Eradicating Chronic Ear, Nose, and Throat Infections: A Systematically Conducted Literature Review of Advances in Biofilm Treatment

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Eradicating Chronic Ear, Nose, and Throat Infections: A Systematically Conducted Literature Review of Advances in Biofilm Treatment

Angelia Smith, MD, Farrel Joel Buchinsky, MD, and J. Christopher Post, MD, PhD, MSS

Abstract

Objective. Bacteria can grow as individual, planktonic organisms or as complex biofilm communities that are more resistant to treatment. This review was designed to systematically search to identify recent laboratory studies on eradication of biofilms in otolaryngologic infections to highlight promising advances in biofilm treatment.

Data Sources. A systematic electronic literature search of Medline/PubMed, CINHAL, and Web of Science was conducted for articles describing the treatment of biofilm infections in ear, nose, and throat (ENT) diseases through March 2010. English-language articles and articles with an English abstract that focused on biofilm treatment were considered for review.

Review Methods. Each included article was reviewed by one of the authors for study design, treatment intervention, and outcome. Data from in vitro and animal studies were considered separately from human studies.

Results. A total of 30 articles were identified for this review, including 5 studies that included a human treatment component. In general, antibiotics were relatively ineffective for eradicating biofilm infections. Markedly higher antibiotic dosages were required to reduce biofilm presence compared with doses that were effective in eradicating planktonic bacteria. Mupirocin irrigation, gentian violet, and thiamphenicol glycinate acetylcysteine effectively eradicated biofilms. Physical disruption, surfactants, and probiotics were also shown to be beneficial in both nonhuman and human studies.

Conclusion. Eradicating ENT biofilms is difficult when treating single-organism or mixed flora biofilms. Antibiotic therapy is often ineffective against biofilms, and clinical treatment may need to focus on nonantibiotic therapies that reduce, disrupt, or eradicate ENT biofilms.

Keywords
biofilm, treatment, ENT, review, otitis, voice, tonsil, sinusitis, rhinitis

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Bacteria can exist in 2 forms: free-floating planktonic bacteria and complex, integrated communities called biofilms. Planktonic bacteria are independent, individual organisms, while biofilms are sophisticated networks of pathogens living within a protective glyocalyx formed by extracellular polymeric substances (EPS). While bacteria evaluated in clinical laboratories exist in planktonic form, clinical infections often include biofilm bacteria. Biofilms augment bacteria resistance and survival by several mechanisms. Deeper layers contained within the biofilm become increasingly less active, more anaerobic, and less exposed to superficial treatments. Antibiotics that target metabolically active cells, such as β-lactams, may be ineffective against the inactive, deep bacteria biofilm layers. Key antigens and ligands can also become hidden within biofilm, masking antibiotic target sites. Furthermore, genetic material can be exchanged among members of the biofilm, increasing diversity, permitting adaptation to new pathological niches, and improving survival.

Biofilms form on moist biotic and abiotic surfaces, making them common for infections of the ears, nose, and throat (ENT). For example, biofilms were identified in sinus tissue in 36 of 50 (72%) patients with chronic rhinosinusitis (CRS) compared with zero of 10 control patients undergoing endoscopic procedures for...
transphenoidal hypophysectomy, optic nerve decompression, and cerebrospinal fluid leak repair. Cultured organisms from CRS biofilms included *Staphylococcus aureus* (50%), *Haemophilus influenzae* (28%), *Pseudomonas aeruginosa* (22%), and *fungus* (22%). Otitis media (OM) is also commonly caused by biofilms, although standard testing may fail to identify bacteria. An analysis of middle ear mucosa biopsies from 26 children (mean age, 2.5 years; range, 0.5-14 years) undergoing tympanostomy tube placement for the treatment of OM cultured 1 of 3 major pathogens (*H influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*) in only 19% of effusions using traditional culture methods; however, more specific biofilm polymerase chain reaction testing identified bacteria in all effusions.

Furthermore, ENT devices frequently become contaminated with biofilms, with biofilm growth identified on middle ear ventilation tubes, speech valve prostheses, tympanostomy tubes, cochlear implants, bone-anchored hearing aid implants, and ossicular chain reconstruction prostheses; these biofilm growths may result in device failure or recurrent infections.

Biofilms have a negative impact on the effectiveness of infection treatment. Bacterial isolates from sputum samples obtained from 110 cystic fibrosis patients with acute exacerbations were grown planktonically and as biofilms. Antibiotics selected for treating acute exacerbations were effective against planktonically grown bacteria for 60% of patients and biofilm bacteria for only 22% of patients. As expected, patients treated with antibiotics to which biofilm-grown bacteria were susceptible were significantly more likely to have a reduction in sputum bacteria and length of hospitalization.

Difficulty in eradicating biofilm infections with systemic antibiotics has led to interest in adding nonantibiotic therapy. Generally, these therapies are directed toward physical biofilm disruption. For example, low-frequency, high-intensity ultrasound has been shown to improve antibiotic efficacy when treating biofilms by a variety of possible mechanisms, including increasing antibiotic transport to bacteria, permeability of cell membranes, and metabolic activity of biofilm bacteria to increase susceptibility to antibiotics affecting active organisms. Low-intensity, pulsed ultrasound was used to effectively treat chronic sinusitis in 57 patients treated 3 days per week for 5 weeks. Total improvement of symptoms was 81%, with significant improvements in postnasal drip and nasal obstruction noted. Although biofilms were not specifically assessed in this study, biofilms are typical of CRS.

This review was designed to gain insight into the effectiveness of antimicrobial therapies against common ENT infections. The usefulness of both antibiotic and nonantibiotic therapies was evaluated by conducting a systematized and thorough search of the available literature on in vitro and in vivo data for animal and human infections. This literature review is unique in its methodology in that the wide variety of articles were gathered in a highly systematic fashion, unlike traditional narrative reviews.

**Methods**

**Search Methodology**

A comprehensive electronic Medline literature search (www.pubmed.org) evaluating the treatment of biofilms for ENT infections was conducted to include journal articles published through March 2010. As shown in Figure 1, candidate articles were identified by searching for articles that included the keyword *biofilm* and at least 1 of the following additional terms: otolaryngology, tonsil, adenoid, cochlear, sinusitis, rhinitis, otitis, tympanostomy, or voice.

Following identification of articles to be included in this review, additional references were sought through both backward and forward tracking, using the citations identified through the initial search. Backward reference tracking was performed by reviewing citations provided in the list of references in each article meeting inclusion criteria. Forward citation tracking was accomplished by reviewing related articles identified by PubMed for each of the included articles. Abstracts of identified articles were evaluated for inclusion using the same criteria described below. In addition, another search was performed using the primary author’s name for each included article and the keyword *biofilm*. After identifying the initial set of articles with this extensive Medline search...
method, the same initial keyword search with the same criteria was conducted in CINHAL and on Web of Science. No additional articles were identified for inclusion.

**Study Selection Criteria**

Articles for potential selection were screened using the following inclusion criteria: focus on biofilm, relevant to ENT infections, original data, treatment of existing infection, and availability of an English-language abstract. Articles were excluded if they were review articles, evaluated biofilm pathophysiology or formation, described infection pretreatment or prevention, or described biofilms in noninfectious conditions. All identified titles and abstracts were evaluated by one of the authors (A.S.).

**Methodological Quality of Selected Studies**

Study design was reviewed for all studies, with design details provided in study summary tables. For studies that included a human treatment component, methodology quality of the human study portion was evaluated using a standardized checklist designed for assessing the methodological quality of both randomized and nonrandomized studies of health care interventions. This checklist directs assessment of study methods presentation, data reporting, and external and internal validity. Possible scores range from 0 to 32, with higher scores signifying higher quality.

**Data Abstraction and Analysis**

The following data were extracted from included studies: study design (including inclusion criteria for human studies), data analysis, and results. Formal meta-analytic techniques were not planned because of the anticipated variety of methods used in the included studies.

**Results**

**Literature Search Results**

The Medline literature search yielded 250 potential candidate citations. Screening of titles and abstracts using the inclusion criteria identified 27 studies (Figure 1). Six additional studies were identified through searching related articles, references, and publications by the same primary author. English full text was available for review for all but 1 article. This article included a detailed English abstract for a Japanese article; the abstract provided sufficient detail for inclusion in this review. After review of articles (or, for the 1 citation, the abstract only), 3 of the candidate citations were excluded, yielding a total of 30 articles for inclusion in this review. In addition to the search results shown in Figure 1, a CINAHLL keyword search was performed that identified 64 potential selections, and a Web of Science search identified 331 potential selections. These selections were screened using the same inclusion criteria, and they identified no additional articles.

**Study Quality**

Study designs for in vitro and animal studies are all described in Table 1, and Table 2. Among those studies provided in Table 1, 8 studies were produced by a shared set of researchers, with most of these studies generated from within the same research facility in the Netherlands. In most cases, in vitro studies used organisms obtained from clinical samples for biofilm development. The human studies scored low (9-16 of a possible 32) when assessed on the standardized methodological quality checklist (Table 3). None of the human studies reported a conflict of interest. Two studies included both an in vitro and a human study component and are therefore included in both Table 1 and Table 3.

**In Vitro and Animal Studies**

The most widely studied organisms for ENT infections include *H influenzae, S aureus*, and *P aeruginosa*. *H influenzae* biofilms are commonly associated with OM and are also found in pediatric adenoid and tonsillar tissue. *S aureus* is most commonly associated with CRS. *P aeruginosa* has been implicated as a biofilm former in CRS and on devices such as voice prostheses.

The in vitro studies in this review included studies evaluating individual organisms or mixed bacteria and yeast biofilms. Yeasts are often implicated in the failure of prosthetic devices because ingrowths into the silicone, perhaps assisted by certain bacterial species, are believed to increase the pressure required to activate vocalization.

Microbial biofilm reduction was evaluated using antibiotic and nonantibiotic therapies, including mechanical biofilm disruption, salivary peptides, probiotic bacteria, and surfactants. Antibiotic studies generally showed an inability to eradicate biofilms, although bacterial growth was often inhibited. Minimum inhibitory concentrations (MICs) for treating biofilms were generally substantially higher than those effective against planktonic bacteria. For example, moxifloxacin dosages of 1000 times the MIC were needed to reduce *S aureus* biofilms, although even this high concentration failed to eradicate biofilms. In a rabbit model, tobramycin washes at 400 times MIC eliminated *Pseudomonas* biofilms in vivo in one study but not in another.

Both clarithromycin and erythromycin inhibited *Pseudomonas* biofilm formation, with inhibition increasing as the dose increased. Hexose, a marker of biofilm formation, also decreased with increasing doses of clarithromycin and erythromycin. Decreases in hexose were noted at lower than MIC levels with no decrease in bacterial load, suggesting that low antibiotic levels may inhibit biofilm formation without killing bacteria, possibly through as yet undetermined actions that block EPS formation.

Novel nonantibiotic treatments may reduce biofilms more effectively than antibiotics, although some treatments, such as buttermilk, have been shown to reduce bacteria while increasing yeast. Physical disruption, including surfactant washes and laser shockwave pulsing, seem to hold promise for eradicating biofilms. While the percentage surface area covered by biofilm was reduced to <1% with mupirocin flushes, the authors point out that sheep sinuses are more septated than human sinuses and

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**Table 1**

<table>
<thead>
<tr>
<th>Title</th>
<th>Authors</th>
<th>Year</th>
<th>Study Design</th>
<th>Inclusion Criteria</th>
<th>Biofilm Organism(s)</th>
<th>MICs</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Study 1</td>
<td>A. S.</td>
<td>2010</td>
<td>Randomized</td>
<td>Focus on biofilm, relevant to ENT infections</td>
<td><em>H influenzae</em></td>
<td>0.1</td>
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**Table 2**

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<th>Inclusion Criteria</th>
<th>Biofilm Organism(s)</th>
<th>MICs</th>
<th>References</th>
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<tbody>
<tr>
<td>Study 2</td>
<td>A. S.</td>
<td>2011</td>
<td>Nonrandomized</td>
<td>Focus on biofilm, relevant to ENT infections</td>
<td><em>S aureus</em></td>
<td>0.05</td>
<td>33-36</td>
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</table>

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**Table 3**

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<tr>
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<th>Inclusion Criteria</th>
<th>Biofilm Organism(s)</th>
<th>MICs</th>
<th>References</th>
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<tr>
<td>Study 3</td>
<td>A. S.</td>
<td>2012</td>
<td>Randomized</td>
<td>Focus on biofilm, relevant to ENT infections</td>
<td><em>P aeruginosa</em></td>
<td>0.01</td>
<td>34-35</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism Evaluated</th>
<th>Methods</th>
<th>Outcome Analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kondoh et al (1996)</td>
<td>Pseudomonas aeruginosa</td>
<td>Clinically isolated bacteria were grown on Teflon and treated with clarithromycin.</td>
<td>Biofilms were evaluated using scanning electron microscopy compared with a no-treatment control. Hexose content was also evaluated after treatment compared with no treatment.</td>
<td>Biofilms decreased following treatment. Clarithromycin lacks bactericidal effect against <em>P. aeruginosa</em>; however, it reduced biofilm ingredient hexose by inhibiting polysaccharide synthesis.</td>
</tr>
<tr>
<td>Kondoh and Hashiba (1998)</td>
<td>P. aeruginosa</td>
<td>Bacteria obtained from patients with chronic sinusitis, OM, or tonsillitis were cultured with clarithromycin, erythromycin, or midecamycin for 7 days.</td>
<td>Not reported</td>
<td>Biofilm growth was decreased with clarithromycin or erythromycin but not midecamycin. None of the drugs, however, eradicated biofilms using the doses selected.</td>
</tr>
<tr>
<td>Free et al (2000)</td>
<td>Mixed yeast and bacteria</td>
<td>Clinically isolated mixed microflora from explanted voice prostheses were cultured and grown as biofilms on voice prostheses. Prostheses were perfused with soft drinks or saline 3 times daily for 9 days and biofilms were evaluated.</td>
<td>Bacterial growth was compared with control specimen.</td>
<td>Perfusion with caffeinated soft drinks reduced bacteria to 1% to 5% of control amount, while yeast amount was 81% to 197% of control. Noncaffeinated and sugar-free drinks did not inhibit bacteria.</td>
</tr>
<tr>
<td>Elving et al (2000)</td>
<td>Four yeasts and 8 bacteria</td>
<td>Clinically isolated organisms from explanted voice prostheses were cultured and then exposed to synthetic salivary peptides derived from histatin.</td>
<td>Agar plates were rated for the presence or absence of growth inhibition.</td>
<td>Only dhvar4 inhibited growth of all tested oropharyngeal microorganisms.</td>
</tr>
<tr>
<td>van der Mei et al (2000)</td>
<td>Yeast</td>
<td>Silicone rubber voice prostheses were inoculated with microflora cultured from an explanted prosthetic device. Devices were then perfused with probiotic bacteria and biofilm growth was subsequently evaluated for the presence of yeast.</td>
<td>Growth was compared with a control device not treated with probiotics.</td>
<td>Thick biofilm consistently covered control device, with the number of yeast in the control device set at 100%. Yeast was significantly reduced with <em>Streptococcus thermophilus</em> and <em>Lactococcus</em> and <em>Lactobacillus</em> strains, with no significant change after treatment with <em>Bifidobacterium infantis</em> or <em>Enterococcus faecium</em>. The greatest reduction occurred with <em>Lactococcus lactis</em> (4% of control).</td>
</tr>
<tr>
<td>Free et al (2003)</td>
<td>Mixed bacteria and yeast</td>
<td>Clinically isolated mixed microflora from explanted voice prostheses were cultured and grown as biofilms on voice prostheses. Prostheses were treated with Provox flushing, airflow blowing, or imitated cough 3 times daily for 12 days, and biofilms were evaluated.</td>
<td>Bacterial growth was compared with control specimen.</td>
<td>Bacteria were reduced by 45% to 71% of control values with Provox flush, with no effect on yeast. Bacteria and yeast decreased to 45% to 87% of control values with airflow or imitated cough.</td>
</tr>
<tr>
<td>Oosterhof et al (2003)</td>
<td>Mixed bacteria and yeast</td>
<td>Microflora were grown on Groningen voice prostheses. Devices were exposed to salivary substitutes, antisepsics, mucolytics, or ascorbic acid. Biofilms were evaluated on day 8.</td>
<td>Bacterial quantity was compared with treatment vs a saline-exposed control. Air resistance was also measured. Significant differences were evaluated using a t test with significance set at <em>P</em> &lt; .01.</td>
<td>Dhvar5 effectively reduced the number of bacteria and yeasts, without associated reduction in air flow resistance. Mucolytic N-acetyl cysteine and antiseptic Triclosan significantly decreased air flow resistance compared with the control. Nonsignificant air flow reductions occurred with salivary substitutes, chlorhexidine digluconate, and ascorbic acid. The antiseptic-positive controls did reduce bacterial count or air flow resistance.</td>
</tr>
</tbody>
</table>
Table 1. (continued)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Organism Evaluated</th>
<th>Methods</th>
<th>Outcome Analyzed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodriguez et al (2004)17</td>
<td>Mixed bacteria and yeast</td>
<td>Biofilms were grown on silicone rubber voice prostheses. The effect of biosurfactants was evaluated.</td>
<td>Biofilm quantity after biosurfactant was compared with a control.</td>
<td>Biofilm bacteria and fungi decreased, respectively, to 4% to 13% and 15% to 26% of control levels with biosurfactants.</td>
</tr>
<tr>
<td>Schwandt et al (2004)18</td>
<td>Mixed bacteria and yeast</td>
<td>Clinically isolated mixed microflora from explanted voice prostheses were cultured and grown as biofilms on Groningen voice prostheses. Prostheses were flushed with fermented milk drink, buttermilk, N-acetylcysteine, or saline 3 times daily for 6 days, and biofilms were evaluated.</td>
<td>Bacterial growth was compared with control specimen.</td>
<td>Microflora reduction was greatest with buttermilk, which significantly reduced bacteria to 0.1% and yeast to 25% of control values. Fermented milk drink and N-acetylcysteine significantly reduced bacteria to 32% and 2.4% of control, respectively, with a nonsignificant reduction in yeast.</td>
</tr>
<tr>
<td>Schwandt et al (2005)19</td>
<td>Mixed bacteria and yeast</td>
<td>Clinically isolated mixed microflora from explanted voice prostheses were cultured and grown as biofilms on Provox2 voice prostheses. Prostheses were treated with flushed with fermented milk drink, buttermilk, or saline 3 times daily for 6 days, and biofilms were evaluated.</td>
<td>Bacterial growth was compared with control specimen.</td>
<td>Fermented milk drink reduced bacteria to 22% of control but increased yeast up to 201%. Buttermilk reduced bacteria to 60% but increased yeast up to 483%.</td>
</tr>
<tr>
<td>Slinger et al (2006)20</td>
<td><em>Haemophilus influenzae</em></td>
<td>Clinical isolates from patients with perforated tympanic membranes or who were able to provide surgically obtained middle-ear aspires were cultured. MICs were performed on planktonic and biofilm-forming strains.</td>
<td>Statistical comparisons between planktonic and biofilm assays were done using the McNemar test to compare paired proportions.</td>
<td>Bacteria eradication was more likely with planktonic bacteria. Differences in effectiveness of antibiotics against biofilms were not readily apparent with traditional susceptibility testing. Combining 2 to 3 antibiotics was more effective for treating biofilms. Antibiotic combinations containing rifampin and ciprofloxacin were most effective against biofilms.</td>
</tr>
<tr>
<td>Cross et al (2007)21</td>
<td><em>P aeruginosa</em></td>
<td>Biofilms were grown and then challenged with furosemide.</td>
<td>Inhibition of growth of planktonic and biofilm bacteria was determined.</td>
<td>Furosemide did not affect planktonic bacteria; however, biofilms were disrupted.</td>
</tr>
<tr>
<td>Desrosiers et al (2007)22</td>
<td><em>Staphylococcus aureus</em></td>
<td>MICs to moxifloxacin were determined. Biofilms were grown and then incubated with moxifloxacin or saline.</td>
<td>Biofilm quantity was compared at varying antibiotic concentrations against the control.</td>
<td>Viable bacteria quantity was similar for controls and moxifloxacin-treated biofilms at MIC and sub-MIC levels. Bacteria were reduced with antibiotic concentrations above MIC, with peak reduction occurring at 1000 times MIC. Even at this concentration, however, biofilm bacteria were not eradicated.</td>
</tr>
<tr>
<td>Desrosiers et al (2007)23</td>
<td><em>S aureus</em> and <em>P aeruginosa</em></td>
<td>Clinical isolates from patients with refractory CRS were plated and untreated (control) or treated with citric acid/zwitterionic surfactant, surfactant delivered hydrodynamically, or saline delivered hydrodynamically.</td>
<td>Mean percentage reduction in bacteria quantity from controls was compared using paired t test with significance set at P &lt; .05.</td>
<td>All treatments significantly reduced bacterial quantity and biofilm growth, with the greatest reduction in bacteria and biofilm disruption seen with citric acid/zwitterionic surfactant delivered hydrodynamically.</td>
</tr>
<tr>
<td>Oxley et al (2007)24</td>
<td><em>P aeruginosa</em></td>
<td>Clinically isolated bacteria were grown as biofilm on tympanostomy tubes. Tubes were treated with combinations of ciprofloxacin and dexamethasone, ofloxacin, or saline.</td>
<td>Changes in biofilm over 21 days of treatment were evaluated with each drug therapy and the control.</td>
<td>Ciprofloxacin treatments reduced biofilm thickness, although the biofilm was not eradicated and continued to mature and grow despite antibiotic therapy.</td>
</tr>
<tr>
<td>Reference</td>
<td>Organism Evaluated</td>
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<td>Results</td>
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</tr>
<tr>
<td>Chiu et al (2008)²⁵</td>
<td><em>P. aeruginosa</em></td>
<td>Clinical isolates from patients with refractory CRS added to plates containing diluted baby shampoo or saline. After 20 hours of incubation, plates were processed for biofilm detection.</td>
<td>Bacterial growth was compared with control.</td>
<td>Baby shampoo eradicated planktonic but not biofilm bacteria.</td>
</tr>
<tr>
<td>Kaji et al (2008)²⁶</td>
<td>Nontypeable <em>H. influenzae</em></td>
<td>Clinical isolates from patients with respiratory infections were tested for susceptibility to ampicillin, cefotaxime, erythromycin, clarithromycin, levofloxacin, and gatifloxacin, and biofilm growth was measured.</td>
<td>Significance was evaluated using paired t test with significance set at $P &lt; .05$.</td>
<td>Levofloxacin and gatifloxacin significantly inhibited biofilm formation by β-lactamase–negative ampicillin-susceptible and -resistant bacteria. Only gatifloxacin completely eradicated β-lactamase–negative ampicillin-resistant bacteria.</td>
</tr>
<tr>
<td>Ha et al (2008)²⁷</td>
<td><em>S. aureus</em></td>
<td><em>S. aureus</em> and bacteria from the nasal passages of patients with CRS were cultured. MICs were performed on planktonic and biofilm-forming strains.</td>
<td>Biofilm inhibition was evaluated by using control comparators.</td>
<td>MICs for planktonic bacteria were markedly lower than those effective for biofilm bacteria. Biofilm mass was reduced by at least 90% with mupirocin but not ciprofloxacin or vancomycin.</td>
</tr>
<tr>
<td>Krespi et al (2008)²⁸</td>
<td><em>P. aeruginosa</em></td>
<td>Bacteria were obtained from clinical otorrhea and grown on culture plates, stainless steel screws, tympanostomy tubes, and sutures. Biofilms were exposed to laser pulses delivered with shockwave probes.</td>
<td>Biofilms were photographed before and after laser exposure.</td>
<td>Laser-generated shockwaves resulted in biofilm oscillation and detachment.</td>
</tr>
<tr>
<td>Starner et al (2008)²⁹</td>
<td>Nontypeable <em>H. influenzae</em></td>
<td>Inhibition of biofilm formation and persistence was measured following exposure to varying concentrations of azithromycin, erythromycin, and gentamicin.</td>
<td>Significance was evaluated using paired t test. Significant differences, all $P \leq .001$.</td>
<td>Subinhibitory concentrations of azithromycin (but not gentamicin or erythromycin) significantly decreased biomass and maximal thickness of new and established biofilms. Gentian violet effectively disrupted planktonic and biofilm bacteria. Ferric ammonium citrate did not affect planktonic bacteria. The effect of ferric ammonium citrate on biofilms was strain dependent.</td>
</tr>
<tr>
<td>Wang et al (2008)³⁰</td>
<td><em>P. aeruginosa</em></td>
<td>Bacteria were isolated from an infected cholesteatoma. Biofilm and planktonic growth were assessed when exposed to gentian violet or ferric ammonium citrate.</td>
<td>Significance evaluated using t test with significance set at $P &lt; .05$.</td>
<td>Antibiotic doses required to inhibit biofilm growth were above MICs. Ciprofloxacin more effectively disrupted biofilms at typical patient doses compared with azithromycin.</td>
</tr>
<tr>
<td>Wang et al (2009)³¹</td>
<td><em>H. influenzae</em></td>
<td>Bacterial strains were isolated from sputum samples from patients with acute exacerbations of chronic obstructive pulmonary disease, with biofilm response to azithromycin and ciprofloxacin evaluated.</td>
<td>Significance evaluated using paired t test with significance set at $P &lt; .05$.</td>
<td>Antibiotic doses required to inhibit biofilm growth were above MICs. Ciprofloxacin more effectively disrupted biofilms at typical patient doses compared with azithromycin.</td>
</tr>
<tr>
<td>Alandejani et al (2009)³²</td>
<td><em>S. aureus</em> and <em>P. aeruginosa</em></td>
<td>Bacteria were obtained from the clinical microbiology laboratory of a pediatric hospital. Planktonic and biofilm growth was tested after exposure to a control or honey.</td>
<td>Growth compared against control. Significance evaluated using Fisher exact test with significance set at $P &lt; .05$. A comparison was also made against antibiotic bactericidal rates against these same bacteria identified in an earlier study.</td>
<td>All planktonic bacteria were killed by honey. Although resistance was greater among biofilms, 63% to 90% of biofilms were killed with honey. Bactericidal rates were significantly higher with honey compared with antibiotic monotherapy from a previous study.</td>
</tr>
</tbody>
</table>

Abbreviations: CRS, chronic rhinosinusitis; MIC, minimum inhibitory concentration; OM, otitis media.

*Available only as abstract.
that the hydraulic washes may not have treated all of the sheep mucosa, thereby skewing the results toward less biofilm clearance than may occur in clinical practice in humans. Combining citric acid/zwitterionic surfactant with physical disruption by jet lavage effectively removed biofilms isolated from CRS grown on a film in the laboratory. However, a soak in baby shampoo, which contains zwitterionic (cocomidopropyl betaine) and anionic (sodium trideceth sulfate) surfactants, did not eliminate preformed biofilms in the laboratory. The novel treatments honey and topical gentian violet were effective in the lab.
Smith et al

The few human studies included in this review support the conclusions from in vitro and animal studies. Patients in these studies presumably had biofilm-based infections since they were refractory to conventional antibiotic therapy. Similar to in vitro data, treatment with mupirocin irrigation and gentian violet effectively eradicated biofilms. Thiamphenicol glycinate acetylcysteine, the combination of broad spectrum antibacterial thiamphenicol and n-acetylcysteine, also eradicated CRS. N-acetylcysteine is a mucolytic agent, antioxidant, and indirect precursor of glutathione, with anti-inflammatory effects. Glutathione has also been shown to have an important role in the growth of some biofilms. An in vitro study included in this review likewise showed a positive effect of n-acetylcysteine against biofilm grown on voice prostheses, with removal of biofilm EPS, reduction in both bacteria and yeast, and a 34% decrease in prosthesis air flow resistance.

### Table 3. Human Studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Methods</th>
<th>Inclusion Criteria</th>
<th>Intervention</th>
<th>Outcome</th>
<th>Validity Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schwandt et al (2005)</td>
<td>18 patients with long-standing voice prosthesis; measured outcome: time to prosthesis clogging</td>
<td>Experienced voice prosthetic patients</td>
<td>Patients drank buttermilk daily after each meal; if unable to tolerate buttermilk taste, patients drank fermented milk</td>
<td>Lifetime of prosthesis ↑ 3.8 times with fermented drink (P &lt; .01) and replacements ↓ 39% (P &lt; .01); no significant change with buttermilk</td>
<td>10</td>
</tr>
<tr>
<td>Kayama et al (2006)</td>
<td>Retrospective review of 52 ears with otorrhea from canal or mastoid bowl</td>
<td>Failed systemic antibiotics and/or ototopicals or saline irrigation; MRSA cultured ± other organisms; all ages</td>
<td>Weekly aural cleaning and 1% gentian violet application; no additional treatment</td>
<td>Resolution in 47 ears (90%) after an average of 5 topical treatments; resolution rate 96% with MRSA alone and 50% with MRSA plus Pseudomonas aeruginosa</td>
<td>15</td>
</tr>
<tr>
<td>Macchi et al (2006)</td>
<td>102 patients with recurrent URI; all had mucosal biopsies taken before (plus some after) the intervention that were cultured and evaluated by SEM</td>
<td>Healthy patients with acute exacerbation of CRS, adenoiditis, or pharyngotonsillitis; excluded if received antibiotics in the preceding 15 days; adult outpatients</td>
<td>Intramuscular thiamphenicol glycinate acetylcysteinate for 1 day, then aerosol for the next 9 days</td>
<td>Biofilms present in 24 patients at baseline; after treatment, clinical and bacteriologic cure occurred in 21 cases (88%)</td>
<td>14</td>
</tr>
<tr>
<td>Solares et al (2006)</td>
<td>Retrospective review of 42 episodes of MRSA-positive CRS exacerbations</td>
<td>MRSA-positive sinus cultures treated with mupirocin ± other antibiotic irrigations; single clinic, adults</td>
<td>7 episodes treated with mupirocin nasal irrigation alone, 24 treated with mupirocin plus doxycycline, 4 with mupirocin plus TMP-SMX</td>
<td>Patients were followed for 3 to 27 months, with recurrent infection in 12 patients; at the end of the study, only 1 clinically symptomatic patient remained MRSA positive out of 15 (with complete data, 47%) with subjective improvement; posttreatment data were available for 15 patients; 7 of 11 (with complete data, 64%) with improved olfaction</td>
<td>16</td>
</tr>
<tr>
<td>Chiu et al (2008)</td>
<td>18 patients with refractory CRS treated with shampoo irrigation; evaluated by pre/post smell function + pre/post quality-of-life scores</td>
<td>History of sinus surgery with continuing symptoms</td>
<td>Irrigation with 1% baby shampoo in water twice daily for 4 weeks + usual care</td>
<td>7 out of 15 (with complete data, 47%) with subjective improvement; posttreatment data were available for 15 patients; 7 of 11 (with complete data, 64%) with improved olfaction</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviations: CRS, chronic rhinosinusitis; URI, upper respiratory infection; MRSA, methicillin-resistant Staphylococcus aureus; SEM, scanning electron microscopy; TMP-SMX, trimethoprim-sulfamethoxazole.

*Based on Downs and Black checklist. Maximum score for highest validity is 32.*

**Human Studies**

The few human studies included in this review support the conclusions from in vitro and animal studies. Patients in these studies presumably had biofilm-based infections since they were refractory to conventional antibiotic therapy. Similar to in vitro data, treatment with mupirocin irrigation and gentian violet effectively eradicated biofilms. Thiamphenicol glycinate acetylcysteine, the combination of broad spectrum antibacterial thiamphenicol and n-acetylcysteine,
Surfactants also showed promise in human studies. Although baby shampoo failed to eradicate biofilms in the laboratory portion of the study by Chiu and colleagues,\(^2\) 50% of subjects with CRS completing the human study portion of the trial had subjective improvement in symptoms by posttreatment scores, and 64% had objective improvement in smell testing after 4 weeks of twice-daily rinses. Despite its failure to eradicate biofilms, baby shampoo may be a promising surfactant treatment of CRS as it is well tolerated and inexpensive.

Probiotic drinks were also shown to reduce clinically important biofilms in patients with voice prostheses requiring frequent changes.\(^{19}\) Eighteen patients consumed a commercial *Lactobacillus casei*–based fermented milk drink or buttermilk 3 times daily for 6 months in a nonrandom, open-label trial. During the in vitro portion of this study, both drinks reduced bacteria but stimulated yeast growth. After 6 months in the human study, the clinical results showed that patients drinking the fermented milk drink had significantly longer prosthesis lifetimes and required fewer replacements compared with their history prior to participating in the trial. The buttermilk group showed nonsignificant improvements.

**Discussion**

Biofilms are difficult to eradicate, and novel, nonantibiotic modalities identified through in vitro and animal studies have shown promise in studies with human patients with refractory ENT infections. Not only are results different when evaluating treatment effects against planktonic versus biofilm bacteria, but clinical response to both surfactant\(^2\) and probiotic drink therapy\(^{19}\) was better in human studies than might have been predicted from earlier, nonhuman data. In other words, clinical outcomes for these studies were better than predicted by the laboratory arms. One possible explanation for at least part of the dissimilar response in human and nonhuman studies may be the laboratory use of single bacteria strains or combinations of mixed flora that differ from those present in the humans participating in clinical trials.

Overall, however, focus on treating established biofilms may need to shift from antibiotic to nonantibiotic therapy to effectively eradicate established biofilms. Physical disruption of biofilms seems to have the most promise, and laser-produced pressure waves, pulsed ultrasound, or hydrodynamic flushing may soon replace traditional surgical techniques of gross debridement. Researchers have also demonstrated ENT biofilm prevention using many of the same techniques that have been shown in this review to disrupt established biofilms, including the use of probiotics and surfactants.\(^{1,2,4,8,17,48,49}\) These data suggest the future direction for clinical ENT practice: moving away from traditional systemic antibiotics toward nonantibiotic antimicrobial therapies for both the prevention and treatment of common biofilms.

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**Author Contributions**

Angela Smith, author, researcher (conception and design, acquisition of data, and analysis and interpretation of data); Farrel Joel Buchinsky, editor, guidance in interpretation; J. Christopher Post, editor, guidance in interpretation.

**Disclosures**

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**References**


