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The impact of intraoperative saline irrigations on bacterial load within the maxillary sinus

Kristin A. Seiberling, MD, Richard K. McHugh, MD, PhD, Wilson Aruni, PhD, and Christopher A. Church, MD

Loma Linda University School of Medicine, Department of Otolaryngology–Head and Neck Surgery, Loma Linda, CA

Abstract

Background—Saline irrigations are routinely employed during endoscopic sinus surgery to remove mucous and debris from the sinus cavities. What is unknown is whether this results in a quantitative reduction in pathologic bacteria within the sinus mucosa. The objectives of this study were to quantify the amount of 5 different bacteria (*Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, coagulase-negative *Staphylococcus* (CNS), and *Streptococcus pneumoniae*) within the maxillary sinus and to determine the impact of saline irrigations on bacterial counts.

Methods—Twenty patients with chronic rhinosinusitis were prospectively enrolled. After bilateral maxillary antrostomies, biopsies were taken of the maxillary sinus mucosa prior to any irrigation. In each patient, the left maxillary sinus was then irrigated with 250 cc of normal saline (NS) with a pressurized pulse-irrigation device and the right side was irrigated with 250 cc of NS using a 30-cc syringe attached to a curved suction tip. Repeat maxillary sinus mucosal biopsies were then taken from each side. Each biopsy was analyzed using quantitative polymerase chain reaction to determine the presence and amount of each of the bacteria.

Results—Saline irrigations were found to significantly reduce the amount of *S. aureus*, *P. aeruginosa*, and *S. pneumoniae* found within the maxillary sinus mucosa. No difference was found for *H. influenzae* or CNS. No difference in bacterial load reduction was able to be shown between the pressurized saline flushes and manual saline rinse methods.

Conclusion—Intraoperative saline irrigations are able to significantly reduce the amount of potentially pathogenic bacteria within the diseased sinus mucosa.

Keywords

maxillary sinus; endoscopic sinus surgery; bacteria; polymerase chain reaction; saline irrigations; hydrodebrider; in-traoperative irrigations; bacterial load

Chronic rhinosinusitis (CRS) is an inflammatory disease of unknown etiology. Bacterial infection and colonization has been thought to promote CRS pathogenesis as well as contribute to postoperative persistence of disease. Therefore, medical management of CRS includes long-term antibiotics and nasal saline irrigations, both which are thought to reduce bacterial load. Nasal saline irrigations facilitate the evacuation of allergens and irritants. Daily isotonic or hypertonic saline nasal irrigation has been shown to improve quality of life, decrease antibiotic and nasal steroid use, and improve sinus symptoms in patients with a history of frequent infections and chronic complaints.^{1–3} Saline irrigations are also used intraoperatively during endoscopic sinus surgery (ESS) to remove mucus or debris from the diseased sinuses. Traditionally, saline irrigations are introduced into the maxillary sinus using a syringe attached to a curved suction tip. The irrigations are thought to aid clearance of disease particularly when there is pus, thick inspissated mucus, or fungal debris in the sinus. What is unknown is whether this results in a quantitative reduction in potentially pathologic bacteria within the sinus mucosa. New devices have also been developed which apply pressurized irrigations into the maxillary sinus through a rotating 360-degree tip. These devices are thought to penetrate and irrigate all areas of the maxillary sinus, resulting in more thorough removal of disease. With use of the syringe technique for irrigation, it is unknown whether all areas of the sinus are equally addressed. Incomplete sinus penetration may contribute to persistent disease and continued bacterial growth in certain patients.

The aims of this study were 2-fold. Our first aim was to quantitate the amount of 5 different bacteria present in the maxillary sinus using real-time polymerase chain reaction (rPCR). We chose to evaluate 5 of the most common bacteria identified in CRS: *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. Our second aim was to see if saline irrigations effectively reduce bacterial load using quantitative rPCR. Saline irrigations were applied using both a 30-mL syringe and a pressurized irrigation device.

Patients and methods

Patients

After approval from the Loma Linda University Institutional Review Board, 20 consecutive patients with CRS were prospectively recruited from our tertiary rhinology practice. The diagnosis of CRS was made according to the signs and symptoms as outlined by the Rhinosinusitis Task Force.⁴ Only those patients who had failed medical management (nasal steroid sprays, antibiotics, saline rinses, and oral steroids when indicated) and were undergoing ESS were recruited for this study. Inclusion criteria included a Lund Mackay score of at least 1 in each maxillary sinus and nasal endoscopy demonstrating mucosal inflammation and/or infection. Only those patients with symmetric bilateral maxillary sinus disease as noted by computer tomography (CT) and endoscopy were included. Patients were excluded if they had fungal ball, antrochoanal polyp, tumor, or asymmetric disease in the maxillary sinus. All patients but 4 had the same preoperative medication, which included nasal steroid sprays and saline rinses. In 4 patients perioperative antibiotics were given, 3 with polyps and 1 with known methicillin-resistant *Staphylococcus aureus* (MRSA).

Surgical technique

During surgery, a standard maxillary antrostomy was created on both sides and enlarged so that the majority of the maxillary sinus could be visualized. Mucosal biopsies were taken along the posterior wall prior to any instrumentation except for removal of any nasal polyps obstructing visualization of the mucosal surface. The samples were then sent for analysis for bacterial load using rPCR. In each patient, the right maxillary sinus was irrigated with 250 cc of normal saline using a 30-cc syringe and a curved olive tip suction. The left side was irrigated with 250 cc of normal saline using the Xomed Hydrodebrider Endoscopic Sinus Irrigation System (Medtronic, Inc., Jacksonville, FL). The tip of the maxillary sinus hydrodebrider handpiece was placed into the maxillary sinus and the sinus was irrigated with normal saline while rotating the blade 360 degrees to improve sinus penetration. Repeat maxillary sinus mucosal biopsies were then taken in a location adjacent to the pretreatment biopsy.

Tissue storage and DNA extraction

The biopsies were placed on ice, then rapidly transferred to a –80-degree freezer. No additional freeze/thaw cycles occurred prior to processing. DNA extraction was carried out as per the manufacturer's method for the DNeasy Qiagen kit (Qiagen, Inc., Valencia, CA). A total of 1.8 mL of phosphate buffered saline (PBS) was added to the sample and centrifuged at 1800g for 5 minutes. The supernatant was decanted and resuspended in 180 μ L of PBS and incubated for 2 minutes at room temperature. A total of 25 μ L of proteinase K solution and 200 μ L Buffer AL were then added. These were mixed thoroughly by vortexing, and incubated at 56°C for 10 minutes, then 200 μ L of ethanol (96–100%) was added to the sample, and mixed thoroughly by vortexing. The mixture was then pipetted (including any precipitate) into the DNeasy Mini spin column and placed in a 2-mL collection tube. This was then centrifuged at 6000g (8000 rpm) for 1 minute. The DNeasy Mini spin column was then placed in a new 2-mL collection tube, 500 μ L Buffer AW1 was added, and the mixture was centrifuged for 1 minute at 6000g (8000 rpm). The flow-through and collection tube were discarded and the DNeasy Mini spin column was placed in a new 2-mL collection tube, 500 μ L Buffer AW2 added, and centrifuged for 3 minutes at 20,000g (14,000 rpm) to dry the DNeasy membrane. The flow-through and collection tube were again discarded and the DNeasy Mini spin column again placed in a clean 1.7-mL microcentrifuge tube and 200 μ L Buffer AE pipetted directly onto the DNeasy membrane. Incubation at room temperature for 1 minute was allowed to occur, and then the fluid was centrifuged for 1 minute at 6000g (8000 rpm) to elute.

rPCR

The QuantiTet SYBR Green I fluorescent dye detection system (Qiagen) was used with the following primers (organism, primer sequence, and product size):

Haemophilus influenzae (F: 5'-GGAATCTGGCTTATGGAG-3'; R: 5'-CACATCAACCTTCCTCAA-3'; 336)

Streptococcus pneumoniae (F: 5'-AACCATATAGGCAAGTACAC-3'; R: 5'-ATCATGCTTAAACTGCTCAC-3'; 429)

Pseudomonas aeruginosa (F: 5'-GACGGGTGAGTAATGCCTA-3'; R: 5'-CACTGGTGTTTCCTTCCTATA-3'; 617) Coagulase-negative *Staphylococcus* (F: 5'-AAGAGTTTGATCCTGGCTCAG-3'; R: 5'-GGTTACCTTGTTACGA CTT-3'; 102) *Staphylococcus aureus* (F: 5'-AAGGGCGAAATAGAAGTGCCGGGC-3'; R-5'-CACAAGCAACTGCAAGCAT-3'; 294)

Reaction mix

The following were then mixed together on ice (reagent volumes are for one 25- μ L reaction): 12.5 μ L of 2 $\times$ master mix, 1 μ L forward primer, 1 μ L reverse primer, 1 μ L DNA template, 9.5 μ L DNase/RNase-free water for a total volume of 25.0 μ L. Two PCR reactions were prepared for each sample.

Quantification of bacterial DNA

The SmartCycler qPCR device (Cepheid, Sunnyvale, CA) using the SYBR Green I fluorescent dye detection system was used and reactions were performed in a 25- μ L final volume. Amplification conditions consisted of initial denaturation at 95°C for 10 minutes followed by 45 three-step cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The threshold line was set at 30 as an arbitrarily chosen point within the log-linear phase of amplification, significantly greater than the variability in background fluorescence and well before the reaction plateau. Baseline was set for each probe set. After completion of the PCR, a melting curve was obtained by heating the amplicon from 60 to 95°C at a rate of 0.2°C/second. An average of the 2 samples was reported.

Statistical analysis

The difference between pretreatment and posttreatment bacterial load was calculated using a paired *t* test and unpaired *t* test. Statistical analysis was carried out using GraphPad Prism (GraphPad Software, La Jolla, CA) and statistical significance was defined as $p < 0.05$. Power analysis determined that 20 to 25 samples would be appropriate to determine statistical significance for this study. Power analysis was calculated for all 5 treatments separately and also collectively. The standard error of the proportion and sample distribution was calculated based on individual experiments.

Results

Twenty patients, 12 males and 8 females were enrolled into this study. All patients had a diagnosis of CRS as described above and bilateral symmetric maxillary sinus disease with CT imaging showing a grade 1 Lund Mackay score in 17 patients and a grade 2 score in 3 patients. Of the 20 patients, 8 had nasal polyps, 1 had allergic fungal sinusitis (AFS), and 3 had Samter's triad. Nine patients underwent primary surgery whereas 11 underwent revision surgery, with an average of 2.7 prior surgeries (range 1–6). Four of the 20 patients were given perioperative antibiotics. Using quantitative rPCR all 5 bacteria were detected in each patient. Results were produced in cycle time (cT), which represents the number of PCR cycles required to hit a threshold number of copies and is inversely proportional to the concentration of DNA in the tissue (Fig. 1). Saline irrigations using the syringe technique

were found to significantly reduce the amount of bacteria for *S. aureus*, *P. aeruginosa*, and *S. pneumoniae* (Fig. 2). Using the pressurized irrigation device, a significant reduction in *S. aureus* and *P. aeruginosa* was seen (Fig. 2). Saline irrigations using the syringe technique produced a 2.3, 2.0, 0.98, 1.27, and 1.19 log reduction in bacterial counts for *S. pneumoniae*, *Pseudomonas*, CNS, *H. influenzae*, and *S. aureus*, respectively. Using the powered irrigation device resulted in a 0.93, 1.78, 1.14, 1.02, and 1.23 log reduction in bacterial counts for *S. pneumoniae*, *Pseudomonas*, CNS, *H. influenzae*, and *S. aureus*, respectively. No difference between the powered irrigations and syringe technique were found for any of the 5 bacteria (Table 1).

Discussion

In this study we were able to detect and quantify the amount of 5 different bacteria within the maxillary sinus mucosa. We chose to use biopsy specimens rather than swabs of the mucosal surface as previous studies have identified bacteria both within biofilms and intracellularly.⁵ We were able to show that intraoperative saline flushes significantly decreased the amount of bacterial load within biopsies of the maxillary sinus mucosa for *S. aureus*, *P. aeruginosa*, and *S. pneumoniae*. No difference in bacterial load was found for *H. influenzae* and CNS after saline rinses. Overall, we found that saline rinses produced a log reduction between 0.93 and 2.3 for the 5 different bacteria tested. The findings of this study provide important information regarding a simple technique (saline flushes) that may be used during ESS to effectively reduce bacterial load. This may prove to be of importance in the postoperative course of certain patients. Theoretically, those patients who experience a substantial decrease in bacterial load with saline rinses might heal better and faster than those who did not. Although in this study we did not examine postoperative outcomes, other studies have looked at the course of patients with persistent bacterial infections postsurgery. Jervis-Bardy et al.⁶ have shown that the intraoperative presence of *S. aureus* with failure to clear the bacteria postoperatively leads to persistent mucosal disease at the 90-day postoperative visit. This study suggests that the presence of *S. aureus* at the time of ESS and the lack of clearance of the bacteria during surgery may be a predictor for surgical failure. Persistence of disease and bacterial load may be the result of inadequate surgery, incomplete disease removal, and biofilm formation. Therefore, during ESS it is desirable to not only open the sinuses widely but to eliminate the bacteria and/or biofilms within the mucosa. We have shown that saline irrigations are a simple and effective way to reduce bacterial load. While the 5 organisms detected in this study are frequently associated with acute exacerbations of CRS and possibly associated with pathogenesis, it cannot be determined whether reductions in bacterial load of the magnitude found in our patients have a significant clinical impact.

In this study we did not find any difference on the effect of bacterial load between the pressurized irrigation device and syringe technique used to rinse the sinus. This may be due to several reasons. First, the biopsies were taken in a location that was easily accessed through a standard maxillary antrostomy (the posterior maxillary wall). This area is likely effectively rinsed with saline using both the syringe technique and hydrodebrider handpiece. It is possible that the hydrodebrider is more effective in reducing bacterial load in areas of the sinus that are more difficult to reach. It is unknown whether the syringe approach allows

the saline to effectively treat all aspects of the sinus mucosa. On the contrary, the maxillary sinus hydrodebrider delivers a rotating spray of pressurized saline at 5 mL/second in a 360-degree manner, enabling direct irrigation of all areas of the maxillary sinus. It is possible that if biopsies were taken in another location (such as the anterior wall of the maxillary sinus), one would see a greater reduction in bacterial load using the hydrodebrider compared to the syringe technique. In vitro research shows that the hydrodebrider is more effective at disrupting bacteria than traditional saline lavage when directly applied to the target tissue.⁷ When used in vitro, a 99% reduction in bacterial coverage was shown in chronic sinusitis specimens. Second, the flow rate produced by both the powered irrigator and the syringe techniques may have been above the threshold required for removal of organisms along the posterior maxillary wall.

Although not done in this study, the irrigant may be impregnated with antibiotic solutions, antibiofilm treatments, or detergents. Nasal lavage with 0.05% mupirocin has been shown to decrease *S. aureus* colonization and improves symptom score in those with recalcitrant CRS.⁸ In addition, in vitro studies have shown that mupirocin lavages may effectively treat *S. aureus* growing as a biofilm.⁷ While this study did not look at biofilm formation, pressurized irrigations are known to disrupt the biofilm matrix and decrease bacterial colony forming units. Powered irrigations have also been shown in vitro⁹ and in a sheep model¹⁰ to reduce biofilm formation when combined with citric acid zwitterionic surfactant. Although this study did not show a difference between changes in bacterial load when treated with syringe vs powered irrigation device, it is likely that the results would differ if we looked for changes in biofilm matrix. Bacterial biofilms are thought to be a contributor to disease persistence in CRS patients and are most effectively removed with pressurized rinses that disrupt the biofilm matrix. Future in vivo studies are needed to determine the effect of saline irrigations, antibiotic impregnated solutions, and surfactant solutions on bacterial load when used with a powered irrigation device.

Conclusion

This study provides evidence that saline irrigations applied to the sinus at the time of endoscopic sinus surgery decrease load of certain potentially pathogenic bacteria.

Acknowledgments

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FIGURE 1.

Standard curve showing inverse correlation between DNA concentration and cT value. Ct = cycle time.

FIGURE 2.

Changes in bacterial load as reflected by PCR cT time. p value < 0.05 considered significant. L1 = pretreatment bacterial load; L2 = posttreatment with pressurized device bacterial load; R1 = pretreatment bacterial load; R2 = posttreatment with syringe technique bacterial load.

TABLE 1

Log reduction in amount of bacteria seen after washing with saline

Bacteria	Average log reduction L	Average log reduction R	<i>p</i>
<i>S. aureus</i>	1.23	1.19	0.73
<i>S. pneumoniae</i>	0.92	2.3	0.15
<i>Pseudomonas</i>	1.78	2.0	0.688
<i>H. influenzae</i>	1.02	1.27	0.331
CNS	1.14	0.98	0.158

CNS = coagulase-negative *Staphylococcus*; L = pressurized saline side; R = syringe side.