Targeting Post-Surgical Staphylococcus aureus in Chronic Rhinosinusitis

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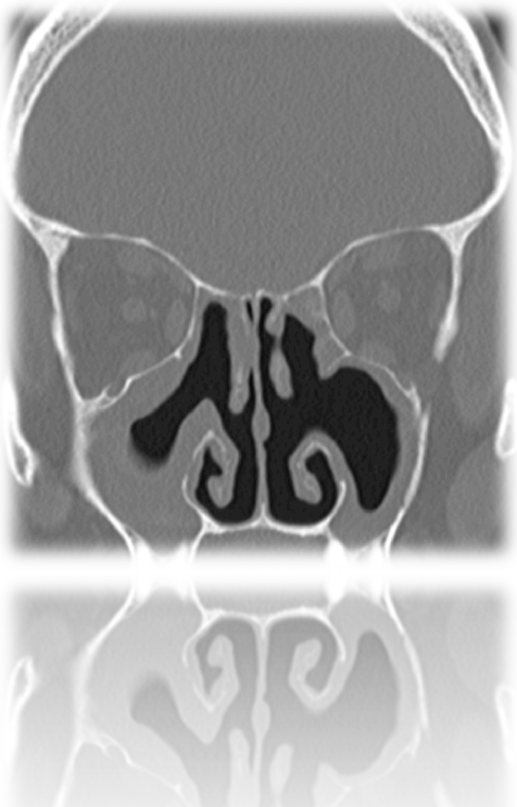

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Cover image:

Axial non-contrast CT image of a 54 yo female patient with surgically-recalcitrant chronic rhinosinusitis, with maxillary sinus mucosal thickening evident. *Staphylococcus aureus* is frequently cultured from swabs taken from both her maxillary sinuses.

To my darling Maggie, the kindest person I know

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Joshua Jervis-Bardy and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Dr. Josh Jervis-Bardy

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Jervis-Bardy J, Foreman A, Bray S, Tan L, Wormald PJ.
Laryngoscope 2011;121:1104-7.

What is the origin of Staphylococcus aureus in the postoperative sinonasal cavity?
Jervis-Bardy J, Foreman A, Boase S, Valentine R, Wormald PJ.
International Forum of Allergy and Rhinology 2011;1:308–312.

Microbiological outcomes following mupirocin nasal rinses for symptomatic, Staphylococcus aureus–positive chronic rhinosinusitis following endoscopic sinus surgery.
Jervis-Bardy J, Wormald PJ.
International Forum of Allergy and Rhinology 2012;2:111-5.

A randomised trial of mupirocin sinonasal rinses versus saline in surgically-recalcitrant staphylococcal chronic rhinosinusitis.
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Manuka Honey: A treatment for chronic rhinosinusitis?

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The in vitro activity of Manuka Honey on S. aureus biofilms is time and dose dependent: Potential implications for treatment of persistent mucosal infection following endoscopic sinus surgery.

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Methyloglyoxal-infused honey mimics the anti-S. aureus biofilm activity of Manuka Honey: Potential implications in Chronic Rhinosinusitis.

Australasian Rhinological Society ASM, Sydney, September 2010.

The etiology of sinonasal Staphylococcus aureus following surgery for Chronic Rhinosinusitis.

American Rhinologic Society ASM, Boston, USA, September 2010.

Understanding CRS and novel topical therapies.

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Targeting post-surgical Staphylococcus aureus in Chronic Rhinosinusitis: current and future treatment modalities.

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Abbreviations used in this thesis

AFRS	Allergic fungal rhinosinusitis
ATCC	American Type Culture Collection
CAZS	Citric acid/Zwitterionic surfactant
CRS	Chronic Rhinosinusitis
CRSsP	Chronic Rhinosinusitis *sans* (without) polyposis
CRSwP	Chronic Rhinosinusitis with polyposis
CSF	Cerebrospinal fluid
EDTA	Ethylenediaminetetraacetic acid
EM	Eosinophilic mucous
EML	Endoscopic modified Lothrop
ESS	Endoscopic sinus surgery
FDA	Federal Drug Authority
FESS	Functional endoscopic sinus surgery
FISH	Fluorescence *in situ* hybridisation
HIV/AIDS	Human immunodeficiency virus/Acquired immunodeficiency syndrome
IQR	Inter-quartile range
MGO	Methylglyoxal
MRSA	Methicillin-resistant *Staphylococcus aureus*
NIR	Near infra-red
PMN	Polymorphonuclear
RCT	Randomized controlled trial
SNOT-20	Sino-Nasal Outcome Test (20)
SW	Shock-wave
TGA	Therapeutic Goods Administration
VAS	Visual analogue scale

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Thesis summary

The research contained within this thesis is an investigation of topical antimicrobial treatments in a subset of patients with Chronic Rhinosinusitis (CRS). For the purposes of this manuscript, our 'patient of interest' has persistent disease following sinus surgery ('surgically-recalcitrant disease') and a sinonasal cavity that similarly persistently cultures *Staphylococcus aureus*.

To begin with, an extensive literature review is presented in three parts. Firstly, the definition, epidemiology, socioeconomic burden, aetiopathogenic theories and the management of CRS are discussed. From the literature review, it is clear that CRS is disease without a unifying, underlying aetiopathogenic factor, nor does there exist a universal panacea for the treatment of the surgically-recalcitrant patient. Of promise, however, recent research suggests that there may be merit in aggressively targeting the presumed *S. aureus* biofilm bioburden in these patients with topical antimicrobials. Secondly, therefore, we progressed to explore the myriad of possible antimicrobial agents for use as topical treatments in CRS. This exhaustive list includes a number of anti-biofilm strategies that have unknown treatment potential in CRS, as many have not previously been mentioned, let alone evaluated, in the Rhinological literature to-date. Thirdly, recognizing the importance of device selection in delivering topical treatment to the sinuses, we reviewed the potential delivery modalities currently available for this purpose.

The research investigation commenced with two studies evaluating the efficacy of mupirocin sinonasal rinses in recalcitrant *S. aureus*-positive CRS. Following from two small studies reported in the literature, we felt it was important to firstly evaluate this treatment in a prospective randomized control trial, and secondly, to retrospectively assess a much larger cohort. The former study revealed that mupirocin treatment was greatly superior compared to placebo in removing culturable *S. aureus* from the sinuses. Additionally, it improved both the endoscopic appearance of the sinonasal cavity and patient-reported symptoms

following treatment, although only the endoscopic examination results were significantly different when compared to those observed in the placebo arm. The latter study demonstrated that long-term, well after the mupirocin treatment is complete, *S. aureus* is again readily cultured in these patients; it appears, therefore, that whilst mupirocin is a promising treatment, there is a significant rebound following cessation of treatment. We also determined that thankfully, however, the rate of induced resistance mupirocin is very low.

The third study performed was an in vitro assessment of the anti-biofilm activity of Manuka (*Leptospermum scoparium*) honey. In this study we demonstrated that Manuka honey is not active against *S. aureus* biofilms at concentrations amenable to delivery using a rinse bottle; however, there is sufficient activity when Manuka honey is fortified with exogenous methylglyoxal (MGO). MGO has recently been identified as the active constituent in Manuka honey. These finding are significant, because Manuka honey may be suitable as a long-term treatment option by virtue of its excellent resistance profile. Whereas fears of inducing treatment-resistant bacterial strains limit the long-term use of traditional antibiotics (such as mupirocin), Manuka honey may be a suitable long-term or even maintenance therapy in surgically-recalcitrant *S. aureus*-positive CRS.

Our final study aimed to evaluate the origins of sinonasal *S. aureus* following sinus surgery, as previous studies have shown culture rates of this organism to increase in the post-operative period. We had previously hypothesized that this increase in culture-rate may be a result of biofilm activity. In this current study, we indeed identified biofilm dispersal as the likely underlying causal factor. As a result, we now further suggest that the early post-operative period may be an ideal treatment window in which to treat with antimicrobials given the vulnerable state of the dispersed biofilm during this time. Rather than being a *treatment agent* study like the other papers in this thesis, this *treatment time* evaluation may ultimately precipitate early anti-biofilm intervention trials in the future.

Chapter One: Systematic Review of Literature

1.1 DEFINING THE DISEASE: STAPHYLOCOCCUS AUREUS, CHRONIC RHINOSINUSITIS AND POST-SURGICAL RECALCITRANCE

1.1.1 CHRONIC RHINOSINUSITIS: DEFINITIONS

Rhinosinusitis is considered to comprise a spectrum of inflammatory and infectious disorders concurrently affecting the nose and paranasal sinuses[1]. Rhinosinusitis can be differentiated from conditions involving the nose and paranasal sinuses by careful history, endoscopic examination and radiological findings. CRS is a sub-group of rhinosinusitis in which symptoms (and/or signs[1]) are present for >12 consecutive weeks[2]. Other sub-groups include acute, sub-acute and recurrent-acute rhinosinusitis[3]. Importantly, if symptoms *completely* resolve following medical therapy then a diagnosis of CRS should not be made; rather, this response to medical therapy is indicative of sub-acute rhinosinusitis[3]. CRS itself can be further subdivided, most commonly as the to presence or absence of nasal polyposis (chronic rhinosinusitis with polyposis [CRSwP] versus chronic rhinosinusitis *sans* polyposis [CRSsP])[2]. Allergic fungal rhinosinusitis (AFRS) is an important, clinically-challenging sub-group of CRSwP. These CRSwP patients have the additional key features of culture- and/or silver stain-proven fungus, mucous rich in eosinophilia and a systemic fungal allergy[4]. Patients with Samter's triad (see 1.1.3), another sub-group of CRS, also experience a severe disease phenotype.

To make a diagnosis of CRS, both subjective and objective criteria must be met[1, 2], whereas in the past a diagnosis could be made on history alone[3]. Thus, a diagnosis of CRS (European guidelines[2]) currently requires the following:

1. Duration of disease > 12 weeks continuously
2. Clinical symptoms consistent with CRS (>1)
    a. Nasal blockage and/or congestion
    b. Nasal discharge
        i. rhinorrhoea and/or
        ii. post-nasal drip
    c. Facial pain and/or pressure
    d. Reduction or loss of smell
3. Objective evidence, either
    a. Endoscopic signs

i. Polyps
ii. Mucopurulent discharge from the middle meatus
iii. Oedema/mucosal obstruction primarily in the middle meatus

OR

b. CT changes
   i. Mucosal changes within the osteomeatul complex and/or sinuses.

4. Absence of cystic fibrosis, gross immunodeficiency, congenital mucociliary disorders, invasive fungal sinusitis, vasculitis and granulomatous diseases.

Whilst very generally very similar, current North American diagnostic guidelines further stratify symptoms into 'major' and 'minor' groups, and also allow provisions for the use of plain X-ray[1].

To make a diagnosis of CRSwP, criteria for CRS must be met in the presence of bilateral, endoscopically visualized polyps in the middle meati[2]. In patients in whom sinus surgery has previously been performed and the middle meatus altered, polyps are defined as pedunculated lesions (in contrast to cobblestoned mucosa, although this differentiation can sometime be difficult clinically) in the sinonasal cavity persisting > 6 months after surgery[2]. It is possible to have nasal polyposis without CRS (ie. a patient with asymptomatic nasal polyposis), just as it is possible to have CRS without polyposis.

1.1.2 CHRONIC RHINOSINUSITIS: BURDEN OF DISEASE

Epidemiology

United States National Health Survey data estimated a 12.6% prevalence of *chronic sinusitis* in 1996[5] and of *sinusitis* 16% in 1997[6]. These estimations may not be an accurate representation of current CRS prevalence, however, as it is possible that patients better fitting other sub-group classifications of rhinosinusitis (such as recurrent-acute rhinosinusitis) may have been included. Furthermore, it is likely that these figures may even further over-estimate CRS prevalence, as purely symptom-based diagnosis of CRS can be innacurate[1]. Regardless, a similar prevalence was reported in the 2009 survey, with 13% of respondents answering in the affirmative when asked whether they had been told by a doctor (or other health professional) in the past 12 months that they had sinusitis[7]. In contrast to

survey data, a 1991 Korean health assessment in which more than 9,000 subjects underwent a physician-led history and physical exam (including nasal endoscopy), revealed only 1.01% of subjects to be suffering from chronic sinusitis[8]. Likewise, the prevalence of physician-diagnosed CRS based on a review of ICD-9 coding in Minnesota (US) was only 2.0%[9]. The true rate of chronic rhinosinusitis, fitting both European and North American diagnostic criteria, is therefore likely to be somewhere between 1-16%.

Regarding nasal polyposis, the prevalence in Europeans is estimated to be 2.1-4.3%[10-12], with a similar 4.2% reported in the United States[13], and a slightly lower 0.5% reported in Koreans[8]. In two autopsy studies Larsen and Tos found nasal polyps in a surprisingly high 32%[14] and 42%[15] of cadavers, suggesting that mild nasal polyposis might be a highly asymptomatic condition. These studies, however, included polyps found anywhere in the sinonasal cavity during endoscopic surgery as a positive finding, whereas the strict definition requires polyps to be present and obvious in the middle meatus[2]. Again, the true prevalence of nasal polyposis is likely to be somewhere between these figures.

Socioeconomic impact

There is evidence that chronic rhinosinusitis significantly impacts quality of life similarly to chronic debilitating diseases such as diabetes and congestive heart failure[16]. In 1996, it was estimated that rhinosinusitis costs the United States $3.4 billion in annual direct health-care expenditure, with an additional 12.5 million lost work days[17].

1.1.3 Chronic Rhinosinusitis: Theories of Aeitiology

Rather than being a disease with a single genetic, infectious or environmental aetiological factor (such as cystic fibrosis, tuberculosis or mesothelioma respectively), CRS is increasingly recognized as a syndrome with numerous potential causes that may be numerous, disparate, and frequently overlapping[1]. Indeed, a single unifying aetiological mechanism may never be found[1]. Numerous contributing disease factors have been explored in the literature. These factors either directly or indirectly relate to the host *inflammatory* response or to the

external *infectious* trigger. In addition, host anatomical variations, such as concha bullosa, may be important[2]. Whilst the presence of one factor in isolation may not confer disease, it is likely the CRS syndrome develops as a result of multiple contributing factors. The disease phenotype, therefore, can be thought of as the end result of interactions between a pre-disposed host and common- if not ubiquitous- microbiological organisms (Figure 1). Furthermore, symbiotic relationships between microbes of different species can potentially perpetuate infection and drive disease[18].

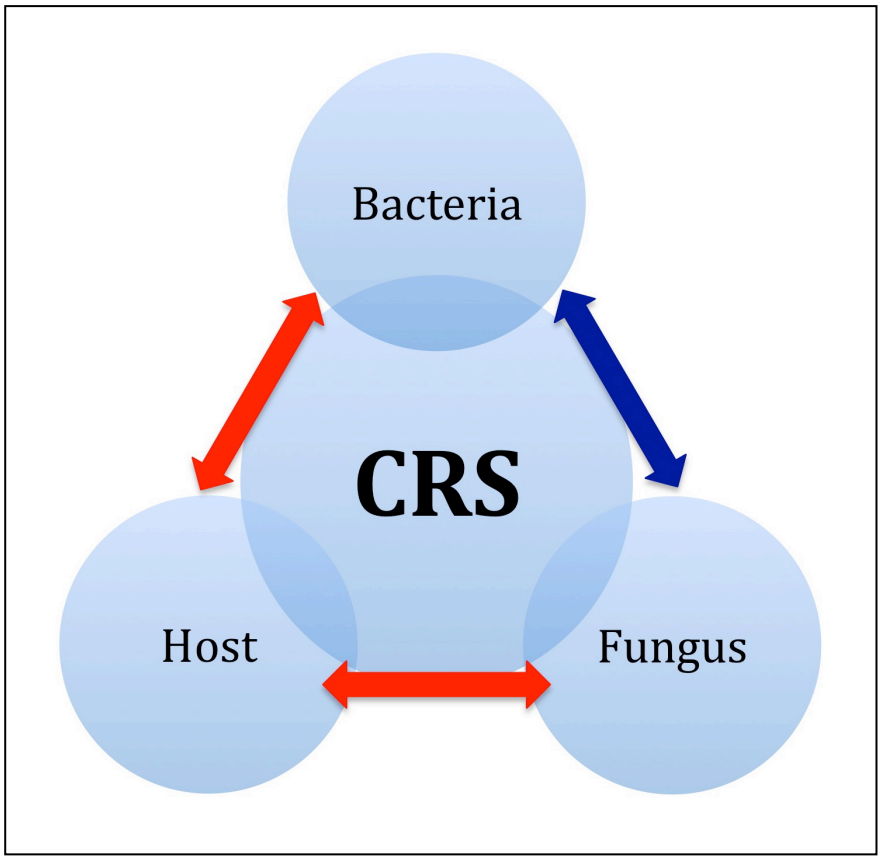

Figure 1. The aetiopathogenic relationships behind CRS. The disease is driven by a complex interplay between a predisposed host and common microbes (red arrows). Boase et al.[18] have suggested that interactions between microbes of differing species (blue arrow) may also be important.

Below is a list of host and microbiological factors thought to be aetiologically-linked to CRS, adapted from Fokkens et al.[2] Whilst there are differences at the molecular level between the host response in CRSsP and CRSwP with regards to classical cytokine profiles[19], the aetiological factors discussed here are of a general nature, rather than specific to any sub-type of CRS (except where mentioned).

Host inflammatory response

Ciliary impairment

Cilia play an important role in maintaining sinonasal homeostasis, ensuring mucous is transported out the sinus ostia, through the osteomeatal unit into the nasal cavity and ultimately into the post-nasal space and down the oesophagus. Secondary cilia dysfunction (as opposed to the primary dysfunction of cystic fibrosis or Kartagener's syndrome) leads to relative mucous stasis and promotes bacterial activity. Sustained infection further damages ciliary function, potentiating a vicious cycle[2].

Allergy

Positive skin-prick tests- demonstrating a systemic IgE response- are common in patients with CRS. Likewise, asthma is also a common association[20]. Patients with AFRS, an especially severe CRS phenotype, have systemic allergy to fungus[4]. AFRS diagnostic criteria requires evidence of systemic anti-fungal IgE[21], although recent work by Boase et al (unpublished data, personal communication) suggests local IgE to be just as important (although a more invasive measure to quantify). Whilst the presence of eosinophilic mucous (EM) seems to be a more important prognostic marker than systemic anti-fungal IgE, it is likely that many EM-positive but systemic anti-fungal IgE-negative patients actually have 'hidden' local anti-fungal IgE. This may explain the similarly poor clinical course often experienced by these 'AFRS-like' patients[22]. The presence of systemic allergy to aeroallergen alone does not confer rhinosinusitis; rather, it is far more commonly associated with allergic rhinitis.

Immunocompromised state

Immunodeficiency is associated with a severe CRS phenotype, with a majority of surgically-recalcitrant CRS patients in a series by Chee et al. demonstrating an abnormal *in vitro* T lymphocyte proliferation pattern in response to antigen[23]. HIV/AIDS, a classical CD4+ deficiency, is associated with rhinosinusitis, with two series reporting an incidence of 34%[24] and 54%[25] respectively. In isolation, therefore, it might be hypothesized that a hypofunctioning local (T cell) immune

response is important in CRS. These results, however, must be balanced by an appreciation of the important and undeniable management role that corticosteroids play in CRS. Corticosteroids modulate the immune response via a number of mechanisms (including depleting the circulating T lymphocyte pool[26]), and are capable of causing both profound disease regression in CRS and also profound immunodeficiency. Rather than a result of decreased local immune function, therefore, it would that indicate that the host response in CRS is one that is inappropriate or dysregulated.

Genetic

Whilst mutations in single genes are responsible for cystic fibrosis and Kartagener's syndrome, no individual genetic abnormality responsible for CRS has been identified. Rather, as numerous gene polymorphisms have been associated with CRS[27], it appears that any genetic predisposition may just increase the chance of an individual developing CRS rather than necessarily ensuring it. Accordingly, Rugina et al. found that 53% of patients with CRSwP reported a positive family history for nasal polyposis[20].

Environmental and socioeconomical factors

Cigarette smoking has been associated with a higher prevalence of chronic CRS[28]. Similarly, *in vitro* experiments have unsurprisingly demonstrated the toxicity of pollutants on respiratory epithelium[29]. In addition, low income has also been associated with higher population rate of rhinosinusitis[28].

Aspirin sensitivity

Samter's triad- concurrent nasal polyposis, asthma and aspirin (salicylic acid) sensitivity- was first described in 1967 by Sampter and Beers[30]. Upon administration of aspirin (or a similar non-steroidal anti-inflammatory), cyclo-oxygenase-1 inhibition shunts arachadonic acid metabolism down the 5-lipoxygenase pathway, with a resultant increased production of leukotreines. The arachadonic acid metabolism pathway in aspirin-sensitive individuals is skewed towards leukotreine production at baseline; additionally, with administration of aspirin in these patients, leukotreine production is increased far beyond normal

levels[31]. Clinically apparent symptoms, namely bronchospasm, result. Presumably, nasal polyposis occurs as a result of the elevated levels of baseline leukotrienes, because simply avoiding aspirin and other non-steroidal anti-inflammatory medications does not ameliorate disease; likewise, desensitization achieves only modest results[32]. Surprisingly, however, strict avoidance of dietary salicylates has not yet been formally trialed in patients with Samter's triad.

Microbiological trigger

Bacteria

The resident paranasal microbiology in patients with CRS differs from that of healthy controls. Abou-Hamad et al. attempted to isolate pathogens from the maxillary sinuses of healthy controls undergoing orthognathic surgery, and successfully cultured bacteria from only 18% of swabs using conventional microbiological techniques[33]. Kostamo et al. performed nasal lavage on healthy controls, and found a higher culture rate of 70%. By contrast, sinus swabs from patients undergoing surgery for CRS generally yield higher culture rates of pathogenic organisms[34, 35], although this is not always the case[36]. More sensitive bacterial detection techniques have also been used to evaluate sinonasal microbiology in both CRS and control patients. These techniques have the advantage of being able to potentially detect bacteria in biofilm form whereas standard culture techniques are only sensitive for free-living planktonic form (see 1.1.6-7). These techniques utilize involve either microscopy of tissue or mucous samples (general stains such as hematoxylin and eosin, electron microscopy and/or fluorescence in situ hybridization [FISH] or molecular assessment techniques on extracted DNA or RNA (such as PCR or microarray)[37]. Using FISH, for example, Foreman et al. identified bacterial biofilms in 36/50 (72%) of CRS patients compared to 0/10 (0%) of normal controls[38]. Certain bacteria, such as *Staphylococcus aureus*, are more readily implicated as pathogenic in CRS[39], whilst others, such as *Haemophilus influenzae*, are more-recognized as pathogens in acute rhinosinusitis[40]. Two observations suggest a role for bacteria in CRS: firstly, the resident microbiological flora in CRS appears to be different compared to healthy controls and secondly, administering a short-course of antibiotics in CRS often temporarily improves symptoms[41] (but does not, by definition, provide a cure[3]).

Mupirocin, a targeted anti-staphylococcal antibiotic with no systemic absorption and presumably no immunomodulation activity, has been shown to be clinically effective in recalcitrant post-surgical CRS (see also 1.2.1)[42]. This would suggest that the bacterial load removed by mupirocin must be important in the underlying disease process. The virulence mechanisms of *S. aureus*, and its association with a more severe disease phenotype, are discussed further in 1.1.5-8.

Bacteria alone, however, cannot be solely responsible for CRS. It has been long known that up to 44% of the population are colonized at the nasal vestibule with bacteria, namely *S. aureus*[43]. In non-CRS colonized patients, the bacterial load is largely confined to the anterior nose[44], and is presumably prevented from colonizing and infecting the paranasal sinuses by an effective host immune response. If *S. aureus* were solely responsible for CRS, it would stand to reason that in colonized patients the paranasal sinuses would soon be seeded by bacteria from the vestibule and subsequent progression to CRS would be inevitable; this is clearly not the case.

Fungi

In the immunocompromised patient, there is a clear association between fungi and sinus disease in invasive fungal sinusitis. In the immunocompetent CRS patient, however, fungus (such as *Aspergillus* and *Alterneria*) remains a controversial atiological agent[45]. Ponikau et al. felt strongly that fungus was responsible for the CRS phenotype, arguing for a change in nomenclature in order to better reflect this supposed aetiology[46]. Interestingly, however, whilst fungus was isolated (using a very sensitive culture technique) from 96% of patients with CRS, it was also found in 100% of normal controls[46]. Whilst initial trials of topical anti-fungals in CRS achieved promising results[47, 48], subsequent studies[45, 49-51] and a recent meta-analyses[52] have questioned their clinical effectiveness. Today, only a small minority of Rhinologists continue to prescribe topical anti-fungals for their patients[53], although small percentage of patients with severe fungal disease may benefit from systemic delivery[54]. It appears, therefore, extremely unlikely that fungus is indeed the underlying, unifying aetiopathogenic agent in CRS.

1.1.4 CHRONIC RHINOSINUSITIS: MEDICAL AND SURGICAL MANAGEMENT

Medical management

The management of CRS initially consists of medical therapy[2, 55]. Functional endoscopic sinus surgery (FESS; or simply endoscopic sinus surgery [ESS]), therefore, is reserved for patients that fail a trial of maximal medical therapy. What exactly constitutes 'maximal medical therapy' is a somewhat elusive concept, however most Rhinologists would considers this to entail a short course of oral antibiotics, a short burst of oral corticosteroids and maintenance nasal corticosteroids and saline irrigations[53]. Antibiotics should only prescribed when an accurate swab of mucopus can be obtained, in order to facilitate best-practice culture-directed prescribing[55]. This combination of medical therapy targets multiple aetiological factors, targeting both the host response (oral and nasal corticosteroids) and the presumed microbiological trigger (antibiotics).

Surgical management

When CRS cannot be satisfactorily management by medical therapy, ESS is advocated. The Messerklinger technique[56], popularized by Stammberger and Kennedy in the 1980s, aims to normalise the diseased sinus mucosa by enabling ventilation through the natural ostia and restoring mucociliary clearance. In addition, the post-ESS cavity allows for improved sinus penetration of irrigant (normal saline, hypertonic saline or otherwise), which serves to facilitate clearance of mucous and/or mucopus and also allows better delivery of topical treatment to the target end-organ[57]. For CRS patients in whom medical therapy is insufficient and ESS has not previously been performed, the standard of practice in Adelaide, Australia is for these patients to be offered bilateral middle meatal antrostomies, bilateral complete spheno-ethmoidectomies and bilateral frontal recess clearance. This 'full-house FESS' approach is considered a first-step, conservative procedure. It is worth noting, however, that some authors advocate trialing long-term medical therapy before ESS[58]; however, this is not currently our practice in Adelaide, Australia.

Medical management after surgery

The patient who continues to have signs and symptoms of CRS despite ESS is a challenging one. Generally, patients fail conservative sinus surgery for one of three reasons; firstly, due to incomplete surgery or post-operative scarring (stenosis, adhesions and/or synechiae) leaving the sinus ostia obstructed; secondly, because of persistent mucosal disease or thirdly, because of unfavorable anatomy (such as a narrow frontal sinus drainage pathway despite frontal recess clearance). The former are likely to require revision ESS (although, again, maximal medical therapy should always be attempted as a first measure) whilst medical therapy is the mainstay of treatment in patients with largely mucosal disease. There are, however, aggressive surgical options that can be considered for the latter group. For patients who fail frontal recess clearance, the endoscopic modified Lothrop procedure[59] (EML) is a safe and efficacious procedure when performed by an experienced surgeon[60]. Additionally, endoscopic medical maxillectomy creates a 'mega-antrostomy' which may be beneficial in patients with severely disease maxillary sinuses[61]. As demonstrated in Figure 2 however, medical therapy remains the only chance of 'cure' in patients with persistent symptoms after aggressive surgery, as there remains no further conventional surgery that can physically be performed (assuming the sinuses remain widely opened). These patients now have a wholly mucosal disease and require medical treatment. In these truly surgically-recalcitrant patients a number of options (in addition to the standard 'maximal medical therapy') have been proposed. Ferguson et al. advocate trialing agents classically used in allergic rhinitis, such as antihistamines, anticholinergics and leukotreine-receptor antagonists, although evidence for this strategy is limited[62]. In the absence of firm treatment protocols for these surgically-recalcitrant patients, the clinician is simply left with a list of treatment options; in practice, whilst selected therapies may provide clinical benefit in some patients, no option is regarded as a universal panacea. The following alternative options are based on an excellent review by Desrosiers and Kilty[63]:

Long-term macrolide therapy

Based on the immunomodulatory effect of macrolides in Japanese patients with diffuse panbronchiolitis[64], long-term therapy has been proposed as efficacious in

recalcitrant CRS[65-67]. This therapy probably works best in patients without eosinophilic mucous/infiltrate[63, 66, 67].

Topical antimicrobials

Following promising studies by Solares et al.[68] and Uren et al.[42] (see 1.2.1) sinonasal antimicrobial rinses have become a popular treatment option in surgically-recalcitrant CRS. Although nebulized antibiotics are now thought to be generally ineffective[69], it is likely that the delivery limitations of the nebulizer[70-72], rather than the antibiotics itself, are most to blame for the underwhelming results in these studies (see also 1.3.4). Topical antimicrobial rinse treatment should be culture-directed, with endoscopically-guided swabs preferred over blind sampling[63]. Topical delivery allows for far greater concentration of antimicrobial to the sinuses compared to oral administration, allowing for treatment protocols to be delivered with an anti-biofilm intent. For an exhaustive discussion of different antimicrobial classes, specifically with anti-*S. aureus* biofilm activity, see 11.2.

Topical surfactant

Chiu et al. trialed baby shampoo irrigations in 18 patients with surgically recalcitrant CRS[73]. A 1% shampoo in saline dose regime was chosen because this concentration was able to prevent *Pseudomonas aeruginosa* biofilm formation in vitro, but ineffective against formed biofilms at any concentration. Curiously, no microbiological end-points where reported in this study, and it was not reported how many patients, if any, had *Pseudomonas* mucopurulence on enrollment.

Steroid irrigations

Steroid irrigations are thought to be superior to conventional steroid sprays by virtue of the increased sinonasal penetration afforded by irrigation devices (see 1.3). Whilst head-to-head clinical trials have yet to confirm this assumption of superior clinical efficacy, it does appears that this treatment agent/device concept is at least safe[74, 75].

Allergy desensitization

Aspirin desensitization in Samter's triad is only modestly successful[32]; this therapy is only weakly recommended by Desrosiers and Kilty[63]. Fungal immunotherapy has been trialed with promising results, albeit in small patient numbers[76].

Intravenous immunoglobulin

Ramesh et al. trialed IVIG in children with chronic sinusitis, again with promising results but small patient numbers (n=6)[77]. Chee et al. used IVIG in 19% of patients referred to a tertiary Allergy & Immunology Clinic with recalcitrant CRS however did not report post-treatment outcome results[23].

Bacterial lystates

German studies have used bacterial lysates as a topical treatment in CRS[78, 79]. The aim of this treatment is to induce changes in the local host immune response by introducing foreign (*Enterococcus*) antigen. It is not known whether this treatment modality has ever been used in Australia; it certainly is not currently part of our recalcitrant-CRS treatment armamentarium.

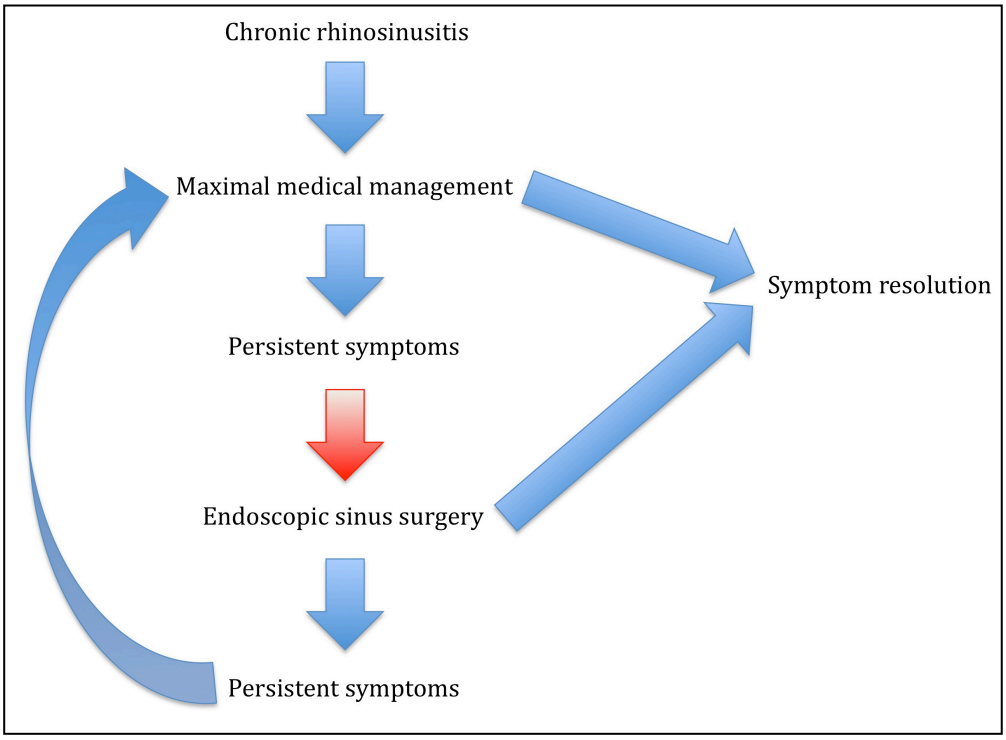

Figure 2. Chronic rhinosinusitis treatment algorithm, adapted from Palmer et al.[80] The red arrow indicates a potential rate-limiting step; after aggressive surgeries such as the endoscopic modified Lothrop procedure and/or medial maxillectomies, a point is reached at which no further surgeries can physically be performed. If disease persists despite aggressive surgery, the only option is to consider medical treatment.

1.1.5 Staphylococcus aureus: The microbiology of Chronic Rhinosinusitis

Whilst the bacterial pathogens in acute rhinosinusitis are well recognized (eg. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*.), there remains a lack of consensus as to the important bacterial species in CRS. For example, the role of anaerobes is felt to be important by some authors[81], whereas others studies suggest anaerobes to be of far lesser significance compared to aerobic bacteria[82, 83]. Comparing culture results between different studies can be difficult, partly because of differing sampling methods[81], but more importantly because of the inherent limitations of standard microbiology culture techniques[37, 81]. Historically, the standard means to identify pathogens relies on conducive environmental conditions that allow a pathogen to thrive outside of the body. The growth media selection and incubation conditions may bias towards cultivation of one species at the expense of another[37]. For instance, many studies do not report anaerobic bacteria, presumably because samples were never cultured under these conditions. More sensitive microscopy and molecular detection techniques (as previously mentioned in 1.1.3) may

correlate poorly to standard culture, but have the advantage of being able to identify bacteria that would otherwise be missed. Nonetheless, the large body of literature pertaining to purely standard culture results cannot be ignored. *S. aureus* was the most common pathogenic isolated in our series of 48 patients with evidence of infection at the time of ESS for CRS[84]. Other authors to have reported a similar high rate of culture of this organism in patients presenting for ESS include Kostamo et al.[35] and Bhattacharyya et al.[85] Whilst other authors report a preponderance of *S.epidermidis* over *S. aureus*[34, 83], the former is regarded as an unlikely pathogen in CRS. Regarding the more sensitive techniques, Foreman et al. reported a higher rate of *S. aureus* biofilm detection using FISH compared to *Pseudomonas* and *Haemophilus* in patients presenting for ESS. In an important recent study, Stephenson et al., using a molecular detection technique, found that whilst *S. aureus* was the predominant aerobe in CRS, anaerobes were even more prevalent[86].

1.1.6 STAPHYLOCOCCUS AUREUS: VIRULENCE MECHANISMS

S. aureus is well equipped for the causing of infection- and also for survival- in the sinonasal cavity. It is the pathogen thought most likely to be of aetiopathogenic consequence in CRS, not only because of its common isolation in patients with CRS (see 1.1.5) and its association with a more severe disease phenotype (see 1.1.8), but also because of its various survival and disease-causing mechanisms. These virulence mechanisms can be broadly categorized as either active secretory (such as cytolytic exotoxin and superantigen production), passive secretory (peptides that inhibit phagocytosis and allow intracellular residence) or structural adaptation (biofilm formation)[87, 88].

Cytolytic exotoxin production

S. aureus secretes a variety of exotoxins that cause host cell lysis. These cytolytic toxins disrupt host cell membrane integrity by the insertion of pores into the host cell membrane. Examples of secretory exotoxins capable of being produced by certain *S. aureus* strains include alpha-hemolysin, beta-hemolysin, gamma-hemolysin, leukocidin, and Panton-Valentine leukocidin[89].

Superantigen production

Superantigen exotoxins are unique in their ability to induce a T-cell proliferation (with massive cytokine release) by cross-linking an antigen presenting cell and a T-cell by binding outside the normal peptide-binding groove[87, 90]. They are also robust molecules, being relatively heat- and protease-resistant[87]. Whilst umbrella term 'staphylococcal superantigens' may imply these toxins are produced by both *S. aureus* and *S. epidermidis*, in actuality far more *S. aureus* strains code for superantigens than do *S. epidermidis*[91, 92]. Bachert et al. first suggested a pathogenic role for staphylococcal superantigens in CRSwP in 2001[93]. However, a subsequent study by Heymans et al., in which *S. aureus* strains isolated from CRSwP, CRSsP and control sinonasal cavities were analysed for the presence of exotoxin genes, found no correlation between exotoxin gene detection and polyposis[94].

Phagocytosis prevention and intracellular residence

Phagocytosis by polymorphonuclear leuckocytes (PMNs or neutrophils) is an important function of the host innate immune system in the eradication of invading micro-organims[88]. This response is the primary cellular defense against *S. aureus* infections in humans[88]. This process progresses in a step-wise manner (PMN recruitment, phagocytosis, killing of ingested micro-organisms and finally PMN apoptosis); *S. aureus* has evolved mechanisms to interfere with each step[88].

A first step in the eradication of invading microorganisms is active recruitment of PMNs to the site of infection by chemotaxis. Whilst a number of proteins, cytokines and exotoxins produced by *S. aureus* are chemotactic, the chemotaxis inhibitory protein of *S. aureus* (CHIPS), found in >60% of clinical isolates, specifically aims to inhibit this step[95].

The second step is phagocytosis, a process whereby neutrophils bind and ingest invading microorganisms. Opsonization the process in which microbial peptides are 'tagged' by complement (amongst other molecules, such as immunoglobulin), which facilitates identification of the foreign microbe by PMNs. *S. aureus* produces

a number of molecules that block the complement system, thereby inhibiting phagocytic binding[88].

Thirdly, human neutrophils employs oxygen-dependent and oxygen-independent mechanisms to kill ingested microorganisms[88]. In turn, *S. aureus* secretes detoxifying molecules, such as catalase and superoxide dismutase, in order to combat the hostile intracellular environment[88].

Lastly, the neutrophil induces apoptosis on the removal of the foreign microorganisms. Certain strains of *S. aureus* have been shown to promote early neutrophil apoptosis, effectively disseminating only partially apoptosed (ingested but not destroyed) bacteria[88]. Alternatively, by delaying apoptosis, the partially apoptosed bacteria may be able to survive long-term within the neutrophil ('intracellular residence')[88]. *S. aureus* is also able to survive within cells that also perform phagycytosis, such as the epithelial cell lining of the sinonasal mucosa.

Biofilm formation

In order to facilitate survival in the hostile host environment, it is now known that bacteria form organized communities rather than simply existing as a singular bacterium in isolation. These organized communities are called biofilms, and consist of bacteria adherent to a mucosal surface or foreign body surrounded by an extensive protective extracellular polymeric substance (EPS, or glycocalyx)[96, 97]. The EPS is composed primarily of polysaccharides, but also contains protein and extracellular DNA[98]. Adoption of a biofilm phenotype (in contrast to individual, free-living, planktonic bacteria) is an important virulent step for a number of reasons, as outlined in reviews by a number of authors[96, 97, 99]:

Primarily, the EPS matrix enables the bacteria to adhere to a surface that would normally be hostile. For example, in the nasal cavity the constant flow of mucous and cilia activity would normally simply sweep free-swimming bacteria away, but adoption of biofilm phenotype allow for the bacteria to become sticky and remain adherent to the sinonasal mucosa.

Secondly, the three-dimensional biofilm structure is relatively resistant to antimicrobials, itself for a number of reasons; firstly, the EPS matrix offers a physical barrier to antibiotic penetration and secondly, the close contact of bacteria within the biofilm allows for rapid cell-to-cell transfer of antibiotic resistance genes or plasmids. Additionally, the low-nutrient state within the biofilm ensures bacteria exhibit a low metabolic rate, which confers relative resistance against growth-dependent antimicrobial killing. This latter technique is similar to the small-colony variant phenomenon, in which a sub-population of bacteria- not necessarily in a strict biofilm phenotype- adopt a low-metabolic state akin to hibernation[100].

Finally, a biofilm phenotype confers a survival advantage against the host immune response. Adopting the biofilm phenotype allows bacteria to hide from the host immune system, avoiding detection and hence phagocytosis. Importantly, *S. aureus* in biofilm form continues to secrete active and passive virulence substances as previously outlined.

1.1.7 STAPHYLOCOCCUS AUREUS: THE BIOFILM LIFE-CYCLE

A potential causal relationship between biofilms and CRS has been suggested by a number of authors following the appreciation by Cryer et al. in 2004 that biofilms could be found on the sinus mucosa surfaces of human subjects with recalcitrant chronic sinusitis[101]. Whilst early studies formed a loose association between the microscopic appearance of detected biofilm and simultaneous culture results[101, 102], subsequent authors have been able to determine the biofilm species, using advanced imaging and detection techniques such as FISH[103]. More recently, it has been appreciated that biofilms of different species confer a differing prognostic outcome[39]. As a result, treating patients with recalcitrant CRS within an anti-biofilm paradigm has become a popular CRS research focus. Before outlining the myriad of topical anti-biofilm treatments that have been proposed for use in recalcitrant CRS (see Table 2) or may be of potential use in the future (see 11.2), it is important to consider how biofilms form, mature and adapt to the surrounding environment.

The formation of a biofilm occurs in a number of successive phases: surface conditioning, docking, locking, maturation and finally dispersal[97]. This process is dependent upon several factors, such as bacterial species, surface composition (medical implant, biological surface etc..) and the surrounding host environment [97, 104].

Surface conditioning

This is the process in which a surface is altered either by the host environment or bacterial secretory products, facilitating adhesion[104]. The former relates to surface changes of medical implants upon introduction into the host environment and is as such unrelated to CRS; the latter, however, may be important in recalcitrant CRS (although has not been previously explored in the literature).

Docking or primary bacterial adhesion

Bacteria contact the surface and become irreversible adherent as a result of a number of factors including electrostatic and hydrophobic interactions, Van der Waals forces and changes temperature. Bacteria that have docked are easily removed[97, 104].

Locking or secondary bacterial adhesion

In this phase, reversibly bound bacteria start producing EPS. As a consequence, adhesion strengthens and may become irreversible[104].

Maturation and quorum-sensing

Quorum sensing refers to the phenomenon whereby the accumulation of 'signaling' molecules in the surrounding environment enable a single cell to sense the cell density, so that the population as a whole can make a coordinated response[105]. *Pseudomonas* bacterium that have lost the ability for functional quorum-sensing, form flat, abnormal biofilms in contrast to robust 3-dimentional mature structures[106]. Curiously, however, when *S. aureus* growth is too-highly regulated a paradoxically smaller biofilm results[107]. An important characteristic of a mature biofilm is the presence of water-channels, which allow for the influx of nutrient and the outflux of waste products[96, 97].

Dispersal

Dispersal is the process by which the biofilm actively breaks down a portion of its own EPS matrix, in order to release free-living planktonic bacteria. These dispersed clones may, therefore, seed a new biofilm and perpetuate the biofilm life-cycle. It is generally thought that biofilms disperse in favorable conditions[108], although this may not always be a correct assumption[109, 110]. Presumably, this *active dispersal* is in an effort to further populate an already nutrient rich environment. Regardless of trigger, the dispersed biofilm is a vulnerable target for the host-immune defenses and antimicrobials alike. Having lost the protective EPS matrix, a dispersed biofilm is therefore likely to respond much more favorably to conventional antibiotic treatment. For this reason, one strategy to combat biofilms is targeting the EPS matrix in an effort to force dispersal. This is termed *passive dispersal* because the biofilm itself is not actively initiating this phase[111]. Stimulating passive dispersal as an antibiofilm strategy is discussed further in 1.2.5 and 1.2.6; manipulation of the environmental surrounds in order to coerce the biofilm into active dispersal is also discussed, in 1.2.9.

1.1.8 Staphylococcus aureus: Outcomes following Sinus Surgery

After the discovery that bacterial biofilm were often present in CRS, studies evaluating the prognostic implications of sinonasal mucosal biofilms soon followed. Psaltis et al. identified biofilms in 20/40 (50.0%) of patients undergoing ESS using a non-species specific detection technique[112]. In this study, a strong correlation between biofilm-positivity and poorer post-operative outcomes was then found when the authors assessed clinical outcomes retrospectively. Subsequently, Singhal et al. performed a similar, wholly prospective study with similar outcomes[113]. More recently, two species-specific clinical outcomes studies have been performed. Foreman et al. demonstrated that intra-operative *S. aureus* biofilm detection correlated to poorer post-ESS outcomes, whereas *Haemophilus* biofilm detection conferred a better post-operative outcome[39]. Singhal et al. again performed a similar prospective study, and confirmed the strong correlation between *S. aureus* biofilm detection at the time of ESS and poorer objective and subjective post-operative outcomes[114]. As seen in Figure 3, *S. aureus* biofilm-positive patients reported similar symptoms scores at 12 months post-operatively

compared to their baseline, pre-operative scores; in essence, these patients were surgically-recalcitrant. Although these studies did not specifically evaluate the response of the *S. aureus* biofilm itself to ESS, we have previously hypothesized that the increase culture-rate of *S. aureus* often observed following surgery[34] might indicate active biofilm dispersal during this early post-ESS period[84]. If this hypothesis was to be true, this period may represent a serendipitous window for antimicrobial treatment at a point biofilm life-cycle phase in which the bacteria are most vulnerable. In theory, therefore, treatment during this dispersal-friendly period may improve the poor post-ESS outcomes associated with the presence of intra-operative *S. aureus* biofilm.

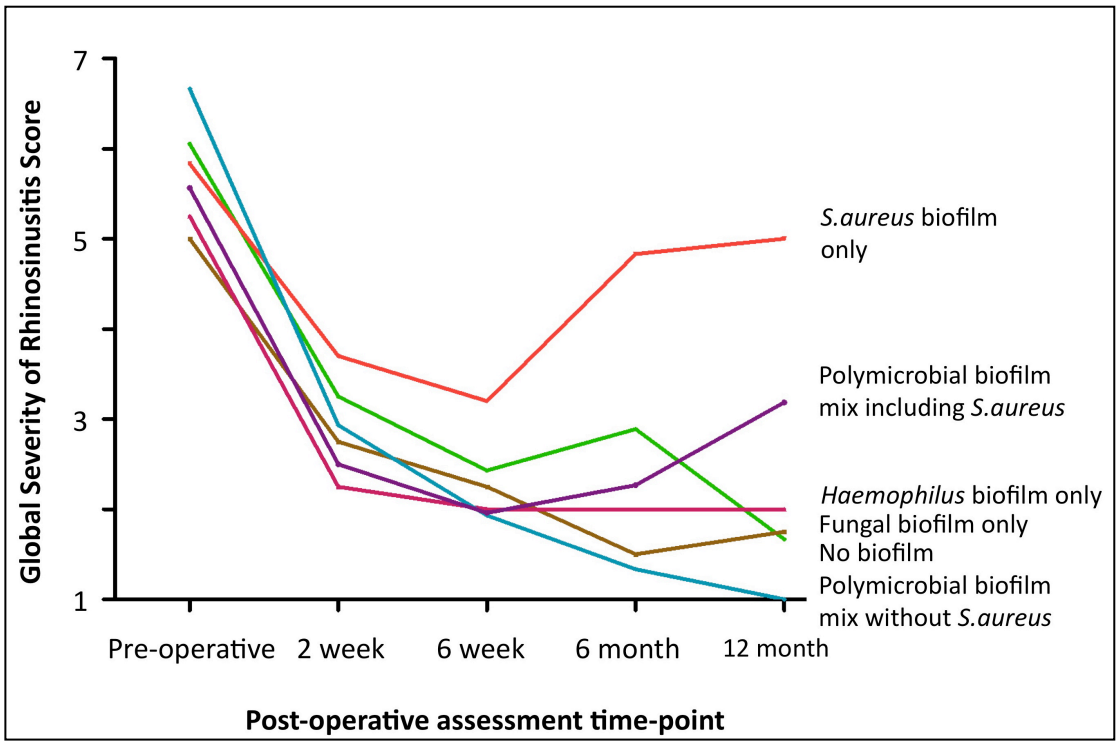

Figure 3. Biofilm sub-group analysis of patient-reported symptoms before and after surgery, using the Global Severity of Rhinosinusitis Score[21] (GARS). It is apparent that patients with either *S. aureus* biofilm only, or *S. aureus* in a polymicrobial biofilm mix, fare worse than those without during the 12 months post-operative period. Adapted from Singhal et al[114], with permission.

The landmark species-specific studies by Foreman et al. and Singhal et al. strengthened the argument that *S. aureus* has an important role in CRS, whilst also inferring the obvious question- would specific anti-biofilm treatments in these patients improve clinical outcomes? A small pilot study by Uren et al.[42] (see 1.2.1) would suggest that the answer is in the affirmative.

1.1.9 STAPHYLOCOCCUS AUREUS: NASAL AND EXTRA-NASAL INFECTION

From a purely rhinological point-of-view, the aforementioned studies by Foreman et al. and Singhal et al. elegantly demonstrate the clinical sequelae of sinonasal *S. aureus* biofilm[39, 114]. There is, however, potentially another important consequence relating to this bacterium in the nose- the conferred risk of extra-nasal nosocomial infection.

Nasal colonization with *S. aureus* has long been associated with an increased risk of extra-nasal infection, such as boils and styes in non-hospitalised patients[115], or post-operative nosocomial wound infection in inpatients[116, 117]. Nasal colonization refers to the ability of *S. aureus* to reside in the anterior nasal cavity ('the nasal vestibule') in healthy individuals without causing any apparently local tissue response; this contrasts to infection, which implies there is a host response[118] (with oedema, mucopurulence etc..) and patient-reported symptoms consistent with such. Recent studies have demonstrated that rapid screening and decolonization with topically applied mupirocin to the anterior nasal vestibule (plus a chlorhexidine body rinse) reduces the risk of iatrogenic infection in high-risk patients[119]. These important findings have potential profound public health repercussions, as *S. aureus* iatrogenic infection is a billion-dollar problem.

Unfortunately, however, no studies have previously assessed the extra-nasal complication risk of known *S. aureus*-positive CRS patients. Numerous studies evaluating *S. aureus* colonization prevalence, site of colonization, and potential reasons for decolonization failure have, however, collected data on 'throat' or oropharynx carriage[120-123]. Of consequence, Amerlaan et al. found that throat carriage of *S. aureus* predicted for colonization failure[123]. Whilst this study attempted to evaluate for the presence of otorhinological pathology in the included patients, it is possible that some of these throat-carriage positive patients had either clinical or subclinical CRS, with the culture results simply a reflection of post-nasal drip. Given that the disease in CRS is one of infection- with oedema and often mucopurulence- it would stand to reason that the risk of nosocomial infection in these patients is at least as high as their non-CRS vestibule-colonized counterparts. Satisfactorily treating the *S. aureus* bioburden in post-ESS CRS,

therefore, may be equally as important for an extra-nasal reason as it is for the improvement of the patient's rhinological symptoms.

1.2 Defining the treatment agent: Staphylococcus aureus and the antimicrobial treatment spectrum

Why do we advocate treating with an anti-biofilm intent? Although the sampling of tissue from patients in the post-operative period is not performed in our department, one can assume that in a patient from whom *S. aureus* is cultured (from planktonic clones, by definition), either an underlying, non-culturable *S. aureus* biofilm is likely to be present (dispersing the clones) or no biofilm is present simply because environmental conditions are so favorable that a wholly dispersed, planktonic phenotype exists. We believe, therefore, that antimicrobial therapy should be delivered with an anti-biofilm intent. The manifestly poor microbiological outcomes observed when recalcitrant *S. aureus* culture-positive CRS patients are treated with oral antibiotics (in ignorance of an anti-biofilm treatment intent), as observed in 12.2, ads strength to this viewpoint.

Strategies targeting the *S. aureus* biofilm can be classified into three theoretical interventions. Firstly, steps can be taken to prevent biofilm formation. Secondly, where a mature biofilm exists, then attempts are made to either kill the biofilm or remove it from the surface to which it is adherent. If these fail, then thirdly the surface to which the biofilm is attached is itself removed[96]. In CRS, as the biofilm is attached to the host sinus mucosal surface, preventing biofilm formation (a strategy more applicable to the coating of medical implant devices such as indwelling urinary catheters) or removing the surface to which it is attached (such as removing an infected hip prosthesis) are less applicable measures than employing strategies to kill or removal the biofilm biomass. These *direct* 'kill or remove' strategies can be further classified with regards to the intended target-site of the antimicrobial agent, which can be the bacteria, the matrix, or both (Table 1). Other *indirect* antibiofilm strategies involve environmental manipulation, aiming to make the surrounds hostile to the biofilm. Potential candidates for manipulation include the surrounding gaseous environment, the surrounding bacterial flora and the host immune response. For the purposes of this text, any agent acting directly

or indirectly to either kill, remove or otherwise disrupt the biofilm biomass is deemed to be exerting an antibiofilm effect.

Table 1. Antimicrobial agents that directly target the biofilm can be classified according to the targeted biofilm component/s.

Targeted biofilm component		
Bacteria	**Matrix**	**Bacteria & Matrix**
Antibiotic	Enzymatic destruction	Laser
Biocide	Mechanical disruption	
Bacteriophage	Surfactant	
Iron competition		

Regardless of anti-biofilm mechanism, only those agents able to be delivered topically are discussed in this thesis. Topical delivery bypasses hepatic metabolism, therefore minimizing any adverse effects that may be associated with systemic absorption whilst affording maximal drug delivery to the site of infection. Highly concentrated level of antibiotics are generally required to have an antibiofilm effect[124], and as such topical therapies stand the best chance of success against the biofilm. Topical anti-*S. aureus* biofilm treatments previously evaluated in the Rhinology literature to-date are demonstrated in Table 2.

1.2.1 STAPHYLOCOCCUS AUREUS AND ANTIBIOTICS

Antibiotics are chemical substances, generally with an excellent safety profile for oral, intravenous or topical use in humans, which have the specific "capacity to inhibit the growth of and even to destroy bacteria and other micro-organisms[125]". Antibiotics can be classified with regards to their specific mechanism of action or metabolic target; they can interfere with cell wall, protein or nucleic acid synthesis, or act to disrupt the cell wall membrane itself. The action of the antibiotic on the bacteria is further classified as either bacteriostatic or bacteriocidal, depending as to whether the agent stops bacteria from growing and dividing (-static) or kills the bacteria (-cidal).

Common topical antibiotics used to treat *S. aureus* skin infections in Australia include sodium fusidate and mupirocin[126]. Ophthalmic topical preparations of gentamicin (solution) and tetracycline (ointment) are also available[127]. Currently, no antibiotics have Therapeutic Good Administration (TGA) approval for topical use in the sinuses. Sodium fusidate and mupirocin are further discussed here because these are the two agents indicated for treatment of cutaneous (skin) *S. aureus* infection according to the Australian Medicines Handbook[126]; mupirocin has also been used in two previous small cohort studies in post-ESS CRS[42, 68]. Gentamicin and tetracycline also warrant further discussion, as gentamicin has also been trialed in the post-ESS CRS setting[128, 129], whilst oral doxycyline, a derivative of tetracycline, has previously been studied in CRSwP[130]. Other topical antibiotics assessed in the rhinology literature have either a weak-anti staphylococcal effect, are not available topically in Australia, or have been shown to be ineffective in recalcitrant CRS with *S. aureus.*

Sodium fusidate (inhibits protein synthesis, bacteriostatic) ointment is commonly used to treat staphylococcal skin infections, and is safe, efficacious and well-tolerated in this setting[131]. Akiyama et al. showed in a rat model that *S. aureus* biofilm biomass formed on damage skin could be reduced but not eradicated with 2% fusidic acid cream[132]. No studies using sodium fusidate have been reported in the rhinology literature, however, perhaps reflecting the fears of induced antibiotic-resistance associated with this agent which generally limits treatment duration to 14 days[131].

Mupirocin (principly inhibits protein and RNA synthesis[133], can be bacteriostatic or bacteriocidal) cream or ointment is primarily used for topical decolonization of the *S. aureus*-positive nasal vestibule, a practice which reduces the risk of extra-nasal nosocomial infection in at-risk individuals[119, 134]. The rate of mupirocin resistance following treatment has been reported to be 0-2.4%[135, 136], although high rates of community resistance have been observed with unregulated use[137]. Mupirocin is an ideal topical treatment in CRS as it is stable in nasal secretions, retaining 100% of its anti-staphyloccocal activity[138]. Mupirocin solution has been reported to reduce *S. aureus* biofilm biomass by >90% in vitro[139], and has been

shown to be efficacious in a sheep model of sinusitis[140]. Additionally, two authors have reported promising clinical outcomes in recalcitrant post-ESS CRS when delivered as a sinonasal rinse, although neither studies were blinded nor placebo controlled[42, 68]. Solares et al. treated patients with a mupirocin ointment mixed into a saline solution rinse[68], whilst Uren et al. used mupirocin powder dissolved in solution[42]. As a powder, mupirocin is not readily available in Australia, in contrast to the ointment or cream preparations. The latter are not readily dissolvable in solution, however, and as such are likely to result in an unpredictable delivery pattern when used as a wash.

Widely utilized as an intravenous antibiotic, topical gentamicin (inhibits protein synthesis, can be bacteriostatic or bacteriocidal) sinonasal rinses have been previously trialled in the post-ESS setting. Concerns regarding eustachian tube penetration with direct ototoxicity and systemic absorption (with potential for nephrotoxicity)[128, 129] have limited the clinical utility of this agent.

Tetracycline (inhibits protein synthesis, bacteriostatic) ointment is not currently marketed for use in Australia, however is obtainable in certain circumstances[127]. Oral doxycycline, a derivative of tetracycline, has been shown to decrease nasal polyposis size and improve clinical outcomes compared to placebo, with a sustained effect following cessation of treatment[130]. In this study, the authors suggested that clinical efficacy of doxycycline was in part due to an immunomodulatory mechanism[141] as well as its antibiotic effect. A similar mechanism of action is seen with macrolide antibiotics in CRS[65] (see 1.1.4)- indeed, preparations of clindamycin and erythromycin are available for use in Australia to treat acne vulgaris[126]. However, no studies using topical tetracycline, nor topically-delivered macrolides, have been performed in CRS to date.

Various other topical antibiotics have been trialed in the post-ESS setting with varying success. Leonard et al. used ceftazidime rinses in patients with surgically recalcitrant CRS[142], however this antibiotic has only a weak anti-staphylococcal effect and is only available as an intravenous preparation in Australia[143]. Various antibiotics delivered by nebulization by Scheinberg et al.[144] (cefuroxime,

ciprofloxacin, levofloxacin and tobramycin) and Vaughn et al[145] (ceftazidine, ciprofloxacin, levofloxacin, ofloxaxin and tobramycin) have either a weak-anti staphylococcal effect, are not available topically in Australia, or both. In a well-designed cross-over trial, Videler *et al.* concluded that nebulization with bacitracin/colimycin was not an effective treatment in recalcitrant CRS with *S. aureus*.[146] These disappointing results may be partially attributable to the delivery technique, however, with the authors questioning the adequacy of the nebulizing device. Although not readily available in Australia, fusafungine delivered via nasal spray was shown by Mosges et al. to be beneficial in a small study of 20 patients[147]. Finally, topical moxifloxacin has been shown by Desrosiers et al. to be effectively anti-biofilm at very high concentrations[124], however it is not currently commercially available and has not been used clinically.

1.2.2 STAPHYLOCOCCUS AUREUS AND DISINFECTANTS

In contrast to antibiotics, disinfectants generally are active against a broad spectrum of bacteria and have a multiple mechanisms of action. Rather than having a specific metabolic target, disinfectants generally kill indiscriminately and as such are more likely to be toxic to mammalian cells[97, 148]. Disinfectants are classified by chemical structure, and include alcohols, biguanides, halogen releasing agents, heavy metal derivatives, oxidizing agents, phenols and quaternary ammonium compounds[149]. Common disinfectants used in medicine include ethanol (an alcohol), chlorhexidine (biguanide), iodine (halogen-releasing agent) and hydrogen peroxide (oxidizing agent). Whilst less is known about disinfectant action compared to antibiotics, the antimicrobial mechanisms involved in biocide activity include cell membrane damage, denaturing of proteins, inhibition of DNA synthesis and DNA strand breakage[149].

Few studies have been performed evaluating topically delivered disinfectants in CRS. Rombaux et al. demonstrated that a single intra-operative rinse with povidon-iodine solution decreased the rate of bacterial culture from the sinuses compared to simply disinfecting the skin of nose, although there was no statistically-significant change in the rate of *S. aureus* recovery between the two groups[150].

Additionally, povidone-iodine is less stable than mupirocin in nasal secretions[151] which limits it potential clinical utility.

Neher et al. trialed N-chlorotaurine in 12 patients with CRS[152]. N-chlorotaurine is an oxidizing agent, principally produced by activated macrophages[153]. The clinical study was performed in patients who had not previously undergone ESS, however, and as such has questionable applicability in recalcitrant *S. aureus*-CRS. Nonetheless, the authors concluded a possible beneficial effect with this treatment.

Alanjedani et al. demonstrated the in vitro activity of Manuka Honey against both *P.aeruginosa* and *S. aureus* biofilms at a 1:2 dilution[154]. In a study by Kilty et al., histological assessment of the rabbit maxillary sinus following treatment with this concentration of Manuka Honey suggested this treatment modality to be safe[155]. Unfortunately, however, neither of these studies reported the methylglyoxal (MGO) concentration of the Manuka honey used. MGO has recently been shown to contribute the additional antimicrobial effect seen with Manuka honey compared to regular non-MGO honeys[156]. In isolation, MGO disrupts cellular homeostasis by denaturing DNA and intracellular proteins[157]. Manuka honey is therefore classified as a disinfectant, not only because of the phenol compound MGO, but also because of the oxidizing agent hydrogen peroxide, which is found in all honeys regardless of MGO concentration. Whilst the safety profile of Manuka Honey has not been fully established, its degree of consumption in New Zealand, where it is a widely consumed and popular foodstuff, suggests any risk to be minimal at most. Of most promise, however, is the discovery by Blair et al. that microbial resistance to Manuka honey cannot be induced[158]. This unique honey, therefore, may be an exciting treatment option that can be potentially used long-term without fear of developing resistance.

1.2.3 STAPHYLOCOCCUS AUREUS AND BACTERIOPHAGES

Bacteriophages, discovered independently by Twort and d'Herelle almost a century ago[159], are bacterial viruses that attach to and subsequently kill their specific hosts by internal replication and lysis[160]. They are perhaps the most ubiquitous organisms on earth, found in varying environments, in- and on-

humans and animals alike[160]. 'Phage therapy', the use of bacteriophage for therapeutic effect, has until recently been largely ignored as an antimicrobical therapy in preference to antibiotics. Only recently have clinical trials using phage therapy begun to appear in the literature. Wright et al. used an anti-pseudomonal phage in patients with treatment-resistant otitis externa[161]. Gill et al. studied the efficacy of phage therapy in *S. aureus* bovine mastitis[162], a common biofilm-mediated disease of milk-producing cattle[163]. In vitro assessments of bacteriophage activity on formed *S. aureus* biofilms appear promising[164]; whilst similar assessments have not been performed using bacteria taken from patients with CRS, it would seem that an appropriate next step would be assessing phage therapy in a *S. aureus* CRS animal model.

1.2.4 STAPHYLOCOCCUS AUREUS AND IRON COMPETITION

Chelators of iron, such as tetrasodium EDTA, impair iron-related metabolism in bacteria; similarly, gallium nitrate interferes with bacterial DNA and protein synthesis by competing with and replacing Fe^{3+}[165]. EDTA has been shown to decrease *S. aureus* biofilm biomass formed on hemodialysis catheters[166] but to-date has not been studied from a rhinological perspective. Gallium nitrate has been shown in a sheep model of sinusitis to decrease *S. aureus* biofilm biomass when delivered topically[140]. However, no clinical studies of gallium nitrate in CRS have been performed to date, despite it having existing FDA-approved for treatment of cancer-related hypercalaemia[167] and hence an acceptable safety profile. In addition to competing for iron, gallium has anti-proliferative and immunomodulatory activity[165], which may have a theoretical application in CRSwP. This has not been previously explored in the rhinology literature, however, and is outside the scope of this thesis.

1.2.5 STAPHYLOCOCCUS AUREUS AND ENZYMATIC DISRUPTION OF THE BIOFILM MATRIX

Endogenously-produced matrix-degrading enzymes involved in active biofilm dispersal include glycosidases, proteases, and deoxyribonucleases[111]. Similar exogenous enzymes can be used for a therapeutic effect, passively (ie. not a active, biofilm-driven process) dispersing the biofilm into more readily treatable planktonic bacteria. An antibiotic (or disinfectant), therefore, must be used in conjunction to the kill the released bacteria, otherwise these bacteria will simply

seed new biofilms. Lauderdale *et al.* demonstrated this therapeutic technique by using proteinase K and DNaseI to precipitate the dispersal of MRSA biofilms grown on titanium plates *in vitro*; the subsequent free-living bacteria were then successfully treated with traditional antibiotics[168]. To date, no similar studies have been reported in the rhinology literature.

1.2.6 STAPHYLOCOCCUS AUREUS AND MECHANICAL DISRUPTION OF THE BIOFILM MATRIX

Desrosiers *et al.* demonstrated in an *in vitro* model that pressurized saline jet lavage was able to decrease *S. aureus* (and *P.aeuruginosa*) biomass compared to control[169]. Le *et al.* used a mechanical hydrodebrider (Medtronic, Jacksonville, FL) in conjunction with a combined citric acid and zwitterionic surfactant (CAZS; caprylyl sulfobetaine) to treat *S. aureus* biofilms in a sheep model of sinusitis. In this study, however, the hydrodebrider was not used in isolation, and as such attributing any of the reported results to the hydrodebrider (or surfactant) alone was not possible[140]. Subsequently, however, Valentine et al. performed a similar study was able to conclude that that the hydrodebrider was only minimally-efficacious when used with saline alone[170]. Similar orthopedic models have shown high-pressure saline lavage to be efficacious in reducing biofilm biomass, although not to the degree when used in combination with antibiotics or soap[171].

1.2.7 STAPHYLOCOCCUS AUREUS BIOFILM AND SURFACTANT

Destrosiers et al. further demonstrated the anti-biofilm activity of CAZS *in vitro*[169]. In isolation, CAZS reduced *S. aureus* biofilm biomass compared to controls, and was slightly more efficacious than the saline jet lavage technique. Again, *Le et al.* tested the hydrodebrider-CAZS combination in sheep but did not test either in isolation[140], although Valentine et al. did show that CAZS did decrease biofilm biomass- albeit temporarily[170]. CAZS has been subsequently shown by Tamashiro *et al.* to denude cilia in a rabbit model of sinusitis, however, and as a consequence clinical trials have not been performed[172]. Similar findings were echoed by Valentine et al.[170]

1.2.8 STAPHYLOCOCCUS AUREUS BIOFILM AND LASER

Krespi *et al.* used a combination of near-infrared (NIR) and shockwave (SW) laser to treat *in vitro S. aureus* biofilms, with the rationale of dispersing the biofilm with

the NIR laser and then killing the released planktonic cells with SW laser. This combined approach yielded a decreased biofilm biomass that was less metabolically active when compared to controls[173]. Translating these results into clinical practice faces a significant delivery challenge, however, as accessing and treating the entirety of the sinonasal mucosal surface under direct vision is not currently possible with rigid endoscopes.

1.2.9 STAPHYLOCOCCUS AUREUS AND ENVIRONMENTAL MANIPULATION- GAS COMPOSITION

Inhaled air is principally composed of nitrogen, oxygen, water vapour and trace gases (such as argon, carbon dioxide and nitrous oxide). Manipulation of oxygen and certain trace gases such as nitrous oxide has the potential to exert an antimicrobial effect on the *S. aureus* biofilm principally by triggering active dispersal (see1.1.7).

Whilst an aerobic environment is preferred, *S. aureus* is readily adaptable to anaerobic conditions, with changed respiration patterns facilitating continued growth in the absence of oxygen[174]. Furthermore, anaerobic conditions facilitate *S. aureus* biofilm formation, allowing persistence of the bacteria despite the changed environmental stressor[175]. Conversely, increases in oxygen tension can result in biofilm dispersal, demonstrating how oxygen can be used to exert antibiofim effect.

The trace gas nitrous oxide reacts with oxygen to form nitric oxide (NO). Very low concentrations of NO have previously been demonstrated in diseased maxillary sinuses[176, 177]. Barraud et al. demonstrated that NO was able to precipitate *P.aeruginosa* biofilm dispersal[178]. Jardelza et al. similarly demonstrated that NO at similar concentrations to those found in healthy maxillary sinuses disrupted *in vitro S. aureus* biofilms. The authors postulated a link between the low NO concentration found in diseased sinuses and enhanced biofilm growth at similar concentrations in vitro, hypothesizing that efforts to normalize the local nitric oxide concentration in CRS might have a therapeutic effect via a biofilm disruption (ie. dispersal) mechanism[179].

Again, whenever dispersal is triggered, traditional antibiotics (or disinfectants) must simultaneously be used in order to 'mop-up' the now free-swimming, planktonic bacteria. This is regardless of the gaseous trigger (or antimatrix enzyme) utilized. How to reliably deliver the gaseous trigger to the sinonasal cavity also needs to further evaluated.

1.2.10 STAPHYLOCOCCUS AUREUS AND ENVIRONMENTAL MANIPULATION- PROBIOTICS

Probiotics are defined as "live microorganisms that, when administered in adequate amounts (in humans or animals), confer a health benefit on the host"[180]. Extensively studied with regards to manipulation of the gut microflora, probiotics exert a therapeutic effect via a number of mechanisms including remodeling of microbial communities, suppression of pathogens and suppression of proinflammatory factors[181]. Gut probiotics have been trialed in CRS- although delivered orally and not with an antibiofilm intent. Mukerji et al. treated CRS patients with the probiotic *Lactobacillus rhamnosus* and unsurprisingly concluded it was not a beneficial treatment[182]. More promisingly, Iwase *et al.* have pioneered an expansion of the probiotic paradigm by delivering bacteria to the nasal cavity for therapeutic effect[183]. In their study, *S. aureus* colonized volunteers were treated with *S.epidermidis* in an attempt to decolonized the nasal vestibule. This was successful in 80% of volunteers in whom a matrix-degrading strain of *S.epidermidis* was inoculated. The same study also reported similar *in vitro* findings in which *S.epidermidis* inhibited *S. aureus* biofilm formation[183].

1.2.11 STAPHYLOCOCCUS AUREUS AND ENVIRONMENTAL MANIPULATION- ADJUNCTS TO THE HOST IMMUNE RESPONSE

Another indirect anti-biofilm strategy is enhancement of the host immune response, by vaccination or promotion of opsinisation[184]. Neither approach has previously been assessed from a rhinological perspective. Vaccination aims to produce a long-term protective immune response against a pathogen. Weizmann *et al.* proposed vaccines blocking bacterial adhesion to be an ideal strategy[185]. Current anti-biofilm vaccine research largely focuses on preventing biofilm formation on implant devices[184], however, rather than the treatment of mature biofilm on human mucosal surfaces; the former is far less potentially relevant to CRS than the latter. Opsonization is the process where microorganisms are coated

with host-produced molecules (such as immunoglobulins, complement factors and mannose-binding lecithin) that enhance phagocytic activity. Gyimesi at al. demonstrated enhanced *S. aureus* phagacytosis by macrophages with the addition of a bispecific monoclonal antibody complex dually specific for the complement C3b receptor and *S. aureus* capsular polysaccharide[186]. The bispecific antibody was designed to bind the macrophage at one site (the complement receptor) and the *S. aureus* capsule at the other. Krishnamurthy et al. have also employed a similar approach[187].

Table 2. Antimicrobial agents proposed in the rhinology literature, specifically for treating with an anti-*S. aureus* biofilm intent. Adapted from Foreman et al.[188]

Agent	**Antimicrobial Classification**	**Study Design**	**Outcome**
Mupirocin[139]	Antibiotic	*in vitro*	*Greater cidality than Ciprofloxacin or Vancomycin*
Mupirocin[140]	Antibiotic	Animal *in vivo*	*96.4% reduction in biofilm biomass compared to control following 5 day treament protocol*
Mupirocin[42]	Antibiotic	Human *in vivo*	*Pilot study; objectively, subjectively efficacious & well tolerated. 15/16 patient S. aureus culture-negative after treatment*
Gallium Nitrate[140]	Iron competition	Animal *in vivo*	*68.5% reduction in biofilm biomass compared to control following single dose*
Manuka Honey[154]	Disinfectant	*in vitro*	*Biocidal against 16/22 S. aureus strains*
Moxifloxacin[124]	Antibiotic	*in vitro*	*Biocidal at very high concentrations (10^3xMIC planktonic)*
CAZS +/- Hydrodynamic force[169]	Surfactant +/- mechanical disruption	*in vitro*	*Significant reduction in CFUs post treatment with & without hydrodynamic force*
CAZS + Hydrodynamic force[140]	Surfactant + mechanical disruption	Animal *in vivo*	*30.9% reduction in biofilm biomass compared to control following single dose*
Laser[173]	Laser	*in vitro*	*34% reduction in biofilm biomass following SW and NIR laser*
Hydrodynamic force[169]	Mechanical disruption	*in vitro*	*Significant reduction in CFUs post treatment*
Hydrodynamic force[170]	Mechanical disruption	Animal *in vivo*	*Reduction in biofilm following treatment, although not statistically-significant*

1.3 DEFINING THE TREATMENT TECHNIQUE: MAXIMISING TOPICAL DELIVERY TO THE SINUSES

Having first explored the myriad of agents with anti-*S. aureus* potential, it is [189]imperative to consider the various products and techniques available to deliver solutions topically to the sinonasal cavity. Regardless of device, sinonasal treatment distribution is improved significantly following endoscopic sinus surgery[57]. With each modality it is important to recognize the sinonasal distribution that can be expected and how patient positioning may affect the distribution. Many older studies use penetration of the middle meatus as the delivery end-point, rather than noting penetration of individual sinuses[189-193]. More recent publications, however, assess delivery to each sinus individually[57, 70-72, 194, 195], allowing a much better appreciation of what can be expected from a particular device when used clinically.

Other 'practicality' factors that should also be considered include the ease of use and cleaning, as well as the relative cost[196]. Studies assessing these factors are unfortunately scarce, although delivery techniques in which the neck is extended are probably more comfortable than those is which neck flexion is required[197]. Cleaning is important, as microbe contamination from soiled rinse bottles may contribute to clinically relevant disease, although direct evidence is limited[198-200]. Just as bacteria exist in the biofilm phenotype on human sinus tissue, biofilm growth on irrigation devices has also been demonstrated[200]. As a cleaning method, Keen *et al.* advocate rinsing with boiled water or a disinfectant[200], however evidence from other fields suggest scrubbing in conjunction with disinfectants is superior to disinfectant alone in removing biofilm from medical devices[201]. Hence, devices that can easily be easily cleaned with scrubbing (versus just rinsing with boiled water or disinfectant) are preferred. An estimation of overall technique/device practicality versus relative sinonasal distribution for different delivery modalities is demonstrated in Figure 4.

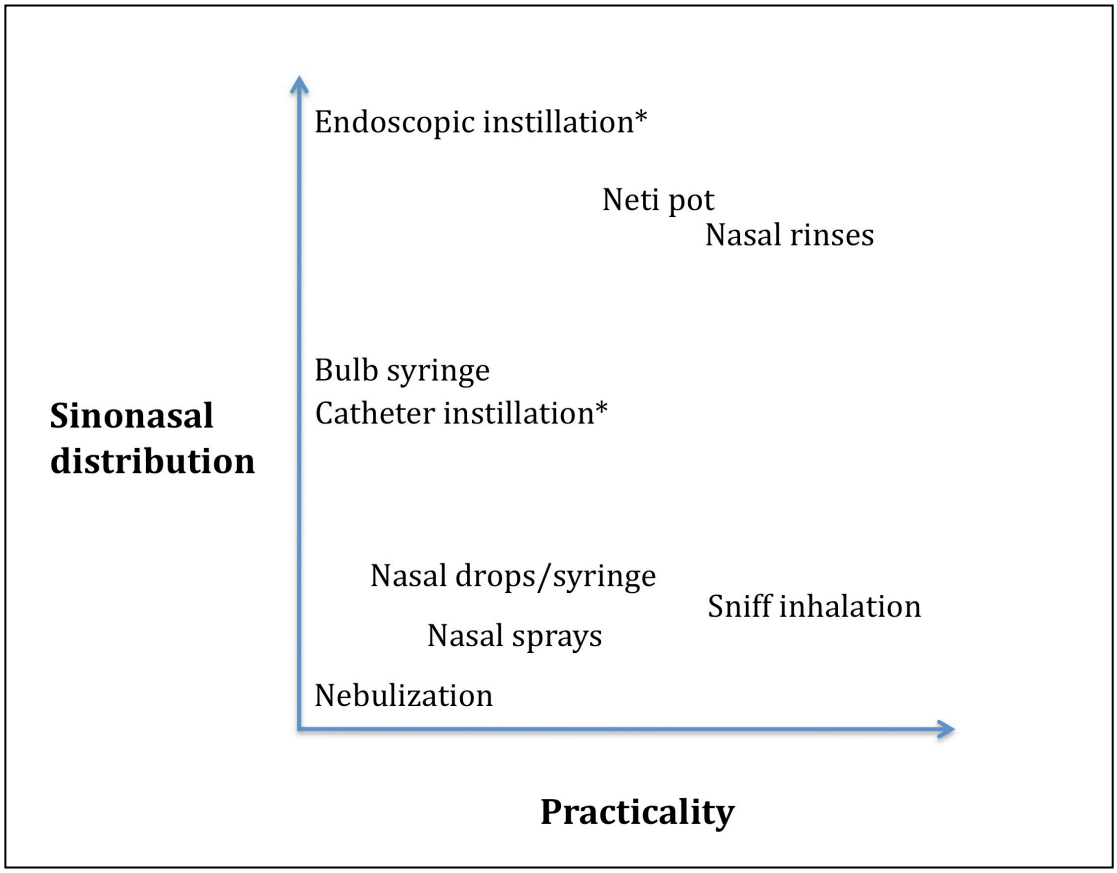

Figure 4. Relative sinonasal distribution versus the practicality (cost, cleaning, ease of technique) of various topical delivery techniques. *= sinonasal distribution not previously studied

1.3.1 SINUS RINSE BOTTLE

Volume: 100-240mls

Sinus penetration:

Several authors have assessed the sinonasal penetration afforded by the sinus rinse bottle. Olson et al. used three techniques (sniff inhalation, nebulizer and sinus rinse bottle) to deliver CT-contrast to the sinuses in unoperated volunteers, and concluded that the plastic squeeze nasal rinse bottle was the best delivery method[71]. Valentine et al. performed a sinus penetration study in maximally-dissected cadavers, and found that the rinse bottle superior compared to nebulization[202]. In another cadaver study, Harvey et al. demonstrated that the rinse bottle afforded only slightly inferior sinus penetration compared to the Neti-pot, and was far superior compared to an atomization spray[57]. The authors felt that in the clinical setting, however, any distribution differences between the sinus rinse bottle and the Neti-pot were likely to be minimal, and further postulated that

the rinse bottle might have an additional mechanical debridement advantage due to its high-pressure nature of delivery (also see below). Beule et al. have demonstrated that positioning is important when using this technique, having found that adopting the vertex-to-floor position maximized delivery to the frontal sinuses[203]. Singhal et al. also favour positions in which the head is tilted forward from a vertical plane in order to maximize frontal sinus penetration[204]. Additionally, Harvey et al. and Grobler et al. have demonstrated that penetration is maximized with ESS[57], when the sinus ostia are greater than 3.95mm in area[195].

Practicality of use:

Sinus rinse bottles consist of a squeezable plastic bottle with a removable plastic nipple that inserts into the nasal vestibule during use. Most devices also contain a plastic straw that allows for the bottle contents to be fully expelled from the nipple during use in an over-the-sink position. These three components can be cleaned individually, although the narrow nature of the straw makes this slightly more challenging. It is important to remember to remove the straw when using the vertex-to-floor position advocated by Beule et al.[203], as the bottle becomes inverted during rinsing in this position and will not expel solution with the straw *in situ*. Additionally, the vertex-to-floor position is more difficult to perform than the over-the-sink position; formal studies, however, are required in order to properly quantify this difference.

1.3.2 NETI-POT

Volume: 240mls

Sinus penetration:

Originally part of the Yogic and Ayurvedic traditions, the Neti-pot (Figure 5)is a centuries-old nasal irrigation device[196]. In their cadaver study, Harvey et al. found the Neti-pot to be a better delivery device that the sinus rinse bottle when a head-over-sink position was adopted. The authors did conclude, however, that reflex velopharyngeal closure in live patients using sinus irrigation (obviously not reproducible in the cadaver) might mean that there is actually little difference between the two devices. Additionally, as the Neti-pot is a gentle, 'slow-flow' device, any potential mechanical debridement effect that might be seen with high-

pressure devices is not present with Neti-pot use. Again, whilst ESS increases the sinus penetration with the Neti-pot[57], it is not know whether particular delivery positions with this device are better than others.

Practicality of use:

The Neti-pot interior can easily be accessed for scrubbing by simply removing the lid; however, the narrow neck and head of the spout makes accessing this particular area difficult. Whether or not the Neti-pot is clinically more comfortable or easy to use compared to other delivery modalities has not been previously assessed.

Figure 5. The Neti-pot. Image courtesy of the Department of Otorhinolaryngology Head & Surgery, The Queen Elizabeth Hospital, Adelaide.

1.3.3 BULB SYRINGE

Volume: 3oz/89mls

Sinus penetration:

Using a blue-dye solution, Miller et al. examined the sinus distribution of a number of delivery devices in patients > 6 weeks post ESS[194]. Bulb syringe, atomization,

nasal spray and nebulization were trialed. Independent observers scored the dye penetration to a number of regions, however penetration of the sphenoid and frontal sinuses were not assessed. The bulb syringe was superior to nebulization with regard to penetration of the ethmoid region and maxillary sinuses, and superior to the spray & atomization devices with regards to ethmoid penetration. Studies assessing the effect of patient positioning on bulb syringe distribution have not been performed.

Practicality of use:

The bulb syringe consists of a hollow rubber bulb with a fixed cannula projection. The undetachable nature of the cannula ensures that cleaning of the inner surface of the bulb (and the lumen of the cannula) is extremely difficult- scrubbing is impossible. Williams et al. demonstrated how bulb syringes can easily become contaminated with bacterial pathogens under normal use conditions[205]. Similar results have also been reported by Patel et al[206].

1.3.4 NEBULIZATION

Volume: <10mls

Sinus penetration:

Nebulization is the process by which a gas is used to break up a medication (usually initially a liquid or a powder) into a fine mist for inhalation. Various nebulization devices have been trialed in the rhinology literature. Negley et al[70], Olson et al.[71], and Wormald et al.[72] all assessed the sinonasal penetration of a continuous nebilization device using either radiological or radionucleotide imaging. Whilst the studies by Olson et al. and Negley et al. used healthy unoperated volunteers, Wormald et al. the nebulizer to be inferior in delivering treatment to the maxillary sinus and frontal recess in post-ESS CRS patients when compared to lavage with a 5ml syringe (performed in the mecca position, see Chapter 1.3.7). Subsequently Valentine et al. assessed a pulsed nebulizer to be an inferior delivery device[202]. Similarly, Hwang et al. evaluated two different nebulizer devices in both healthy volunteers and post-ESS patients and concluded that both afforded minimal sinus penetration only[207]. Very recently, Manes et al. evaluated a powered nebulizer in maximally dissected cadavers, and

demonstrated that satisfactory maxillary, frontal and sphenoid sinus penetration could be achieved- but only after EML had been performed[208]. Studies assessing the effect of patient position on nebulization distribution have not been performed, although devices that emit a finer mist have been shown to have better sinus penetration compared to those that have a larger particle size[209].

Practicality of use:
The nature of the nebulization device, with various attachable parts and lengths of tubing, makes cleaning difficult but possible. The monetary costs involved with nebulization (ie. purchasing, maintenance and replacement), however, are considerably greater than that of the simpler treatment modalities.

1.3.5 SNIFFING INHALATION

Volume: <1ml
Sinus penetration:
The sniffing inhalation method involves filling a cuffed hand with solution, occluding the contralateral nostril and then inhaling the solution with maximal inspiratory effort. This technique was commonly prescribed following endoscopic surgery in the past, however more recently has been superseded by other methods. Surprisingly, Olson et al. found sniffing inhalation to be superior to nebulization and only slightly inferior to the rise bottle with regards to overall sinus penetration in healthy un-operated volunteers[71].

Practicality of use:
Promisingly, one study has found this technique to be similarly well tolerated as rinse bottle irrigation[210]. Additionally, sniffing inhalation is highly practical as there is no apparatus that must be purchased; provided the hands are thoroughly clean pre-use, the risk of introducing contaminants to the sinonasal cavity is also probably very low.

1.3.6 NASAL SPRAYS

Volume: 0.05ml-20ml
Sinus penetration:

Sprays can be simple pump devices, pressurized or breath-activated (on maximal expiration). Atomization refers to sprays that deliver medication in a fine mist much like nebulization; the difference being that atomized sprays deliver a pressurized mist, whilst nebulization involves mainly voluntary inhalation. Nasal spray devices work by placing solution (contained in a bottle or canister) under pressure, and then allowing solution to escape down a pressure gradient via a small opening. The pressure in a simple nasal spray bottle is temporarily increased above normal atmospheric pressure by a pump action (allowing solution release via a nozzle which is always open), whilst solution in an atomizer canister is housed at a constant supra-atmospheric pressure (allowing solution release when a nozzle is temporarily opened; the longer the nozzle is opened, the greater the volume of solution released). Breath-activated device delivery is driven by the forced expiration of air against a closed soft palate, which expels air through the device mouthpiece and into the device proper; contained solution is thus aerosolized and released into the nasal cavity[211]. Nasal sprays are comparable to drops in their ability to reach the middle meatus[189, 192, 212], afford similar sinus deposition to nebulizers[207], but are substantially inferior to the large volume devices (Neti-pot[57], sinus rinse bottle[57], bulb syringe[194]). Although breath-activated devices have been shown to be slightly superior to simple pump devices[211], these are likely to be similarly inferior to the larger volume rinse devices, although head-to-head studies have not been performed.

Practicality of use:

Nasal sprays are relatively simple to use. Whilst spray bottles tips can become contaminated with bacteria, device design seems to prevent contamination of the contained solution[213]. Decontaminating the bottle tip, therefore, is all that is required in order to prevent cross-contamination.

1.3.7 NASAL DROPS/SYRINGE

Volume: 0.05ml-10ml

Sinus penetration:

Direct instillation of solution into the nasal cavity via a modified Pasteur pipette device or syringe is simple and inexpensive. The challenge with this approach is

ensuring the treatment solution arrives at the sinus ostia, rather than draining directly anteriorly out the nose or down into the nasopharynx. Various eponymous positions have been proposed in order to maximize the penetration of nasal drops to the middle meatus/sinuses; some even require multiple changes of position[190, 212, 214, 215]. The inspiration for such techniques likely stem from the Proetz method, a technique that uses a combination of gravity and negative pressure to replace sinus contents with instilled medication via a series of positioning maneuvers[216]. Raghavan and Logan preferred their 'Ragan' positioning to the mecca (more middle meatus penetration) and Mygind (more comfortable) positions, although their study was performed on a non-operated cadaver[190]. Merkus et al. performed a study in healthy volunteers, and concluded that not one of several positions was superior to any other in affording middle meatus penetration[193]. Whilst Wormald et al. felt installation via a 5ml syringe was superior to nasal sprays[72], most other authors feel nasal drops and sprays afford comparable middle meatus/sinus delivery[189, 192, 212].

Practicality of use:

Using a Pasteur pipette-type device or a syringe to deliver treatment is possible without contacting the skin if the nasal alar or engaging the nasal vestibule. With careful technique, therefore, nasal soiling of the delivery device is easily prevented and as a result cleaning may not be necessarily required. Some delivery positions are better tolerated and easier to perform (neck extended, head back position) compared to more difficult techniques (mecca position)[197].

1.3.8 CATHETER INSTILLATION AND ENDOSCOPIC INSTILLATION

Volume: >20mls

Sinus penetration:

Catheter instillation can be performed using the Yamik device or alternatively under direct endoscopic guidance. The Yamik catheter (Figure 6) occludes the nasal vestibule and choana with two inflatable cuffs, allowing both drainage of the sinus contents and delivery of treatment by alternating negative and positive pressure[217, 218]; it is similar in nature, therefore, to the Proetz method. Whilst proponents of the Yamik method have not performed sinus penetration studies,

direct endoscopic instillation theoretically allows for complete sinonasal mucosal coverage (provided ESS has been performed).

Practicality of use:

Whilst the Yamik technique has been used clinically in both the pre- and post-ESS settings[152, 218], there are a number of barriers to widespread clinical applicability. Primarily, the device is invasive and requires placement by a Rhinologist familiar with the device for each use. Additionally, the device may be uncomfortable and compliance may be affected as a result[218].

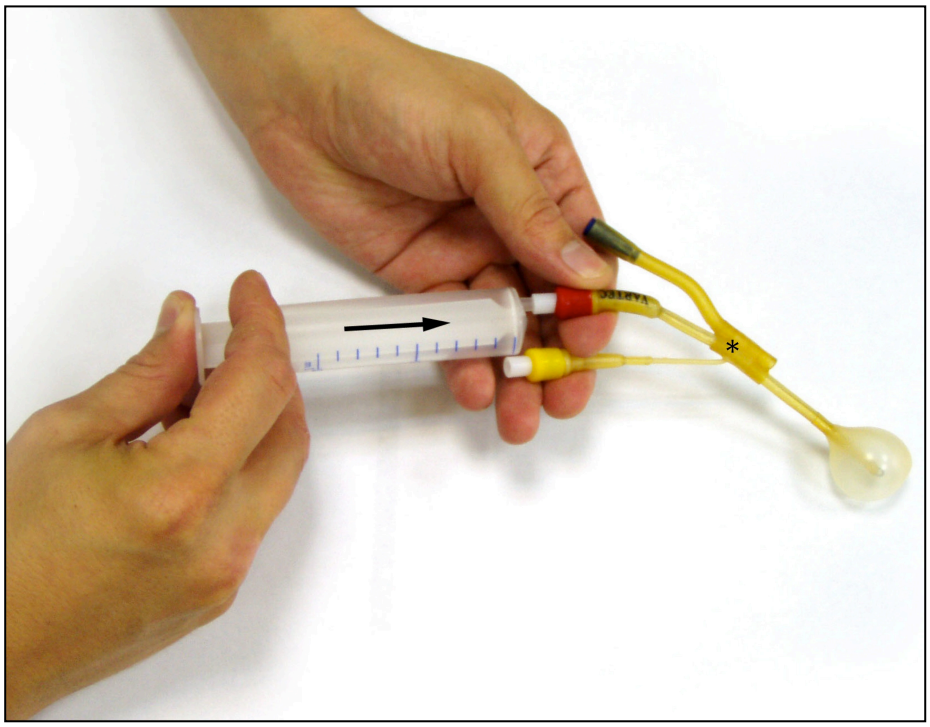

Figure 6. The Yamik catheter device. Once the nasal vestibule balloon (*) is inflated (black arrow), the contents of the sinonasal cavity can be altered by delivering treatment or removing secretions via the yellow and blue ports. (Image adapted from www.yamik.ru).

Alternatively, sinus contents can simply be suctioned and treatments instilled under direct endoscopic vision in patients post-ESS. Regardless, a consultation with a Rhinologist is required for both techniques, making any treatment requiring catheter instillation relatively cost- and resource-ineffective and therefore less desirable.

1.3.9 GENERAL DEVICE CONSIDERATIONS

It is important to ensure that whatever treatment is introduced into the nasal cavity is as sterile as possible. The two sources of possible contamination are the solution itself and the device containing the solution. Firstly, nasal irrigations should be used with water that has been pre-boiled- before the addition of salts- in order to kill and potential pathogens that may be inadvertently present. Tap water delivered fresh and without pre-boiling has very recently been associated with two deaths in the United States due to amoebic encephalitis with *Naegleria fowleri*[219]. Secondly, as previously mentioned, keeping the delivery device clean and pathogen-free appears to be important as device-to-host contamination is possible[200].

1.4 CHAPTER ONE: SUMMARY AND STUDIES TO BE PERFORMED

Summary

It is evident that CRS is a common condition with a relatively poorly understood, multifactoral aetiopathogenesis. Despite surgical intervention, clinically-significant disease persists in a subset of patients. Unfortunately, treatment protocols in these recalcitrant patients are lacking.

The recent appreciation of *S. aureus* biofilms as a predictable marker (and potential cause) of surgically-recalcitrant disease, however, has opened the door to new and exciting treatment options for these clinically-challenging patients. Although CRS is acknowledged as a multifactorial disease, insights gained from the study by Uren et al. suggests that aggressively treating a singular aetiopathogenic factor (bacteria, specifically *S. aureus*) in a subset of recalcitrant patients (those with culturable *S. aureus*, and therefore presumed underlying dispersed biofilm) may be efficacious where other treatments have failed. In addition, satisfactory treatment of the *S. aureus* bioburden in these patients may have the added benefit of reducing the risk extra-nasal *S. aureus* infection. In order to maximize the potential success of this singular treatment strategy, it is evident, therefore, that several key criteria that should be met:

Criterion #1.
The ideal treatment agent should have activity against *S. aureus* in biofilm form. If purely a biofilm dispersal agent, then this treatment agent should be delivered in conjunction with a traditional antibiotic or antiseptic.

Criterion #2.
The ideal treatment agent should have a satisfactory safety and efficacy profile for topical use in the human sinonasal cavity

Criterion #3.
The ideal treatment agent should be able to be delivered by high-volume nasal rinse devices (such as the rinse-bottle or Neti-pot), as these devices have been shown to afford superior sinonasal delivery compared to other methods.

In addition, whilst the optimal timing of treatment is assumed to be when the patient reveals herself/himself to have not responded to ESS (and 'maximal medical therapy' again fails), a better understanding of the biofilm response to surgery is required. If this response indeed involves active dispersal, then the post-ESS period may represent an ideal, serendipitous anti-biofilm treatment window

<u>Studies to be performed</u>

Mupirocin

As mentioned elsewhere, mupirocin nasal rinses in recalcitrant *S. aureus* culture-positive CRS have been suggested as efficacious in two small cohort studies. Aside from the uncontrolled nature of these studies, two major criticisms can be made; neither study reported, firstly, patient outcomes long-after treatment is ceased (ie. is there a rebound effect following cessation?) or secondly, post-treatment culture sensitivities (ie. is mupirocin-resistance induced by treatment?). The former question is addressed in 12.2 and the latter in 12.1.

Manuka Honey

In 13.1, we evaluate Manuka honey as a potential antimicrobial for topical delivery in recalcitrant *S. aureus* CRS. Manuka honey is an attractive potential agent,

possessing not only a high anti-staphylococcal activity, but also an ideal resistance profile. In fact, microbial resistance to Manuka honey has never been shown nor able to be induced.

Biofilm behavior following sinus surgery

Having previously suspected that *S. aureus* biofilms may disperse in the early post-ESS period, we evaluate this hypothesis in 14.1. As previously mentioned, biofilm dispersal represents a timely, ideal treatment opportunity. In contrast to the *treatment agent* evaluations presented elsewhere is this thesis, this observational study aims to evaluate whether or not this post-operative window may indeed represent a serendipitous, ideal *treatment time.*

Chapter Two: An Evaluation of Mupirocin

2.1 MICROBIOLOGICAL OUTCOMES FOLLOWING MUPIROCIN NASAL RINSES FOR SYMPTOMATIC, *STAPHYLOCOCCUS AUREUS*-POSITIVE CHRONIC RHINOSINUSITIS FOLLOWING ENDOSCOPIC SINUS SURGERY

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Jervis-Bardy, J.

Project design, data collection and analysis, manuscript preparation

Wormald, P.J.

Project design, manuscript preparation

By signing this document, I (the co-author) hereby acknowledge these to be accurate descriptions of the contribution I made to this paper and give permission for it to be included in the candidate's thesis.

2.1.1 ABSTRACT

Background

Persistent infection following endoscopic sinus surgery (ESS) for Chronic Rhinosinusitis (CRS) is a frustrating entity for the patient and Rhinologist alike. Mupirocin nasal washes been proposed as an efficacious treatment in such patients. Two small studies have reported excellent short-term post-treatment outcomes, however the long-term microbiological outcomes following treatment are not known; likewise, the rate of mupirocin-resistance following treatment has not been explored.

Methods

This was a retrospective chart review of 61 patients with *S. aureus*-positive surgically-recalcitrant CRS having undergone 0.05% mupirocin nasal rinse treatment, twice-daily for 4 weeks. Specific outcomes reported included post-treatment culture results, time to first post-treatment *S. aureus* culture, and mupirocin-sensitivity following treatment.

Results

Of 57 patients meeting minimal post-treatment follow-up criteria, 42 (73.7%) progressed to microbiological failure by subsequently cultured *S. aureus*. Mean time to first positive culture was 144 days. Of the 42 patients who progressed to microbiological relapse, full antibiotic-sensitivity data was available for 41; of these, only 1 was found to subsequently harbor a mupirocin-resistant strain of *S. aureus* thus yielding a post-treatment resistance rate of 2.4%.

Conclusion

Treatment with mupirocin nasal washes in *S. aureus*-positive, surgically recalcitrant CRS has a high microbiological failure rate, with 73.7% of patients subsequently re-culturing *S. aureus*. Our current treatment regime of 0.05% nasal washes twice-daily for 4-weeks is associated with a post-treatment resistance rate that is consistent with other studies of topical mupirocin use, suggesting that

mupirocin washes are no more likely to induce resistance than nasal vestibule decolonization in the high risk medical or surgical patient.

2.1.2 INTRODUCTION

Persistent infection following endoscopic sinus surgery (ESS) for Chronic Rhinosinusitis (CRS) is a frustrating entity for the patient and Rhinologist alike. The high culture-rate of *S. aureus* amongst surgically-recalcitrant patients[220, 221] strongly suggests a contributing role for this persistant aerobe. Multiple virulence mechanisms such as superantigen production[222], biofilm formation[223], and intramucosal residence[224] enable this organism to not only precipitate an altered immune response but also evade conventional antibiotic therapy and potentially contribute to post-surgical recalcitrance.

Numerous topical agents, targeting not only the free-living bacteria but also the supposed biofilm nidus, have been proposed. Our group has recently published a promising pilot study of mupirocin nasal rinses in *S. aureus* culture-positive post-ESS patients[42]. Similarily, Solares et al. treated patients with MRSA-associated exacerbations of CRS and reported subsequent culture-proven MRSA *symptomatic* persistence following mupirocin in only 1/21 patients (4.8%)[68]. Despite these encouraging results, however, the long-term outcomes following topical mupirocin have not previously been reported.

Furthermore, mupirocin-resistance is a growing community concern. First reported in 1987[225, 226], mupirocin-resistance is thought to be low in the community setting[227], however in selected populations resistance-rates of up to 13.2% have recently been reported[228]. A proportional relationship between community/institution rates of mupirocin use (as a cream or ointment) and resistance has been demonstrated by a number of authors in various population groups[137, 229, 230]. Only Caffrey and colleagues, however, have been able to demonstrate a direct relationship (between previous use and subsequent resistance) on an individual patient level[231]. Resistance following treatment with mupirocin rinses in CRS, however, has not previously been explored.

This study, therefore, was designed to assess firstly the long-term re-culture rate of sinonasal *S. aureus* following treatment with mupirocin and secondly to assess the rate of mupirocin-resistance following treatment.

2.1.3 MATERIALS AND METHODS

This was a retrospective case-note review of surgically-recalcitrant, *S. aureus*-positive patients who were treated with one or more courses of 0.05% w/v mupirocin nasal rinses, twice-daily for one month. This study was approved by our local IRB. Mupirocin was prescribed where there was ongoing *S. aureus* infection in a symptomatic, post-ESS patient despite patent sinus ostia. Data was pooled from patients completing the previously-published pilot study between July 2006 and July 2007[42] (drug delivery via a 200ml nasal rinse) and from subsequent patients (drug delivery via a 10ml nasal syringe) treated between August 2008 and June 2010. Although high-volume, low-pressure nasal-rinse delivery offers superior sinonasal distribution[203], the 10ml syringe protocol was adopted following the pilot study to limit the financial burden on the patient. Patients from the published pilot study[42] were followed-up immediately following completion of the treatment course, with an endoscopically-guided 'surveillance' swab sent in all cases at this visit regardless of clinical indication. At subsequent clinic visits, swabs were taken depending on clinical merit and only where evidence of infection was observed. For patients in the syringe group, however, an immediate post-treatment clinic assessment was not routinely performed, as they were not part of any trial. In these patients, an endoscopically-guided swab for microscopy, culture and sensitivities was only sent at the first post-treatment visit if there was no observable improvement in symptoms and/or endoscopic appearance. Likewise, at subsequent clinic visits swabs were taken depending on clinical merit and only when the patient complained of symptoms of sinus infection (nasal obstruction, rhinorrhea, post nasal drip, facial pain and loss of sense of smell) and the treating surgeon endoscopically viewed evidence of infection. Mupirocin resistance/sensitivity was determined for all *S. aureus* cultures using standard microbiological techniques.

All patients who received mupirocin for symptomatic infection following ESS were included if *S. aureus* was culture-proven by a positive swab within 21 days prior to the start of treatment.

Where patients received multiple courses of mupirocin during the study period, the time to specific outcome (re-culturing *S. aureus*, most recent clinic visit) was measured from the first day of the initial treatment, rather than any subsequent course. If patients were followed up on only one occasion and/or for less than 90 days following initiation of mupirocin therapy only demographic data was collected; these patients were not included in the final analysis.

Data analysis

Fisher's exact test was used for contingency testing.

2.1.4 RESULTS

Demographic data

61 patients (26 females, 35 males) met the inclusion criteria during the study period, including 16 patients from the original pilot study. Mean age was 56.5 years (range: 27-83). As demonstrated in table 1, a high percentage of patients had previously been known to have nasal polyposis and/or histologically-proven eosinophilic mucin (EM), reflecting the extreme disease severity seen in our practice. Demonstrated also in Table 3, a high percentage of patients were previously found to be *S. aureus* culture-positive at the time of their most recent surgery; we have previously demonstrated the post-ESS persistence of *S. aureus* detected intra-operatively[84, 232]. 57/61 patients were seen in clinic at least twice following treatment with at least one visit being outside the 90-day window and were included in further analysis; the mean time from start of treatment to most recent clinic review of these patients was 748 days (range: 96-1631) demonstrating the long-term nature of follow-up.

Table 3. The percentage of patients previously known to have nasal polyposis, eosinophilic mucin, and/or a previous intra-operative *S. aureus* culture amongst those included in this study.

	Nasal Polyposis?	Histologically-proven EM?	*S. aureus* cultured at surgery?
Yes	48 (78.7%)	31 (50.8%)	34 (55.7%)
No	12 (19.7%)	13 (21.3%)	17 (27.9%)
Unknown	1 (1.6%)	17 (27.9%)	10 (16.4%)

Re-culture rate

First visit

Mean time from start of treatment to first follow-up visit was 42 days (range 15-126). A swab was taken at this visit in 37/57 (64.9%) patients; of these swabs, 17/37 (45.9%) were positive for *S. aureus*.

All visits

Post-treatment swabs were taken from 52/57 (91.2%) patients, at a mean time of 98 days (range: 20-611) following start of treatment. Of these 52 patients, clinically-indicated swabs were taken from 48 (92.3%) whilst the remaining 4 patients never required a clinically-indicated swab but did have a single post-treatment 'surveillance' swab sent only as part of the pilot study. 5/57 (8.8%) of patients were infection-free at all post-treatment visits and hence did not have a swab sent at any visit.

In all, 42/57 (73.7%) patients subsequently cultured *S. aureus* following mupirocin treatment at one or more post-treatment visits. In all patients culturing *S. aureus* post-treatment, at least one of these swabs was taken from a non-surveillance visit and in the setting of infection. Mean time from start of treatment course to first positive *S. aureus* culture was 144 days (range: 20-561). The cumulative percentage of patients progressing to post-treatment microbiological failure is seen in Figure 7; 40/57 (70.0%) of patients had failed treatment within 1 year.

15/57 (26.3%) of patients, therefore, did not have *S. aureus* cultured from their sinonasal cavity following treatment. Of these, no post-treatment swab was sent in 5/15 (33.3%) as there was no clinical indication. A further 4/15 (26.7%) were from the pilot study; these patients had the immediate post-treatment surveillance swab as their only post-treatment microbiological assessment (as outlined above). The remaining 6/15 (40%) of patients had swabs send on clinical grounds following treatment however did not culture *S. aureus*. Overall, the mean time from start of treatment course to most recent clinic review in the 15 negative patients was 705 days (range: 191-1631). There was no difference in the rates of microbiological failure between patients from the pilot study (12/16, 75.0%) and the subsequent syringe cohort (30/41, 73.2%; p=1.00, Fisher's exact test).

Resistance rate

Of the 42 patients who progressed to microbiological failure, full antibiotic-resistance data was available for 41. Of these, only 1 was found to subsequently harbor a mupirocin-resistant strain of *S. aureus*, thus yielding a post-treatment resistance rate of 2.4%. This patient was a 65 year-old female with a history of ongoing infection following ESS (bilateral complete sphenoethmoidectomy, middle meatal antrostomy and frontal recess clearance) two years previously. Despite culturing a sensitive strain 5 months after the initial 4-week treatment regime, it was following a second course of mupirocin (12 months after the first) that a resistant strain of *S. aureus* was first isolated. 200 µg disc-diffusion susceptibility testing confirmed high-level resistance[233]. The most-recent *S. aureus* strains isolates from the other 40/41 (97.6%) patients were all mupirocin-sensitive.

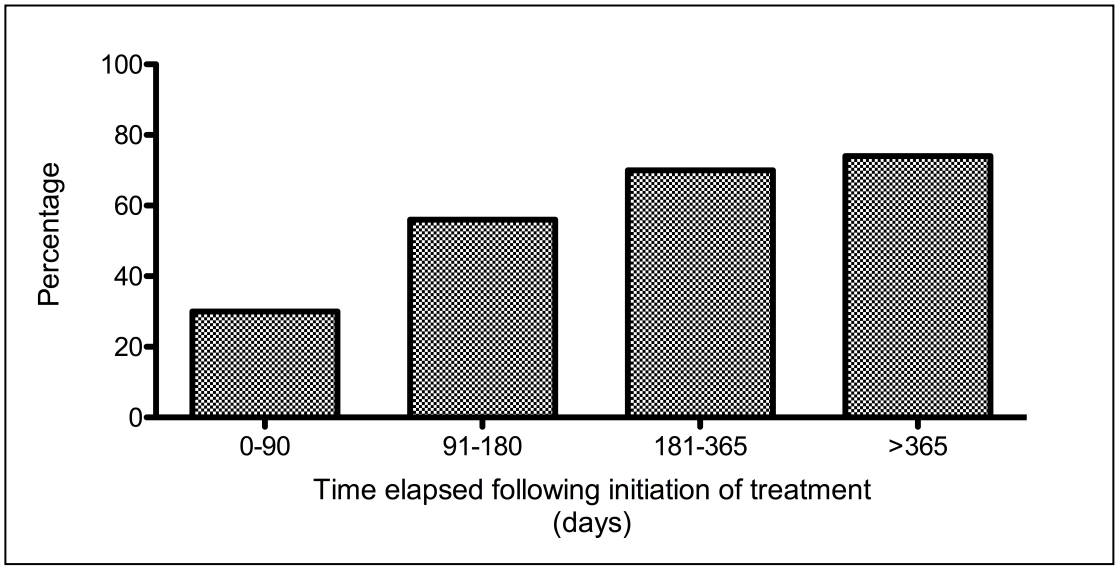

Figure 7. The cumulative percentage of patients progressing to post-treatment microbiological failure following topical mupirocin.

2.1.5 DISCUSSION

In this study we have shown that microbiological failure following mupirocin nasal rinses in *S. aureus*-positive, surgically recalcitrant CRS is 73.7%. The rate of resistance following treatment is 2.4%.

Mupirocin in CRS

Various topical treatments for surgically-recalcitrant CRS have been proposed in the literature[73, 140, 154, 234]. Such agents treat with an 'anti-biofilm' intent, reflecting the hypothesised role of biofilms- and in particular *S. aureus* biofilms- in the pathogensis of CRS[39, 188, 235]. Mupirocin has been demonstrated in vitro to be able to reduce the biomass of mature biofilms by >90%[139] and on the strength of two separate open-label studies (Solares et al. in 2006[68] and subsequently Uren et al. in 2008[42]) has become a popular anti-biofilm treatment in recalcitrant CRS.

Microbiological failure

As a topical agent, mupirocin nasal vestibule decolonization has recently been shown to decrease hospital-acquired infections in high-risk patients[119]. Whilst mupirocin decolonization is successful in the short-term, high long-term failure rates have been reported[236-238]. In this study, as patients were not routinely followed-up at the immediate cessation of treatment, it is not possible to

determine whether subsequent culture of *S. aureus* represented persistence of bacteria throughout and following treatment or rather microbiological relapse following a period of satisfactory 'sterility'. Hence, all patients culturing *S. aureus* post-treatment were simply deemed to have failed treatment.

There are a number of potential reasons that may explain the high rate of microbiological failure seen in our patient cohort. Firstly, whilst mupirocin may eradicate a large proportion of the biofilm biomass, any surviving bacteria following subtotal eradication may simply regenerate biofilm following treatment. Secondly, without mucosal penetration of antibiotic, intracellular and/or interstitial *S. aureus* can theoretically serve to regenerate any extra-mucosal biofilm nidus. The efficacy of topical mupirocin against intracellular and interstitial *S. aureus* is not known. Thirdly, whilst certain delivery techniques allow penetrance of solution into and through the post-ESS sinus ostia[195], complete sinus mucosal coverage is impossible; hence bacteria in difficult access areas (such as within the frontal sinus) are unlikely to receive treatment. Lastly, the post-treatment nose can be re-colonizised by both extra-nasal and extra-corporal bacteria[239, 240].

Microbiological failure, however, cannot be viewed as absolute treatment failure. Regardless of subsequent culture result, some patients anecdotally report long-term symptom improvement after mupirocin treatment. In the post-ESS period, however, as *S. aureus* culture is associated with poorer outcome measures[232], it is likely that many patients re-culturing *S. aureus* also report a relapse of symptoms. As objective and subjective outcome measures were not collected in this study, however, the effectiveness of topical mupirocin treatment – and the relationship between a positive culture and patient symptoms - can only be inferred.

<u>Mupirocin resistance</u>

High-level mupirocin resistance is mediated by the MupA plasmid[241] whereas low-level resistance is due to mutations in the isoleucyl RNA synthetase gene, *ileS*[242]. Where mupirocin usage has been poorly controlled and regulated in the past very high community-wide resistance-rates have been observed. For instance, in New

Zealand in 1999 the rate of resistance was 28%; this was directly attributable to the over-the-counter availability of mupirocin in the decade prior[137]. Responsible drug prescription yields much lower rates of resistance; in Western Australia mupirocin resistance-rates amongst MRSA isolates dropped from 15% to 0.3% following adoption of strict Health Department guidelines[229]. On an individual patient level, two studies have examined the rate of induced resistance post treatment. Raz et al. reported 1/34 (2.9%) patients developed resistance following varied courses of intranasal mupirocin ointment[135]. In a similar study of 199 US Army personnel, a single 5-day course of ointment did not subsequently precipitate a single mupirocin-resistant strain[136]. The rate of resistance in our cohort (2.4%) is similarly low. In addition to recent mupirocin treatment, our isolated case of mupirocin-resistance also had a history of pseudomonas infection (sinonasal, treated with tobramycin nasal rinses) within the previous 12 months; both features have been reported as risk factors for developing mupirocin resistance by Caffrey et al.[231]

2.1.6 CONCLUSION

Treatment with mupirocin nasal rinses in *S. aureus*-positive, surgically recalcitrant CRS has a high microbiological failure rate, with 73.7% of patients subsequently re-culturing *S. aureus*. Our current treatment regime of 0.05% nasal rinses twice-daily for one month is associated with a post-treatment resistance rate that is consistent with other studies of topical mupirocin use, suggesting that mupirocin nasal rinses are no more likely to induce resistance than nasal vestibule decolonization in the high risk medical or surgical patient.

2.2 A RANDOMISED TRIAL OF MUPIROCIN SINONASAL RINSES VERSUS SALINE IN SURGICALLY-RECALCITRANT STAPHYLOCOCCAL CHRONIC RHINOSINUSITIS

Jervis-Bardy, J., Boase, S., Psaltis, A., Foreman, A. & Wormald, P-J. (2012) A randomized trial of Mupirocin sinonasal rinses versus saline in surgically recalcitrant staphylococcal chronic rhinosinusitis.
The Laryngoscope, v. 122(10), pp. 2148-2153

Statement of Authorship

A randomised trial of mupirocin sinonasal rinses versus saline in surgically-recalcitrant staphylococcal chronic rhinosinusitis

Laryngoscope. 2012

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By signing this document, I (the co-author) hereby acknowledge these to be accurate descriptions of the contribution I made to this paper and give permission for it to be included in the candidate's thesis.

2.2.1 ABSTRACT

Background

Chronic rhinosinusitis recalcitrant to surgery is a frustrating clinical entity. Recently, mupirocin sinonasal rinses have been suggested as an efficacious treatment alternative in these patients where *Staphylococuccus aureus* infection is demonstrated. To our knowledge, how best to treat this *S. aureus* reservoir has not been previously evaluated in a double-blind randomised, placebo-controlled trial.

Materials and Methods

25 S.aureus-positive chronic rhinosinusitis patients with persistent sinonasal infection despite endoscopic sinus surgery received either a one-month, twice-daily treatment course of mupirocin sinonasal rinses (MUP) or saline rinses (CON). The primary outcome was *S. aureus*-culture negativity at the conclusion of treatment; secondary Rhinological outcomes included subjective and objective measures of rhinosinusitis.

Results

22 patients satisfactorily completed the treatment period. 0/13 (0.0%) of CON patients returned a *S. aureus*-negative sinonasal culture at one month, compared to 8/9 (88.9%) of MUP patients ($p<0.01$). Improvements in Rhinological outcomes observed in MUP patients following treatment were not subsequently evident when these patients were followed up at a delayed assessment 2-6 months after completing treatment.

Conclusion

Mupirocin sinonasal rinses are an effective short-term anti-*S. aureus* treatment in surgically recalcitrant CRS as assessed by microbiological and selected Rhinological outcomes, although the latter improvements may not be durable with time.

2.2.2 INTRODUCTION

Chronic rhinosinusitis (CRS) has a estimated prevalence of up to 16% in the community[1] and at least 350,000 endoscopic sinus surgery (ESS) procedures are performed annually in the United States alone[243]. A subset of CRS patients, having first failed medical therapy, subsequently fail ESS by progressing to a disease state in which the wide-open sinonasal mucosal cavity remains chronically infected, with mucosal oedema and mucopurulence evident. In our experience, *Staphylococcus aureus* is the most commonly identified organism by standard culture in these recalcitrant patients. Accordingly, *S. aureus* is increasingly recognised as a critical disease-modifier CRS, with pathogenicity related to biofilm formation[223], intracellular residence[224], and superantigen production[222]. Not surprisingly, it is also the most commonly cultured bacteria following ESS[34]. Topical antibiotics have therefore been proposed as potentially efficacious in the surgically-recalcitrant patient[63]. This strategy should always be employed with an 'anti-biofilm intent', reflecting the relative antimicrobial resistance achieved by bacteria when in the biofilm form[124]. As satisfactory coverage of the infected sinonasal cavity cannot be achieved with simple ointments or sprays due to inadequate penetration into the sinuses[57, 203], there is a need for agents to be delivered as a solution via a nasal rinse device. Promisingly, two uncontrolled cohort studies have recently suggested that high-volume, low-pressure nasal rinses with mupirocin may be efficacious in the chronically infected, post-ESS patient, with excellent immediate post-treatment microbiological and clinical outcomes reported[42, 68]. To our knowledge, no placebo controlled, randomised trials have evaluated the efficacy of mupirocin rinses as treatment for recalcitrant CRS with *S. aureus*.

The aim of this study was to assess whether mupirocin rinses were effective in precipitating a *S. aureus* culture-negative sinonasal cavity in patients with chronic treatment-resistant sinonasal *S. aureus* infection. Post-treatment microbiology, therefore, was the primary outcome measure in this study. Rhinological outcomes (patient symptoms, examination findings and a quality-of-life assessment) following treatment were also assessed as a secondary, clinical measure.

2.2.3 MATERIALS AND METHODS

Participants

Full inclusion criteria are outlined in Table 4. Twenty-five patients were enrolled in the study (15 men and 10 women; median age 57.6 years). Baseline demographic and clinical characteristics are demonstrated in Table 5. The most recent surgical intervention in all patients was a combined bilateral middle meatal antrostomy, spheno-ethmoidectomy and frontal recess clearance, either with (14/25, 56.0%) or without (11/25, 44.0%) an endoscopic modified Lothrop (EML) procedure. Although most patients reported a previous history of nasal polyposis (21/25, 84.0%), in no patients were frank nasal polyps evident at the time of enrollment. The use of intranasal corticosteroid (INC) was not controlled for, as all patients had previously trialed INC and the majority were currently using either a steroid spray or rinse on enrollment (23/25, 92.0%). Those taking INC on enrollment were instructed to continue doing so throughout the duration of the study; likewise patients regularly taking antihistamine tables or sprays were advised to continue doing so. One of the two patients not currently taking INC was inadvertently started on one upon enrollment; this patient was not excluded as he had previously used a similar preparation in the past with minimal symptomatic improvement only.

Table 4. Inclusion and exclusion criteria. ESS= endoscopic sinus surgery; CRS=chronic rhinosinusitis.

Inclusion criteria	Exclusion criteria

• ESS no less than 12 weeks prior to enrollment
• Ongoing signs and symptoms of CRS despite at least one trial of oral antibiotics
• Open sinus ostia on endoscopic examination
• Positive *S. aureus* swab no more than 8 days prior to enrollment
  o No additional bacteria cultured
  o Sensitive to mupirocin and penicillin
	• Antibiotics in previous 2 weeks
	• Immunocompromised
	• Smoker
	• Less than 18 years of age
	• Pregnant
	• Taking oral corticosteroids
	• Penicillin and/or mupirocin allergy

Table 5. Baseline patient demographics and clinical characteristics. Data are medians (interquartile range) or numbers (%). EML= endoscopic modified Lothrop procedure, SNOT-20= 20-point sinonasal outcomes test, *= 1 patient with nasal polyposis status unknown, **= 2 patients with nasal polyposis status unknown.

	CON (n=14)	MUP (n=11)
Age in years	55 (41-64)	59 (47-71)
Sex (females)	5 (35.7%)	5 (45.5%)
History of nasal polyposis	12** (85.7%)	9*(81.8%)
Previous EML	7 (50.0%)	7 (63.6%)
Visual analogue score	28.5 (11.25-37.0)	22.5 (9.0-34.0)
SNOT-20 score	20.5 (8.8-29.5)	16.0 (11.0-37.0)
Lund-Kennedy endoscopic score	2.0 (2.0-6.0)	7.0 (3.0-8.0)

Study design

This was a prospective, double-blinded, placebo-controlled study conducted at the academic teaching hospitals in Adelaide, Australia. Ethics approval was granted by our local Institutional Review Board. Patients with ongoing signs and symptoms of CRS[3] despite complete ESS were recruited for this study between October 2009 and August 2011. Patients were randomised to either the mupirocin arm (MUP n=11) or control arm (CON n=14) and provided with an unlabelled 28-day treatment kit as outlined in Table 6. Unlabelled sachets containing 125mg mupirocin + a proprietary buffered salts blend (mupirocin arm [MUP]) or a proprietary buffered salts blend only (control arm [CON]) were provided by NeilMed Pharmaceuticals (Santa Rosa, CA). As part of the enrollment process, patients were informed that the treatment kits had been prepared in such a manner to ensure the MUP and CON kits were as similar as possible. Patients were instructed to heat tap water to boiling point, fill the rinse bottle to 240ml and dissolve the contents of sachets once the water had cooled to a tepid temperature. All patients were given Sinus Rinse bottles (Neilmed, Santa Rosa, CA) for use in the trial and were instructed to use one bottle for 2 weeks before discarding and using the second bottle. Patients were also instructed to clean the rinse bottle at least once per day with boiling water and detergent; these measures were an attempt to minimise the potential confounder of sinus bottle bacterial contamination[198, 200]. In

order to not completely withhold treatment from patients in CON, which would be ethically inappropriate, they were given a course of amoxycillin + clavulanic acid tablets, whereas patients in MUP were given a placebo (dextrose tablets).

Table 6. Contents of treatment kit.

Item	Instructions
56 sachets • 125mg mupirocin + salts (MUP) OR • Salts only (CON)	1 sachet dissolved in boiled water, cooled to a tepid temperature, twice per day.
40 tablets • Dextrose (MUP) OR • 500mg amoxicillin + 125mg clavulanic acid (CON)	1 tablet swallowed whole with food, three times per day.
2 Sinus Rinse bottles	Use one bottle for rinses twice per day for 2 weeks and then discard.

Pre-treatment assessment

Patients attended the clinic and completed a pre-treatment baseline questionnaire, which included routine demographic data and two subjective measurement tools: a modified 20-item Sino-Nasal Outcome Test (SNOT-20) quality-of-life index (20 items, each scored from 0-3; total score range 0–60) and a sinus visual analogue symptom (VAS) scoring pro forma[244] (summation of 5 individual symptom scores plus an "overall symptomatology score" to give a total score range 0–60). The sinonasal cavity was then examined endoscopically and digitally recorded for subsequent objective analysis and scoring (Lund-Kennedy (LK) endoscopic score[244]; score range 0-20).

Immediate post-treatment assessment

Following 28 days treatment, all patients returned to the clinic for assessment; the SNOT-20 and VAS battery was again completed, and a digital recording of the sinonasal cavity was conducted. In addition, an endoscopically-guided culture swab was taken in all patients and sent for routine microbiological analysis of *S. aureus*. Treatments instituted at this visit, if any, were made on clinical grounds only and were not standardised.

Delayed post-treatment assessment

Patients who returned a *S. aureus*-negative culture swab from the immediate post-treatment assessment returned to the clinic between 2 and 6 months following the immediate post-treatment visit for a delayed post-treatment assessment. At this visit the SNOT-20 and VAS battery were again completed, the sinonasal cavity was digitally recorded, and a culture swab taken for microbiological analysis of *S. aureus*.

At the conclusion of the trial, the digital examination recordings for all visits were analysed by a blinded, independent observer (S.B.) and scored endoscopically[244].

Data analysis

The results of this study were analysed using GraphPad Prism 5.0 software (San Diego, CA). Data were examined for normality prior to hypothesis testing. Comparisons between treatment groups (MUP vs. CON) were performed using Mann-Whitney *U* tests for unpaired continuous variables, and Fisher's exact test for count data. The pre- vs. immediate post treatment changes, and the immediate post-intervention vs. delayed post intervention changes were performed using Wilcoxon matched-pairs sign ranked tests for continuous variables and Fisher's exact test for count data.

Data are Median and interquartile ranges (IQR) unless otherwise specified. A p-value of <0.05 was considered significant.

Statistical power was calculated for the primary end-point of culture-negativity at the immediate post-treatment assessment. Power analysis estimates determined a sample size of 11 patients per group would be required to achieve statistical significance (80%, P=0.05) based on response rates of 25% and 90% in the CON and MUP groups respectively. The 90% response rate assumption for MUP was based on the results from the pilot study in which 15/16 patients (93.8%) of patients were culture-negative following treatment with mupirocin rinses. For CON, the 25% response rate estimation was based on the observation that chronically infected patients have a poor response to conventional therapy by

definition, often receiving multiple courses of oral antibiotics per year. This number may actually represent an over-estimation, given that the inclusion criteria for this study requiring culture-positivity despite at least one course of oral antibiotics.

2.2.4 RESULTS

Immediate post-treatment assessment

As outlined in Figure 8, two patients from MUP were lost to follow-up prior to the immediate post-intervention assessment; one developed a unilateral otomastoiditis (on a background of previous chronic suppurative otitis media necessitating modified radical mastoidectomy) requiring intravenous antibiotics, whilst the other became unwell with viral gastroenteritis and declined follow-up due to general malaise. One patient from CON was given additional oral antibiotics by his general practitioner and was excluded from analysis.

Mupirocin vs. control analysis

S. aureus negative cultures were returned from 8/9 (88.9%) of mupirocin patients, compared to 0/13 (0.0%) placebo controls (Figure 9; p=<0.01).

The LK endoscopic score change from baseline was significantly different between groups (MUP -4.0 [-7.0 to -1.3] vs. CON 3.0 [-1.0 to 4.0], p<0.01; Figure 10). The change from baseline in the SNOT-20 scores approached significance (MUP -7.0 [-10.0 to -1.0] vs. CON -1.0[-4.5 to 3.0], p=0.06), whilst the VAS scores were not significantly different (MUP -6.0 [-10.0 to -3.5] vs. CON -1.0 [-7.5 to 1.0], p=0.17).

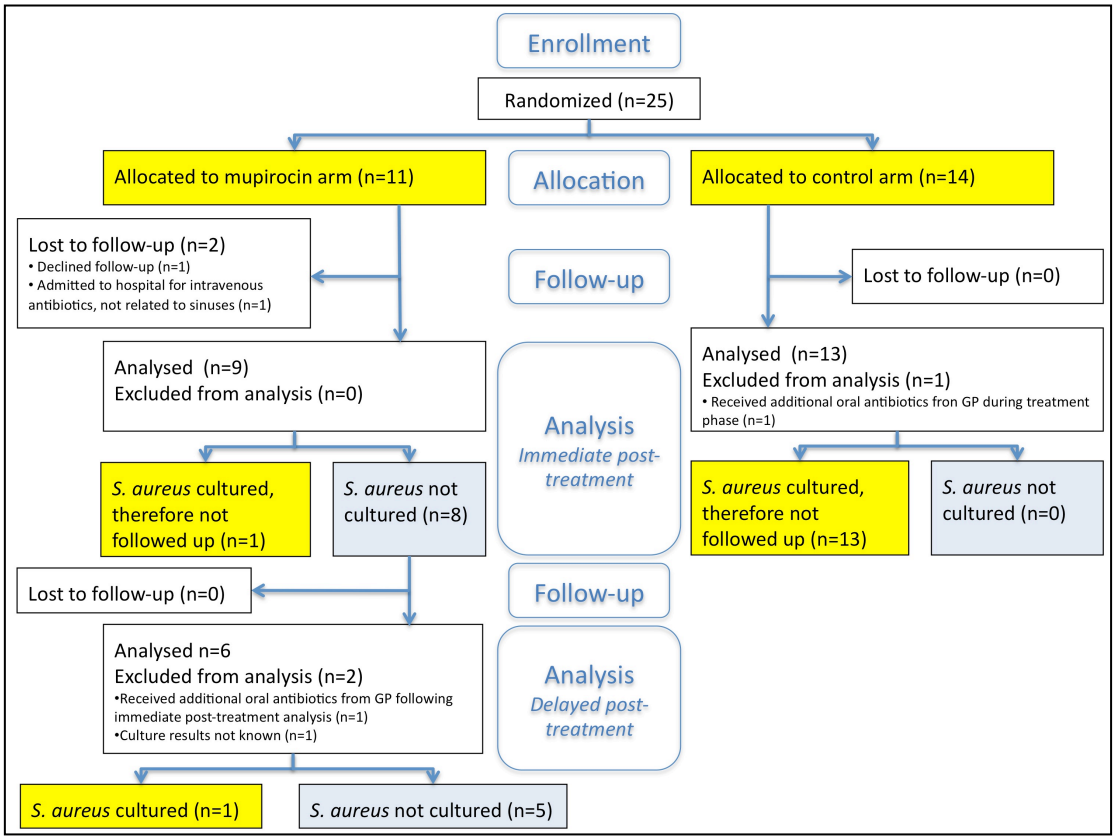

Figure 8. Flow chart from enrollment to analysis.

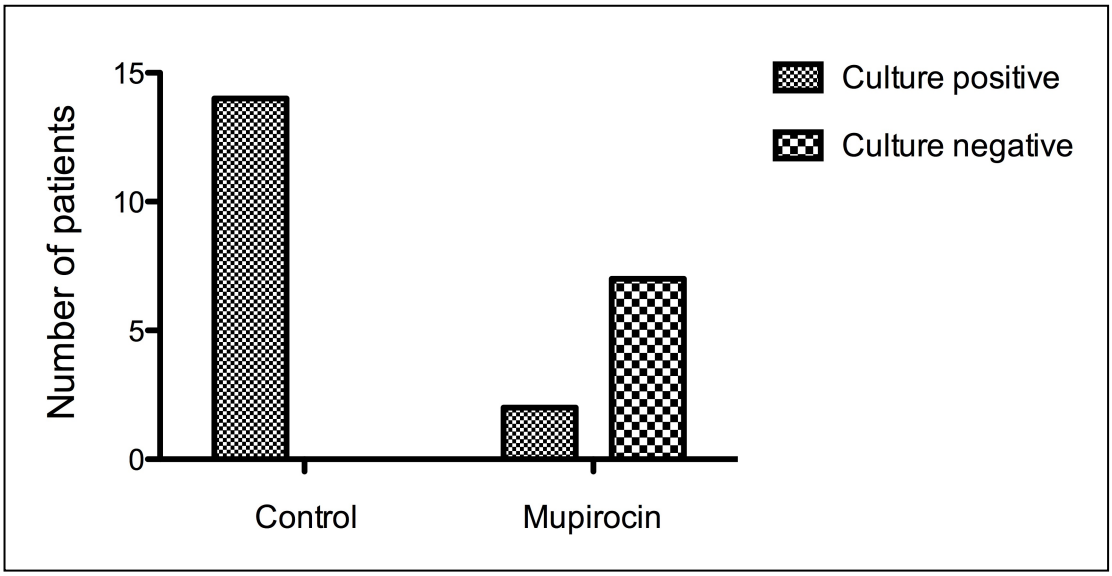

Figure 9. Immediate post-treatment culture results from patients in both the mupirocin and control arms.

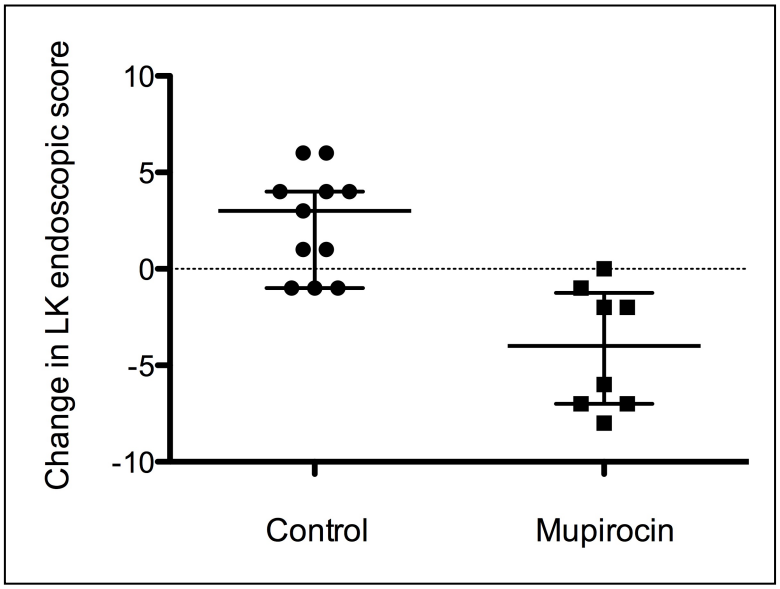

Figure 10. The change in Lund-Kennedy endoscopic score from baseline to immediately following treatment. Values below the dotted line demonstrate improvement and vice- versa. The difference between the two treatment groups is statistically significant (p=<0.01; Fisher's exact test). Horizontal lines represent the median values and interquartile ranges.

Mupirocin pre-treatment vs. post-treatment analysis

Within MUP, there were immediate post treatment improvements compared to baseline for VAS (17.0 [8.0-27.5] vs. 8.0 [1.0-23.0], p=0.01) and LK endoscopic scores (7.0 [2.5-7.5] vs. 1.0 [1.0-1.0], p=0.02). The difference in pre- and post-treatment SNOT-20 approached significance (14.0 [9.0-18.5] vs. 5.0 [3.5-22.5]; p=0.08). Within MUP, there was no difference in the score change from baseline in patients who had not previously undergone EML compared to those that had, as measured by either LK endoscopic score (-6.5 [-7.8 to -1.5] vs. -2.0 [-5.8 to -1.3], p=0.56), VAS (-10.0 [-13.8 to -5.8] vs. -5.0 [-6.8 to -1.5], p=0.09) or SNOT-20 (-6.5 [-10.0 to 4.5] vs. -7.0 [-10.5 to -1.0], p=0.81).

Delayed post-treatment assessment

No placebo patients progressed to the delayed visit as all patients had earlier re-cultured *S. aureus* and hence failed treatment. All remaining eligible patients from the mupirocin arm (Figure 8) were re-assessed at a mean 89 days following start of treatment (range: 49-191), with a culture result available in all but one case. One patient had received oral antibiotics from her general practitioner following the immediate post-treatment visit, and as such her outcomes (including the culture result) from the delayed assessment were excluded from final analysis; the

remaining patients had not received any new treatments in the interim. Otherwise, all eligible patients completed the subjective assessment battery and had a digitally-recorded endoscopic examination at this visit.

Overall, *S. aureus*-negative cultures were returned from 5/6 (83.3%) eligible patients (all MUP). The LK endoscopic scores had deteriorated since the immediate post-treatment assessment (p=0.02), and were no longer significantly different compared to baseline (7.0 [2.5-7.5] vs. 2.0 [2.0-5.0], p=0.15; Figure 11). Similarly, the VAS scores had also deteriorated (17.0 [8.0-27.5] vs. 4.0 [2.0-26.0], p=0.27). Again, within MUP there was no difference in the score change from baseline in patients who had not previously undergone EML compared to those that had, as measured by either LK endoscopic score (-3.0 [-6.0 to 0.0] vs. -2.0 [-3.5 to 1.5], p=0.55), VAS (-4.5 [-20.5 to -3.0] vs. -3.5 [-4.5 to 3.5], p=0.36) or SNOT-20 (8.0 [-10.0 to 11.0] vs. -4.0 [-10.0 to -2.0], p=0.40).

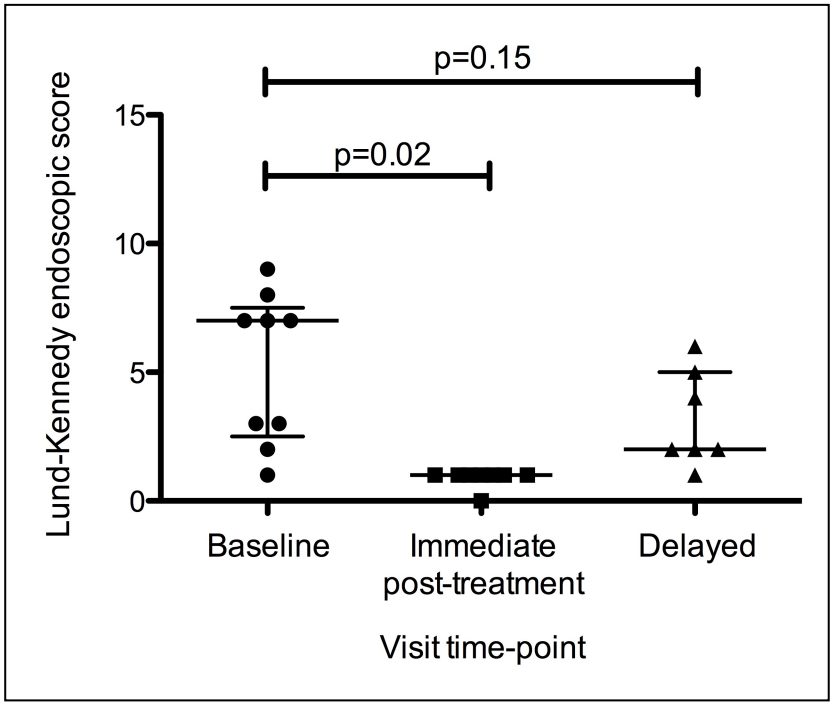

Figure 11. Comparison of the Lund-Kennedy endoscopic score (Fisher's exact test) at baseline, immediate post-treatment and delayed post-treatment visits in patients from the mupirocin group. Horizontal lines represent the median values and interquartile ranges.

In both patients who re-cultured *S. aureus* following mupirocin (one immediately and one delayed), the re-cultured isolate demonstrated preserved mupirocin-sensitivity. Regarding INC use, both patients not taking INC upon enrollment (one who was concurrently started on INC, whilst the other refrained from use throughout the study period) were in the MUP treatment group, vis-à-vis both were *S. aureus* culture-negative at the immediate post-treatment assessment.

2.2.5 DISCUSSION

In this study, mupirocin rinses yielded an immediate post-treatment *S. aureus* culture-negative cavity in 8/9 patients (88.9%), in contrast to CON patients who returned a negative culture in 0/13 (0.0%) cases (p=<0.01). Objectively, treatment with mupirocin resulted in a significant improvement in endoscopic improvement compared to baseline (p=0.01), and this change from baseline was also significantly different compared to that observed in CON patients. Subjectively, whilst MUP patients experienced a statistically-significant improvement in symptoms following treatment (VAS score), their change from baseline was not statistically different from that observed in CON. At the delayed post-treatment visit, whilst the cultures remained negative in 5/6 (85.7%) eligible MUP patients, earlier improvements in objective and subjective measures were not sustained.

Treatment rationale

Symptom relief

A constellation of various aetiopathogenic factors are contributory in CRS, with no single factor in isolation predicating disease[2]. Although outcomes following ESS are generally excellent[245], a subset of patients experience persistent disease despite complete surgery. Treatment options in these patients are numerous[63], although none in isolation is considered a universal panacea. Aggressively treating the microbiological bioburden is one such strategy; for example, mupirocin nasal rinse therapy in *S. aureus*-positive patients has become popular in recent times following the single cohort, unlabelled studies by Solares et al.[68] and Uren et al.[42]

Prevention of extra-nasal infection

Anterior nasal carriage with *S. aureus* has been long associated with poorer in-hospital outcomes as a result of extra-nasal sequelae[117]. That patients with *S. aureus*-positive CRS are at a similar risk with regards to extra-nasal infection as their non-CRS nasal carriage counterparts can only be inferred, however, despite CRS being a common chronic illness with a prevalence second only to arthritis[5], there have been no specific studies evaluating this risk reported in the literature to date. A recent multicentre observational cohort study found that throat carriage of *S. aureus* predicted future failure of *S. aureus* decolonization; this may have occurred in the setting of post-nasal drip in CRS in some cases (be it clinically apparent or sub-clinical), however it is not possible to further speculate as no examination of the nasal cavity was undertaken[123]. Regardless, it is reasonable to assume that patients who fail ESS by progressing to a disease state in which the wide-open sinuses are chronically infected with *S. aureus* are at least at a similar risk of extra-nasal infection compared to healthy, asymptomatic carriers of *S. aureus* at the vestibule.

Delivery technique

Prior to ESS sinus penetration is minimal regardless of the delivery technique[57]. For this reason alone, any effort to topically treat *S. aureus* in the sinuses cannot be satisfactorily achieved without ESS. ESS involves removal of bone and mucosa to extirpate disease, improve aeration of the sinuses, and maximize the dimensions of the natural sinus ostia to facilitate mucociliary clearance[56]; and as a consequence allow satisfactory post-ESS sinus topicalisation. High-volume, low-pressure nasal lavage has been shown to afford maximal penetration of the post-operative sinus ostia[57, 203], hence this was the delivery technique chosen for this study.

S. aureus eradication

In this study, the low post-mupirocin *S. aureus* culture rate is similar to those reported in studies in which uncomplicated nasal vestibule carriage is treated with mupirocin ointment[238]. These findings demonstrate that effectively delivering mupirocin rinses to the sinuses in patients with chronic sinonasal *S. aureus* infection has an immediate and effective result. Our low microbiological relapse rate observed at the delayed visit (1/6; 16.7%) may simply be a reflection of low

patients numbers and/or the short duration of follow-up. In contrast we have previously demonstrated a *S. aureus* re-culture rate following mupirocin rinses of 73.7% in patients followed-up over the long-term [246].

Rhinological outcomes

All patients

The immediate post-mupirocin improvements in examination findings and patient symptoms are mostly consistent with the findings of an earlier preliminary study conducted in our department[42]. However, there was no significant improvement in the quality-of-life measure (SNOT-20). That microbiological clearance can be obtained with only a modest clinical improvement suggests that the intranasal *S. aureus* bioburden is only partly causal in the underlying disease process.

The initial improvements in endoscopic examination and patient symptoms, however, were no longer apparent at the delayed visit. This rebound of disease-related signs and symptoms may be a reflection of increased, but unculturable, bacterial activity following cessation of antimicrobial treatment. However, further well-controlled studies with larger sample sizes are required before any definitive conclusions can be drawn for the effect of MUP treatment on clinical outcomes over the longer term.

Post-EML patients

As ostial penetration is roughly proportional to ostial size[195], one may have hypothesized that patients in whom EML had previously been performed may have had greater (and more durable) improvement with mupirocin compared to those that have had only conventional surgery. This was not, however, observed in this study, although this may be in part to small patient numbers in each sub-group.

INC use

One (MUP group) patient was INC-free throughout the period of the study yet was *S. aureus*-free at both the immediate- and delayed post-treatment assessment. This observation questions the contribution of maintenance anti-inflammatory therapy

to outcomes (both short- and long-term) following topical antimicrobials in CRS. Dedicated studies are suggested, therefore, in order to properly assess this relationship.

2.2.6 CONCLUSION

Mupirocin nasal rinses are an effective short-term anti-*S. aureus* treatment in surgically recalcitrant CRS as assessed by microbiological and selected clinical criteria. Immediate post-treatment culture rates are comparable to those observed when uncomplicated anterior nasal carriage is treated with mupirocin ointment. The contrasting success of mupirocin in eliminating culturable *S. aureus* yet failing to improve quality-of-life outcomes supports a multifactoral aetiology at play in underlying disease state of these recalcitrant patients, rather than a wholly staphylococcal-centric one.

Chapter Three: An Ideal Treatment

3.1 METHYLGLYOXAL-INFUSED HONEY MIMICS THE ANTI-STAPHYLOCOCCUS AUREUS BIOFILM ACTIVITY OF MANUKA HONEY: POTENTIAL IMPLICATION IN CHRONIC RHINOSINUSITIS

Jervis-Bardy, J., Foreman, A., Bray, S., Tan, L. & Wormald, P-J. (2011) Methylglyoxal-infused honey mimics the anti-*Staphylococcus aureus* biofilm activity of Manuka honey: potential implication in chronic rhinosinusitis.
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Statement of Authorship

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3.1.1 ABSTRACT

Objectives/Hypothesis

Low pH, hydrogen peroxide generation, and the hyperosmolarity mechanisms of antimicrobial action are ubiquitous for all honeys. In addition, manuka honey has been shown to contain high concentrations of methylglyoxal (MGO), contributing the relatively superior antimicrobial activity of manuka honey compared to non-MGO honeys. In high concentrations, manuka honey is effective in killing *Staphylococcus aureus* biofilms in vitro. Lower concentrations of honey, however, are desirable for clinical use as a topical rinse in chronic rhinosinusitis in order to maximize the tolerability and practicality of the delivery technique. This study, therefore, was designed to evaluate the contribution of MGO to the biofilm-cidal activity of manuka honey, and furthermore determine whether the antibiofilm activity of low-dose honey can be augmented by the addition of exogenous MGO.

Study Design

In vitro microbiology experiment.

Methods

Five *S. aureus* strains (four clinical isolates and one reference strain) were incubated to form biofilms using a previously established in vitro dynamic peg model. First, the biofilm-cidal activities of 1) manuka honey (790 mg/kg MGO), 2) non-MGO honey supplemented with 790 mg/kg MGO, and 3) MGO-only solutions were assessed. Second, the experiment was repeated using honey solutions supplemented with sufficient MGO to achieve concentrations exceeding those seen in commercially available manuka honey preparations.

Results

All honey solutions containing a MGO concentration of 0.53 mg/mL or greater demonstrated biofilm-cidal activity; equivalent activity was achieved with !1.05 mg/mL MGO solution.

Conclusions

MGO is only partially responsible for the antibiofilm activity of manuka honey. Infusion of MGO-negative honey with MGO, however, achieves similar cidality to the equivalent MGO-rich manuka honey.

3.1.2 INTRODUCTION

Chronic Rhinosinusitis (CRS) is a common, debilitating condition with a potential for medical and surgical recalcitrance. In surgically recalcitrant cases, *Staphylococcus aureus* is often the implicated organism[221]. The biofilm form of *S. aureus* is increasingly recognized as relevant in CRS pathogenesis and is frequently associated with poorer post-surgical outcomes[39]. Given the persistence of this organism in the post-operative period[84], additional medical therapies are required to treat this organism if we want to improve patient outcomes, especially in surgically-recalcitrant cases. Numerous anti-biofilm topical treatments have recently been proposed, with many specifically targeting *S. aureus* biofilms[42, 73, 139, 154].

Manuka (*Leptospernum scoparium*) Honey is active against a broad spectrum of gram-positive and gram-negative bacteria[247]. Using an *in vitro* model of *S. aureus* (and *Pseudomonas aeruginosa*) biofilms, Alandejani et al. recently demonstrated the biocidal activity of Manuka Honey at a concentrations of 33% v/v (equivalent to approximately 50% w/v)[154]. The phenol compound methylglyoxal (MGO) has recently been shown to be uniquely present in Manuka Honey[156] (and certain other selected honeys), affording greater antibacterial activity compared to non-MGO honeys.

Promisingly, *in vitro* attempts at inducing resistance to Manuka Honey have similarly not been successful[158]. Manuka Honey, therefore, may be an ideal topical agent for use in surgically recalcitrant *S. aureus*-positive CRS.

In order to maximize the affordability and tolerability of Manuka Honey, lower concentrations of honey are preferred than those tested by Alandejani et al-

provided the anti-biofilm activity is preserved. Additionally, the contribution of MGO to the anti-biofilm activity of Manuka Honey has not previously been established. This study, therefore, was designed to evaluate this contribution, and furthermore determine whether the anti-biofilm activity of low-dose honey can be augmented by the addition of exogenous MGO.

3.1.3 MATERIALS AND METHODS

Bacterial Strains

Four clinical isolates taken from patients with severe CRS were selected. The in vivo presence of S. aureus biofilms on sinonasal mucosa harvested during surgery was previously determined in all patients using an established Fluorescence In-Situ Hybridisation (FISH) protocol[248]. Bacterial strains were isolated from each patient either at the time of tissue harvest or in the early post-operative period and were stored in 80% glycerol at -80°C for future use. American Type Culture Collection (ATCC) reference strain 25923, a known biofilm-forming strain[139], was used for the purpose of quality control.

Honey

Manuka Honey was supplied by Watson & Sons (Masterton, New Zealand). Non-MGO honey was purchased from Capilano Honey (Inala, Queensland). Independent MGO concentration and pH analysis of both honeys was performed by Hill Laboratories (Hamilton, New Zealand) (Table 7). After delivery, all honey was stored a 4°C in the dark prior to use.

Table 7. pH and MGO concentration of tested honeys.

	Capilano/Non-MGO honey	**Manuka Honey**
pH	4.0	3.8
MGO concentration	<10.0mg/kg	790mg/kg

Biofilm Assay

All biofims were grown using a modified version of the Calgary biofilm device protocol[249] as outlined below.

Bacterial Innoculation

Prior to inoculation of the device strains were transferred from stock cultures to Columbia horse blood agar (Oxoid, Adelaide, Australia). After incubation for 24h at 37°C, a single colony was inoculated in 1.5mls of 0.45% saline and adjusted to a turbidity of 2.0-2.2 MacFarland opacity standard. Individual wells of a sterile 96-well plate (Nunc, Rosklide, Denmark) were inoculated with 16.67ul of bacteria solution and 133.33ul of cerebrospinal fluid (CSF) broth (Oxoid, Adelaide, Australia). A sterile 96-pin plate lid (Innovotech, Calgary, Canada) was inserted into the inoculated wells, and incubated at 35°C on a gyro-rotary platform (Ratek, Melbourne, Australia) at 70rpm for 30h.

Honey challenge

Manuka Honey and Non-MGO honey in CSF broth solutions were prepared immediately prior to use. Additional honey solutions augmented with MGO (Sigma-Aldrich, St. Louis, MO) solutions were also prepared, representing quadruple-strength Manuka Honey, augmented non-MGO honey equivalent to Manuka Honey and augmented non-MGO honey equivalent to quadruple-strength Manuka Honey.

A challenge plate was constructed by inoculating a new 96-well plate with 200ul of the 5 honey solutions at 66.00%, 33.00%, 16.50% and 8.25% w/v concentrations in CSF broth. Wells containing MGO (in CSF broth) and CSF broth only were also prepared.

The 96-pin lid was removed from the bacterial inoculation plate, washed twice with physiological saline, inserted into the challenge plate and returned to the incubated gyro-rotator for 24h at 35°C.

Recovery

Following the challenge, the 96-pin lid was washed twice with physiological saline and placed into a new 96-well plate with wells containing 150ul CSF broth and sonicated for 5 minutes to dislodge the peg-bound biofilm. The pin-lid was then

removed and replaced with a standard 96-well lid and incubated at 37°C for 24h under static conditions.

Assessment of biocidal activity

Following incubation, recovery wells were visually assessed (and verified using a BioRad Microplate spectrophotometer; Hercules, CA) for turbidity and macroscopic biofilm. Non-turbid wells reflected anti-biofilm activity/biocidality of the corresponding challenge solution well.

All treatments were run in duplicate and the experiment was performed twice to ensure consistency of results.

3.1.4 RESULTS

The biocidality or non-biocidality of all honey and/or MGO solutions was uniform for all strains tested, suggesting that the anti-biofilm activity of honey and MGO may be broadly effective against a range of *S. aureus* strains.

Manuka Honey

Biocidal activity against all strains was seen at a 66.00% and 33.00% w/v concentration of Manuka Honey (equivalent MGO concentrations 0.53mg/ml and 0.26mg/ml). 16.50% w/v (0.13mg/mg MGO) was not biocidal against any strains (Table 2).

Non-MGO honey

Biocidal activity was not demonstrated against any strains at any concentration tested (Table 2).

MGO only

Biocidal activity against all strains was uniformly demonstrated at concentrations ≥1.05mg/ml MGO (Table 3).

Manuka and Non-MGO honey augmented with MGO

Non-MGO honey augmented with MGO demonstrated equivalent biocidal activity to Manuka Honey. Biocidal activity against all strains was achieved with 16.50% w/v augmented honey solution, with addition of MGO to an equivalent concentration of that found in 66.00% w/v Manuka Honey (0.53mg/ml; Table 2).

Table 8. Biocidality of various honeys at differing concentrations in CSF broth. Cell colour corresponds to equivalent MGO-only concentration seen in Table 9.'+' biocidal; '-' not biocidal; '*' not tested; MGO- methylglyoxal; CH- Capilano/Non-MGO honey; MH- Manuka Honey

	Equivalent solid honey				
% Honey Concentration (w/v)	CH (<10mg/kg MGO) only	MH (790mg/kg MGO) only	CH + 790mg/kg MGO	MH +2,370mg/kg MGO	CH +3,160 mg/kg MGO
66	-	+	+	*	*
33	-	+	+	+	+
16.50	-	-	-	+	+
8.25	-	-	-	-	-

Table 9. Biocidality of methylglyoxal-only solution. MGO- methylglyoxal; '+' biocidal; '-' not biocidal

MGO Concentration (mg/ml)	Biocidality
2.11	+
1.05	+
0.53	-
0.26	-
0.13	-
0.06	-
<0.01	-

3.1.5 DISCUSSION

Overall, all honey solutions containing a MGO concentration of 0.53mg/ml or greater demonstrated biofilm-cidal activity; equivalent activity was achieved with ≥1.05mg/ml MGO-only solution. Manuka Honey at 33.00%, or both honeys at 16.50% with additional MGO, were therefore biocidal. Non-MGO honey was not biocidal at any concentration tested.

An increased appreciation of the role of *S. aureus* biofilms in CRS has simultaneously generated interest in potential biocidal agents to treat this condition[73, 124, 139, 140]. *S. aureus*-associated CRS is often recalcitrant to our current medical and surgical paradigms, making the presence of this organism a marker for severe disease. This subgroup may well require additional targeted therapy to improve their outcomes. Several novel treatments have been proposed to serve this role. Nasal lavage with mupirocin, for example, has been proposed as an efficacious, well-tolerated topical treatment, with a recent pilot study reporting impressive subjective and objective outcomes following a 4-month treatment protocol[42].

However, mupirocin-resistance, first reported in 1987[225, 226], is an emerging concern. Whilst rates of mupirocin resistance are thought to be low in the community setting[227], in selected population groups rates up to 13.2% have been reported[228]. With increasing interest in mupirocin as a methicillin-resistant *S. aureus* (MRSA) de-coloniser of the nasal vestibule[119] similar high rates of mupirocin-resistance may potentially be seen in the future. The ideal alternative to mupirocin, therefore, should be likewise well tolerated and efficacious as a nasal lavage, with minimal or no potential for the development of resistance; Manuka Honey certainly fulfills the latter criteria[158].

Honey is not a new topical antibacterial agent. By contrast, the understanding of Manuka Honey's enhanced and unique antibacterial activity[250], now shown to be proportional to the MGO concentration[156], is relatively new. MGO targets protein and DNA synthesis; *relatively* resistant bacteria generally have an intrinsic ability to withstand the toxic effects of MGO by possessing a robust capacity for DNA repair and sufficient levels of detoxification enzymes[157, 251]. The exact mechanisms involved in MGO activity against *S. aureus*, and the reasons why *S. aureus* is particularly sensitive to MGO-rich honey[247] have not been explored.

This study has shown that the biocidal activity of MGO is enhanced when in honey solution, despite the non-biocidality of non-MGO honey. The reasons for this enhanced activity are unclear. In the clinical setting, therefore, a combined MGO and honey solution (whether from Manuka or fortified non-MGO honey) yields a stronger biocidal activity compared to an equivalent MGO-only solution and would hence be the preferred treatment option in CRS. Given the hyper-osmolarity and acidity of concentrated honey solutions, a 16.50% honey solution augmented with MGO is likely to be better tolerated as a nasal lavage compared to a 33.00% Manuka Honey solution, although both solutions are similarly biocidal. A pilot study conducted by our department suggests that 16.50% honey is the upper-limit of tolerability when delivered by nasal lavage (unpublished data).

3.1.6 CONCLUSION

MGO is only partially responsible for the anti-biofilm activity of Manuka Honey. Non-MGO honey augmented with MGO, however, achieves similar biocidal activity to the equivalent MGO-rich Manuka Honey.

Clinical trials assessing the safety, tolerability and efficacy of Manuka Honey at 33.00%, or both honeys at 16.50% with additional MGO, are suggested.

Chapter Four: Is there an ideal treatment window?

4.1 WHAT IS THE ORIGIN OF STAPHYLOCOCCUS AUREUS IN THE EARLY POST-OPERATIVE SINONASAL CAVITY?

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By signing this document, I (the co-author) hereby acknowledge these to be accurate descriptions of the contribution I made to this paper and give permission for it to be included in the candidate's thesis.

4.1.1 ABSTRACT

Background

Despite increasing evidence of a role for *Staphylococcus aureus* (*S. aureus*) biofilms in chronic rhinosinusitis (CRS), the origin of this organism in the postsurgical sinonasal cavity had been unclear. Recently, we suggested that the increased culture rate *of S. aureus* following endoscopic sinus surgery (ESS) may be related to biofilm activity. This study, therefore, was designed to evaluate the origin of early postoperative sinonasal *S. aureus* and assess the early postoperative outcomes in patients culture- positive for this organism.

Methods

Twenty-nine patients undergoing ESS for medically-recalcitrant CRS were prospectively enrolled. A comprehensive intraoperative *S. aureus* screening protocol was followed for all patients (including swabs for culture and tissue for fluorescence in situ hybridization [FISH] *S. aureus* biofilm analysis); early postoperative management included endoscopically-guided swabs for culture in all patients.

Results

Twenty of 29 (69.0%) patients cultured *S. aureus* postoperatively, of which 17 of 20 (85.0%) were screen-positive at surgery. Seven of 11 (63.6%) intraoperatively biofilm-positive but culture-negative patients progressed to culture S. aureus post-ESS. *S. aureus* culture was associated with selected poorer early post-ESS outcomes.

Conclusion

S. aureus persists in the sinonasal cavity despite ESS. The postoperative culture of sinonasal *S. aureus* in patients previously biofilm-positive but culture-negative may reflect the dynamic ability of *S. aureus* to adapt to the surgically-altered microenvironment with subsequent biofilm dispersal and release of planktonic clones.

4.1.2 INTRODUCTION

The pathogenic role of *Staphylococcus aureus* in Chronic Rhinosinusitis (CRS) has been the subject of much interest and debate in the recent literature. *S. aureus* is both the most common bacteria isolated from the sinonasal cavity following endoscopic surgery (ESS) for CRS[35] and also the most common isolate in patients with surgically recalcitrant disease[220]. Virulence mechanisms such as superantigen production[222, 252], biofilm formation[38], and intramucosal residence[224, 253] enable this organism to not only precipitate an altered immune response but also evade conventional antibiotic therapy. Targeted anti-staphylococcal therapy in surgically recalcitrant, culture-positive patients has been reported to yield impressive post-treatment outcomes[42]. *S. aureus*, therefore, is thought to play an important role not only in the aeitopathogensis of CRS but also in the persistent disease of patients non-responsive to surgery.

S. aureus appears to have a dynamic role in the peri-operative period. Culture rates of this organism consistently increase following surgery. For example, Jiang et al. recently cultured *S. aureus* from 15/71 (21.1%) patients undergoing ESS; postoperatively, however, sinonasal *S. aureus* was isolated from 12/31 (38.7%) patients completing a standard course of post-ESS oral antibiotics and an alarming 28/40 (70.0%) patients without antibiotic prophylaxis, an overall culture rate of 40/71 (50.6%)[34]. The aetiology of this post-ESS increase has previously been thought to represent *de novo* colonization (or infection)[85], however we have previously hypothesized this to be a biofilm-driven phenomenon whereby surgical disruption of the biofilm or alternatively a biofilm-driven phenotypic change results in culturable, planktonic bacteria[84]. Accordingly, we have recently demonstrated the strong correlation between *S. aureus* biofilm detection and poorer post-ESS outcomes, a relationship not observed with biofilms of other commonly isolated flora[39].

This study, therefore, was designed further evaluate the origin of sinonasal *S. aureus* in the post-operative setting, and secondly to assess the early post-operative outcomes in this patient group.

4.1.3 MATERIALS AND METHODS

Consecutive patients undergoing ESS for CRS were included in this prospective study. All patients met the American Academy for Otolaryngology-Head and Neck Surgery diagnostic criteria for CRS[3] and had failed medical therapy necessitating surgical intervention. This study was approved by the Human Ethics Research Committee of the Central Northern Adelaide Health Service and was conducted in the tertiary referral practice of the senior author (P-J.W.).

Pre-operative data collection

All patients completed a pre-operative questionnaire, which included routine demographic data, the SNOT-20 quality-of-life index (score range 0-60)[254] and a sinus symptoms visual analogue scoring (VAS) proforma (summation of 5 individual symptom scores plus an 'overall symptomatology score' to give a total score range 0-60)[244]. Lund-Mackay (LM; score range 0-24) scoring was performed for all pre-operative CT-scans[255].

Intra-operative protocol

Immediately following anesthetic induction, a single swab was taken of the nasal vestibule by encircling both nares (including the septum anterior to the internal nasal valve) under direct vision.

All patients had standardized ESS, undergoing bilateral complete spheno-ethmoidectomy, frontal recess clearance and middle-meatal antrostomy (+/- endoscopic modified Lothrop (EML) procedure/frontal drillout). Intra-operatively, all patients had an endoscopically guided guarded swab taken, performed by first inserting the swab into a sterile 8G otology metal suction tip (Mediplast, Malmo) and then introducing this suction tip/swab combination into the nasal cavity. Following sampling, the swab tip was then retracted into the body of the suction tip and removed from the nose. This process ensured no contamination of the swab from the vestibule. Swabs were taken from any area of mucous encountered during the procedure; where no secretions were evident a swab was taken from the left or right maxillary sinus. Additionally, where thick mucous was observed, this was extracted from the nose under endoscopic vision and sent for standard

cultures and histological examination. The latter was specifically performed to assess presence or absence of profound eosinophila (with or without Charcot-Leyden crystals), diagnostic of eosinophilic mucous (EM). No nasal packing was used at the completion of surgery. Sinus tissue was harvested for Fluorescence *In situ* Hybridization (FISH) *S. aureus* biofilm detection using our standard protocol[248].

Post-operative follow-up

Following ESS, all patients were treated with a course of oral amoxycillin and clavulanic acid (15 days for standard ESS and 20 days where EML was performed). Patient with nasal polyposis were given a 3-week tapered course of oral prednisolone. Routine follow-up was at 2- and 6-10 weeks post-operatively. A guarded swab was taken at the 2-week visit where there was evidence of thick and purulent discharge; at the 6-10 week visit all patients had a guarded swab taken. Endoscopic examination of all patients at the 6-10 week visit was digitally recorded and subsequently scored by 2 independent observers (A.F., S.B.) blinded to the patients' subjective and microbiological post-operative outcome. At the second visit, patients also completed a SNOT-20 quality-of-life index and the VAS proforma. The microbiological, objective and subjective data obtained from the 6-10 week visit was taken as a reflection of the early post-operative period. Antibiotic treatment following the 2-week visit was not standardized; patients with infection despite oral antibiotics were generally started on topical antibiotic rinses at this visit.

Patients with penicillin allergy were excluded from the study, as were immunosuppressed patients and those on antibiotics and/or steroids within 2 weeks pre-operatively. Patients not followed up at least twice within 90 days post-operatively with at least one sinus swab during this time were also excluded from the study.

Statistical analysis

Comparisons between groups were performed using Mann-Whitney *U* tests; count data was analysed using Fisher's exact test. Trend analysis was performed using

the Chi-squared test for trend in proportions. Median and inter-quartile ranges (IQR) were used for non-parametric data.

4.1.4 RESULTS

A total of 29 patients completed the study (17 men and 12 women; mean age 49.9 years). 11 patients had primary ESS with 18 undergoing revision ESS (including 12 who had additional EML procedure). Nasal polyposis was evident in 17/29 (58.6%) patients. Mean Lund-Mackay (LM) score was 15.6/24, reflecting the severe disease demographic encountered in our practice.

Intra-operative S. aureus

As shown in Figure 12, 9/29 (31.0%) patients returned a positive sinus culture for *S. aureus* (from swab and/or fresh mucous) whilst 16/29 (55.2%) patients were FISH-positive for this organism. Overall, 20/29 (69.0%) patients were 'screen-positive', having had *S. aureus* identified by at least one modality- sinus culture, vestibule swab and/or FISH biofilm analysis. These isolation rates were consistent with previous published data by our department[84, 248]. Only 1 patient was found to be *S. aureus*-positive at the nasal vestibule whilst simultaneously culture- and biofilm-negative from the sinuses; as the remaining 8 vestibule-positive patients were concurrently culture-positive from the sinuses it was presumed that vestibule-positivity in these patients simply reflected the clearance of *S. aureus*-positive mucous from the sinonasal cavity and out the nose.

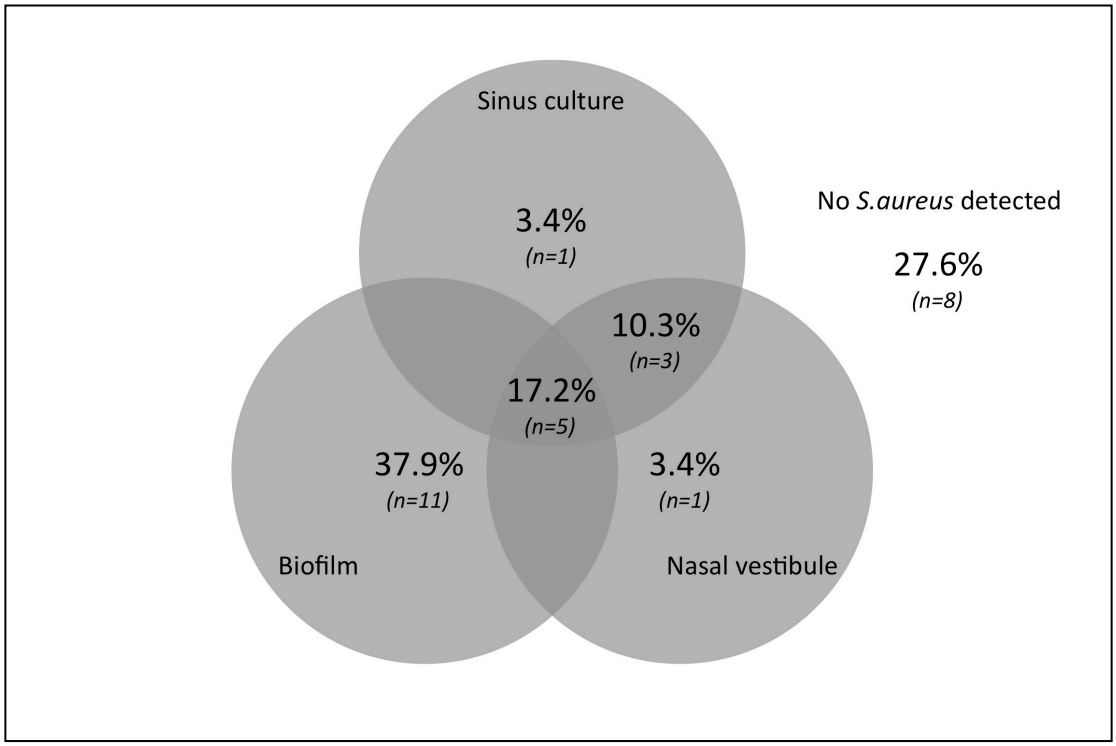

Figure 12. Intra-operative *S. aureus* screen results distribution..

Interestingly, sinus culture-positive patients had no greater LM scores compared to culture-negative patients (Mann-Whitney *U* test p=0.14), however the difference in LM scores between biofilm-negative and –positive patients approached significance (Mann-Whitney *U* test p=0.055).

There was a strong correlation between *S. aureus* sinus culture and eosinophilic mucous (EM); *S. aureus* was cultured from 8/14 (57.1%) patients with histologically-proven EM compared to only 1/15 (6.7%) without (Fisher's exact test, p=0.0052). The rate of *S. aureus* biofilm identification, however, was similar in both eosinophilic mucin-positive and –negative patients (9/14 (62.3%) vs. 7/15 (46.7%); Fisher's exact test, p=0.46).

S. aureus was cultured from the sinuses in a similar proportion of patients undergoing primary compared to revision surgery (3/11 (27.3%) vs. 7/18 (38.9%); Fisher's exact test, p=0.69); the biofilm detection rate was likewise similar (6/11 (54.5%) vs. 10/18 (55.6%); Fisher's exact test, p=1.00).

There was no difference in the rate of *S. aureus* sinus culture in patients with polyposis compared to those without (7/17 (41.2%) vs. 2/12 (16.7%); Fisher's exact test, p=0.23), however the rate of biofilm detection in patients with polyposis was significantly higher (12/17 (70.6%) vs. 4/12 (33.3%); Fisher's exact test, p=0.015).

Pre-operative subjective assessment

Screen-negative patients reported similar baseline VAS and SNOT-20 scores compared to their screen-positive counterparts (VAS median scores 19.50 (IQR 12.50-39.31) vs. 35.50 (IQR 29.44-41.81); Mann-Whitney *U* test, p=0.13. SNOT-20 median scores 16.00 (IQR 11.25-31.75) vs. 28.00 (IQR 17.50-36.50); Mann-Whitney *U* test, p=0.27). Likewise, biofilm-positive patients reported similar baseline VAS and SNOT-20 scores compared to biofilm-negative patients (VAS median scores 35.50 (IQR 29.44-40.94) vs. 29.75 (IQR 15.00-40.38); Mann-Whitney *U* test, p=0.34. SNOT-20 median scores 27.50 (IQR 16.75-35.75) vs. 21.00 (IQR 11.50-35.00); Mann-Whitney *U* test, p=0.42). Again, there was no difference in the baseline VAS reported by patients culturing *S. aureus* compared to their culture-negative counterparts, although the difference in SNOT-20 scores did approach significance. (VAS median scores 37.88 (IQR 28.88-46.75) vs. 35.00 (IQR 18.75-37.19); Mann-Whitney *U* test, p=0.18. SNOT-20 median scores 30.00 (IQR 24.00-43.50) vs. 21.00 (IQR 11.25-31.50); Mann-Whitney *U* test, p=0.077).

Post-operative S. aureus

A total of 20/29 (69.0%) patients returned one or more post-operative positive *S. aureus* cultures, including the isolated vestibule-positive patient.

Where *S. aureus* was cultured from the sinuses intra-operatively, this organism was cultured in the post-operative period in all cases (9/9, 100.0%). Culture-negative but biofilm-positive patients had a greater propensity to demonstrate culturable *S. aureus* post-operatively compared to those both culture- and biofilm-negative (7/11, 63.6% versus 3/8, 37.5%) however this was not a statistically significant difference (Fisher's exact test, p=0.37). As demonstrated in Table 10, however, the trend to culture *S. aureus* post-operatively along a continuum of the

three groups was strongly significant (Chi-squared test for trends in proportions; p=0.0017). As shown in Table 11, nasal polyposis and eosinophilic mucous were associated with an increased rate of *S. aureus* culture post-ESS whereas CT LM score and previous surgery were not.

Table 10. Trend to culture *S. aureus* post-ESS depending on swab and biofilm status (Chi-squared test for trends in proportions, p=0.0017). *Vestibule-positive but biofilm-negative patient excluded.

Intra-operative *S. aureus* status*	**Number culturing *S. aureus* post-ESS**
Culture-negative, biofilm-negative	3/8 (37.5%)
Culture-negative, biofilm-positive	7/11 (63.6%)
Culture-positive	9/9 (100%)

Table 11. Proportion of patients with (present) or without (absent) pre-operative risk factors progressing to culture *S. aureus* post-ESS (Fisher's exact test; '*' indicates statistical significance).

Risk Factor	**Present**	**Absent**	**p=**
	Number culturing *S. aureus* post-ESS		
Nasal polyposis	15/17 (88.2%)	5/12 (41.7%)	0.014*
Eosinophilic mucous	14/14 (100.0%)	6/15 (40.0%)	0.0007*
LM score > 16	9/10 (90.0%)	11/19 (57.9%)	0.11
Previous surgery	14/18 (77.8%)	5/11 (45.5%)	0.11

<u>Subjective early post-operative outcome</u>

A total of 28/29 patients returned the post-operative questionnaires and hence were included in this sub-analysis.

There was no significant difference observed between patients returning *S. aureus*-positive swabs compared to those culture-negative as measured by the SNOT-20 (median scores 6.00 (IQR 2.00-14.00) vs. 5.00 (IQR 2.50-12.00); Mann-Whitney *U* test, p=0.77), however culture-positive patients recorded a higher VAS compared to their swab-negative counterparts (median scores 10.00 (IQR 5.00-18.00) vs. 3.00 (IQR 1.00-9.50); Mann-Whitney *U* test, p=0.046).

Objective early post-operative outcome

As seen in Figure 13, the LK endoscopic score for *S. aureus*-positive patients was greater than those *S. aureus*-negative (median 8.75 (IQR 5.50-9.50) vs. 4.50 (IQR 2.50-6.25); Mann-Whitney *U* test, p=0.013).

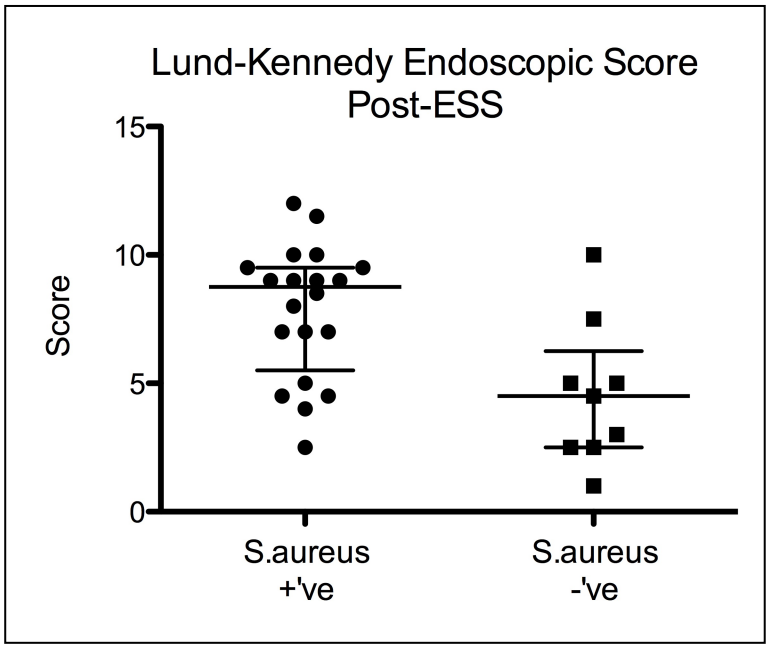

Figure 13. Post-ESS, The Lund-Kennedy score is significantly greater where *S. aureus* is cultured (Mann-Whitney *U* test p=0.013).

4.1.5 DISCUSSION

In this study, a decreasing trend to culture *S. aureus* post-operatively along a continuum bookended by patients intra-operatively *S. aureus*-culture positive (9/9, 100.0%) and patients dually biofilm- and culture-negative 3/8 (37.5%) was observed (p=0.0017). Intermediately, intra-operatively biofilm-positive but culture-negative patients progressed to culture *S. aureus* post-operatively in 7/11 (63.6%) cases. Overall, 17/20 (85.0%) patients culturing sinus *S. aureus* post-ESS had evidence of *S. aureus* colonisation/infection intra-operatively as detected by our rigorous screening process. Where *S. aureus* was cultured post-ESS, this was associated with a poorer endoscopic appearance and total visual analogue symptom score.

Recalcitrant CRS following appropriately performed surgery is a frustrating entity for clinicians and patients alike. Given the propensity for *S. aureus* to be cultured in this setting, much recent research has been dedicated to potential topical agents for use in patients recalcitrant to conventional treatments[42, 73, 154]. Early identification of at-risk individuals, however, should allow for early intervention and potentially afford better outcomes. Understanding the aetiology of *S. aureus* in the post-operative setting is the first step in such a process.

Post-ESS infections have previously been thought to be caused by opportunistic *de novo* bacteria[85]. Alternatively, Plouin-Galdon *et al.* demonstrated a propensity for intracellular *S. aureus* to persist in the sinonasal cavity despite ESS[224], suggesting the post-surgical culture of *S. aureus* to instead reflect the insidious nature of this organism. We have previously proposed that the increased culture rate seen following ESS may be due to surgical disruption of the biofilm[84]. Given the poorer post-operative mucosal appearance and symptomatic discharge in this patient group, some component of this process is likely due to biofilm dispersal, that is the process by which biofilm sheds planktonic bacteria in an effort to the seed surrounding tissues in a response to favourable conditions[111]. The strongly persistent nature of culturable *S. aureus* despite ESS may therefore represent persistent, active biofilm dispersal. 7/11 (63.6%) of intra-operative biofilm-positive but culture-negative patients returned a positive culture during the early post-operative period, suggesting this setting to be a relatively *S. aureus*-friendly environment facilitating dispersal. The similar rate of intra-operative *S. aureus* culture in patients undergoing primary vs. revision surgery, however, suggests this phenotypic shift to be limited to the early post-operative period- otherwise one would expect to observe a higher culture rate in the revision group. Hai et al. showed that bacteria recovered from the early post-ESS cavity generate less biofilm (when compared to bacteria recovered intra-operatively[256]), similarly suggesting this setting to favour a dispersed biofilm phenotype. Denuded mucosa, slow cilia recovery and immunologically-inert clot may contribute to this process. Given topical delivery to the sinuses is greatly maximized following ESS[57], however, targeted pharmacotherapy during this period has the potential to

strongly augment the host immune response, halt dispersal and exert biofilm-cidal effects.

3/8 (37.5%) of patients dually biofilm- and culture-negative at operation cultured *S. aureus* during the early post-operative period. Whilst these patients may represent detection error (ie. false-negative FISH and/or swab sampling error), it also is possible that rather de novo colonization of susceptible post-operative mucosa is causal in this group. Given the rate of post-ESS *S. aureus*-culture was in this group was not significantly different compared to patients intra-operatively biofilm-positive but culture-negative (3/8, 37.5% vs 7/11, 63.6%; p=0.37), both explanations are plausible, Regardless, the unexpectedly high culture rate in this dually-negative group may suggest that early post-ESS anti-*S. aureus* prophylaxis may ultimately be advisable regardless of operative culture or biofilm detection results.

The increased propensity for *S. aureus* culture post-ESS seen in patients with nasal polyposis and EM may be a contributing factor to the poorer post-ESS outcomes associated with these risk factors[22, 257]. Whilst high post-operative culture rates may indicate an increased superantigen-generating bacterial load serving to perpetuate polyposis, an underlying mechanism linking EM and *S. aureus* is less clear.

Whilst poorer-early post-ESS outcomes were observed in conjunction with *S. aureus* culture, longer follow-up is certainly required in order to determine whether patients culture-positive during this early period persistently culture *S. aureus* long-term. Additionally, further research is required to investigate the relationship between early post-ESS *S. aureus* culture and long-term subjective and objective outcomes. Finally, whilst these results certainly suggest biofilm dispersal is responsible for the increased culture rates following ESS, without genotyping studies it is not possible to definitely conclude this is the case rather than extra-nasal or *de novo* colonization.

4.1.6 CONCLUSION

The post-operative culture of sinonasal *S. aureus* in patients previously biofilm-positive but culture-negative may reflect the dynamic ability of *S. aureus* to adapt to the surgically-altered microenvironment with subsequent biofilm dispersal and release of planktonic clones. Given the association between *S. aureus*-culture and a poorer early post-ESS outcome, the early post-operative window may be an ideal setting for aggressive topical antibiotic prophylaxis in the high-risk patient; prospective interventional clinical studies are required.

Synopsis

The research contained within this thesis has advanced our knowledge of the advantages, but also the limitations, of treating surgically-recalcitrant CRS with an entirely antimicrobial approach. As outlined in this thesis, CRS is a complex disease, with a broad, multifactorial aetiopathogensis. Bacterial biofilms represent one important, and potentially treatable, aetiopathogenic factor. Despite complete surgery, a subset of patients continue to be bothered by persistent staphylococcal infection; it is in this patient group that *S. aureus* biofilms are thought to have an important role. A myriad of antimicrobial agents are potentially available for use as topical treatments in this setting; a similary wide range of delivery devices are also available. Following an exhaustive literature review, it was decided that further assessment of the anti-biofilm agents mupirocin and Manuka Honey were warranted, and that the sinus rinse bottle was the most effective treatment delivery method The research contained within this thesis, therefore, includes firstly an evaluation of mupirocin rinses, an agent commonly used to treat these patients and secondly, an investigation of the in vitro anti-biofilm effect of an ideal treatment agent (Manuka Honey). Lastly, an assessment of biofilm behavior in the early post-operative period was conducted- posing the question as to whether this time period may represent an ideal treatment window in which anti-biofilm rinses may be used with the potential for greatest antimicrobial effect.

An evaluation of Mupirocin

Two studies evaluating mupirocin were conducted. Firstly, post-treatment outcomes were assessed retrospectively in 57 symptomatic, *S. aureus*-positive post-surgical patients treated with mupirocin rinses. Secondly, a prospective randomised controlled trial (RCT) was conducted, enrolling 25 patients. The results from the two studies of mupirocin reveal that adopting this antibiotic-only approach yields excellent immediate post-treatment outcomes. In the RCT, mupirocin nasal rinses were far superior in achieving a *S. aureus* culture-negative sinonasal cavity immediately following treatment compared to contols

(8/9, 88.9% vs. 0/13, 0.0%; p<0.01). In addition, the endoscopic appearance of the sinonasal cavity improved with treatment compared to controls, as did patient quality-of-life as assessed by a modified SNOT-20 assessment tool. Importantly, however, once the mupirocin treatment is ceased outcome measures trend back towards pre-treatment levels. In the retrospective study, despite excellent short-term outcomes, we identified a long-term (over a year) microbiological failure rate of 73.7% following treatment. In this analysis, however, it is important to note that the remaining 26.3% of patients in fact achieved long-term cure following treatment. In the retrospective study, we identified a mupirocin-resistance rate induced by treatment of 2.3%- a similarly small percentage comparable to mupirocin use in other settings.

In evaluating the two studies together, we can conclude that mupirocin nasal rinses are an effective short-term anti-*S. aureus* treatment in surgically recalcitrant CRS non-responsive to traditional systemic antibiotic treatment. This success is assessed by microbiological, endoscopic and clinical criteria. However, following treatment there is a trend back toward re-colonization in the long term with approximately 3 out of 4 patients culturing *S. aureus* following a single 1 month treatment course of mupirocin rinses. This, however, demonstrates that in this highly recalcitrant group- in which traditional cultured directed systemic antibiotic treatment have failed repeatedly- a long-term cure is achieved in 1 out of 4 patients. In addition, treatment carries a small risk of inducing mupirocin resistance.

An ideal treatment agent

In order to achieve microbiological cure in this highly recalcitrant group of patients other agents are sought to either augment or replace mupirocin. Manuka honey was identified as such an agent with a high level of antimicrobial potential and low toxicity. The study in this thesis evaluates the *in vitro* potential of Manuka Honey solution as an anti-biofilm agent against the most important bacteria found in recalcitrant CRS, *S. aureus*. Manuka Honey is an attractive potential agent for use in CRS, as it has a very favourable resistance profile that allows for long-term use without the risk of inducing resistance. This is in direct

contrast to mupirocin- where although the resistance rate is small, it increases with treatment duration and multiple courses. Manuka Honey is active against *S. aureus* biofilms but Manuka honey cannot be used without dilution as a topical agent in CRS. What has not been previously established is whether the antibacterial activity is preserved when the honey is diluted into solution that is able to be rinsed though the sinonasal cavity. In this study, the anti-biofilm activity of methyglyoxal (MGO) the 'active ingredient' in Manuka Honey- was also assessed without the presence of honey.

The results of this study demonstrated that at a concentration amenable to rinsing (16.5% w/v), Manuka Honey must be fortified with extra MGO in order to preserve anti-biofilm activity. When tested in isolation, however, MGO-only solution was not as potent when compared to an MGO-in-honey solution. As a result, it was determined that 16.5% w/v Manuka Honey fortified with extra MGO was the solution most appropriate for use in the clinical setting, as this solution required the minimum total MGO required whilst preserving anti-biofilm activity. Before this solution can be used in patients, however, in vitro animal trials are needed to ascertain clinical safety and efficacy.

An ideal treatment window

For topical treatment to be effective the sinuses need to be open so that the nasal and sinus rinse can penetrate the sinus and eradicate the biofilm. The opportunity for such treatment is in the post-operative period. The question as to whether patients who have *S. aureus* at the time of surgery should be treated immediately after surgery or rather when infection is clinically evident as some post-operative time point is unclear. This study was conducted to evaluate the behavior of *S. aureus* biofilms in response to surgery. Previously, we had hypothesized that biofilm dispersal may occur in the early post-operative period[84]. In this current study, patients with CRS having ESS underwent an intra-operative screen for *S. aureus*, with cultures sent from the both the nasal vestibule and sinonasal cavity and tissue analyzed for the presence of *S. aureus* biofilm using fluorescence *in situ* hybridisation. Post-operatively, sinus swabs were taken from all patients at regular intervals, with the culture results

correlated to the intra-operative screen findings. The results from this study revealed that 7/11 (63.6%) intra-operatively *biofilm-positive but culture-negative* patients cultured *S. aureus* post-operatively. This suggested that, in these patients, the quiescent intra-operative biofilm had been dispersed by surgery into its planktonic form. The planktonic bacteria are more susceptible to antimicrobials and potentially this immediate post-operative period should be the most effective period to target such bacteria before they can re-establish their biofilm form and develop resistance to treatment. Prospective interventional clinical studies are required to confirm this hypothesis.

Concluding statement

This thesis establishes the role of *S. aureus* in recalcitrant CRS and assesses new topical agents, mupirocin and Manuka honey, as potential treatments for this very difficult clinical problem. While mupirocin is a highly effective short-term treatment, long term re-colononization and the development of resistance may limit its widespread use as a single agent. Manuka honey, has been shown to be highly effective in vitro when diluted to a level that is suitable for topical application as long a MGO is added to this solution. Its superior resistance-profile compared to traditional antibiotics may make it more appropriate for long-term therapy. This thesis also establishes the likely outcomes of patients with *S. aureus* found at the time of surgery and provides insight into possible *aggressive early-intervention* antimicrobial treatment protocols. These protocols may be of benefit to treat the high-risk patient (ie. *S. aureus*-positive at the time of surgery) before he or she might progress to chronic infection and surgical recalcitrance. Finally the role of combination therapies (mupirocin and Manuka honey) is still to be evaluated. Failing this, however, it is likely that greater treatment success may be gained with multimodal treatment, in which the multiple disease components (as demonstrated in Figure 1) are all targeted simultaneously. After all, it is entirely possible- if not likely- that a disease with an acknowledged multifactoral

aetiopathogenesis requires treatments targeted at both bacteria and the immune system.

Ultimately, however, a greater volume of research is required in order to assess the merits of all these strategies in well-conducted safety and clinical efficacy trials. Whilst a myriad of excellent antimicrobial and anti-inflammatory topical treatments- in addition to effective and easy-to-use delivery devices- have either been proposed in the literature or are even already clinically available, there remains a large void of good quality clinical studies evaluating these treatments.

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