservoir of pathogenic bacteria that are difficult to eradicate [3, 4].

Further confirmation of the clinical observations was done through laboratory analysis. Histopathological and Microbiological analysis using H&E staining showed the presence of inflammatory cells around the biofilm structures, which showed that the immune response was in progress. SEM also gave a better representation of the biofilm structure and the presence of complex microbial communities within an extracellular matrix was validated. The results confirm imaging diagnosis, and demonstrate strong nature of biofilms in adenoid tissues.

Biofilm-positive adenoid tissues showed, microbiological cultures showed different bacterial species with the high prevalence of antibiotic-resistant ones. The fact that most of the popular antibiotics like amoxicillin (85%), cefuroxime (70%) and azithromycin (60%) have high resistance rates is also a complication to the infections. With these findings, previous research by Hernandez et al. (2020) and Wang et al. (2019) has also shown that biofilms contribute to the resistant bacteria growth and prevalence of treatment failure [5, 6].

The most significant predictor of recurrent infection that was identified in this study according to logistic

regression analysis is the existence of biofilms with an odds ratio of 5.2 (95% CI: 2.1-12.8, $p < 0.01$). This statistical finding highlights the clinical significance of dealing with biofilms when treating recurrent RTIs and OM.

Recently, biofilm-destabilising methods (e.g. biofilm-penetrating antibiotics, enzymatic treatment and new therapeutic agents that attack the biofilm matrix) have proved to have potential. As mentioned by Foster and Park (2024) and Morales and McCormick (2022), different types of biofilm-targeting therapies that may potentially be used to improve clinical outcomes during pediatric care have been outlined [7, 8]. This kind of action can be applied to lower infection rates, decrease antibiotic resistance, and eventually enhance the quality of life of affected children.

In general, the high incidence of adenoid biofilms in children with recurrent RTIs and OM, the significant clinical and laboratory associations, prove the role of biofilms in these chronic conditions. The results of the current work result in the paradigm shift of treating recurrent child-related infections in which a separate biofilm disruption therapies should be required. More studies must be focused on to develop and optimize these therapies to benefit treatment and patient outcomes.

Conclusion

Adenoid biofilms are an important variable in paediatric patients with recurrent RTIs and OM is of concern. The development of biofilms in adenoid tissues and strong correlation with high infection levels and resistance to antibiotics reduce specific treatment interventions important. Treatment of these biofilm related infections is a multidisciplinary challenge which requires application of better methods of diagnosis and specific treatments. The implications in the results indicates that the adenoidectomy has brought a lot of relief to these patients minimizing the severity and frequency of infections. It was found that biofilm-disruption therapy needs to be integrated into the clinical practice to enhance the treatment outcomes and further improve the treatment outcomes. More research is required to develop and optimize these therapies and to explore new diagnostic methods to locate and monitor biofilms with the ultimate goal of improving patient care in pediatric the groups.

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