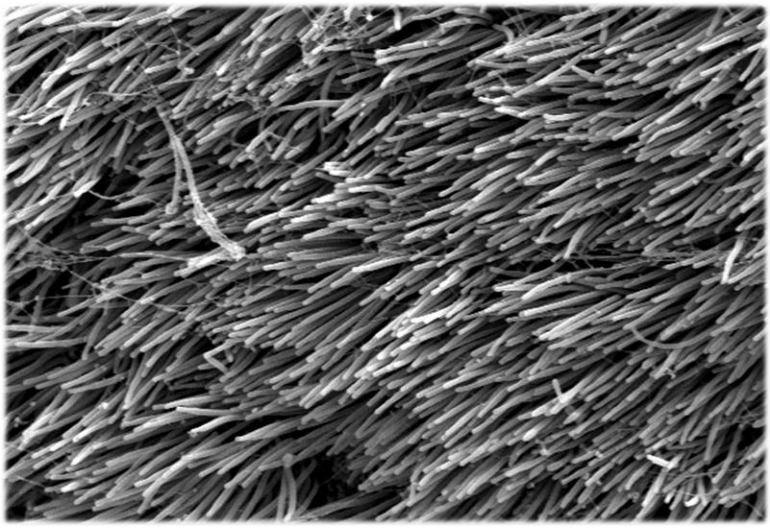
Bacteriophage therapy for application against Staphylococcus aureus infection and biofilm in chronic rhinosinusitis



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The enemy of my enemy is my friend

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I. Abstract

Chronic rhinosinusitis (CRS) is a debilitating condition characterised by critical inflammation of the mucosa of the nose and paranasal sinuses. Effecting up to 14% of the world's population CRS severely impacts a patient's quality of life. The aetiology of CRS is complex and relatively undefined encompassing a multitude of contributing factors. Bacterial infection is one factor thought to play a role in the pathogenesis of CRS. More specifically biofilm forms of the bacterial species *Staphylococcus aureus* have been shown to negatively influence post-operative progression. Current practice treatment strategies often fail to remove biofilms from the mucosa of the nose. It is therefore of import to develop novel anti-biofilm therapeutics. Our understanding of the epidemiology of *S. aureus* infections and biofilms in CRS is also limited. Increasing our epidemiological knowledge would help in the development of effective treatment strategies against recurrent infections.

Investigation into the epidemiology of *S. aureus* infections was undertaken by collecting *S. aureus* isolates from mucous and biofilm structures of CRS patients. The clonal type of each isolate was then compared to the other isolates using pulse field gel-electrophoresis. Results of this study indicated that the majority of patients experiencing recurrent infections maintained the same clonal type. Furthermore the study suggested that long-term antibiotic therapy in some patients can lead to the development of bacterial antibiotic resistance. Therefore development of a novel antibacterial therapy outside of antibiotics is required.

A potential anti-biofilm therapy both eliminating and preventative in nature is the application of bacteriophage. Bacteriophage (phage) are viruses that specifically target, infect and destroy bacterial cells. Initially *in vitro* study was undertaken to assess the anti-biofilm activity of a phage cocktail specific for *S. aureus* (CT-SA) using a minimal biofilm eradication assay plate. *S. aureus* isolates from CRS patients were grown to mature

biofilm form and treated with CT-SA for 48hrs. Following treatment biofilm biomass was determined by staining bacteria with a Live/Dead BacLight stain, imaging the biofilm using confocal scanning laser microscopy and determining biofilm biomass using software COMSTAT2. Results showed CT-SA significantly reduced *S. aureus* biofilms of susceptible strains. Results also indicated that a cocktail of phage was superior to use of a single phage as it reduced the frequency of bacterial resistant to the phage treatment.

Following on from *in vitro* work, the safety and efficacy of CT-SA was assessed *in vivo* using a sheep model of frontal sinusitis associated with *S. aureus* infections. CT-SA was also combined with ethylenediaminetetraaceticacid (EDTA) to observe if these therapies would synergise. Results indicated both CT-SA and EDTA were safe for short term topical application to the sinus regions. Furthermore both CT-SA and EDTA individually significantly reduced *S. aureus* biofilm levels in the frontal sinus, but were not seen to synergise.

Work conducted in this thesis has helped lead towards development of a novel anti-*S. aureus* biofilm agent. Future translation of CT-SA to a clinical trial setting may not only reduce or remove *S. aureus* biofilm from CRS patient noses but also improve their symptomatology and quality of life.

II. 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 Amanda J Drilling 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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IV. Presentations and Awards Arising from this thesis

Presentations:

Basil Hetzel Institute post-graduate presentation, Adelaide, Australia, **Bacteriophage as a Novel Treatment of Recalcitrant Chronic Rhinosinusitis,** August 2011

Australian Society of Otolaryngology Head & Neck Surgery Annual Scientific Meeting, Adelaide, Australia, **'Bacteriophage as a Novel Treatment for Staphylococcus aureus Biofilm,'** April 2012

Basil Hetzel Institute post-graduate presentation, Adelaide, Australia, '**Bacteriophage treatment of biofilm in an in vivo sheep model of sinusitis,'** August 2012

TQEH Research Foundation Research Day (Poster presentation), Adelaide, Australia, '**Bacteriophage reduces biofilm of *Staphylococcus aureus ex vivo* isolates from chronic rhinosinusitis patients,' October 2012**

Australian microbiological Society Microbiological updates seminar, Adelaide, Australia, **'Can Stalin's forgotten cure be used to treat sinusitis?'** October 2012

University Engagement and the Florey Medical Research Foundation Friends & Benefactors presentation, Adelaide, Australia, **'Bacterial Therapeutics: The enemy of my enemy is my friend,'** July 2013

Australian Microbiology Society Conference, Adelaide, Australia, '**Bacterial Therapeutics: The enemy of my enemy is my friend'** July 2013, invited speaker.

Basil Hetzel Institute post-graduate presentation, Adelaide, Australia, '**Cousins, siblings or copies: the genomics of recurrent *Staphylococcus aureus* infections in chronic rhinosinusitis,'** August 2013

American Rhinolgic Society Annual Meeting, '**Safety and efficacy of topical bacteriophage and EDTA treatment of *Staphylococcus aureus* infection in a sheep model of sinusitis,'** Vancouver, Canada, October 2013

TQEH Research Foundation Research Day (Poster presentation), Adelaide, Australia, '**The enemy of my enemy is my friend: Assessing bacteriophage treatment of *S. aureus* biofilm in vivo,'** October 2012

Awards:

Finalist in Poster Presentation,

TQEH Research Foundation Research Day, Adelaide Australia, 2012

Best Presentation Senior PhD researchers

TQEH Research Foundation Research Day, Adelaide Australia, 2013

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VII. Abbreviations

Abbreviation	Description
AR	Acute rhinosinusitis
ATCC	American type culture collection
BIM	Bacteriophage insensitive mutant
C	Confluent
CI	Clinical isolate
CPC	cetylpyridinium chloride
CRS	Chronic rhinosinusitis
CRSwNP	CRS with nasal polyps
CRSsNP	CRS without nasal polyps
CT4	Cocktail of *Staphylococcus aureus* specific phage concentration 6 x 10^4 PFU/mL
CT6	Cocktail of *Staphylococcus aureus* specific phage concentration 6 x 10^6 PFU/mL
CT8	Cocktail of *Staphylococcus aureus* specific phage concentration 6 x 10^8 PFU/mL
CT*hi*	Cocktail of *Staphylococcus aureus* specific phage (heat inactivated)
CT-SA	Cocktail of *Staphylococcus aureus* specific phage
CTSA-EDTA	Combination of cocktail of *Staphylococcus aureus* specific phage and ethylenediaminetetraaceticacid
eDNA	Extracellular DNA
EDTA	Ethylenediaminetetraaceticacid
EPOS	European position paper on rhinosinusitis and nasal polyps committee
EPS	Extracellular polymeric substances

FESS	Function endoscopic sinus surgery
IgE	Immunoglobin type E
MRSA	Methicillin resistance *Staphylococcus aureus*
NP	Nasal polyps
NT	No treatment
PFGE	Pulse Field gel electrophoresis
PFU	Plaque forming units
O	Opaque
QOL	Quality of life
SAgs	Superantigens
SEB	Staphylococcal enterotoxin B
SEM	Scanning electron microscopy
SC	Semi-confluent
TEM	Transmission electron microscopy