

Adherent Biofilms in Adenotonsillar Diseases in Children

Khalid A. Al-Mazrou, MD; Abdulaziz S. Al-Khattaf, MSc, PhD

Objective: To study biofilm formation on the epithelial surfaces of tonsils and adenoids in children undergoing adenotonsillectomy (T&A).

Design: Prospective study.

Setting: Tertiary academic hospital.

Patients: Between September 2005 and August 2006, 76 patients (mean [SD] age, 5.7 [3.3] years; age range, 1-18 years; male-female ratio, 1.8:1) undergoing T&A to treat infection, obstruction, or both were included. Of these, 44 had obstruction (58%), 26 had infection (34%), and 6 had both (8%).

Interventions: Scanning electron microscopy was used to assess for the presence of biofilms.

Main Outcome Measure: Presence of adherent biofilms on the surface epithelium of tonsils and adenoids.

Results: Adherent biofilm formation was demonstrated in 46 patients (61%). Among 26 patients with infections, adherent biofilm formation was detected in 22 (85%), whereas in the group of 44 patients with obstruction only 18 were found to have biofilms (41%). Comparative analysis of the data revealed that the difference was statistically significant ($P=.01$).

Conclusions: Biofilms were identified on the surfaces of infected or enlarged tonsils and adenoids in most patients undergoing T&A. The presence of biofilms in a significantly higher proportion of patients with chronically inflamed tonsils and adenoids vs patients with obstruction indicates an association between the presence of biofilms and chronic inflammation.

Arch Otolaryngol Head Neck Surg. 2008;134(1):20-23

ADENOTONSILLAR DISEASES present a major problem in children and may require surgical intervention. Adenotonsillar hypertrophy causing obstructive sleep apnea syndrome (OSAS) and/or chronic adenotonsillitis are the commonest indications for adenotonsillectomy (T&A) in pediatric practice. It is estimated that worldwide 44 to 120 per 10 000 children (7.5%-17.3% of all children) younger than 15 years undergo T&A.¹ Apart from the associated surgical complications, T&A also has a psychological and financial impact on the patients and their families. Medical therapy for the eradication of infections, therefore, appears to be a more suitable option.

Chronic adenotonsillar infection and/or hypertrophy are thought to be caused by multiple and sometimes resistant bacteria.² Many of these bacteria have the ability to form biofilms that are distinct, matrix-encased communities specialized for surface persistence. This formation of biofilms involves participation of the extracellular-matrix and cell-surface mol-

ecules, including membrane proteins. A considerable amount of bacterial energy and resources are also required for the formation of biofilms. Bacterial cells attach to a suitable surface, replicate, spread, and mature to form biofilms.³

Biofilm-forming bacteria are notoriously resistant to antibiotics, up to 500 times more resistant than their free-swimming counterparts.⁴ In addition, these bacteria also resist the host's killing mechanisms (eg, phagocytosis), thereby becoming persistent colonizers and sources of chronic infection.⁵ Bacteria are released from biofilms as individual planktonic cells or as a result of the sloughing of the biofilms. Biofilms are also formed on biotic surfaces (eg, medical devices), and some may develop on living tissues, as in the case of chronic adenotonsillitis.⁶ Since the presence of biofilm may play a major role in bacterial resistance, it may have a significant contribution to the morbidity associated with adenotonsillar diseases.

Studies reporting biofilm formation on the surfaces of tonsils and adenoids are limited.^{6,7} In addition, there are insufficient

Author Affiliations: Division of Pediatric Otolaryngology, Department of Otolaryngology-Head and Neck Surgery (Dr Al-Mazrou), and Division of Microbiology, Department of Pathology (Dr Al-Khattaf), King Saud University and University Hospitals, Riyadh, Saudi Arabia.

data regarding the presence of biofilms on the surfaces of tonsils and adenoids, especially in children. The present study was performed to determine the presence of biofilms and associated bacterial infections on adenoid and tonsil surfaces.

METHODS

STUDY POPULATION

This study was approved by the ethics committee of the College of Medicine, King Saud University. Tissue samples from tonsils and adenoids were obtained from 76 children (mean [SD] age, 5.7 [3.3] years; age range, 1-18 years; male-female ratio, 1.8:1) undergoing T&A to treat infection, obstruction, or both at King Abdul Aziz University Hospital during the period between September 2005 and August 2006. Forty-four of these patients had OSAS only (mean [SD] age, 5.2 [3.1] years) (58%); 26 had only chronic infections (mean [SD] age, 6.4 [3.4] years) (34%); and 6 had both OSAS and infection (8%). Data were matched for age and sex between the groups. At the time of surgery, tonsils and adenoids were clinically not inflamed, and none of the patients had received antibiotic therapy for at least 1 month prior to surgery.

SAMPLE COLLECTION AND PROCESSING

Tissue samples from adenoids and tonsils were obtained after T&A. The techniques used for the removal of adenoids and tonsils were curettage and cold steel dissection, respectively. Specimens of tonsils and adenoids were collected separately in sterile containers soon after the T&A. Under sterile conditions each specimen was cut into 2 equal halves. One half was used for scanning electron microscopy, and the other half was used for microbiological assessment.

SCANNING ELECTRON MICROSCOPY

The specimen was immediately placed in 2.5% to 3.0% glutaraldehyde (prepared in 0.1M phosphate buffer, pH 7.4) for 24 hours as a prefixation step. It was then rinsed twice with 0.1M phosphate buffer (pH 7.4), postfixed using 1% osmium tetroxide for 1 hour, and finally rinsed with distilled water. Following that, the specimen was dehydrated using graduated concentrations of ethyl alcohol (50%, 70%, 80%, 90%, and 95%) for 15 minutes each followed by absolute alcohol for 30 minutes. After that, the specimen was dried using the critical point dryer (Samdri-pvt-3B; Tousimis Research Corporation, Rockville, Maryland). For mounting, carbon conductive paint was used; for specimens, gold coating with Fine Coat Ion Sputter was used (JFC-1100; JEOL, Tokyo, Japan). Finally, each specimen was examined using a JEOL scanning electron microscope (JSM-6360 LV).

MICROBIOLOGIC ANALYSIS

Specimens were processed and inoculated within 20 minutes. Under aseptic conditions, the specimen was pulverized and then inoculated onto aerobic and anaerobic media. For isolation of aerobic organisms, the specimen was inoculated onto 5% sheep blood, MacConkey, and chocolate agar. For anaerobic organisms, enriched thioglycolate broth was used. The plate and the broth bottles were transferred to aerobic, carbon dioxide, and anaerobic incubators at 37°C. For the provision of aerobic and facultative conditions, incubation was performed for 48 to 96 hours.

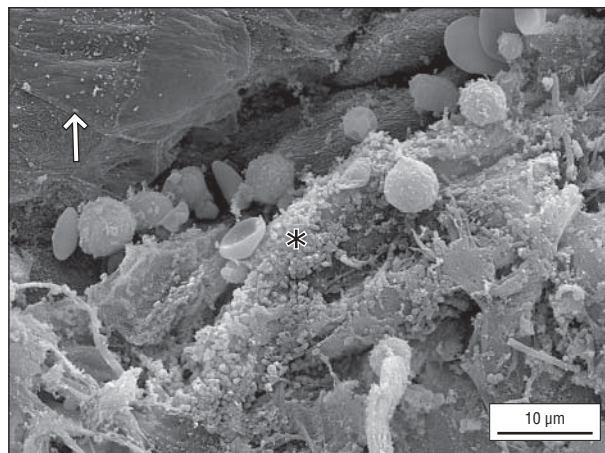


Figure 1. Scanning electron microscopy image showing mixed bacteria (asterisk) and adherent biofilm on the surface epithelium (arrow) of an infected tonsil.

STATISTICAL ANALYSIS

Statistical analysis of the data was performed using SPSS software, version 12.0 (Chicago, Illinois) for Windows (Microsoft Corporation, Redmond, Washington). Statistics for continuous data are reported as mean (SD). The analysis of variance test was used to compare infections and obstruction as predictors of producing biofilms. Age and sex were matched between the groups. Data were considered statistically significant when the *P* value was either equal to or less than .05.

RESULTS

SCANNING ELECTRON MICROSCOPY

Biofilms were identified as acellular deposits among the crypts. Small clusters of bacterial colonies were also seen in the tonsils and in adenoid tissue. The adherent biofilms had a varying number of attached bacteria, ranging from a few cells to a proliferative growth. Mixed-species biofilms were observed in several samples and were distinguished by morphologic characteristics demonstrated in the photomicrograph shown in **Figure 1**. The appearance of the biofilm was inconsistent. Some areas sampled were covered completely with microbial growth, whereas others had few attached microbes, as evidenced by the presence of areas of normal mucosa (**Figure 2** and **Figure 3**).

Of the total of 76 specimens examined, adherent biofilm formation was observed in 46 (61%) with 31 having adherent biofilms on the epithelial surfaces of both the adenoids and the tonsils (67%). Among 26 patients with infections, biofilms were detected in 22 (85%), whereas in the group of 44 patients with obstruction only 18 were found to have biofilms (41%). Comparative analysis of the data revealed that the difference was statistically significant (*P* = .01).

MICROBIOLOGIC ANALYSIS

Pathogenic organisms were isolated from 50 of 76 patients (66%), while the specimens from the remaining

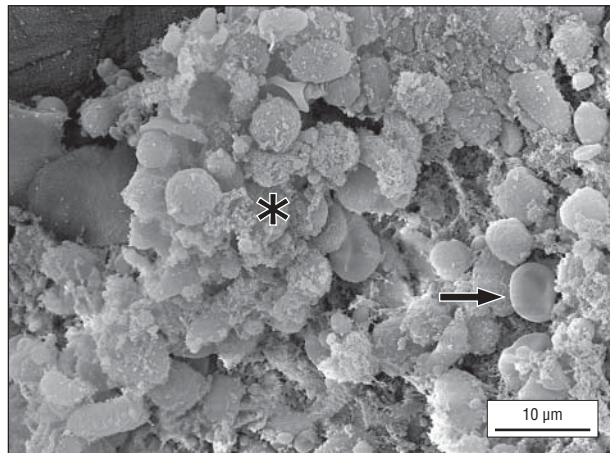


Figure 2. Scanning electron microscopy image of an infected adenoid covered with biofilm (asterisk). Red blood cells are few (arrow).

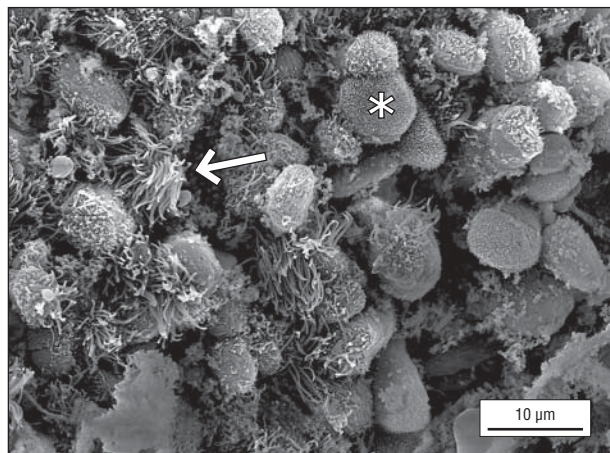


Figure 3. Scanning electron microscopy image of an adenoid in a child with obstructive sleep apnea syndrome showing stripped epithelium (asterisk) and disorganized cilia (arrow).

26 patients revealed no growth in cultures (34%). The **Table** summarizes the results of microbiologic cultures for all the patients. Although the numbers of patients differed among the groups, no significant difference was found in the percentage of positive cultures yielding the growth of pathogens. Microbiologic cultures from some patients yielded mixed growth. In all of the groups, gram-positive organisms were predominant. *Staphylococcus* species were the most frequently isolated, followed by *Streptococcus* species.

COMMENT

This study reports the presence of biofilms on the tonsils and adenoids of 61% of children undergoing T&A (n=46). We found that biofilms were present on 85% of tissue samples from patients with chronic infection, 41% of samples from children with obstruction, and 5% of samples in children with both infection and obstruction. The presence of biofilms on these organs has been reported in the past. In a study of 19 patients, Chole and Faddis⁶ found biofilms in 15 patients with chronically diseased tonsils (74%). Another study reported the pres-

Table. Culture Results According to Indications for Adenotonsillectomy

Culture	Patients, No. (%)		
	Infection	Obstruction	Both Indications
Positive	17 (65)	30 (68)	3 (50)
Negative	9 (35)	14 (32)	3 (50)
Total	26 (100)	44 (100)	6 (100)

ence of biofilms on adenoids in 6 of 16 patients with sinusitis (44%).⁷ Marked differences in the percentages of patients having biofilms on their tonsils or adenoids in these studies may reflect the smaller number of patients investigated. This explanation is supported by another study of a larger group of patients (n=28) in whom biofilms were observed on surgical specimens from the upper respiratory tract in 66% of the individuals.⁸ These findings are in agreement with those of the present study of 76 patients.

To our knowledge, this study reports for the first time the simultaneous presence of biofilms on 2 separate anatomic locations (the tonsils and the adenoids) in 31 of 46 individuals (67%). This may have resulted from both the tonsils and adenoids becoming infected simultaneously or from extension of the infection from one organ to another. Kaplan and Fine⁹ and Hall-Stoodley and Stoodley¹⁰ have recently shown that biofilm colonies are capable of releasing a single cell or small clusters of cells into liquid medium and that these released cells can attach to the surface of the culture vessel forming new biofilm colonies, which enable the biofilm to spread.

A notable finding in the present study was the presence of biofilm on tonsils and adenoids in a significantly higher percentage of patients with infection (85%; n=26) than in those with obstruction (41%; n=18) (P=.01). Chole and Faddis⁶ have attributed chronic tonsillitis to the presence of biofilms. In chronically diseased tonsils, sessile bacteria within biofilms are resistant to host defenses and antibiotics, and these bacterial biofilms within tonsils may explain the chronicity and/or recurrent nature of some forms of tonsillitis. Thus biofilms by virtue of their ability to provide protection to the bacteria against host defenses and antimicrobial therapy may serve as a carriage.¹¹ This may explain the presence of greater numbers of pathogenic bacteria on hypertrophied adenoids and tonsils than on normal ones.¹²⁻¹⁴

Both gram-positive and gram-negative organisms were isolated from patients with both infection and obstruction; *Staphylococcus* species were the most frequently isolated, followed by *Streptococcus* species. The findings of our study are in agreement with those of Brook and Shah,¹⁵ who reported that gram-positive organisms were frequently present in the tonsils and adenoids of children. This previous study was performed in children with a history of streptococcal infections, so a higher number of *Staphylococcus* and *Streptococcus* species, and to lesser extent other gram-negative and gram-positive organisms, were isolated. Brook and Shah¹⁵ found several anaer-

obes in the tonsils and adenoids of children, which is in contrast to our present findings of no patients with anaerobes.

The present article may assist future studies in determining management strategies based on the presence of biofilms for children with adenotonsillar enlargement and/or infection. Stewart¹⁶ has proposed 4 methods to deal effectively with biofilms: prevent attachment, stop growth, disrupt communications, and dissolve the biofilm matrix. Lactoferrin, an innate immunity protein, has recently been shown to inhibit the formation of biofilms by preventing attachment.¹⁷ In addition, Schwaab et al¹⁸ have demonstrated an association between the microbial biofilm, the concentration of lactoferrin, and recurrent tonsillitis. Most or all of the antibiotics in current use were identified on the basis of their activity against free-floating bacteria. Since the presence of biofilms has been associated with chronic infections, development of an antibiotic that acts directly on biofilms may provide a breakthrough in treating many chronic bacterial infections. Future therapies may include enzymes that disrupt the matrix polymers of biofilms,¹⁹ chemical reactions that block biofilm matrix synthesis,²⁰ and analogues of microbial signaling molecules that interfere with the cell-to-cell communication required for normal biofilm formation.²¹

In conclusion, adherent biofilms were more often identified on the surfaces of infected adenotonsillar tissues than on the surfaces of enlarged tissues. This difference may support changes in treatment for these 2 different conditions.

Submitted for Publication: January 29, 2007; final revision received September 6, 2007; accepted September 21, 2007.

Correspondence: Khalid A. Al-Mazrou, MD, Division of Pediatric Otolaryngology, Department of Otolaryngology–Head and Neck Surgery, King Abdul Aziz University Hospital, PO Box 86118, Riyadh 11622, Saudi Arabia (kalmazrou@gmail.com).

Author Contributions: Dr Al-Mazrou had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Al-Mazrou and Al-Khattaf. *Acquisition of data:* Al-Mazrou and Al-Khattaf. *Analysis and interpretation of data:* Al-Mazrou and Al-Khattaf. *Drafting of the manuscript:* Al-Mazrou and Al-Khattaf. *Critical revision of the manuscript for important intellectual content:* Al-Mazrou and Al-Khattaf. *Statistical analysis:* Al-Mazrou. *Administrative, technical, and material support:* Al-Khattaf. *Study supervision:* Al-Mazrou and Al-Khattaf.

Financial Disclosure: None reported.

Previous Presentation: This article was presented at The American Society of Pediatric Otolaryngology 2007 Annual Meeting; April 29, 2007; San Diego, California.

REFERENCES

1. Van Den Akker EH, Hoes AW, Burton MJ, Schilder AG. Large international differences in (adeno)tonsillectomy rates. *Clin Otolaryngol Allied Sci.* 2004;29(2):161-164.
2. Don DM, Goldstein NA, Crockett DM, Ward SD. Antimicrobial therapy for children with adenotonsillar hypertrophy and obstructive sleep apnea: a prospective randomized trial comparing azithromycin vs placebo. *Otolaryngol Head Neck Surg.* 2005;133(4):562-568.
3. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. *Annu Rev Microbiol.* 1995;49:711-745.
4. Slavkin HC. Biofilms, microbial ecology and Antoni Van Leeuwenhoek. *J Am Dent Assoc.* 1997;128(4):492-495.
5. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science.* 1999;284(5418):1318-1322.
6. Chole RA, Faddis BT. Anatomical evidence of microbial biofilms in tonsillar tissues: a possible mechanism to explain chronicity. *Arch Otolaryngol Head Neck Surg.* 2003;129(6):634-636.
7. Zuliani G, Carron M, Gurrola J, et al. Identification of adenoid biofilms in chronic rhinosinusitis. *Int J Pediatr Otorhinolaryngol.* 2006;70(9):1613-1617.
8. Galli J, Ardito F, Calo L, et al. Recurrent upper airway infections and bacterial biofilms. *J Laryngol Otol.* 2007;121(4):341-344.
9. Kaplan JB, Fine DH. Biofilm dispersal of *Neisseria subflava* and other phylogenetically diverse oral bacteria. *Appl Environ Microbiol.* 2002;68(10):4943-4950.
10. Hall-Stoodley L, Stoodley P. Biofilm formation and dispersal and the transmission of human pathogens. *Trends Microbiol.* 2005;13(1):7-10.
11. Gray E, Peters G, Versteegen M, Regelmann W. Effect of extracellular slim substance from *Staphylococcus epidermidis* on the human cellular immune response. *Lancet.* 1984;1(8373):365-367.
12. Kuhn JJ, Prook I, Waters CL, et al. Quantitative bacteriology of tonsils removed from children with tonsillitis hypertrophy and recurrent tonsillitis with and without hypertrophy. *Ann Otol Rhinol Laryngol.* 1995;104(8):646-652.
13. Kielmiovitch IH, Keleti G, Bluestone CD, et al. Microbiology of obstructive tonsillar hypertrophy and recurrent tonsillitis. *Arch Otolaryngol Head Neck Surg.* 1989;115(6):721-724.
14. DeDio RM, Tom LW, McGowan KL, et al. Microbiology of the tonsils and adenoids in a pediatric population. *Arch Otolaryngol Head Neck Surg.* 1988;114(7):763-765.
15. Brook I, Shah K. Bacteriology of adenoids and tonsils in children with recurrent adenotonsillitis. *Ann Otol Rhinol Laryngol.* 2001;110(9):844-848.
16. Stewart PS. New ways to stop biofilm infections. *Lancet.* 2003;361(9352):97.
17. Rogan MP, Taggart CC, Greene CM, Murphy PG, O'Neill SJ, McElvaney NG. Loss of microbicidal activity and increased formation of biofilm due to decreased lactoferrin activity in patients with cystic fibrosis. *J Infect Dis.* 2004;190(7):1245-1253.
18. Schwaab M, Euteneuer S, Lautermann J, Sudhoff H. Muramidase and lactoferrin in adenoidal hypertrophies, hypertrophic and chronic infected tonsil tissue: a quantitative analysis [in German]. *Laryngorhinootologie.* 2005;84(9):660-664.
19. Nemoto K, Hirota K, Ono T, et al. Effect of Varidase (streptokinase) on biofilm formed by *Staphylococcus aureus*. *Chemotherapy.* 2000;46(2):111-115.
20. Yasuda H, Ajiki Y, Koga T, Kawada H, Yokota T. Interaction between biofilms formed by *Pseudomonas aeruginosa* and clarithromycin. *Antimicrob Agents Chemother.* 1993;37(9):1749-1755.
21. Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci U S A.* 2000;97(16):8789-8793.