INTRACELLULAR *STAPHYLOCOCCUS AUREUS* IN CHRONIC RHINOSINUSITIS

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Degree of Doctor of Philosophy

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Dedicated to my wonderful wife, Harriet

and our precious children, Thomas and Arabella.

DECLARATION

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PUBLICATIONS ARISING FROM THIS THESIS

Identifying intracellular *Staphylococcus aureus* in chronic rhinosinusitis: A direct comparison of techniques
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Intracellular *Staphylococcus aureus*: The Trojan horse of recalcitrant chronic rhinosinusitis?
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ABBREVIATIONS

APC	Antigen presenting-cells
ARS	Acute rhinosinusitis
ABRS	Acute bacterial rhinosinusitis
AEC	Airway epithelial cells
AERD	Aspirin-exacerbated respiratory disease
ATCC	American Type Culture Collection
CDS	Codon sequences
ClFA	Clumping factor A
ClFB	Clumping factor B
CF	Cystic fibrosis
CRS	Chronic rhinosinusitis
CSLM	Confocal scanning laser microscopy
CSR	Cell surface receptors
CT	Computed tomography
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
EB	Elementary body
ECF	Extracellular fluid
ECM	Extracellular matrix
EM	Electron microscope
EPS	Extracellular polymeric substances
ESS	Endoscopic sinus surgery
F(ab′)	Fragment antigen-binding region of antibody
FACS	Fluorescence-activated cell sorting
FC	Flow cytometry
Fc	Fragment crystallisable
FcR	Fc receptor
FCS	Fetal calf serum
FISH	Fluorescence in situ hybridisation
FESS	Functional endoscopic sinus surgery
FnBPA	Fibronectin-binding protein A
FnBPB	Fibronectin-binding protein B
HGT	Horizontal gene transfer
HNP	Human neutrophil peptides
HRQoL	Health-related quality of life
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL	Interleukin
INCS	Intranasal corticosteroids
IQR	Interquartile range
LDH	Lactate dehydrogenase
LLO	Listeriolysin O
MEM	Minimum essential medium
MHC	Major histocompatibility complex
MOI	Multiplicity of infection
MMP	Matrix metalloproteinases
MSCRAMM	Microbial surface components recognizing adhesive matrix molecules
NOD	Nucleotide Oligomerization Domain
OCT	Optimal cutting medium
PAMP	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PFGE	Pulsed field gel electrophoresis
PI	Propidium iodide
PMN	Polymorphonuclear leukocytes
PNA	Peptide nucleic acid
PRR	Pattern recognition receptors
PV	Panton-Valentin
QS	Quorum Sensing
RAST	Radioallergosorbent test
RB	Reticulate body
RCT	Randomised controlled trial
RNA	Ribonucleic acid
rRNA	Ribosomal RNA

SaPI	Staphylococcal pathogenicity islands
Sbi	Staphylococcal binder of immunoglobulin
SCV	Small colony variant
SE	Staphylococcal enterotoxins
SEA	Staphylococcal enterotoxin A
SE-L	Staphylococcal enterotoxin-like toxins
SFB	Serum free protein block
SPA	Staphylococcal protein A
TB	Tuberculosis
TBS-T	Tris-buffered saline and 0.05% tween-20 buffer
TCR	T-cell receptor
TGF-β	Transforming growth factor-beta
TIMP	Tissue inhibitor of MMP-1
TLR	Toll-like receptor
TMB	(3,3′,5,5′-Tetramethylbenzidine
TSS	Toxic shock syndrome
UIFM	Upright immunofluorescence microscopy

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ABSTRACT

Chronic Rhinosinusitis (CRS) is a heterogeneous disease characterised by recurrent and persistent episodes of nasal obstruction, discharge and facial pain or pressure. Patients suffering from CRS experience considerable morbidity and have impaired qualities of life. The gold standard treatment of cases that fail medical therapy is endoscopic sinus surgery (ESS). Despite the proven efficacy of ESS, the modern sinus surgeon will see a subset of patients who persistently fail any attempt to improve their disease profile. Recent research into CRS had identified bacterial biofilms, in particular those mediated by *Staphylococcus aureus* to hold a potential role in the aetiopathogenesis of this disease. Patients with biofilms suffer from more severe preoperative symptoms and have worse postoperative outcomes. As a consequence, numerous anti-biofilm therapies have been developed including biofilm dispersal agents and biocidal agents. Despite showing early promise *in vitro*, the use of these therapeutic agents *in vivo* has not translated to a conclusive clinical benefit. Recent studies have identified that *S. aureus* can invade non-professional phagocytic cell types such as epithelium with the ability to survive and replicate intracellularly. This led to the hypothesis that by exploiting the intracellular environment, bacteria may evade host immunity, topical antimicrobial therapy and establish a niche for survival with potential reservoirs for chronic or relapsing *Staphylococcal* infections. Therefore, this PhD thesis set out to investigate whether intracellular *S. aureus* plays a disease modifying role in CRS.

Chapter 1 critically reviews the context of the work included in this thesis pertaining to CRS, *S. aureus*, biofilms and intracellular infections.

Chapter 2 validates a novel imaging technique using confocal scanning laser microscopy (CSLM) coupled with dual staining of fluorescence in situ hybridisation (FISH) probes and nucleic acid counterstains (propidium iodide, [PI]), to identify the presence of intracellular *S. aureus* in whole mucosal specimens, with a direct comparison to previously reported techniques of immunohistochemistry (IHC). The study reported the benefits and drawbacks of each technique, and identified specific roles for their use when examining tissue specimens. The major advantage of CSLM-FISH/PI was that simultaneous biofilm analysis was possible in the same piece of tissue.

Chapter 3 investigated the unexpected phenomenon of false-positive antibody binding in *S. aureus* infected tissue specimens when performing IHC in paraffin embedded tissue sections. This was hypothesised to be caused by protein A expression in the bacterial cell wall that continued to bind IgG-class antibodies with high affinity. A methodology was developed and validated to overcome this issue, with significant implications when performing future IHC experiments.

Chapter 4 utilised the previously reported CSLM-FISH/PI protocols for intracellular *S. aureus* detection in a cohort of CRS and control patients. For the first time the association between biofilms and intracellular infection was reported, suggesting that the biofilm may offer a conditioned environment to allow invasion of *S. aureus* to deeper tissue layers.

Chapter 5 followed a wider cohort of patients in their postoperative course in order to ascertain whether a relationship between intracellular infection and disease recalcitrance could be identified. The results found that intracellular *S. aureus* infection at the time of surgery was significantly associated with failure of medical and surgical therapy in the

postoperative patients. This reinforced the theory that the intracellular location provides bacteria with a protective niche where they can avoid host elimination and topical antimicrobial therapy.

Chapter 6 investigated whether the concept of bacterial phenotype switching following intracellular infection in airway epithelial cells occurs as a mechanism of allowing these organisms to decrease their virulence and evade innate immunity. It was found that *S. aureus* reduces production of its superantigenic enterotoxins as a consequence of internalisation; however, this reduction in virulence was reversible after lysing the host cells and a single sub-culture step. Additionally, for the first time we demonstrated that intramucosal organisms harvested from sinonasal biopsies demonstrate altered phenotypic growth patterns and lack of coagulase activity consistent with small colony variants (SCV). This represented another potential explanation for why bacteria are so capable of internalising and persisting in epithelial tissues.

The findings of this thesis have provided novel insights alluding to a role of intracellular *S. aureus* in CRS. The versatility of *S. aureus* in altering its phenotypic characteristics to take advantage of the local environment makes it troublesome to fully eradicate and significant associations can be made between intracellular infection and recalcitrant disease. Future research should be directed towards identifying novel treatment strategies that can effectively target intracellular organisms.