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 ethylenediaminetetraacetic acid (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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I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue, and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Date: ___________
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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 Rhinologic 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


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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AR</td>
<td>Acute rhinosinusitis</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>BIM</td>
<td>Bacteriophage insensitive mutant</td>
</tr>
<tr>
<td>C</td>
<td>Confluent</td>
</tr>
<tr>
<td>CI</td>
<td>Clinical isolate</td>
</tr>
<tr>
<td>CPC</td>
<td>Cetylpyridinium chloride</td>
</tr>
<tr>
<td>CRS</td>
<td>Chronic rhinosinusitis</td>
</tr>
<tr>
<td>CRSwNP</td>
<td>CRS with nasal polyps</td>
</tr>
<tr>
<td>CRSsNP</td>
<td>CRS without nasal polyps</td>
</tr>
<tr>
<td>CT4</td>
<td>Cocktail of <em>Staphylococcus aureus</em> specific phage concentration $6 \times 10^4$ PFU/mL</td>
</tr>
<tr>
<td>CT6</td>
<td>Cocktail of <em>Staphylococcus aureus</em> specific phage concentration $6 \times 10^6$ PFU/mL</td>
</tr>
<tr>
<td>CT8</td>
<td>Cocktail of <em>Staphylococcus aureus</em> specific phage concentration $6 \times 10^8$ PFU/mL</td>
</tr>
<tr>
<td>CTHi</td>
<td>Cocktail of <em>Staphylococcus aureus</em> specific phage (heat inactivated)</td>
</tr>
<tr>
<td>CT-SA</td>
<td>Cocktail of <em>Staphylococcus aureus</em> specific phage</td>
</tr>
<tr>
<td>CTSA-EDTA</td>
<td>Combination of cocktail of <em>Staphylococcus aureus</em> specific phage and ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>eDNA</td>
<td>Extracellular DNA</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EPOS</td>
<td>European position paper on rhinosinusitis and nasal polyps committee</td>
</tr>
<tr>
<td>EPS</td>
<td>Extracellular polymeric substances</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>FESS</td>
<td>Function endoscopic sinus surgery</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobin type E</td>
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<td>MRSA</td>
<td>Methicillin resistance <em>Staphylococcus aureus</em></td>
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<tr>
<td>NP</td>
<td>Nasal polyps</td>
</tr>
<tr>
<td>NT</td>
<td>No treatment</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulse Field gel electrophoresis</td>
</tr>
<tr>
<td>PFU</td>
<td>Plaque forming units</td>
</tr>
<tr>
<td>O</td>
<td>Opaque</td>
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<tr>
<td>QOL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>SAgs</td>
<td>Superantigens</td>
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<tr>
<td>SEB</td>
<td>Staphylococcal enterotoxin B</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SC</td>
<td>Semi-confluent</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
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