The efficacy of topical antibiotic agents in a sheep model of rhinosinusitis

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The efficacy of topical antibiofilm agents in a sheep model of rhinosinusitis

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ABSTRACT

Background: Biofilms have been shown to be resistant to conventional antibiotic treatment. This study uses a sheep biofilm model developed by our department to investigate several novel topical anti-biofilm treatments.

Methods: Staphylococcal biofilms were grown in 54 sheep frontal sinuses over 8 days. Each sinus was randomized to (1) no intervention, (2) single mupirocin flush, (3) regular 12-hourly mupirocin flushes for 5 days, (4) Citric Acid Zwitterionic Surfactant (CAZS) via syringe, (5) sodium nitrate, (6) CAZS with sodium nitrate, (7) CAZS with mupirocin, and (8) saline regular flushes. Sheep were sacrificed and the sinus mucosa harvested 1 or 8 days after treatment to assess treatment and any biofilm regrowth. Confocal scanning laser microscopy was used to confirm the presence or absence of biofilms, and the extent of biofilm reduction was quantitated using fluorescent in situ hybridization and colony forming unit counts.

Results: In the control sheep biofilm coverage averaged 31.7%. Saline and mupirocin b.d. washes for 5 days had 23% and 0.84% coverage, respectively, when harvested on day 8. A single mupirocin and sodium nitrate wash had 7.7% and 16.2% on day 1 and 5.88% and 16.0% on day 8. CAZS with sodium nitrate had 6.66% on day 1 but 21.55% on day 8 whereas CAZS with sodium nitrate and sodium nitrate had 13.3% on day 8.

Conclusion: This study shows that regular treatment with mupirocin produced the most marked reduction in biofilm surface area coverage (0.84% and 1.25%) with sustained effects over the 8-day follow-up period.


Key words: Antibiofilm, biofilms, citric acid, chronic rhinosinusitis, confocal microscopy, fluorescence in situ hybridization, gallium nitrate, mupirocin, treatment, zwitterionic surfactant

The often resistant nature of chronic rhinosinusitis (CRS) to both medical and surgical intervention has inspired a growing body of research into the role that bacterial biofilms may play in the pathogenesis of this condition. Today, multiple studies exist documenting the presence of biofilms in CRS patients with a recent confocal scanning laser microscopy (CSLM) study by our department showing direct visual evidence of such structures on the sinus mucosa of 44% of CRS patients undergoing surgery. More recently, the presence of biofilms has also been shown to have an adverse effect on the postoperative outcomes of CRS patients.

It is thought that the protection conferred by the encasing self-produced exopolymeric matrix of biofilms provides bacteria with a method of evading host defenses and facilitates their successful colonization of the sinonasal cavity. This matrix coupled with the altered genotypic and phenotypic characteristics of biofilm bacteria also makes them extremely resistant to conventional antibiotic therapies. For this reason, current research has focused on alternative treatments of biofilms and include treatments that inhibit interbacterial quorum sensing signals or disrupt the biofilm’s three-dimensional structure or increase the biofilm’s susceptibility to antibiotics. The aim of this study was to evaluate three alternative antibiofilm treatments: mupirocin, citric acid combined with zwitterionic surfactant (CAZS; delivered under high pressure), and sodium (Ga) nitrate, in a previously established sheep biofilm sinusitis model.

METHODOLOGY

Bacterial Inoculum

Reference strain ATCC 25923, a well-documented biofilm forming strain of Staphylococcus aureus,53 was kindly supplied by the Department of Microbiology at The Queen Elizabeth Hospital for use in this study. To prepare the inoculum, S. aureus was subcultured anaerobically overnight on Columbia Horse Blood Agar (Oxoid, Thebarton, South Australia) at 35.4°C. Subsequently, single colonies were added to 5 mL of sterile 0.9% normal saline and diluted to a 0.5 McFarlane standard. The inoculum was then transferred to test tubes and placed on ice ready for instillation.

Anesthetic

Ethics approval for this project was obtained from the Animal Ethics Committee of the University of Adelaide and the Institute for Medical and Veterinary Science in Adelaide, South Australia.

Twenty-seven merino cross-breed sheep were used in this study. The animals were of 2–4 tooth-age and of similar size and weight. Each was preoperatively drenched to ensure eradication of the parasite oestrus ovis. The sheep were transported from an animal holding facility 2 days before the surgical procedure and fasted overnight.

Each sheep received i.v. phenobarbitone (19 mg/kg) as an induction agent and the anesthesia was maintained with 1.5–2% inhalational halothane. The animal was placed in a supine position, with its head supported in an upright plane...
using sandbags. Its forehead was shaved and prepared with half strength Betadine solution (Mundipharma Pharmaceuticals B.V., Amstaf, The Netherlands) and sterile drapes were placed around its snout and torso. Three sprays (300 μL) of topical Cophenycaine forte (ENT Technologies, Victoria, Australia) were used to decongest each nasal cavity 10 minutes before surgery. Intraoperative oximetry was used to monitor the sheep oxygen status throughout the surgical procedure.

Endoscopic Sinus Surgery

The method of in vitro biofilm growth in this animal model is outlined in this article and is based on the standardized operative technique described in a previous study by our department. First, a middle turbinatectomy was performed and the bleeding remnant edges were cauterized using monopolar diathermy. Using specifically elongated endoscopic equipment, removal of the anterior ethmoid terminus was then performed to allow endoscopic identification of the frontal ostium. Its location was confirmed by instilling fluorescein (0.1 mL diluted in 10 mL of normal saline) into the frontal sinus through externally placed minitrumpheins. The site of trephination was 1 cm lateral to the sagittal plane, along a line marked between the midpoints of each bony orbit. After confirmation of the frontal ostial site, each sinus was flushed with 50 mL of sterile saline through the minitrumpheine. Two 30-cm long Vaseline-coated gauze (Tyco Healthcare, Mansfield, MA) ribbons were then carefully packed into the frontal recess to ensure a water-tight seal of the frontal sinus. The frontal sinus was then inoculated with 1 mL of the prepared dilution of S. aureus via the minitrumpheine. To exclude intranasal leakage of inoculum, the gauze plug was visualized endoscopically during this time. The minitrumpheines were securely capped to prevent any external leakage of bacteria. Postoperatively, the sheep were held in their pens for a period of 8 days. This was determined as the ideal duration for incubation and peak growth of bacterial biofilms, according to previous in vitro data.

Treatment Groups

After the initial 8 days of bacterial inoculation each frontal sinus was treated according to preoperative randomization. All sheep underwent general anesthesia and ventilation of their frontal sinuses by removal of the Vaseline-coated gauze.

No Treatment (Maximal Biofilm Group). These sheep did not receive any treatment and were killed on day 8 after initial sinus inoculation. Previous in vitro data showed that after 8 days of biofilm growth, a plateau phase of growth was reached where biofilm mass remained stable.

Control Treatment Group. After achieving peak biofilm growth by day 8, these sheep received twice daily 100-mL normal saline flushed through the minitrumpheines, for a period of 5 days. On day 16, they were killed following this regular treatment regime.

Mupirocin (ENT Technologies) Regular Flashes. After achieving peak biofilm growth by day 8, this group received regular flashes of mupirocin via the minitrumpheines twice daily for a period of 5 days. On day 16, they were killed following this regular treatment regime. One hundred milliliters of Ringer’s lactate was used to reconstitute 50 g of powdered mupirocin into solution.

Mupirocin Intraoperative Flush. This group received a single intraoperative flush of mupirocin via the minitrumpheines on day 8 after initial inoculation and then killed on either day 9 or day 16. The purpose of this latter group was to assess for biofilm regrowth after initial treatment.

The 5.5% CAZ (Medtronic, Jacksonville, FL). The solution was delivered in pulsatile fashion via a handpiece designed to access the frontal sinus (Xomed Hydrodebrider, Medtronic). For the CAZ groups it should be noted that all CAZ treatments were administered transnasally via the Hydrodebrider. This electronically activated instrument was connected to a mechanically driven pump that delivered CAZ solution under high pulsatile pressure through a stainless steel arm measuring 19 cm in length and 2 mm in diameter, to a bent distal tip that contained a rotating nozzle designed to produce a target spray angle of 30° in one direction. The distally controlled rotating nozzle allowed an effective spraying angle of 60° within the frontal sinus cavity. Treatment was delivered as a one-off intraoperative dose and sheep were harvested on day 9 or day 16.

The 5.5% CAZ with Mupirocin. This group received CAZ treatment transnasally and a single dose of mupirocin via the minitrumpheine. They were then killed on day 9.

The 5.5% CAZ with Gallium Dihydro-Nitrate (Sigma Aldrich, NSW, Australia). This group received CAZ treatment transnasally with a single dose of 100 mL of gallium dihydro-nitrate (5 g/L) via the minitrumpheine and were killed on day 9 or day 16.

Gallium Dihydro-Nitrate Only. A single dose of 100 mL of 5 g/L of gallium dihydro-nitrate solution was delivered into the frontal sinus via the minitrumpheine, and this group was killed on day 9 or day 16.

Acquisition of Specimens

Mucosal specimens from the frontal sinus were collected for tissue analysis 24 hours or 8 days posttreatment. The sheep were killed on the predetermined time points and decapitated. A flap of skin overlying the frontal bone was raised using a sterile scalpel blade and a periosteal elevator was used to remove excess connective tissue. The 2 × 2-cm bony windows were created using a Dremel drill equipped with disposable sterile rotary steel-cutting blades to access the frontal sinus. Bacterial swabs were taken of the intrasinus contents. The mucosal specimens were carefully lifted off the periosteal wall, removed en bloc, and immediately placed in Dulbecco’s Modified Eagle Media (Sigma Aldrich, NSW, Australia) for transport to the department laboratory for analysis. Notes and digital photographs were taken of the harvested mucosa for assessment of the macroscopic features. The samples were divided into three segments measuring 1 × 1 cm for three separate tissue analysis procedures.

Colonies-Forming Units (CFUs)

A mass of 0.1 g of harvested tissue was measured out and placed in 1 mL of sterile water. The immersed tissue was homogenized with disposable sterile tissue-raptor probes rotating at 8 K rpm in 30-second bursts until the supernatant was of a homogeneous texture. Serial dilutions of 1/10 were performed until a concentration of 1/1000 was obtained. Two hundred micrograms were plated out on Columbia blood
agar plates and incubated for 24 hours at 37°C. The CFUs were manually counted and digitally recorded.

**Confirmation of Biofilm Presence using CSLM**

Immediately after collection, a mucosal sample was processed to confirm the presence of biofilms. The specimen was washed thoroughly in three separate beakers of sterile MQ water to remove any planktonic bacteria. Two sections, each measuring 1 cm², were excised for Baclight staining and fluorescence in situ hybridization (FISH). One piece was immersed in 1 mL of sterile MQ water to which 1.5-µL aliquots of the component A (syo 9) and component B (propidium iodide) of the Baclight Live/Dead kit (Invitrogen, Molecular Probes, Carlsbad, CA) were added. The sample was incubated in this solution in darkness at room temperature for 15 minutes. After incubation the sample was rinsed in sterile MQ water to remove excess Baclight and was mounted on coverslips for analysis with a Leica SPS Spectral scanning laser confocal microscope (Leica Microsystems, Wetzlar, Germany) at 20× and 63× magnification using a water immersion lens. The samples were examined for biofilms using previously documented morphological criteria. Biofilms were determined by the presence of immobile, irreversibly attached, live bacteria of appropriate size (0.5-2 µm in diameter) occurring in clusters and towers of microcolonies.

**Quantification of Biofilm Surface Area Coverage Using Peptide Nucleic Acid FISH Assay**

A modified version of a commercially available S. aureus peptide nucleic acid (PNA) FISH kit (AdvanzDx, Woburn, MA) was used to fluorescently label biofilm bacteria adherent on the mucosal surface of the harvested specimens. The extent of biofilm growth was determined by the area of mucosal involvement using scanning laser confocal microscopy. The assay is based on the hybridization of an Alexa 488-labeled Peptide Nucleic Acid (AdvanzDx) probe to a species-specific 16S ribosomal sequence of S. aureus. One drop of the fixation solution (phosphate-buffered saline with detergent) and 20 minutes of heating at 55°C was used to fix each specimen to coated microscope slides (SnowCoat; Surgipath, Richmond, IL). The fixed slides were then immersed in 90% ethanol for 5-10 minutes and allowed to air-dry for 10 minutes. One drop of the Alexa 488 S. aureus FISH probe was used per sample. A coverslip was placed on the mucosal surface and the slides were incubated in darkness at 55°C in a humidified chamber for 90 minutes. After hybridization, the coverslips were removed and the slides underwent a stringent wash procedure with preheated wash solution (tris-buffered saline with detergent) at 55°C for 30 minutes. After 10 minutes of air-drying, the slides were transported to Adelaide Microscopy for analysis by CSLM. The slides were mounted using one drop of mounting medium and a thin coverslip. At an excitation wavelength of 488 nm, the presence of fluorescent green microcolonies and the milder green haze that surrounds such structures were determined to be biofilm. An appropriate z level determined to be 10 µm above the epithelial lining was selected and fixed for serial scanning of the specimen. The software available on the Leica Application Suite—Advanced Fluorescence (Leica Microsystems)—allowed the generation of 100 fields of view, at 20× magnification, of random sites across the entire surface of the mucosal specimen. The offset and gain settings were kept fixed at -0.3 and 850%, respectively. Two independent blinded observers analyzed each captured image by manually outlining the areas of biofilm involvement using the mapping tool available on the software. A total surface area, expressed in squared micrometers, was generated by the software. The sum of all areas of biofilm involvement was accrued for 100 randomly selected fields of view and then averaged to calculate the average percentage of biofilm surface area coverage per visual field analyzed.

**RESULTS**

Of the 54 sinuses included in this study, 52 received the complete treatment course they had been preoperatively randomized to while 2 sinuses did not. The reason for this deviation from preoperative randomization was the following: in one sinus the minitreserved blocked after 1 day and so the sinus only received two flushed of mupirocin (rather than four more days of b.d. flushing) while in the other sinus, the trephine fell out after 3 days resulting in the sinus missing out on 2 extra days of twice-daily mupirocin flushing. It is important to note, however, that despite not receiving the full treatment course, both of these sinuses were harvested at 8 days after treatment commencement in accordance with their preoperative treatment randomization.

**Biofilm Surface Area Coverage**

Table 1 summarizes the average percent biofilm surface area coverage per 100 visual fields and the CFU count for all eight groups. All sinuses receiving treatment, including those randomized to normal saline flushes showed a statistically significant reduction in biofilm surface area coverage when compared with the untreated control group (one-way analysis of variance, p < 0.0001; Dunnett’s multiple comparison test, p < 0.01 for all groups compared with the nontreatment group; see Fig. 1).

The most significant reduction in biofilm coverage was seen in the group treated with twice-daily flushes of mupirocin for 5 days. In this group the average surface area coverage of biofilm per visual field was negligible at 0.84 ± 1.25%. Sinuses only receiving a stat intraoperative dose of mupirocin through a minitreserved had a mean surface area coverage per visual field of 7.51 ± 1.81% when analyzed 1 day posttreatment and 5.87 ± 3.00% on day 8 posttreatment. Although the difference observed in the groups receiving a single dose was not statistically significant for such a small sample size (p > 0.05, Bonferroni’s multiple comparison test), the trend for less biofilm at 8 days posttreatment could suggest either an ongoing antibiofilm action of mupirocin resulting from its extreme stability in nasal secretions or the ability of the body’s immune system and mucociliary system to further reduce biofilm coverage once it reaches a certain manageable level. Interestingly, when examining the percentage surface area of biofilm coverage for the two sinuses not completing the full course of mupirocin flushes because of technical issues, the recorded average surface area biofilm coverages per visual field were 5.87% (for those receiving 1 day of treatment) and 3.43% (for those receiving 3 days of treatment). The foregoing results seem to suggest that the amount of biofilm coverage is inversely proportional to the duration of mupirocin treat-
Table 1  Summary of biofilm surface area coverage and colony-forming unit count for each treatment group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Number of Sinuses Completing Treatment (n)</th>
<th>Average Percentage of Biofilm Surface Area Coverage per Field (±SD)</th>
<th>Average Number of Colony-Forming Units (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>4</td>
<td>31.75 ± 6.58%</td>
<td>267 ± 235</td>
</tr>
<tr>
<td>Saline flush b.d. 5 days (harvested day 8 postop)</td>
<td>4</td>
<td>23.13 ± 2.70%</td>
<td>103 ± 34</td>
</tr>
<tr>
<td>Mupirocin flush b.d. 5 days (harvested day 8 postop)</td>
<td>6</td>
<td>0.84 ± 1.25%</td>
<td>88.8 ± 39.7</td>
</tr>
<tr>
<td>Mupirocin stat flush (harvested day 1 postop)</td>
<td>4</td>
<td>7.70 ± 1.41%</td>
<td>94.7 ± 19.4</td>
</tr>
<tr>
<td>Mupirocin stat flush (harvested day 8 postop)</td>
<td>4</td>
<td>5.88 ± 3.00%</td>
<td>90.7 ± 11.4</td>
</tr>
<tr>
<td>CAZS/HD intraop Rx (harvested day 1 postop)</td>
<td>6</td>
<td>6.66 ± 1.74%</td>
<td>116 ± 24.51</td>
</tr>
<tr>
<td>CAZS/HD intraop Rx (harvested day 8 post-op)</td>
<td>4</td>
<td>21.95 ± 1.35%</td>
<td>128 ± 10.6</td>
</tr>
<tr>
<td>CAZS/HD/mupirocin intraop Rx (harvested day 1 postop)</td>
<td>4</td>
<td>8.40 ± 2.74%</td>
<td>123 ± 55.8</td>
</tr>
<tr>
<td>CAZS/HD/Ga intraop Rx (harvested day 1 postop)</td>
<td>4</td>
<td>14.3 ± 1.81%</td>
<td>94.0 ± 9.90</td>
</tr>
<tr>
<td>CAZS/HD/Ga intraop Rx (harvested day 8 postop)</td>
<td>4</td>
<td>13.3 ± 7.08%</td>
<td>121 ± 11.0</td>
</tr>
<tr>
<td>Ga intraop Rx (harvested day 1 postop)</td>
<td>4</td>
<td>16.2 ± 2.58%</td>
<td>100 ± 27.3</td>
</tr>
<tr>
<td>Ga intraop Rx (harvested day 8 postop)</td>
<td>4</td>
<td>10.0 ± 1.49%</td>
<td>125 ± 9.90</td>
</tr>
</tbody>
</table>

CAZS/HD = citric acid combined with zwitterionic surfactant/hydrodebrider; Ga = gallium; intraop = intraoperatively; postop = postoperatively.

...ment, although the small sample sizes preclude definitive conclusions from being made.

As seen in Table 1, the combination of chemical and physical dissolution of biofilms with the once-off intraoperative treatment with the CAZS/hydrodebrider (HD) system proved initially successful in reducing biofilms. At 1 day postintervention, the average amount of biofilm surface area coverage/visual field for biofilms treated with CAZS/HD was 6.66 ± 1.74% compared with 31.75 ± 6.58% in the untreated groups. This was comparable with the amount of biofilm remaining after single intraoperative mupirocin treatment 7.51 ± 1.81% with no statistically significant difference seen between the two groups (p > 0.05, Bonferroni’s multiple comparison test). Interestingly, combining mupirocin with the CAZS/HD in the single intraoperative treatment did not appear to increase the amount of biofilm reduction seen at day 1 with biofilm coverage in this group averaging 8.40 ± 2.74%. Despite the obvious initial biofilm reduction seen immediately after CAZS/HD treatment, the amount of biofilm present 8 days after treatment was significantly higher at 21.95 ± 1.35%, approaching pretreatment levels. This would suggest that possible regeneration or repopulation of the biofilm had occurred over the 8 days in the absence of repeated applications of the CAZS/HD treatment.
The addition of a single intraoperative flush of the iron chelator Ga to the CAZS/HD treatment was also effective in reducing biofilms but did not appear to augment the immediate antibiofilm action of the CAZS/HD. Interestingly, however, the amount of biofilm regeneration 8 days after initial treatment was minimal, suggesting a possible ongoing biofilm static action for Ga that continues well beyond the initial biofilm reduction caused by the CAZS/HD. The lack of biofilm regrowth 8 days posttreatment also seen in the sinuses only receiving intraoperative Ga treatment without CAZS/HD adds further support to this hypothesis.

Colony-Forming Units

No statistically significant difference was seen between the planktonic load (CFUs) of the untreated sinuses and any of the treatment groups (Fig. 2). This finding not only further supports the biofilm paradigm that states that biofilm bacteria are extremely difficult to culture using standard culturing techniques but also supports the belief that bacterial biofilms may play an important role in the pathogenesis of rhinosinusitis.

DISCUSSION

The established role of bacterial biofilms in chronic diseases, such as CRS, and their formidable resistance to conventional medical and surgical therapies poses a significant problem to the treating physician. This study has evaluated several different proposed treatments, each with varying degrees of immediate and short-term success. All treatment groups investigated showed a statistically significant reduction in biofilm surface area coverage when compared with those sinuses receiving no treatment, with a sustained reduction in biofilm regrowth observed in all groups except for that treated with CAZS/HD. The most marked biofilm reduction was evident in those sheep treated with varying regimens of the antibacterial agent mupirocin.

Mupirocin (pseudomonic acid A) has been reported to have broad antistaphylococcal activity in the in vitro setting as well as in clinically relevant biological infections. The antibacterial action of mupirocin is derived from its inhibition of bacterial iso-leucyl tRNA synthetase, indirectly blocking the synthesis of RNA and hence inhibiting bacterial cell growth. It acts by passive diffusion and attachment to the binding site of tRNA synthetase. Its unique mechanism of action makes the development of cross-resistance with other commercially available antimicrobial classes unlikely.

Mupirocin's marked stability in mucus-laden nasal secretions, its minimal systemic absorption, and its rapid degradation in human serum makes it an ideal antibiotic for topical use. This not only allows for the delivery of greater local concentrations to the affected area but also reduces the risk of systemic side effects. The wide use of mupirocin in the eradication of *S. aureus* colonization of the nasal vestibule has been well documented. More recently, mupirocin has also been shown to possess antibiofilm activity against multiple different bacteria. This study provides additional *in vivo* evidence of its effectiveness in the almost complete eradication of *S. aureus* biofilms grown in the frontal sinuses of sheep. Importantly, the reduction of biofilm surface area coverage was found to be directly proportional to the duration of treatment. Although a single intraoperative treatment with mupirocin resulted in a dramatic and statistically significant reduction in biofilm coverage, a substantial amount of biofilm remained. Although we did not witness a significant regeneration of...
biofilm in the short 8-day period after treatment, it is theoretically possible that the remaining biofilm may serve as a nidus for biofilm regrowth over a longer time interval. More importantly, the possibility of these surviving bacteria developing resistance to mupirocin after incomplete eradication is of significant concern. We believe that this problem may be overcome with the repeated intensive use of mupirocin over a short time period as evidenced by the almost complete eradication of biofilms with twice-daily nasal douching with topical mupirocin. It should be noted, however, that for the purpose of this study, mupirocin was delivered through minitreffines left in situ for 8 days after surgery. Although feasible, it is highly unlikely that such a practice would be applied to the human setting. This problem may be overcome by the incorporation of mupirocin into a nasal delivery device, such as an irrigation bottle or spray, although it may be difficult to achieve the equivalent intrasinus concentrations and mucosal exposure afforded by direct delivery through a trephine. Interestingly, however, a recent small clinical study by Solares et al. reported the effective treatment of methicillin-resistant S. aureus-mediated CRS with mupirocin nasal irrigations, suggesting that this delivery technique may be sufficient.

The intention to use CAZS solution as a method of eradicating adherent biofilms was based on its potential ability to disrupt the protective exopolymeric substance matrix. Theoretically, accounting for 85% of biofilm mass, the encasing exopolymeric substance matrix consists of polysaccharide chains bound together by tight calcium bridges that contribute to its integrity and confers protection against shear forces. Citric acid was posed as a potential sequestering agent to disrupt these calcium bridges, further enabling dissolution of the disrupted polymers by a zwitterionic surfactant, caprylyl sulfobetaine, and, ultimately, resulting in removal of the biofilm. A recent study evaluated the ability of CAZS solution to remove in vitro biofilms grown from clinical isolates under drip-flow reactor conditions. Although effective in reducing the bacterial plate count compared with control, static treatment with CAZS was significantly enhanced by delivering the solution under hydrodynamic force (Medtronic hydrodebrider). Reductions in the order of 3.9–5.2 log_{10} CFU were achieved in S. aureus and Pseudomonas aeruginosa biofilms. However, these biofilms were grown in vitro and a decrease of this magnitude was not replicated in the biological sheep model.

Hydropressure has already been used in orthopedic surgery as a means of mechanically removing biofilms from open wounds or prosthetic devices. Debriding infected soft tissue and bone with jet lavage has resulted in significant reduction of attached biofilms on those target sites and is especially gentle on the native bony tissue if low pressure delivery is used. Soft tissue exhibits greater resilience to traumatic damage as a result of inherent elasticity, and thus a higher pres-
sure setting on the pulsatile jet lavage system is afforded in our treatment of the frontal sinus.\textsuperscript{27} Although our results suggest an initial impressive reduction in biofilm coverage after the use of CAZS/HD, the significant regrowth present at 8 days posttreatment was of concern. One possible explanation for this may be that the residual posttreatment biofilm load may be too large for complete clearance by the body’s immune and mucociliary systems. This may allow the remaining untreated biofilm to completely regenerate in the absence of further treatments with CAZS or longer-acting antibiotic agents such as mupirocin or Ga.

Ga nitrate was tested as an adjunct antibiotic treatment because it is known to have antibacterial properties. This transitional metal is similar in structure to iron (Fe) and is used to exploit Fe uptake mechanisms, which are critical for bacterial metabolism and growth. Ga is thought to interfere with Fe-dependent pathogenic processes, essential enzyme functions such as those involved in DNA synthesis, and electron transfer.\textsuperscript{28} This “Trojan Horse” approach has been shown to be effective in preventing \textit{P. aeruginosa} biofilm development, and in high doses, Ga has also been found to be effective in killing planktonic bacteria and established biofilms.\textsuperscript{29} As with all research, this study had several limitations. One such limitation was the absence of a hydrodebrider-only control group. Without the inclusion of such a group it is difficult to make conclusions on the individual antibiotic contributions of the CAZS and hydrodebrider system themselves. A further limitation of this study was our uncertainty that the hydrodebrider was making direct contact with the entire surface of the affected sinus mucosa because of the significant septations seen within frontal sheep sinuses. Once the instrument was passed endoscopically into the frontal sinus and activated, we were relying on indirect evidence of contact by externally observing for CAZS outflow from the minitrephines. The lack of direct contact with the entire mucosa may lead to some surfaces not receiving the benefit of high-pressure treatment. If these sites are included in the samples taken for analysis, they may not be truly representative of the treatment administered.

CONCLUSION
Recalcitrant CRS is a difficult condition to manage and has been closely linked with the presence of bacterial biofilms in recent scientific research. This \textit{in vivo} study examined a range of proposed antibiofilm treatment regimes in a standardized biofilm sheep model of CRS. Regular treatment with mupirocin flushes over a 5-day period showed an almost complete eradication of biofilms as measured by mucosal surface area coverage, with sustained effects over the 8-day period of follow-up.

REFERENCES