The Relevance of Bacteriophage Therapy in the Era of Antibiotic Resistant Bacteria

By

Sandra Patricia Porteous Morales

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Certificate of Authorship

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Sandra Patricia Porteous Morales

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Abstract

Against a backdrop of rising antibiotic resistance and a dwindling pipeline of new antibacterial drugs, this thesis set out to examine the potential of bacteriophage (phage) therapy as an alternative or complementary means of treating bacterial infection. Phage therapy is used as a frontline antibacterial therapy in the former Soviet bloc countries but remains an unexplored technology in Western science. To investigate the reasons behind this and other aspects of phage therapy, this thesis undertook the development of bacteriophage-based products against two important human bacterial pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Pseudomonas aeruginosa* (MRPA).

To develop phage products in today's scientific and regulatory framework it was necessary to return to basic principles. The first stage in this process involved the assembly of reference collections of the target bacteria. Once these were available, bacteriophages were isolated from a range of environmental sources, and their spectrum of activity and physical characteristics evaluated. Bacteriophages with the appropriate reactivity profile were then tested for stability, morphology, and further analysed using molecular biological techniques. Phages with therapeutic potential were then combined into mixtures or "cocktails" and their activity evaluated against clinical isolates from different geographical regions. Lastly, a commercial phage product was used in one compassionate case study involving a hospital patient to treat a refractory *P. aeruginosa* urinary tract infection.

Fifty-two MRPA and fifty-eight MRSA phages were isolated over a period of 18 months. Selected phage therapeutic candidates were shown to be physically stable and genetically different from each other. They also showed a broad spectrum of activity against the targeted pathogens and this resulted in the production of three prototypes cocktails for each target pathogen. The MRPA cocktails achieved a reactivity of 62%-90% against clinical isolates from four geographical areas while the MRSA cocktails achieved a reactivity of 61%-96% in two geographical areas. In the clinic, a compassionate phage therapy treatment

was well tolerated, produced no adverse side-effects, and in combination with antibiotics, resulted in the complete eradication of a refractory *P. aeruginosa* urinary tract infection.

This thesis has demonstrated, for the first time in Australia, that it is possible to develop stable, fully characterised, broad-spectrum bacteriophage-based products with the potential to treat human infections caused by MRSA and MRPA. It also showed the value of phage therapy in the clinic by eradicating a chronic *P. aeruginosa* infection. Furthermore, though not presented in the main body of this thesis, two of the bacteriophage prototypes developed here (one MRSA and one MRPA) were recently shown to be effective in treating bacterial infections in two separate animal models.

Phage therapy has the potential to play a major role in addressing the serious problems caused by the ever-widening antibiotic resistance crisis. No doubt, there will be production and regulatory hurdles to overcome and an urgent requirement to train a new generation of microbiologists and clinicians skilled at developing and administering these powerful antibacterials. However, it is now obvious that the old paradigm of depending on a constant stream of novel antibiotics is no longer valid and alternative technologies such as this must be fully explored.

Posters, oral presentations and peer reviewed publications in chronological order

Poster Presentations

1 **Morales, S.P.,** *et al.* (2007) Bacteriophage Therapy: An alternative to conventional antibiotic treatment for resistant infections; Proceedings of The Australian Society for Microbiology Conference, Adelaide.

2 **Morales, S.P** *et al*. (2007) Bacteriophage Therapy in Aquaculture: Isolation of *Streptococcus iniae* phages for the treatment of farmed Barramundi fish in Australia: Proceedings of the 17th Evergreen International Phage Biology Meeting, ed. C. Loc-Carrillo, Olympia.

3 **Morales, S.P** *et al*. (2009) In vitro activity of bacteriophage cocktails against *Pseudomonas aeruginosa*: Proceedings of the 18th Evergreen International Phage Biology Meeting, C.Loc-Carrillo, Olympia.

4 **Morales, S.P** *et al.* (2010) Global screening of therapeutic phage preparations against clinical isolates of *Pseudomonas aeruginosa*. Poster and Abstract in the Proceedings of the 17th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Vienna, Austria, 10-13 April.

5 **Morales, S.P** *et al.* (2012) Bacteriophage treatment inhibits and reduces biofilm formation by *Pseudomonas aeruginosa* strains from Cystic Fibrosis patients. Poster and Abstract in the Proceedings of the 22nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), London, England, 31st March-3 April.

6 Pabary, R., Singh, C., **Morales, S**., *et al* (2012) Anti-Pseudomonal bacteriophage cocktail reduces inflammatory responses in the murine Lung. *Thorax* 67 (S103): A50-A51.

7 Drilling, A., **Morales, S.,** *et al* (2013) Developing a novel treatment for sinonasal *Staphylococcus aureus* biofilms: The enemy of my enemy is my friend. Proceedings of The Australian Society for Microbiology Conference, Adelaide.

Oral Presentations

1 **Morales, S.P**., *et al*. (2009) In vitro and in vivo efficacy of phage therapy cocktails for the treatment of multidrug-resistant *Pseudomonas aeruginosa*; Proceedings of The Australian Society for Microbiology Conference, ed. J. Sofronidis, Perth.

2 **Morales, S.P** *et al.* (2010) Prospects of phage therapy. First Colombian Event in Phage Biology and Phage Therapy: keynote speaker. Bogota, Colombia, 17-18 August 2010.

3 **Morales, S.P** *et al.* (2010) Use of bacteriophages for the control of *Salmonella* sp. First Colombian Event in Phage Biology and Phage Therapy: keynote speaker. Bogota, Colombia, 17-18 August 2010.

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5 **Morales, S.P** *et al.* (2011) Development and optimization of a universal MRSA phage cocktail. Oral presentation and abstract in the Proceedings of the 19th Evergreen International Phage Biology Meeting. Olympia, USA, 7-12 August 2011.

6 **Morales, S.P** *et al.* (2013) Bacteriophage therapy for the treatment of *Pseudomonas aeruginosa* lung infections in Cystic Fibrosis patients. Oral presentation and abstract in the Proceedings of the 20th Evergreen International Phage Biology Meeting. Olympia, USA, 4-9 August 2013.

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1 Smithyman, A.M. and **Morales, S.P**. (2007) Bacteriophage Therapy: An alternative treatment in the Era of Antibiotic Resistant Bacteria?. *Syntrophy*,8 (1): 1,9

2 Khawaldeh, A., **Morales, S et al** (2011). "Bacteriophage therapy for refractory Pseudomonas aeruginosa urinary tract infection". *Journal of Medical Microbiology* 60 (11): 1697-1700.

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4 Drilling, A., **Morales, S**., *et al* (2014). Safety and efficacy of topical bacteriophage and ethylenediaminetetraacetic acid treatment of *Staphylococcus aureus* infection in a sheep model of sinusitis. *International Forum of Allergy and Rhinology* 4 (3): 176-186

AWARDS

Becton Dickinson ASM Student Award winner in 2011 for the work done on the subject: "**Development of bacteriophage cocktails for the management of nosocomial and community- acquired MRSA**".

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