# Assessment of the *Streptococcus milleri group* on Adenoid Tissues

**Jerin K. Joseph, BS, Patrick J. Antonelli, MD, William O. Collins, MD, Rodrigo C. Silva, MD, and Carolyn O. Dirain, PhD**

**Department of Otolaryngology, College of Medicine**
**University of Florida, Gainesville, FL**

## ABSTRACT

*Objectives:* To determine if the *Streptococcus milleri* group (SMG) constitutes a greater microbial burden in adenoid tissues from patients with recurrent acute otitis media (RAOM) or chronic otitis media with effusion (COME) compared to patients with obstructive sleep apnea (OSA).
*Study Design:* Controlled, ex vivo.
*Methods:* Human adenoids were obtained from children undergoing adenoidectomy for RAOM (n=7), COME (n=5), or OSA (n=16). Specimens were processed for total deoxyribonucleic acid (DNA) isolation. All specimens were analyzed by real-time polymerase chain reaction for the quantification of the *S. milleri* group and total bacterial load.
*Results:* All adenoid specimens had evidence of microbes with levels that were not different between the three groups (p=0.20). DNA levels of *S. anginosus* and *S. intermedius/S. constellatus* were not different in adenoids from RAOM patients as compared to those with OSA. In contrast, adenoids from patients with COME displayed higher DNA levels of *S.anginosus* (p=0.005) and *S. intermedius/S.constellatus* (p= 0.02) compared to those with RAOM or OSA.
*Conclusions:* The microbial load of the *S. milleri* group appears to be higher in patients with COME. The role of the *S. milleri* group in the pathogenesis of COME warrants further exploration.

## INTRODUCTION

Inadequately treated RAOM or COME is thought to lead to chronic suppurative otitis media.[1] The pathogens commonly involved in RAOM and COME are *Streptococcus pneumoniae* and *Haemophilus influenzae*,[2,3] whereas the pathogens commonly associated with chronic suppurative otitis media include *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[4,5] The presence of chronic pathogens in the middle ear has long been linked to the clinical disease. Human adenoid tissues are thought as a likely reservoir for bacteria[6] and the nasopharynx has been shown in animal models to be a route for tubotympanic infection with chronic pathogens.[7,8] However, it remains unclear what leads to middle ear infection with chronic pathogens

The *Streptococcus milleri* group is a subgroup of viridans streptococci that includes *S. anginosus*, *S. intermedius*, and *S. constellatus*.[9] The *S. milleri* group is commonly found in the upper aerodigestive tract and has been implicated in the exacerbation of a variety of serious infections.[10] Biofilms that harbor *S. milleri* bacteria are thought to be involved in many infectious diseases, such as the exacerbation of cycstic fibrosis (CF). Therefore, we hypothesized that the *S. milleri* group might be involved in the transition from RAOM and COME to chronic suppurative otitis media.

Adenoid tissue is readily available after an adenoidectomy, which is commonly performed for patients with RAOM, COME and obstructive sleep apnea (OSA), allowing for comparison of children at risk of developing chronic suppurative otitis media against those that are not. The purpose of this study was to determine whether the *S. milleri* group constitutes a greater microbial burden in adenoid tissues from patients with RAOM or COME compared to patients with OSA.

***Contact Information:*** Jerin K Joseph, jjoseph101@ufl.edu

## MATERIALS AND METHODS

This study was approved by the University of Florida Institutional Review Board. Specimens obtained during surgery were promptly transported on ice to the Otolaryngology Laboratory and stored at -80°C until later use. Specimens were thawed in 1 molar phosphate buffered saline (PBS) at 4°C and cut into several portions for use for the different assays.

Specimens were processed for total DNA isolation using DNeasy 96 Blood and Tissue Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. After DNA extraction, DNA quantity and quality were determined (Synergy™ HT, BioTek Instruments, Inc., Winooski, VT).

Total bacterial load was assessed using a 16S primer set while real-time qPCR assay for *S. anginosus* and *S. intermedius/S. constellatus* were performed using specific primer sets.[11] Detection of DNA by real-time qPCR was carried out with the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). DNA was amplified in triplicate for each specimen and the mean threshold cycle (Ct) values were determined. Ct is the number of cycles required for the fluorescent signal to cross a threshold, i.e., exceeds background level, and is inversely proportional to the amount of target DNA in the sample. Mean Ct values were compared using one-way ANOVA followed by Student's t-test (JMP Pro 11, SAS Institute Inc., Cary, NC). A $p \leq 0.05$ was considered significant.

Scanning electron microscopy (SEM) was performed on portions of the adenoids (Phenom SEM, NanoScience Science Instruments, Inc., Phoenix, AZ).

## RESULTS

A total of 28 samples were obtained from children undergoing adenoidectomy for RAOM (n=7), COME (n=5), or OSA (n=16). All adenoid specimens had evidence of microbes with levels that were not different between the three groups (p=0.20; data not shown). The threshold cycle (Ct) of 16S was used to normalized the Ct for *S. anginosus* and *S. constellatus/S. intermedius*. When samples were analyzed for *S. anginosus*, we found that the 16S-normalized Ct for *S. anginosus* was significantly lower (p=0.005) in the adenoids from patients with COME compared to adenoids from OSA or RAOM patients, indicating higher DNA levels of *S.anginosus* in COME adenoids (**Figure 1**).

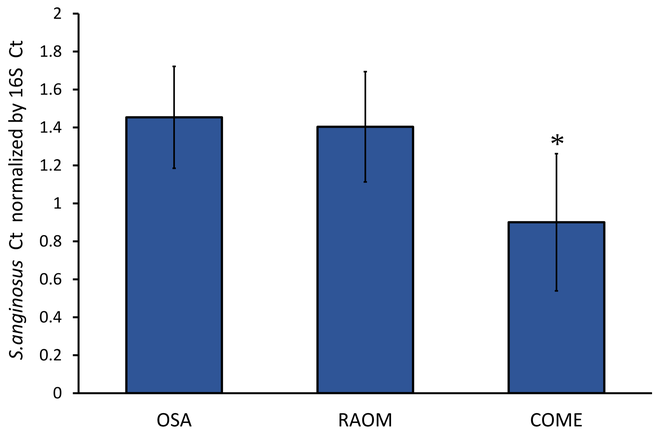


**Figure 1.** *S. anginosus* in adenoids measured by real-time PCR. *S. anginosus* threshold cycle (Ct) values were normalized by total bacterial load (16S Ct). A lower Ct indicates higher amount of target DNA in the sample. Adenoids from COME patients have higher DNA levels of *S. anginosus* compared to adenoids from OSA and RAOM patients (*p=0.005) . Error bars represent standard error.

Using a primer set that detected both *S. constellatus* and *S. intermedius*, we also found that DNA levels of *S. intermedius/S. constellatus* were significantly higher (p=0.02) in adenoids from COME patients compared to adenoids from RAOM and OSA patients (**Figure 2**).

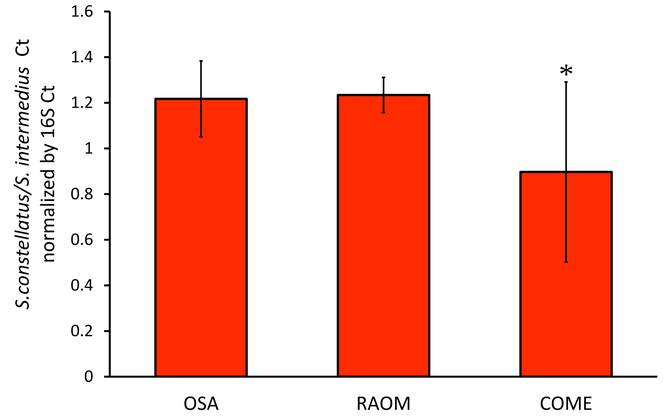


**Figure 2.** *S. intermedius/S. constellatus* in adenoids measured by real-time PCR. Threshold cycle (Ct) values were normalized by total bacterial load (16S Ct). A lower Ct indicates higher amount of target DNA in the sample. Adenoids from COME patients have higher DNA levels of *S. intermedius/S. constellatus* compared to adenoids from OSA and RAOM patients (*p=0.02) . Error bars represent standard error.

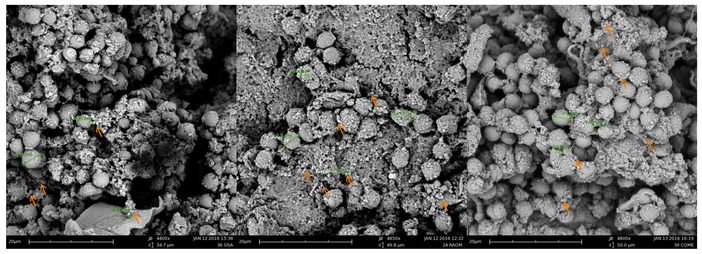


**Figure 3.** Scanning electron micrographs of adenoids. Eukaryotic cells (3.5 to 5μM) predominate but cocci-shaped microbes (about 0.5 to 1 μM, indicated by orange arrows) appear to be present in adenoids from all three groups.

## CONCLUSIONS

The microbial load of the *S. milleri* group appears to be higher in patients with COME. The role of the *S. milleri* group in the pathogenesis of COME warrants further exploration.

## REFERENCES

1. Bluestone C. Epidemiology and pathogenesis of chronic suppurative otitis media: implications for prevention and treatment. *Int J Pediatr Otorhinolaryngol* 1998;42(3):207-223.
2. Bluestone CD, Stephenson SJ, Martin LM. Ten-year review of otitis media pathogens. *Pediatr Infect Dis J.* 1992;11(8):S7-S11.
3. Post JC, et al. Molecular analysis of bacterial pathogens in otitis media with effusion. *JAMA.* 1995;273(20):1598-1604.
4. Fliss DM, Shoham SI, Leiberman A, Dagan R. Chronic suppurative otitis media without cholesteatoma in children in southern Israel: incidence and risk factors. *Pediatr Infect Dis J.* 1991;10(12):895-899.
5. Kenna MA, Bluestone BC. Microbiology of chronic suppurative otitis media in children. *Pediatr Infect Dis J.* 1986;5(2):223-225.
6. Rajeshwary A. Bacteriology of symptomatic adenoids in children. *North American Journal of Medical Sciences.* 2013;5(2):113-118.
7. Antonelli PJ, et al. Pseudomonas otitis media after eustachian tube obstruction. *Otolaryngol Head Neck Surg* 1992;107(4):511-515.
8. Antonelli PJ, et al. Acute otitis media increases middle ear susceptibility to nasal injection of pseudomonas aeruginosa. *Otolaryngol Head Neck Surg* 1994;110(1):115-121.
9. Bert F, Bariou-Lancelin M, Lambert-Zechovsky N. Clinical significance of bacteremia involving the ``streptococcus milleri'' group: 51 cases and review. *Clinical Infectious Diseases* 1988;27:385-387.
10. Aracil B. A study of susceptibility of 100 clinical isolates belonging to the streptococcus milleri group to 16 cephalosporins. *Journal of Antimicrobial Chemotherapy* 1999;43:399-402.
11. Olson AB Shibly CD, Schmidt L, Wilcox MA, Surette MG, Corbett CR. Development of real-time PCR assays for detection of the streptococcus milleri group from cystic fibrosis clinical specimens by targeting the Cpn60 and 16S RRNA genes." *Journal of Clinical Microbiology* 2010;48(4):1150-1160.