
INTRACELLULAR *STAPHYLOCOCCUS AUREUS*
IN CHRONIC RHINOSINUSITIS

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Dedicated to my wonderful wife, Harriet
and our precious children, Thomas and Arabella.

DECLARATION

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Identifying intracellular *Staphylococcus aureus* in chronic rhinosinusitis: A direct comparison of techniques

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ABBREVIATIONS

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

SaPI	Staphylococcal pathogenicity islands	TB	Tuberculosis
Sbi	Staphylococcal binder of immunoglobulin	TBS-T	Tris-buffered saline and 0.05% tween-20 buffer
SCV	Small colony variant	TCR	T-cell receptor
SE	Staphylococcal enterotoxins	TGF- β	Transforming growth factor-beta
SEA	Staphylococcal enterotoxin A	TIMP	Tissue inhibitor of MMP-1
SE-L	Staphylococcal enterotoxin-like toxins	TLR	Toll-like receptor
SFB	Serum free protein block	TMB	(3,3',5,5'-Tetramethylbenzidine
SPA	Staphylococcal protein A	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.