The Skin Sore Trial: Exploring a better treatment option for impetigo in Indigenous children living in remote Australia

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Thesis submitted for the degree of
Doctor of Philosophy

December 2014

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Darwin, NT, Australia
DECLARATION

I hereby declare that the work herein now submitted as a thesis for the degree of Doctor of Philosophy of the Charles Darwin University is the result of my own investigations, and all references to the ideas and work of other researchers have been specifically acknowledged. I hereby certify that the work embodied in this thesis has not already been accepted in substance for any degree, and is not being currently submitted in candidature for any other degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, and online via the University’s Open Access repository eSpace.

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December 2014
ABSTRACT

Impetigo is prevalent among Australian Indigenous children living in the Northern Territory. The cause of this endemic skin infection is multifactorial including household overcrowding, poverty, endemic scabies and fungal skin infections, tropical climate, insect bites and minor trauma. Although impetigo is widespread, affecting up to 50% of children in a community at one time, care seeking for treatment of impetigo does not reflect this burden accurately, possibly due to awareness of the painful injection prescribed. A better treatment is needed.

This thesis presents the first randomised controlled trial conducted amongst remote living Indigenous Australian children on the treatment of extensive impetigo, and one of the largest impetigo trials worldwide. Methodological gaps were addressed, with studies published on the susceptibility of *Streptococcus pyogenes* to trimethoprim-sulphamethoxazole (SXT), the transport of impetigo swabs in remote settings and photography protocols for capturing and scoring sores. The principal findings are:

1. Either of two short courses of SXT are non-inferior to standard treatment of impetigo with intramuscular benzathine penicillin G (BPG) – published in *The Lancet*;

2. *S. pyogenes* remains the key driver of impetigo in our region; and

3. *S. pyogenes* is susceptible *in vitro* to SXT, and SXT is effective *in vivo* for the treatment of skin infections caused by *S. pyogenes*.

These findings will inform the management of children with impetigo in remote Indigenous communities and have already been incorporated into treatment algorithms and guidelines regionally and nationally. This provides a simple, short-course antibiotic regimen that is palatable, inexpensive and has a low side-effect profile, as an alternative to an injection of intramuscular penicillin, which has been the standard of care for more than 20 years. Ongoing surveillance will
be needed to understand how this new regimen is utilised and whether widespread use for a common condition will alter antibiotic resistance profiles.
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Chapter 5: Developing a methodology for capturing and scoring standardised digital images of impetigo

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7.1 Chapter overview

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ACKNOWLEDGEMENTS

There are many people to acknowledge for their support throughout the four years of work that has gone into this thesis. Above all, I thank my Lord and Saviour, Jesus Christ, through whom I am strengthened each day to accomplish what he has called me to do (Philippians 4:13).

It would not have been possible without the steadfast love and support of my husband Darren Westphal. He has been a constant source of encouragement, day in and day out, even when it all seemed impossible. He has been by my side throughout this PhD and has been a great sounding board for my thoughts as they developed. His skills as an amateur photographer and involvement in health research guided me through the early days of developing a method for photographing digital images. He has kept me focused on the bigger world outside my thesis, encouraged me to present my work at international conferences so that he could come along too and kept our family functioning at peak times of my thesis. Our children Zachary and Eliana, who have both been born during my candidature, have grounded me when I felt overwhelmed. They have kept me laughing, kept me busy and are really looking forward to when Mummy no longer needs to work all the time! My family both in Australia and the USA have taken care of my children to give me time to write, encouraged me even when they weren’t too sure what I was talking about and listened along the way to all of my challenges. My mum, Narelle Bowen, has been willing on several occasions to come and care for my family, to give me the opportunity to write, even arriving from the other side of the country with less than 24 hours notice when I needed her.

My supervisors have taught me the art and science of research, motivated me when paving a path forward seemed impossible and corrected endless drafts of papers. To each of them I offer my sincere thanks for taking on a novice researcher to lead this trial. I would like to thank Jonathan Carapetis for his unending belief in my ability to achieve goals that seemed insurmountable; an always open door, telephone line or email inbox to discuss my questions and address challenges; and his vision for the big picture. My involvement in
research was inspired by Jonathan and would not have been possible without him opening this doorway. Likewise, my regular meetings would not have occurred without his excellent personal assistants Caroline Sheridan, Kristy Coulston and Linda Lorimer. Adding Steven Tong as a co-supervisor in my second year has been an enormous blessing. His wisdom has been an asset, his understanding of the PhD journey and timeline important, and his research savvy inspiring. Steve has sat with me on many occasions nutting out statistical analyses that seemed impossible, suggesting creative ways forward with papers and has been available for endless questions. Ross Andrews oversaw the trial management team and answered many questions on epidemiology along the way. He was willing from the outset to consider my input into changes in the study design. I have learned and grown under their collective supervision and am inspired by their ongoing commitment to answering questions that make a meaningful difference to the health of Indigenous Australians.

Alongside my core supervisors sit the two other chief investigators on this trial. Bart Currie has inspired me throughout. His enthusiasm, knowledge and clinical acumen and his endless energy for high quality research to improve the quality of care for Indigenous people makes him an outstanding clinician researcher, who was never too busy for my questions. Bart has also been integral in translating our findings into treatment guidelines in real time and has guided me through this process. Malcolm McDonald left Darwin early in my candidature, but has remained a supporter, encourager and most importantly, an excellent critic of my written work. His ability to edit words out of a manuscript without losing any meaning is a skill I hope I will continue to develop in my future work. These five investigators envisioned this trial, pursued it undeterred through three NHMRC funding rounds and welcomed me to the team. I am humbled by their collective experience, inspired by their enthusiasm and motivated to continue to conduct translational research with meaningful outcomes for Indigenous children.

My PhD is a single, large randomised controlled trial, conducted in very remote communities of the Northern Territory. It is one of the largest impetigo trials ever conducted, and the first in truly remote settings where the burden of disease is the highest. It would not have been possible without the large trial team: the chief
investigators (Jonathan, Steve, Ross, Bart and Malcolm), the project managers (Irene O’Meara and her predecessor Tammy Fernandes) and the research team (Jane Nelson, Melita McKinnon, Therese Kearns, Valerie Coomber, Christine Francais, Colleen Mitchell, Dianne Halliday, Dev Tilikiratne, Neil Kerinaiua, Lynette Johnson, Simone Baker, Doreen Jinggarrabarra, Duncan Wutumbul, Roslyn Gundjirrjirr and Elvira Dhumabuy). Alongside this group were residents, and medical and nursing students, who participated for a brief week or two as research assistants to gain exposure to research in Indigenous communities. Jane, TK, Melita and Margaret Landrigan were critical in the establishment of the trial and oversaw the operations when the team was in the field. The laboratory scientists both at Menzies (Rebecca Towers, Rachael Lilliebridge, Donna Woltring, Jacklyn Ng, Wajahat Mahmood, Bianca Hayes and Vanessa Theobold) and at Royal Darwin Hospital (Jann Hennesey, Greg Haran, Pam Smith, Luke Tennent, Nicole McMahon, Gloria de Castro and Nu Nu Swe) were tireless in their commitment to processing thousands of swabs for culture and antibiotic susceptibility testing.

Irene O’Meara has been an outstanding project manager throughout the trial, keeping track of trial progress, planning trips to communities and endlessly recruiting staff for the study. Irene’s willingness to answer any question, clean data, discuss results and make suggestions has been invaluable. I cannot thank her enough for her drive, dedication and enthusiasm to see this trial through to completion. Irene’s willingness to go the extra mile and her dedication to Indigenous health is highly commendable. I know with certainty that the results reported in this thesis would not have been possible without Irene’s commitment. Irene is the face of the Skin Sore Trial in the remote communities. Children and families were keen to know the results when they saw Irene arrive back in their communities. In addition to numerous recruitment trips, she coordinated all the feedback trips on which we had the exciting opportunity of sharing our findings with families, teachers, clinic staff and community elders. Having Irene in charge saw recruitment completed in the required time frame when even this seemed impossible. Irene has become a good friend and is a dedicated project manager.
Robyn Liddle, Melita McKinnon, Tegan Harris and Linda Walsh were all involved in data management for the study. Robyn’s willingness to teach me Microsoft Access and to answer numerous queries about the data structure was admirable. Robyn was also invaluable in teasing out various challenges in statistical analysis. Her willingness to build databases and modify these until working smoothly for the photography review was fantastic. Without Robyn’s expertise in this area, it would not have been possible to organise and score the thousands of digital images collected over the course of the study. The members of the Area9 team at Menzies throughout my PhD were also incredibly helpful in this process, making sure that the technology for scoring digital images by colleagues in Melbourne, Perth, Auckland, Adelaide or Brisbane worked smoothly. They problem solved and developed solutions to make the process work faster when this was also a challenge.

Kara Burns was integral in developing simple protocols for amateur photographers that gave us sets of digital images of the same sore that could be scored. I thank my paediatric colleagues who willingly looked at the paired digital images. Tom Snelling, Andrew Steer, Claire MacVicar, Deena Parbhoo, Rebecca Cresp, Sarah Cherian, Sarah Martin and Ruth Lennox were all willing and available when I needed their assistance.

Mark Chatfield’s assistance with statistical analysis for the study was invaluable. His advice to the chief investigators on various important statistical decisions strengthened the study. He also guided us through the process of engaging a data safety and monitoring board. I thank Jim Buttery who chaired this group along with Keith Edwards, Stephen Lambert and Peter O’Rourke for their commitment to the study.

During my training to become an infectious diseases specialist, I experienced a brief period of training in a microbiology laboratory. Whilst not being a microbiologist, my PhD candidature has extended my knowledge and skills in microbiology. It would not have been possible to do so without the willingness of microbiology colleagues to share their insights and to answer my questions. These included Rob Baird, Malcolm McDonald, Peter Ward, Phil Giffard, Steven Tong and Jann Hennesey. To the scientists at Menzies and Royal Darwin...
Hospital who have conducted experiments, processed thousands of specimens and provided a robust microbiological data set, I am truly grateful.

Sharing office space with other PhD students has been a privilege and encouragement along the way. Many a problem felt less challenging after discussion with peers, at various stages, on the same journey. The broader higher degree community at Menzies has also been invaluable with their willingness to stop for a chat in the tea room to commiserate, encourage or congratulate! The education office of Caroline Sheridan and Catherine Richardson has helped me keep the funding bodies and university reports timely. The finance department was also integral in administering my scholarships.

Menzies has been a welcoming and productive environment in which to begin my research career. Whilst not being my immediate supervisors, I have been mentored and supported by Anne Chang and Peter Morris. Their experience in conducting randomised trials, and their passion for doing excellent research that makes a difference for Indigenous children, is inspiring. My colleagues in the Infectious Diseases Department of Royal Darwin Hospital should also be thanked. This group of world-class clinician researchers encourage and support junior colleagues, and are in turn able to make ground-breaking discoveries. This would not be possible without the extremely hard work of the research administration and ethics teams. Christina Spargo and Maria Scarlett deserve special mention for their assistance throughout my candidature. Thanks must also go to the Communications team at Menzies who were instrumental in allowing our research findings to gain media attention. Richmond Hodgson, Lucy Barnard and Claire Addinsall were dedicated and their efforts led to local and national media reports on the day our *Lancet* paper was released. I was keen for our findings to be highlighted in the Indigenous media. Lucy coordinated a number of interviews to ensure our research was communicated in plain language to those who may one day need to be treated. I thank my mum, Narelle Bowen and Sue Dibbs for assistance with editing my thesis.
It would not have been possible to enrol in a PhD without financial support. The funding from the National Health and Medical Research Council, Australian Academy of Sciences and Menzies School of Health Research enabled me to train as a researcher and to present our findings at regional, national and international meetings.
DEDICATION

I dedicate this thesis to the participants and their families throughout the Northern Territory who freely consented to join the Skin Sore Trial in the hope that a better treatment for skin sores would be found. It is my hope that an injection will no longer be a deterrent to seeking effective treatment for skin sores, and one day we will report that Indigenous children have the same burden of impetigo as their non-Indigenous counterparts.

To my children, Zachary and Eliana, I hope that one day you might understand the importance of my work in striving to make a difference for Indigenous children, and that you may live in a world where studies like this are no longer needed.
PUBLICATIONS


Submitted Manuscripts under review

**Manuscripts in development**

The global burden of impetigo: A systematic review.

Tasani M, Tong SYC, Holt D, Currie BJ, Carapetis JR, **Bowen AC**. Does the presence of scabies impact treatment efficacy? Further analysis of the Skin Sore Trial results
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Bowen AC, Tong SYC, McDonald MI, Carapetis JR. *In vitro* and *in vivo* susceptibility of *Streptococcus pyogenes* to trimethoprim/sulphamethoxazole in Northern Australia. European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, SPAIN, 10 – 13 May 2014 (Oral and ePoster presentation)

Bowen AC, Tong SYC, Carapetis JR. Epidemiology of impetigo in Indigenous children in remote northern Australia: results from a randomised controlled trial. Annual Meeting of the European Society of Paediatric Infectious Diseases, Dublin, IRELAND, 6 – 9 May 2014 (Oral and poster presentation)

Bowen AC, Tong SYC, Andrews RM, O’Meara IM, Chatfield MD, McDonald MI, Currie BJ, Carapetis JR. Short course oral trimethoprim-sulphamethoxazole is as effective as intramuscular benzathine penicillin G for impetigo: Results of a non-inferiority, randomised controlled trial. Australasian Society of Infectious Diseases Conference, Adelaide, AUSTRALIA, 26 – 29 March 2014 (oral presentation)

Bowen AC. The infectious disease burden of Australia’s Indigenous children living in the Northern Territory. World Congress of the World Society of Paediatric Infectious Diseases, Cape Town, SOUTH AFRICA, 19 - 22 November 2013 (invited speaker)

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Bowen AC, Tong SYC, Carapetis JR. The microbiology of impetigo in Indigenous children of tropical Australia. World Congress of the World Society of Paediatric Infectious Diseases. Cape Town, SOUTH AFRICA, 19 - 22 November 2013 (poster)

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Bowen AC, Tong SYC, Andrews RM, O’Meara IM, Chatfield MD, McDonald MI, Currie BJ, Carapetis JR. Trimethoprim-sulphamethoxazole is non-inferior to benzathine penicillin G for the treatment of impetigo: Results of a Randomised Controlled Trial. Northern Territory Royal Australasian College of Physicians, Alice Springs, NT, 9 November 2013.

Bowen AC, Tong SYC, Andrews RM, O’Meara IM, Chatfield MD, McDonald MI, Currie BJ, Carapetis JR. Trimethoprim-sulphamethoxazole is non-inferior to benzathine penicillin G for the treatment of impetigo: Results of a Randomised Controlled Trial. Paediatric Grand Rounds, Royal Darwin Hospital, Darwin, NT, 1 November 2013.
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<th>Definition</th>
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<tr>
<td>APP</td>
<td>As per protocol</td>
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<tr>
<td>ARF</td>
<td>Acute rheumatic fever</td>
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<td>AST</td>
<td>Antibacterial susceptibility testing</td>
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<td>BHS</td>
<td>beta-haemolytic streptococci</td>
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<tr>
<td>BPG</td>
<td>Benzathine penicillin G</td>
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<td>CARPA</td>
<td>Central Australian Rural Practitioners Association</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
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<tr>
<td>CNA</td>
<td>Collistin and nalidixic acid</td>
</tr>
<tr>
<td>CRF</td>
<td>Case report form</td>
</tr>
<tr>
<td>CTX</td>
<td>Cotrimoxazole (or trimethoprim-sulfamethoxazole)</td>
</tr>
<tr>
<td>DSMB</td>
<td>Data Safety and Monitoring Board</td>
</tr>
<tr>
<td>EUCAST</td>
<td>European Committee on Antimicrobial Susceptibility Testing</td>
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<tr>
<td>GAS</td>
<td>Group A streptococcus (<em>Streptococcus pyogenes</em>)</td>
</tr>
<tr>
<td>HREC</td>
<td>Human Research Ethics Committee</td>
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<tr>
<td>IDSA</td>
<td>Infectious Diseases Society of America</td>
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<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
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<td>ITT</td>
<td>Intention to treat</td>
</tr>
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<td>JPEG</td>
<td>Joint Photographic Experts Group</td>
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<td>MHA</td>
<td>Mueller Hinton agar</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>MHBA</td>
<td>Mueller Hinton agar with horse blood</td>
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<tr>
<td>MHF</td>
<td>Mueller Hinton agar containing defibrinated horse blood and 20mg/liter beta NAD</td>
</tr>
<tr>
<td>MHLHBA</td>
<td>Mueller Hinton agar with lysed horse blood</td>
</tr>
<tr>
<td>MHS</td>
<td>Mueller Hinton agar with sheep blood</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>mITT</td>
<td>modified Intention to treat</td>
</tr>
<tr>
<td>mMRSA</td>
<td>multidrug resistant MRSA</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>MSSA</td>
<td>Methicillin susceptible <em>Staphylococcus aureus</em></td>
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<tr>
<td>NHMRC</td>
<td>National Health and Medical Research Council</td>
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<tr>
<td>nmMRSA</td>
<td>non multidrug resistant MRSA</td>
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<tr>
<td>NT</td>
<td>Northern Territory</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>PhD</td>
<td>Doctor of Philosophy</td>
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<tr>
<td>QC</td>
<td>Quality Control</td>
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<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
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<tr>
<td>SMGGB</td>
<td>Skim milk tryptone glucose glycerol broth</td>
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<tr>
<td>SMZ</td>
<td>sulfamethoxazole</td>
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<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
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<tr>
<td>SSTI</td>
<td>Skin and soft tissue infections</td>
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<tr>
<td>STGGB</td>
<td>Skim milk tryptone glucose glycerol broth</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>---------</td>
<td>-------------</td>
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<tr>
<td>SXT</td>
<td>Trimethoprim/sulphamethoxazole</td>
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<tr>
<td>TMP</td>
<td>trimethoprim</td>
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<tr>
<td>WGS</td>
<td>Whole Genome sequencing</td>
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<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER 1
INTRODUCTION

1.1 THESIS OVERVIEW

This chapter will introduce the main themes of the thesis. To understand the context of this thesis, it will be important to know about both the study setting and impetigo: what it is, how it occurs, what treatments are available and what is known about the natural history of impetigo resolution. This will pave the way for Chapter 2, a systematic review of population-based studies on the prevalence of impetigo to define the global burden. Methodology chapters will follow, defining methods for transporting impetigo swabs in remote contexts (Chapter 3), testing for antibiotic susceptibility of *Streptococcus pyogenes* to trimethoprim/sulphamethoxazole, an antibiotic which has long been thought to be ineffective against this organism (Chapter 4) and capturing digital images for measuring the outcome of a randomised controlled trial (Chapter 5). The results of the major study will then be presented in Chapter 6, followed by the microbiology of impetigo in Indigenous children of Northern Australia in Chapter 7. Chapter 8 will conclude the thesis with future directions for research translation, and highlight the need for ongoing studies to dernormalise skin disease in remote Indigenous children of Australia.
1.2 A BRIEF OVERVIEW OF THE NORTHERN TERRITORY, AUSTRALIA

Figure 1: Map of Australia highlighting the Northern Territory in red.

Source: www.virtualoceania.net/australia/nt_map.png (accessed 19/11/14).

All the work reported in this thesis has been undertaken in the Northern Territory (NT), a federal territory of Australia (Figure 1). This brief orientation is provided to place the work in context. The NT includes about one-sixth of the Australian continent, with an area of 1.35 million square kilometres (www.ga.gov.au/scientific-topics/geographic-information/dimensions/area-of-australia-states-and-territories, accessed 17/11/14). The NT is divided into two regions, the ‘Top End’ where a tropical climate prevails and ‘Central Australia’ with a semi-arid climate. The NT is vast, remote and sparsely populated. It has five major centres, of which Darwin is the largest, and more than 100 remote communities. At the most recent census (2011), the NT population was 211,945 of whom 56,776 (27%) were Indigenous (Aboriginal and Torres Strait Islanders) (www.censusdata.abs.gov.au, accessed 15/11/14). Table 1 provides a limited overview of the social deprivation disproportionately suffered by the NT Indigenous population and consequent health impacts. Nurses and Aboriginal Health Workers, under the guidance of medical practitioners, provide the bulk of primary health care in remote community clinics. The CARPA standard treatment manual is widely used as a clinic manual for the delivery of primary health care (CARPA, 2014).
Table 1: Summary of key demographic differences between the Indigenous and overall population of the Northern Territory, Australia

<table>
<thead>
<tr>
<th></th>
<th>Overall NT residents</th>
<th>Indigenous NT residents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>31 years</td>
<td>23 years</td>
</tr>
<tr>
<td>Proportion aged &lt; 15 years</td>
<td>23.2%</td>
<td>33.2%</td>
</tr>
<tr>
<td>Median household income per week</td>
<td>$1,674</td>
<td>$1,099</td>
</tr>
<tr>
<td>Median number of people per household</td>
<td>2.9</td>
<td>4.2</td>
</tr>
<tr>
<td>Average number of people per bedroom</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>Unemployment rate</td>
<td>4.3%</td>
<td>13.7%</td>
</tr>
<tr>
<td>% Resident remote (outside Darwin)</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Life expectancy at birth (2009)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>75.7y</td>
<td>61.5y</td>
</tr>
<tr>
<td>Female</td>
<td>81.2y</td>
<td>69.2y</td>
</tr>
</tbody>
</table>


1.3 WHAT IS IMPETIGO?

Impetigo contagiosa, also known commonly as skin sores, school sores or shortened to impetigo, is a non-bullous infection of the superficial layers of the epidermis. (Hay et al., 2006) It was first described in 1864. (Fox, 1864) Pyoderma is a term often used interchangeably with impetigo, but pyoderma encompasses a broader definition of superficial bacterial skin infection that includes bullous and non-bullous impetigo, ecthyma, folliculitis, cellulitis and, sometimes, tropical ulcers. (Mahe, 2005) Whilst impetigo is a superficial infection of the epidermis, ecthyma is a deeper infection that extends into the dermis. (Hay et al., 2006) The distinction between these two conditions in terms of clinical recognition and treatment recommendations at a primary care level is poorly understood and as such they are often discussed together, both for microbiology and treatment algorithms. (Stevens et al., 2014, Mahe et al., 2005b,
Steer et al., 2009) This thesis will predominantly use the terms impetigo or skin sores to describe this bacterial skin infection.

1.3.1 Microbiology of impetigo

The gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* commonly cause impetigo. These bacteria may be found independently or in a mixed growth, with the relative proportions varying over time, region and climate. (Koning et al., 2012) Previous studies identified early colonisation of the skin with *S. pyogenes* resulting in the development of sores in the ensuing days through minor breaches in skin integrity. (Ferrieri et al., 1972, Dajani et al., 1972, Dajani et al., 1973) These early studies reported that impetiginous lesions were later colonised with *S. aureus* (Ferrieri et al., 1972, Dillon, 1970) and as such both pathogens were implicated at different time points in the development of impetigo, with *S. pyogenes* causing the infection and *S. aureus* complicating it.

In more recent decades, *S. aureus* has assumed prominence in the global microbiology of skin and soft tissue infections, raising treatment challenges, as this organism has developed resistance mechanisms against the commonly used antibiotics for skin infections. (Moran et al., 2013, Tong et al., 2008) The microbiology of impetigo in the Northern Territory also reflected these trends, with increasing prevalence of methicillin-resistant *S. aureus* (MRSA) as a co-pathogen with *S. pyogenes*, seen in swabs taken from impetigo lesions throughout the Northern Territory (McDonald et al., 2006) and in hospital studies. (Tong et al., 2009) Based on this changing microbiology, it was uncertain whether benzathine penicillin G remained the most effective treatment for impetigo in our context.

1.3.2 Antecedents of impetigo

Intact skin is usually resistant to infection with *S. aureus* or *S. pyogenes* (Tunnessen, 1985). Intact skin may be disrupted by trauma, abrasions, insect bites, scratching, eczema or another dermatosis (e.g., scabies infestation, tinea, pediculosis or varicella zoster). Individuals in contact with others who have a high burden of skin infections may also be colonised, (Ferrieri et al., 1972) followed by the development of impetiginous lesions at the site of skin
disruption. (Currie and Carapetis, 2000, Wannamaker, 1970) Skin pathogens are highly transmissible (Ferrieri et al., 1972) (skin-to-skin and skin-to-fomite) and result in endemic infections where household overcrowding is extreme, (Harris et al., 1992) with epidemic peaks as new bacterial strains are introduced. (Dajani et al., 1973)

Skin on any part of the body can be involved. However, due to trauma and insect bites being the major risk factors in tropical settings, (Leng and Chay, 1982, Kottenhahn and Heck, 1994, Taplin et al., 1973, Allen and Taplin, 1974) lesions are most common on the lower limbs, followed by the upper limbs (Mahe, 2005) and scalp when superinfected pediculosis occurs. (Jinadu, 1985) The predilection for the limbs in tropical contexts may also be due to the wearing of minimal clothes in the heat and humidity; as such, little protection is offered against abrasions and minor trauma. (Belcher et al., 1977) In other populations, lesions on the face, around the nose and mouth are more common. (Koning et al., 2012, Kiriakis et al., 2012)

Impetigo is endemic in hot and humid climates, (Taplin et al., 1973) particularly if there is a lack of ready access to water for cleaning. (Cairncross and Cliff, 1987, Bhavsar and Mehta, 1985, Taplin et al., 1973) Household overcrowding is a suitable environment for transmission of skin pathogens and is more commonly found in regions where poverty prevails. (Harris et al., 1992, Accorsi et al., 2009) Impetigo is a disease of poverty, with burden increasing as socio-economic status decreases. (Masawe et al., 1975) Impetigo also perpetuates where understanding and recognition of the disease as a health priority is poor. (Mahe et al., 2005a, Hay et al., 1994) Where access to dermatologists is limited, primary health care providers may not recognise this (or other) skin diseases and few studies are available to guide effective treatment decisions in these contexts. The integrated management of childhood illness (IMCI) has been developed as an approach to the recognition and treatment of impetigo and is widely used in Fiji, (Steer et al., 2009) but in other regions where impetigo is common, treatment algorithms are less well utilised. (Figueroa et al., 1998, Mahe et al., 1995) Prioritisation of community dermatology is still desperately needed. (Ryan, 2008)
Finally, the normalisation of sores and poor skin health has been raised as a possible explanation for ongoing disease burden. When the majority of children in a community have sores at any one time, and these are recognised to eventually resolve over weeks to months, sores may become an accepted norm in childhood. This normalisation of sores can result in a failure to seek health care, (Dogra and Kumar, 2003) and a failure of health-care practitioners to appropriately treat for impetigo when the child presents with other complaints. On the other hand, skin disease has been found to be the second most common reason for health care seeking in childhood in developing countries. (Estrada Castanon et al., 1992, Figueroa et al., 1996, Walker et al., 2008) This contradictory information makes estimating the true burden of skin disease, including impetigo, difficult. Focusing on episodes of health care sought (through audits of clinic attendance) rather than population prevalence may result in under-reporting of the true burden of impetigo.

1.3.3 Identification of impetigo

Skin sores are identified by the classic characteristics of purulence, erythema and golden crusting, which progresses to form a thick scab. (Wannamaker, 1970) Sores are often painful, itchy and unsightly. Children are aware of sores due to pain and pruritus, but may not complain excessively. (Dogra and Kumar, 2003) Training in the recognition of skin lesions has been identified as a priority for the provision of primary health care in tropical contexts, where impetigo and other skin diseases are common. (Mahe et al., 2005a, Mahe, 2005) Identification of the correct diagnosis is a priority to ensure the most appropriate treatment is offered. For the Skin Sore Trial, research nurses were trained in skin disease recognition using a clinical diagnosis manual developed by our team in earlier work. This diagnostic and treatment manual includes high quality images of dermatological conditions and is widely used throughout the region for education and training of health-care workers.

1.4 WHY IS UNDERSTANDING IMPETIGO IMPORTANT?

While skin sores are rarely fatal, impetigo lesions cause discomfort, inflammation and missed school days. (Hay et al., 1994) In addition, the bacteria are highly transmissible, with early studies showing infection of all other family members with streptococcal pyoderma occurring at a mean of 21 days from the index case. (Ferrieri et al., 1972) More recently in the NT, secondary $S.\ pyogenes$ transmission within the household was detected in 19% of occupants. (McDonald et al., 2008) Likewise $S.\ aureus$, particularly MRSA, colonisation of family members of index cases with skin infections is high. (Miller et al., 2012, Fritz et al., 2012) The break in the skin is the entry point for bacteria that may lead to both severe infectious and post-infectious sequelae. Skin sores have been reported as a risk factor in the development of $S.\ aureus$ (Skull et al., 1999) and $S.\ pyogenes$ (Carapetis et al., 1999) bacteraemia, resulting in hospitalisation. (Engelman et al., 2014) Acute post-streptococcal glomerulonephritis outbreaks occur regularly where skin sores are endemic (Marshall et al., 2011) and contribute to a high burden of chronic kidney disease. (Hoy et al., 2012, White et al., 2001) Finally, the burden of acute rheumatic fever in Indigenous Australians is amongst the highest reported in the world, (Parnaby and Carapetis, 2010, Seckeler and Hoke, 2011) with consequent morbidity and mortality. (Lawrence et al., 2013) This has long been hypothesised to arise from skin infection rather than pharyngitis, as is seen elsewhere in the world. (McDonald et al., 2004) These serious sequelae of impetigo are not reported in this thesis, as the design of the randomised controlled trial and sample size needed were not sufficient to also document these much rarer events. However, the importance of treating impetigo stems from decreasing disease burden, reducing interpersonal transmission and hence reducing these more serious consequences.
1.5 HOW COMMON IS IMPETIGO?

1.5.1 Global burden

A systematic review of population-based studies reported in Chapter 2 confirms the global burden remains high. This updates previous literature that incorporated impetigo into other GAS diseases (Carapetis et al., 2005) or referenced impetigo as one of many skin conditions worthy of attention. (Mahe, 2005, Vos et al., 2012, Hay et al., 2014) The included studies will describe the prevalence predominantly in resource-poor contexts. Due to a paucity of population-based prevalence studies of impetigo in developed countries, estimates from general practitioner surveys (Koning et al., 2006, Rortveit et al., 2011, Shallcross et al., 2013) confirm that impetigo is also a common bacterial skin infection in childhood in industrialised settings.

1.5.2 Indigenous children of the NT

There are ten studies included in the systematic review from Australia, all reporting impetigo prevalence in remote Indigenous children from northern Australia. Seven are studies from the NT, two from Queensland and one from Western Australia. There were no population-based, prevalence studies available for non-Indigenous children of Australia.

Of 4026 children assessed in the seven studies from the NT, the median prevalence of impetigo was 45.7% (inter quartile range, IQR 2.6% – 69.4%). These studies were published between 1992 and 2009, and reflect an ongoing high burden of disease. An estimate of prevalence from the Skin Sore Trial based on episodes eligible for inclusion is similarly high at 870/1715 (50.7%). Research assistants in our trial were seeking to recruit participants with impetigo, which may result in over-estimation, but it does confirm the burden remains high throughout the NT.

1.6 NATURAL HISTORY OF IMPETIGO RESOLUTION

Few studies of the natural history of untreated impetigo, documenting the process and time to resolution, are available. Understanding the natural history of
impetigo resolution is critical in evaluating the end point of treatment studies. Could the changes seen in the appearance of a sore be due to natural resolution alone or is there a treatment effect apparent?

Current knowledge of the natural history of impetigo emerged from observations during outbreaks of impetigo associated with acute post-streptococcal glomerulonephritis at the Red Lake reservation in Minnesota, USA in the 1960s. (Dajani et al., 1973, Ferrieri et al., 1972, Dajani et al., 1972) Professor Dajani was available for correspondence via email during my candidature, to discuss the pathogenesis and healing of impetigo lesions. Having him available to answer my questions expanded my understanding of his observations in children and hamster studies. (Dajani and Wannamaker, 1970) This was critical in developing definitions to formalise objective assessments of the digital images used in our trial, as studies of impetigo resolution have not been ongoing.

Impetigo usually begins with a 1–2cm erythematous macule that rapidly becomes a vesicle or pustule. (Dajani and Wannamaker, 1970) The vesicle ruptures in the first 24–48 hours, leaving a thin crust overlying the erosion. (Dajani and Wannamaker, 1970) Erythema becomes more marked in the initial few days due to underlying neovascularisation, then gradually resolves. Concurrent with erythema, the thin crust thickens over several days and as healing progresses, the crust reduces in size. (Dajani et al., 1973) As the crust dries and contracts, there may be evidence of traction around the edges of the crust and peeling of the surrounding layers of skin. Peeling skin is due to the presence of bacterial proteases that break down the damaged extracellular matrix, providing the opportunity for regeneration. Eventually the lesion becomes flat and dry with peeling around the edge, followed by a slight hypo- or hyper-pigmentation of the skin. This usually resolves with time, and scarring is reportedly uncommon with impetigo (Dajani and Wannamaker, 1970) but more common with the deeper lesion eczema. Untreated, impetigo lesions can remain unresolved for 30 days. (Dillon, 1970)

Hamster models showed resolution of impetigo by 9–11 days from the introduction of bacteria. (Dajani et al., 1971) This is faster than reported in humans. (Dillon, 1970) The hamster model, while useful to understand
pathogenesis, may be a less reliable predictor of the time to resolution in human impetigo, due to the absence of ongoing trauma in an animal laboratory, compared to the real-world experience of children. Beyond this, the natural history of impetigo without treatment remains poorly understood, as few studies have focused on this aspect; treatment is usually recommended and when treated outside of intervention studies, follow-up to determine outcome is rarely included in the clinical algorithm.

**Figure 2: The visual appearance of healing sores with treatment.**

Note: This series of photographs obtained from a participant in the Skin Sore Trial on days 0 (A), 2 (B), and 7 (C) highlight some of the features of sore healing described above. The erythematous macule, pustule and development of thin crusts can be seen in image A. Thickening of the crusts, traction at the edges of the sore and peeling skin are evident in image B. Hypopigmentation can be seen in image C.

**1.7 HOW IS IMPETIGO TREATED?**

**1.7.1 What is the evidence base for treatment of impetigo?**

In developed country settings, impetigo is a common cause for childhood presentations to the general practitioner. (Shallcross et al., 2013) Lesions are often around the mouth and nose, of small diameter (0.5–1cm) and discrete. (Sladden and Johnston, 2004, Hartman-Adams et al., 2014) The Cochrane review on treatment of impetigo (Koning et al., 2012) provides strong evidence for the topical treatment of limited impetigo. These findings have been incorporated into guidelines, which are accessed throughout the world. (Stevens et al., 2014) However, of the 68 included trials, only one includes children with severe
impetigo and that was conducted in a tropical, resource-poor context. (Faye et al., 2007) The remainder studied limited impetigo and were conducted in hospital outpatient departments or general practices, predominantly in developed countries. (Koning et al., 2012) Therefore, while topical therapy with fusidic acid or mupirocin is recommended for limited impetigo based on the synthesis of high quality randomised controlled trials, (Koning et al., 2012, Shim et al., 2014) a major evidence gap remains for the treatment of extensive impetigo.

In regions where impetigo is endemic, lesions are widespread and several body areas are affected at the same time, topical therapies are impractical and expensive. Neither guidelines from the Infectious Diseases Society of America, (Stevens et al., 2014) nor Medecins Sans Frontiers in conjunction with the World Health Organization, (Broek et al., 2013) recommend topical therapy for extensive or endemic impetigo. Both rely on expert opinion to support treatment with oral antibiotics that are active against \textit{S. pyogenes} and methicillin-susceptible \textit{S. aureus} for extensive disease. In addition, the widespread use of topical therapies leads to the rapid emergence of antimicrobial resistance. (Udo et al., 1994) There remains an evidence gap for the most effective treatment for impetigo where the burden is highest (tropical, resource-poor contexts).

1.7.2 How is impetigo treated in Indigenous children in the Northern Territory?

The treatment of impetigo in the NT has historically included a single dose of intramuscular benzathine penicillin G (Table 2). This regimen was recommended in the second edition of the CARPA manual in 1994, (CARPA, 1994) at the time intramuscular benzathine penicillin G (LA-Bicillin®) was first registered for use in Australia. (Currie, 2006) The inclusion of benzathine penicillin G in the NT treatment algorithms was based on non-randomised studies conducted in the USA (Esterly and Markowitz, 1970, Dillon, 1970) in children with extensive impetigo. Cure rates of between 58% and 100% were achieved compared with 20% to 39% for placebo.
Table 2: The CARPA manual and history of recommendations for treatment of impetigo in the NT

<table>
<thead>
<tr>
<th>Edition, date</th>
<th>When to treat?</th>
<th>Treatment Guidelines</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st, 1992</td>
<td>Not stated</td>
<td>Povidine iodine topically to clean sores</td>
<td>Treat scabies when impetigo resolved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bicillin AP intramuscular</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>If no improvement in 48–72 hours, flucloxacillin (with probenecid) or erythromycin for 5–10 days</td>
<td></td>
</tr>
<tr>
<td>2nd, 1994</td>
<td>Not stated</td>
<td>Povidine iodine topically to clean sores</td>
<td>Treat scabies when impetigo resolved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bicillin AP or Bicillin LA intramuscular</td>
<td></td>
</tr>
<tr>
<td>3rd, 1997</td>
<td>&gt;6 infected lesions or the lesions appear severe</td>
<td>Povidine iodine topically to clean sores</td>
<td>Give advice about scabies treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bicillin LA intramuscular</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>If no improvement in 48–72 hours, flucloxacillin (with probenecid)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roxithromycin if penicillin allergic</td>
<td></td>
</tr>
<tr>
<td>4th, 2003</td>
<td>&gt;6 infected sores or the sores look severe</td>
<td>Povidine iodine topically to clean sores</td>
<td>Treat impetigo and scabies together</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bicillin LA intramuscular</td>
<td>Do not use topical mupirocin as resistance develops rapidly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roxithromycin for 10 days if penicillin allergic</td>
<td></td>
</tr>
<tr>
<td>5th, 2009</td>
<td>“Clearly infected sores”</td>
<td>Clean sores with soap and water, sponge off crusts</td>
<td>Check for scabies and treat together</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benzathine penicillin intramuscular or amoxicillin for 10 days if injection not possible</td>
<td>Do not use topical mupirocin as resistance develops rapidly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim-sulphamethoxazole for 5 days if penicillin allergic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Follow up. If not improving, flucloxacillin for 10 days or trimethoprim-sulphamethoxazole for 5 days</td>
<td></td>
</tr>
</tbody>
</table>

Due to the pain of the injection, intermittent BPG stock-outs (Currie, 2006) and uncertainty about the overall number of sores needing treatment, the threshold
for treatment in the NT has varied. In the early CARPA editions, no guidance was provided on when to treat; whereas in the middle editions, more than six purulent or crusted sores were the treatment threshold, unless the “sores look severe”. (Table 2) During the East Arnhem Healthy Skin Program (EAHSP, 2004–2006) when the guideline included the six sores threshold for prescribing treatment, less than 10% of children qualifying for treatment were actually receiving it. (Andrews et al., 2009) In 2009, the recommendations in CARPA for treatment changed to “clearly infected sores”, due to concerns that counting the number of sores was becoming the basis for treatment. (Andrews et al., 2009)

1.8 A RANDOMISED CONTROLLED TRIAL FOR EXTENSIVE IMPETIGO IN INDIGENOUS CHILDREN

Despite some of the highest reported impetigo rates reported in the world in Indigenous children, (Carapetis et al., 2005) a formal evaluation of the efficacy of BPG for impetigo had never been conducted in the NT. In addition, the rising burden of MRSA in skin and soft tissue infections throughout the NT and worldwide led us to hypothesise that an antibiotic active against MRSA was needed. The suspicion that the painful needle was a deterrent to receiving treatment was also important in considering a RCT.

BPG was well established as the standard of care for treatment of impetigo in the NT (Table 2). A placebo has rarely been used in impetigo studies (Koning et al., 2012) and it was not considered ethical in the context of high rates of streptococcal and staphylococcal sequelae seen in children in the NT.

Clindamycin and trimethoprim-sulphamethoxazole were the available, affordable antibiotics with activity against MRSA. The bitter, unpalatable taste of clindamycin in syrup formulation, (Steele et al., 2006) resistance rates in S. aureus at 25% (McDonald et al., 2006) and three times daily dosing, (Group, 2010) meant clindamycin was rejected. Conversely, trimethoprim-sulphamethoxazole is palatable, has excellent skin penetration, (Krolicki et al., 2004) resistance rates below 1% (McDonald et al., 2006), had demonstrated efficacy in a pilot study, (Tong et al., 2010) and is one of the most widely used antibiotics in the world.
Comparing a new treatment to a known standard or active control in a randomised controlled trial, requires a non-inferiority design seeking to determine that the new treatment is not worse than the current treatment, by an acceptable amount. (Piaggio et al., 2012) A robust, measurable outcome is also required.

1.9 ETHICS

This study was approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC09/08). Ethics approval was current throughout the period of the study and concluded on 31 December 2013.

1.10 SUMMARY OF INTRODUCTION

Impetigo is common in childhood, with children living in resource-poor contexts experiencing the highest burden. Despite this, few treatment studies to guide evidence-based treatment algorithms are available for extensive impetigo. Evidence for safe, palatable, simple, effective treatment regimens is needed. The aim of this thesis is to find a better treatment option for impetigo amongst Indigenous children living in remote communities of Australia, and add to the limited available evidence for the treatment of extensive impetigo worldwide.
CHAPTER 2
THE GLOBAL EPIDEMIOLOGY OF IMPETIGO

2.1 SUMMARY
We conducted a comprehensive, systematic review of the population prevalence of impetigo and the broader condition pyoderma. PubMed was systematically searched for impetigo or pyoderma studies published between 1970 and 2014; 66 manuscripts relating to 89 studies met our inclusion criteria. From population-based surveillance, 82 studies included data on children, giving a total of 145,028 children assessed for pyoderma or impetigo. Median childhood prevalence was 12.3% (IQR 4.2–19.4%). Fifty-eight (65%) of the studies were from low or low-middle income countries, where median childhood prevalences were 8.4% (IQR 4.2–16.1%) and 14.5% (IQR 8.3–20.9%) respectively. However, the highest burden was seen in underprivileged children of high-income countries; median prevalence 19.4%, (IQR 3.9–43.3%). Based on data from studies published since 2000 from low and low-middle income countries, we estimate the global population of children suffering from impetigo at any one time to be in excess of 162 million, predominantly in tropical, resource-poor contexts. Impetigo is an under-recognised, neglected tropical disease and in conjunction with scabies, comprises a major dermatological concern in childhood, with potential lifelong consequences of untreated infection.

2.2 STATEMENT OF CONTRIBUTION TO JOINTLY AUTHORED WORK
This chapter is in preparation for submission to a peer-reviewed journal and is authored by myself, Antoine Mahe, Roderick Hay, Ross Andrews, Andrew Steer, Steven Tong and Jonathan Carapetis. I designed this study based on previous work by Antoine Mahe, Roderick Hay, Andrew Steer and Jonathan Carapetis, to
systematically describe the global burden of impetigo. I wrote the protocol, conducted the systematic literature review and assessed all papers for inclusion in the study. Two reviewers, including myself and one of the other co-authors, who all did a fraction of the reviews, conducted all of the full text reviews. I synthesised the results, performed the data analysis and have written this chapter.

2.3 INTRODUCTION

Impetigo is a common dermatosis of childhood. Recent estimates of the global burden of impetigo are in the order of 111 million children from developing countries (Carapetis et al., 2005) to 140 million (Vos et al., 2012, Hay et al., 2014) people affected at any one time. However, these estimates were based on a limited literature review of impetigo in the context of larger studies, have not been recently updated and acknowledge that impetigo estimates are imprecise due to the paucity of published literature from the highest prevalence contexts. This is because impetigo is a disease of poverty, occurring in settings where systematic collection of accurate disease burden data is a lower priority and most available data arise from hospital records, which may under-represent the true population prevalence of skin disease due to selection bias (Hogewoning et al., 2013, Saw et al., 2001, Lawrence et al., 1979, Bechelli et al., 1981, Gibbs, 1996, Bissek et al., 2012) and minimal health-care seeking for skin diseases in resource-poor contexts. (Hay et al., 1994, Behl, 1979, Mahe, 2005) This study will systematically collate, and hence update understanding of, the population prevalence of impetigo in children globally from 1970 to 2014. More precise estimates for impetigo by age group, global region, climate, levels of urbanisation and development are needed to target intervention studies and algorithmic management at the primary health care level.

The bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* typically cause superficial bacterial infections of the skin. (Brook et al., 1997) These superficial skin infections are variously labelled. The term pyoderma is often used to describe all superficial bacterial skin infections in tropical contexts and is inclusive of impetigo, ecthyma and furunculosis. (Mahe et al., 2005b) Impetigo (also known as skin or school sores) is a subset of pyoderma and has both
bullous and non-bullous forms. In addition, impetigo is often divided into primary and secondary impetigo, depending on whether an underlying dermatological (e.g. eczema) or alternative skin infection (e.g. scabies, pediculosis or tinea) preceded the bacterial component of the condition. (Mahe, 2005) Primary impetigo is often preceded by minor trauma or insect bites. (Mahe, 2005) In an attempt to understand the global burden of superficial bacterial skin infection, this study includes reports on both pyoderma and impetigo as the major forms of bacterial skin infection under investigation.

Impetigo is more than a nuisance condition. While mortality estimates for impetigo are low, the frequency of person-to-person transmission in resource-poor communities maintains a high burden of disease and affects well-being. (Murgia et al., 2010) In addition, the infectious (staphylococcal and streptococcal cellulitis, bacteraemia and deep tissue infections) and post-infectious (glomerulonephritis, rheumatic fever) sequelae are well known and cause high morbidity (Carapetis et al., 2005, Hoy et al., 2012, Marshall et al., 2011, Skull et al., 1999) and variable mortality. (Jackson et al., 2011, Tibazarwa et al., 2008) The relative cost to families of missed school days and procuring treatments that may or may not work is also high. (Hay et al., 1994, Yamamah et al., 2012) Skilled diagnosticians are few where the burden is highest. Community health workers often have poor training in skin disease recognition. (Mahe et al., 2005a) Few treatment studies have been conducted in high-burden, endemic settings to guide the development of evidence-based algorithms. (Bowen et al., 2014, van der Wouden and Koning, 2014)

The aim of this systematic review is to evaluate the prevalence of impetigo from studies in the general population and to explore variations in the epidemiology of impetigo. The number of Indigenous Australian children with impetigo in remote communities will also be estimated.
2.4 METHODS

2.4.1 Search Strategy

The systematic review is reported according to PRISMA guidelines. (Moher et al., 2009) References were identified through searches of PubMed for papers published in English between January 1970 and September 2014, which reported population-based studies of skin disorders, with specific reference to impetigo or pyoderma prevalence. We hand-searched the bibliographies of retrieved papers, for additional references. Relevant articles published between 1970 and 2005 were identified through searches by AM and RH (Mahe, 2005) and are synthesised in this manuscript. We used the following search terms ["impetigo" OR "pyoderma"] AND ["Africa" OR "Asia" OR “Latin America” OR “Pacific” OR “Oceania” OR “North America” OR “Europe” OR “Russia” OR “China” OR “India” OR “Developing Country” OR “tropical” OR “Indigenous”]. Duplicates were removed from the search before titles were reviewed for relevance (epidemiology, prevalence, impetigo and pyoderma). If insufficient detail was available in the title, abstracts or entire articles were reviewed to determine whether the study met pre-determined inclusion criteria.

2.4.2 Selection Criteria

Studies were included if they were population-based, prevalence studies, with extractable data on children with pyoderma or impetigo. A physical examination by a clinician was essential for inclusion. Wherever a term was used that inferred a bacterial skin infection (pyoderma, impetigo or sores) and numerator and denominator or proportion-affected data were available, these have been reported. Outpatient dermatology clinic and hospital-based studies from developing countries are rich sources of data on treatment-seeking behaviours for skin diseases. However, due to the management of impetigo occurring predominantly at primary care settings rather than tertiary referral dermatology clinics, these datasets were excluded. Our aim was to present the available baseline data derived from prevalence studies performed in the general population (community or school surveys).
2.4.3 Reviewer Assessment

Papers meeting the inclusion criteria were sourced in full-text and data extracted by two reviewers independently. All papers were assessed by myself. Each of the other co-authors independently assessed a subset of the studies to confirm inclusion criteria and extract data. Data fields extracted included date of study, country, climate, rural/urban environment, study site (e.g. school, household), study design, sampling method, population, age range, gender of participants, qualifications of person conducting the screening, case definition, definition of bacterial skin infection, number of participants, number with impetigo, childhood and adult prevalence, location of lesions, microbiology and presence of scabies.

2.4.4 Definitions

Where the study date was not reported, the year of publication was used. The sampling method was defined as exhaustive if \( \geq 85\% \) of available population were surveyed and non-exhaustive if it was below 85\%. Other options for sampling method included convenience (non-random selection of the available population), targeted (orphanages or institutions) and random selection of participants. Details of the definition used for recording a bacterial skin infection were collected and later categorised as pyoderma (if the text used this term or indicated that impetigo, folliculitis, ecthyma, furunculosis, cellulitis or tropical ulcers were included), impetigo (only impetigo or skin sores were studied) and secondarily infected scabies (the primary focus of the study was scabies with a secondary focus on bacterial skin infection). We have concentrated on reporting impetigo prevalence in children. Adult data were also incorporated where available. If children and adults were included in the study, but separate rates were not provided, then the community wide prevalence of impetigo was reported. It was not possible to ascertain from the studies how representative the study sample was of the broader population.

Countries were categorised into regions according to the United Nations (UN) Population Division (www.esa.un.org/wpp/excel-Data/country-Classification.pdf, last accessed 10 November 2014). Childhood was defined as children aged 0 to 15 years, to calculate population regional and global
prevalence. To assess the total population at risk of impetigo based on the median prevalence estimate, population data from the UN Department of Economic and Social Affairs Population Division for 2012 were used. These were available online at www.esa.un.org/unpd/wpp/unpp/panel_indicators.htm, last accessed 7 December 2014. Each country was also categorised according to the World Development Index as at July 2005 (www.data.worldbank.org) as high (>US$10,725 gross national income (GNI) per capita), upper middle (US$3,466–$10,725 GNI per capita), lower middle (US$876–$3465 GNI per capita) or low (≤US$875 GNI per capita) income.

The Koppen Climate Classification System is the most widely used classification of climates (Peel et al., 2007) and recognises five major climate systems. These include tropical, arid, temperate, cold and polar. Where data on climate was not included in the paper, this classification has been used to code the climate by country and region. (Peel et al., 2007)

2.4.5 Statistical Analysis

The data are synthesized into a narrative summary. Statistical analysis was performed using Stata13 (Statacorp, Texas, USA). Where prevalence estimates have been combined to understand regional and global burden of impetigo, the median prevalence has been used. In order to estimate regional and global burden of impetigo, the median prevalence has been applied to the 2011 Australian Census and 2012 United Nations population estimate for less developed countries. To take into account the variability in study size, the metaregression command in Stata was used to calculate the pooled prevalence. Impetigo and pyoderma are both used commonly to report bacterial skin infections. To assess for any variation in reporting of prevalence based on the chosen term, we assessed the use of each term, and calculated a statistical difference between the median prevalence using a chi squared statistic.
2.5 Results

Figure 1 summarises the results of the search conducted. Of the 1007 titles identified, 952 were from database searching and 55 from additional sources. Two hundred and thirteen duplicate records were removed and 628 papers excluded, mainly due to an absence of data on impetigo or because the studies were conducted in dermatology clinics or hospital settings. The 128 remaining records were subjected to full-text review; 38 papers were excluded with reasons given in Figure 1 leaving 90 papers, each of which was assessed by two reviewers. A further 24 were excluded due to insufficient data reported on impetigo prevalence. The final dataset includes 66 papers reporting on 89 studies (Figure 1, Table 1, Appendix 1) conducted over a 45-year period.

The studies were predominantly from Africa (30/89, 34%), Asia (20/89, 22%) and Oceania (19/89, 21%) and represented populations from 31 countries (Table 1, Figure 2). Studies with data available by decade were: 1970s (28, 32%), 1980s (15, 17%), 1990s (20, 23%), 2000s (23, 26%) and since 2010 (3, 3%).

Data on impetigo prevalence were available for 174,508 individuals of whom 145,028 were children. The study size varied, ranging from 31 to 19,775 participants per study. The median study size was 636 participants (inter-quartile range [IQR] 305–1817), median prevalence 11.2% (IQR 4.2–19.4%) and pooled prevalence 15.5% (95% CI 12.1–19.0%) (Table 3). Extractable prevalence data were available on children in 82 (92%) of the studies. The median impetigo prevalence in children was 12.3%, (IQR 4.2–19.3%) and pooled prevalence 16.6% (95% CI 12.7–20.5%). The median number of children in each study was 534 (IQR 258–1,729).
Figure 1: Flowchart of systematic review according to PRISMA statement

952 Records identified through database searching

55 additional records identified through other sources

794 records after duplicates removed

166 records screened

38 records excluded

128 full-text articles assessed for eligibility

38 full-text articles excluded
- Not in English (n=6)
- Not a community prevalence study (n=14)
- Review paper (n=1)
- Unable to access full text (n=6)
- Microbiology study (n=2)
- No impetigo prevalence data (n=9)

90 papers assessed by 2 reviewers for inclusion

66 papers (89 studies) included in quantitative synthesis

24 papers excluded by 2 reviewers
- Data quality insufficient for reporting prevalence data (n=24)
Table 1: Number of studies of impetigo prevalence by decade, country and region.

<table>
<thead>
<tr>
<th>Decade</th>
<th>Number of studies available</th>
<th>Countries</th>
<th>Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970 - 1979</td>
<td>28</td>
<td>Colombia, Ghana, Tanzania, New Zealand, Brazil, India, USA, Gambia, Panama</td>
<td>Latin America &amp; Caribbean, Africa, Asia, Oceania, North America</td>
</tr>
<tr>
<td>1980 - 1989</td>
<td>15</td>
<td>Pakistan, Solomon Islands, Nigeria, Ethiopia, Vanuatu, India, Fiji, Canada</td>
<td>Asia, Oceania, Africa, North America</td>
</tr>
<tr>
<td>1990 - 1999</td>
<td>20</td>
<td>Australia, Honduras, Mali, Malaysia, Ethiopia, Tanzania, Ecuador, Samoa, Taiwan, Kenya, Solomon Islands</td>
<td>Oceania, Latin America &amp; Caribbean, Africa, Asia</td>
</tr>
<tr>
<td>2000 - 2009</td>
<td>23</td>
<td>Nepal, Australia, India, Fiji, Tanzania, Nigeria, Timor Leste, Turkey, Mali, Ghana, Gabon, Rwanda, Egypt</td>
<td>Asia, Oceania, Africa</td>
</tr>
<tr>
<td>2010 - 2014</td>
<td>3*</td>
<td>Ethiopia, Cameroon, Tanzania</td>
<td>Africa</td>
</tr>
</tbody>
</table>

* Two studies were published in 2010 and did not provide a year of data collection in the manuscript.
Table 2: Summary statistics of available studies by age grouping

<table>
<thead>
<tr>
<th></th>
<th>Total available population (n=89 studies)</th>
<th>Childhood population (n=82 studies)</th>
<th>Adult population (n=11 studies)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median (IQR)</strong> prevalence of impetigo</td>
<td>11.2% (4.2–19.4%)</td>
<td>12.3% (4.2–19.4%)</td>
<td>4.9% (3.1–9.6%)</td>
</tr>
<tr>
<td><strong>Pooled prevalence (95% CI)</strong></td>
<td>15.5% (12.1–19.0%)</td>
<td>16.6% (12.7–20.5%)</td>
<td>9.7% (2.2–7.2%)</td>
</tr>
<tr>
<td><strong>Median (IQR) number of participants per study</strong></td>
<td>636 (305–1,817)</td>
<td>534 (258–1,729)</td>
<td>638 (264–1,645)</td>
</tr>
<tr>
<td><strong>Population with impetigo</strong></td>
<td>23,759</td>
<td>19,811</td>
<td>2,427</td>
</tr>
<tr>
<td><strong>Total population studied (denominator)</strong></td>
<td>174,508</td>
<td>145,028</td>
<td>18,246</td>
</tr>
</tbody>
</table>
2.5.1 Impetigo prevalence in children by region

Reported impetigo prevalence in populations under investigation ranged from 0.2% to 90%. The highest median prevalence of childhood impetigo was reported from Oceania, where from 19 studies, the median prevalence was 40.2% (IQR 17.2–48.1%). Excluding studies from Australia (see box) and New Zealand, of the eight remaining studies from Oceania, the median prevalence remained high at 29.7% (IQR 14.7–42.0%). The median impetigo prevalence in Africa was 7% (IQR 4.1–12.3%), Asia 7.3% (IQR 3–16.1%), resource poor populations in North America 13.3% (IQR 2.1–19.4%) and Latin America and the Caribbean 15.5% (IQR 12.2–20.8%). There was no data available for Europe or China.
### Applying prevalence estimates for impetigo to remote living Australian Indigenous children

There were 10 population prevalence studies available for Australia. All represented data from children living in remote Indigenous communities of northern Australia, with no studies available for non-Indigenous children. The median prevalence reported from these studies was 44.5% (IQR 34.0–49.2%). Four studies were conducted since 2000, with a median prevalence of 43.0% (IQR 40.2–45.7%). We estimated the total number of remote Indigenous children with impetigo at any one time by applying the median prevalence of 44.5% to the remote living Indigenous population from the states of Western Australia, Queensland and Northern Territory aged less than 15 years in the 2011 Australian Census (35,272). We estimate 15,696 Indigenous children are suffering from impetigo at any one time. This is the first time that prevalence estimates have been used to generate a total number at risk amongst Australian Indigenous children. This will be important for local health care planning.

### 2.5.2 Burden of impetigo in low and low-middle income countries

The United Nations Department of Economic and Social Affairs population division estimated the global population below 15 years of age resident in less developed countries in the years 2000–2009 to be at least 1.6 billion children. Utilising the median population prevalence of 9.9%, (IQR 4.1–16.6%) from the studies conducted in developing economies (WDI2005 index of lower-middle or low) since 2000, the estimated population at risk of impetigo at any one time is more than 162 million children. Excluding China, where no studies were available, reduces the estimate to 137 million children with impetigo in low and
low-middle income countries. Table 3 outlines the regional estimates of children with impetigo at any one time using the available data.

**Table 3: Estimates of children with impetigo by regions of the world with available data***

<table>
<thead>
<tr>
<th>Region</th>
<th>Population in 2012 under 15 years</th>
<th>Median impetigo prevalence in children</th>
<th>Estimated number of children with impetigo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>424,072,000</td>
<td>7% (IQR 4.1–12.3%)</td>
<td>29,685,040</td>
</tr>
<tr>
<td>Asia</td>
<td>1,060,076,000</td>
<td>7.3% (IQR 3–16.1%)</td>
<td>77,385,548</td>
</tr>
<tr>
<td>Oceania*</td>
<td>3,653,000</td>
<td>29.7% (IQR 14.7–42.0%)</td>
<td>1,084,941</td>
</tr>
<tr>
<td>Latin American &amp; Caribbean</td>
<td>167,654,000</td>
<td>15.5% (IQR 12.2–20.8%)</td>
<td>25,986,370</td>
</tr>
</tbody>
</table>

*Studies from Australia, New Zealand and North America excluded as all these studies were conducted in small, impoverished populations within these countries that may not reflect the overall burden of impetigo for the childhood population.

### 2.5.3 Childhood population prevalence by age group

Only 13 (15%) studies reported prevalence of impetigo according to age group. Of those that did, the median prevalence in 0–4 year olds studied was 19% (IQR 15–31%), 5–9 year olds 19% (IQR 12–43%) and 10–14 year olds 10% (IQR 7–28%).

### 2.5.4 Childhood impetigo and World Development Index

The majority of studies, 58/89 (65%) (Table 4), were from low or low-middle income countries. The remainder were from middle income or resource-poor populations within high-income countries. Table 5 summarises the median prevalence estimates according to income level of the country, with the highest
estimates coming from underprivileged populations within high-income countries.

**Table 4: Classification of studies by region and World Bank Development Indicator in 2005**

<table>
<thead>
<tr>
<th>Region</th>
<th>High income</th>
<th>Upper Middle income</th>
<th>Low Middle Income</th>
<th>Low Income</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oceania</td>
<td>Australia (10) New Zealand (1)</td>
<td>Fiji* (4) Vanuatu (1) Samoa (1)</td>
<td>Solomon Islands* (2)</td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>Gabon (1)</td>
<td>Egypt* (2)</td>
<td>Ghana* (3) Rwanda (1) Cameroon* (1) Ethiopia (3) Nigeria* (2) Tanzania (9) Kenya (3) Mali (3) The Gambia (2)</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>Taiwan (1) Malaysia* (2) Turkey* (1)</td>
<td></td>
<td>India* (12) Nepal (2) Pakistan* (1) Timor-Leste* (1)</td>
<td></td>
</tr>
<tr>
<td>Caribbean &amp; Latin America</td>
<td>Panama* (2) Honduras* (1) Brazil (2)* Colombia* (1) Ecuador* (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>Canada (6) USA (7)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Median prevalence of impetigo in childhood and overall, categorised by the World Development Index.

<table>
<thead>
<tr>
<th>WDI and number of studies</th>
<th>Median childhood prevalence (IQR)</th>
<th>Median overall prevalence (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N= 82</td>
<td>N=89</td>
</tr>
<tr>
<td>High income (N= 25)</td>
<td>19.4% (IQR 3.9–43.3%)</td>
<td>19.4% (IQR 3.9–43.3%)</td>
</tr>
<tr>
<td>Middle income (n=4 [childhood] OR n=6 [overall])</td>
<td>9.9% (IQR 1.8–18.6%)</td>
<td>9.9% (IQR 2–15.1%)</td>
</tr>
<tr>
<td>Low-Middle (N=12)</td>
<td>14.5% (IQR 8.3–20.9%)</td>
<td>12.8% (IQR 7.8–18.3%)</td>
</tr>
<tr>
<td>Low (n= 41 [childhood] OR n= 46 [overall])</td>
<td>8.4% (IQR 4.2–16.1%)</td>
<td>7.9% (IQR 4.3–16.1%)</td>
</tr>
</tbody>
</table>

2.5.5 Scabies in association with impetigo

Fifty-seven (64%) studies also reported data on the prevalence of scabies. The median prevalence of scabies from all included studies was 3.3% (IQR 0.7–12.9%). Twenty-seven (87%) countries had available data on scabies prevalence. Scabies prevalence varied by region, with the highest median prevalence found in Oceania (n=14) at 16% (IQR 4.9–25%). The median scabies prevalence in Africa (n=28) was 2% (IQR 0.7–7%) and in Asia (n=11) was 3.4% (IQR 0.9–11.9%). The median prevalence of scabies in studies from Latin America and the Caribbean (n=3) was 3% (IQR 0.6–10%). Scabies prevalence also varied by decade. In the 1970s (n=12), the median was 3.2% (IQR 1.4–13%), 1980s (n=8) 1.1% (IQR 0.4–1.8%), 1990s (n=14) 9.2% (IQR 4.9–17%), 2000s (n=20) 1.9% (IQR 0.6–15.1%) and since 2010 (n=3) 1.4% (IQR 0.3–1.8%).
2.5.6 Microbiology of impetigo

Thirty-four (38%) of the studies also reported culture-based microbiology results, predominantly on streptococcal infection (n=31). Only 11/89 (12%) studies reported on the relative contributions of *S. pyogenes* and *S. aureus* from microbiological culture of skin lesions. *S. pyogenes* was identified in a median of 74% (IQR 57–95%) of cultures and *S. aureus* in a median of 64% (IQR 53–80%) of cultures.

2.5.7 Body distribution of impetigo

Data were reported on body distribution of impetigo in 23 studies. Of these, 21/23 (91%) reported the lower limbs as being the most common site. In 11 studies, the proportion of body regions affected was given. These were reclassified as lower limbs, upper limbs and other [scalp, face, neck, torso]. The respective medians for body region distributions (these were not mutually exclusive) were lower limbs 58% (IQR 44–86%), upper limbs 18% (IQR 14–54%) and other 38% (IQR 5–43%).

2.5.8 Impetigo in rural versus urban settings

Studies were classified broadly as rural (n=61), urban (n=15) or both (n=13). Table 6 shows the variation in prevalence when the data were examined by this factor, with a higher prevalence of impetigo reported from rural locations compared to urban settings.
Table 6: Variability in median impetigo prevalence by urban and rural study locations

<table>
<thead>
<tr>
<th></th>
<th>Median impetigo prevalence overall</th>
<th>Median impetigo prevalence in children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural</td>
<td>N=61 studies</td>
<td>N=55 studies</td>
</tr>
<tr>
<td></td>
<td>13.3% (6.7–20.9%)</td>
<td>16.1% (5.9–22.6%)</td>
</tr>
<tr>
<td>Urban</td>
<td>N=15 studies</td>
<td>N=14 studies</td>
</tr>
<tr>
<td></td>
<td>4.8% (2–10%)</td>
<td>4.5% (2–7.3%)</td>
</tr>
<tr>
<td>Both</td>
<td>N=13 studies</td>
<td>N=13 studies</td>
</tr>
<tr>
<td></td>
<td>5.8% (2.4–13.3%)</td>
<td>5.8% (2.4–13.3%)</td>
</tr>
</tbody>
</table>

2.5.9 Childhood impetigo by climatic region

Many studies included details on the climatic conditions of the study region. If not available, these were coded using the Koppen classification (Peel et al., 2007). The majority of studies were from tropical environments, 67/89 (75%). The remaining studies were from cold 9/89 (10%), temperate 9/89 (10%) and arid 4/89 (5%) climates. Median impetigo prevalence in childhood was 12.2% (IQR 4.8–20.8%), 17.5% (IQR 8.2–42.8%), 15.8% (IQR 2.2–30.2%) and 3.9% (IQR 1–13.3%) for tropical, arid, temperate and cold climates respectively.

2.5.10 Variability in sampling technique

There was variability in the sampling techniques employed. The most common technique described was exhaustive sampling. Of the exhaustive population surveillance studies (n=57/89, 64%), the median number of participants was 636 (IQR 305–2528) and median prevalence 10.8% (IQR 4.3–19.4%). The median prevalence was 4% (IQR 2–16.6%) in the 7 studies where random sampling was employed.
2.5.11 Impetigo or pyoderma: is the reporting consistent?

Twenty-six (29%) studies reported on impetigo or skin sores as the primary definition under investigation. Of these, 25 have data available on the prevalence of impetigo in children with the median prevalence of 13.3% (IQR 2.3–22.6%). Pyoderma was reported in 62 (70%) studies with a median prevalence of 11.8% (IQR 5.1–19%). There was no statistical difference in the median prevalence reported based on these definitions, p=0.85. Both definitions were used throughout all decades of the study. There was some variability in the use of definition by region: studies from Africa, Asia and Latin America predominantly reported on pyoderma, whereas studies from Oceania used either definition and North American studies were more likely to report on impetigo (Table 7).

Table 7: Regional variation in the use of pyoderma or impetigo to describe bacterial skin infections

<table>
<thead>
<tr>
<th>Region</th>
<th>Pyoderma reported(%)</th>
<th>Impetigo reported(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa (n=30)</td>
<td>27 (90%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Asia (n=18)</td>
<td>15 (79%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Oceania (n=19)</td>
<td>10 (53%)</td>
<td>9 (47%)</td>
</tr>
<tr>
<td>North America (n=13)</td>
<td>3/13 (23%)</td>
<td>10/13 (77%)</td>
</tr>
<tr>
<td>Latin America &amp; Caribbean (n=7)</td>
<td>7/7 (100%)</td>
<td>0</td>
</tr>
</tbody>
</table>

2.6 DISCUSSION

This systematic review provides comprehensive data and confirms an ongoing, high burden of impetigo in childhood, estimating that more than 162 million children in low and low-middle income countries are affected at any one time. Our study revises upwards the estimate of children impacted by impetigo at any one time, using the same methodology but incorporating more studies, compared to the previous estimate of 111 million children. (Carapetis et al., 2005)
reported burden has remained high throughout the study period, with a median prevalence of 12.3% (IQR 4.2–19.3%). Our estimate derived from 89 studies over 45 years is higher than previously published estimates, which were between 5 and 10%. (Mahe, 2005) Impetigo is more than a benign, nuisance condition and these numbers demonstrate the priority concern impetigo is for public health.

Each decade since 1970 has seen 15–20 new studies published in the peer-reviewed literature. Our search strategy represents a greater depth of coverage and enriches our understanding of the global burden of impetigo. The reported data is derived from prevalence studies performed in the general population, at the community or school level, and provides a more complete understanding of the global burden of impetigo than previous estimates. Each study is valuable in describing the burden of disease for a local or regional population, but collectively they tell a far more compelling story of an under-appreciated disease. These studies are predominantly reported from contexts of poverty or tropical regions.

The data include large regions of the globe over a 45-year interval, suggesting the burden of impetigo remains relatively unchanged over this period. Only three studies were available for the current decade, of which two were published in 2010 (Komba and Mgonda, 2010, Murgia et al., 2010) and likely represent data collected during the previous decade. The remaining study reports data from March 2010. (Bissek et al., 2012) As such, the most precise, recent estimates are from the decade ending in 2009 and have shown no change in the global burden of impetigo. In view of this, we combined the prevalence estimates from all studies published since 2000 in order to estimate the current burden of impetigo.

The data describe a context where progress against control of impetigo appears to be lacking. Including bacterial skin infections in the list of neglected tropical diseases may be one option to refocus attention on the need for this to be a priority target. In addition, the burden experienced by impoverished populations within wealthy countries is high. This is in keeping with version 2.0 of the neglected tropical diseases agenda that utilises ‘blue marble health’ as a phrase to describe the importance of poverty in all countries as a key underlying factor of neglected tropical diseases. (Hotez, 2013) The widespread burden of impetigo
has remained unchanged over more than four decades. By highlighting the global burden, which has previously been estimated at >2% of the global population at any one time, (Hay et al., 2014) an agenda for screening, treatment and further work can crystallise. This will inform primary prevention of kidney and heart disease and gram-positive bacterial sepsis in resource-poor contexts.

A limitation of this systematic combination of datasets is the variability in clinical skills of those performing cutaneous disease surveillance. Studies reported using expert dermatologists, dermatologists in training, paediatricians, general medical officers, nursing staff and community health workers trained in identification of skin conditions, to screen populations. This variability may have resulted in under-reporting of impetigo. In addition, many of the studies were focused on a specific dermatosis e.g., scabies or pediculosis, or conducted in the context of nutrition or child health surveys. Reporting of bacterial infection was a secondary endpoint. Surveys of skin disease were conducted for a variety of reasons, including to demonstrate the substantial burden of disease and significant unmet need. (Walker et al., 2008) Guidelines for skin surveillance studies have recently been developed. (Kotloff, 2008) Only one study to date reported using these in the study design, (Steer et al., 2009) but the availability of these guidelines is likely to inform future surveillance.

The definitions and clustering of conditions used in the studies was variable, but using the two dominant terms of impetigo and pyoderma, there was no statistical difference in the median prevalence. This suggests that our inclusion of both terms as a descriptor of bacterial skin infections is robust.

Our study broadens our knowledge of the global distribution of impetigo, by including studies from countries that were not available in previous disease burden estimates. (Carapetis et al., 2005, Vos et al., 2012) An improved understanding of the global burden may also help to prioritise treatment studies in contexts with the highest burden of impetigo. The Cochrane review on the optimal treatment of impetigo (Koning et al., 2012) includes studies predominantly from high-income countries and references only one study (out of 68) conducted in a similar setting to those reported in our study. Similarly, Karimkhani et al. report bacterial skin infections are under-represented in
Cochrane systematic reviews when matched with corresponding disability-adjusted life years. (Karimkhani et al., 2014)

Initially, we hoped to define the burden of impetigo in developing countries by including studies from low and low-middle income countries using the World Bank Development Index. However, very few studies were excluded on this basis, and the study was broadened to include all countries. It is possible that studies have been conducted in the regions of highest disease burden leading to an over-estimate of the global burden. (Mahe, 2005) We think this is unlikely, given the size, consistency and duration of the estimated burden of impetigo that we have described. However, population-based prevalence studies from Europe, East and South-East Asia, and more recently North America, are under-represented. No studies were available in English from the low or low-middle income countries of Eastern Europe, and this is a significant gap in our global understanding of the burden of impetigo. Recently, the burden of post-streptococcal sequelae in these countries has been reported and is amongst the highest in the world, (Tibazarwa et al., 2008) suggesting that impetigo may also be common here. Despite these gaps, our findings are consistent with the 2010 global burden of disease estimates for impetigo. (Vos et al., 2012, Hay et al., 2014)

This study is limited by the exclusion of unpublished data or grey literature searching. This limitation is more likely to restrict the analysis to developing countries; none of the three studies from developed countries which have been included in the global burden of skin diseases (Hay et al., 2014) were found using the search methods specified, as these studies were all health-care based. Overall, few studies were identified from high-income countries. It is possible that the implementation of a skin disease population prevalence study is because of a pre-specified expectation of high rates of disease. The absence of data may represent lower disease burden, or may just be an unstudied population. In addition, hospital or health care seeking studies were not included.

Our study confirms that the greatest burden of impetigo is in children, with steady decreases in prevalence with increasing age. (Mahe, 2005, Belcher et al., 1977) Despite our study reflecting predominantly impoverished settings,
increases in impetigo have also been reported in children in developed countries attending general practices for care (Shallcross et al., 2013) and it causes a large volume of health care consultations in all regions of the world. (Mohammedamin et al., 2006, Razmjou et al., 2009, Koning et al., 2006) Our summary of data has consistently shown that impetigo predominantly affects the lower limbs in children in resource poor contexts and that both \textit{S. pyogenes} and \textit{S. aureus} are present in swabbed lesions.

Within the 89 studies, there was variability in the prevalence of impetigo reported. Tropical climate, insect bites, scabies, poverty, limited access to water and household overcrowding are all reported factors contributing to the development of impetigo (Currie and Carapetis, 2000) but data on these was not systematically available in the 89 studies. We looked at the burden of impetigo by age group, level of urbanisation and broad climatic region. The individual studies reported prevalence across a range of additional contexts including ethnicity, gender, disability and socio-economic status but these were outside the scope of our review and it is unlikely they would be amenable to systematic analysis given the small numbers of studies addressing sub-groups.

\textbf{2.7 CONCLUSIONS}

Prevalence throughout the study period was highest in Oceania in both resource-poor countries, and underprivileged populations within high-income countries. This synthesis of studies is important for regional and national targeting of healthy skin interventions, as the burden of disease is large. At a global level, this study revises our estimate upwards of the number of children affected with impetigo at any one time from 111 million (Carapetis et al., 2005) to 162 million; this finding alone should drive a comprehensive research agenda for the detection, treatment and prevention of impetigo in resource-poor contexts. It is also important to appreciate that as antibiotics are the backbone of current treatment for impetigo, this disease burden may contribute to burgeoning antibiotic resistance in the absence of evidence-based treatment algorithms. (Tong et al., 2014) This combination of high prevalence and moderate morbidity makes impetigo a high population health priority
CHAPTER 3
IS STREPTOCOCCUS PYOGENES SUSCEPTIBLE TO TRIMETHOPRIM-SULPHAMETHOXAZOLE?

3.1 CHAPTER OVERVIEW

This chapter challenges the conventional wisdom that *Streptococcus pyogenes* is not susceptible to trimethoprim/sulphamethoxazole (SXT). Historically, *in vitro* antibiotic susceptibility testing was confounded by the presence of thymidine, an inhibitor of SXT. As such, *S. pyogenes* appeared resistant to SXT in many earlier assays. The long standing susceptibility of *S. pyogenes* to penicillin obviated the need for an alternative antibiotic for treatment of *S. pyogenes* infections, and exploration of the *in vivo* efficacy of SXT was deemed unnecessary or not considered.

*Staphylococcus aureus* is a key pathogen in impetigo and is often found in conjunction with *Staphylococcus aureus*. The rising prevalence of methicillin-resistant *S. aureus* (MRSA) in remote communities of northern Australia (Tong et al., 2008) was concerning. Both hospital (Tong et al., 2009) and community (McDonald et al., 2006) studies identified MRSA rates above 20%. As such, treatment with antibiotics effective against both pathogens may be required and the Skin Sore Trial was designed to address this question. When the Skin Sore Trial was designed, SXT susceptibility in community *S. aureus* strains was 100%. (McDonald et al., 2006) Susceptibility of *S. pyogenes* was similarly high in unpublished laboratory work.

The work in this chapter demonstrates the *in vitro* susceptibility of *S. pyogenes* to SXT. It was a necessary step in paving the way for our understanding of the results of the Skin Sore Trial. In addition, it alerts the global community dealing with skin and soft tissue infections where both *S. pyogenes* and MRSA are...
implicated, that a simple, oral antibiotic regimen may be effective depending on the local antibiogram.

3.2 STATEMENT OF CONTRIBUTION TO JOINTLY AUTHORED WORK

I designed this study based on preliminary work done by Peter Ward and Malcolm McDonald during the process of achieving funding for this trial. The preliminary work was included as Study 1 in this paper; I then extended that work in Study 2. I wrote the standard operating procedures (SOP) for the conduct of Study 2 and incorporated the recently released European Committee on Antibiotic Susceptibility testing (EUCAST) breakpoints for *S. pyogenes* and SXT. Whilst I planned to assist with the laboratory work, delay in the arrival of supplies meant that Rachael Lilliebridge conducted the susceptibility testing, while I was on maternity leave. I oversaw the microbiology, cleaned the data and analysed the results with the assistance of Steven Tong. I conducted the literature review and wrote all drafts of the manuscript. All co-authors contributed to revisions of the manuscript and approved the final version for publication.

3.3 JOURNAL ARTICLE (FOLLOWING PAGE)

Is Streptococcus pyogenes resistant or susceptible to trimethoprim-sulfamethoxazole?

APPENDIX 2

S. pyogenes was one of the first bacterial infections to be treated with sulfur antibacterials in the 1930s (16) and proved to be clinically effective in the treatment and prophylaxis of S. pyogenes infections (10, 16, 23, 29). However, when sulfadiazine, an early short-acting sulfur antibacterial, was used in mass prophylaxis programs to prevent S. pyogenes tonsillitis and acute rheumatic fever (ARF) in military recruits in the 1940s, the clinical efficacy of this antibacterial was limited due to the presumed development of resistance (13, 15, 27, 31, 42) among some strains. Initial antibacterial susceptibility testing (AST) of S. pyogenes to sulfur antibacterials using a broth dilution method demonstrated that some strains were resistant (25, 53); however, AST was in its infancy and no standardized reference methods existed at that time. This early experience resulted in the belief that trimethoprim-sulfamethoxazole (SXT) is ineffective against S. pyogenes, and its use has been discouraged in clinical practice for decades (35).

Subsequent antibacterial susceptibility experiments showed apparently reduced susceptibility of S. pyogenes (and other bacteria) (6, 40) to the sulfur antibacterials due to antagonism of the inhibition of folate metabolism. Harper and Caswton discovered an inhibitory substance in 1945, eventually identified as thymidine, which was interfering with the ability of sulfur antibacterials to kill the organism (25). Because the activity of SXT is determined by the antibacterial’s ability to deprive an organism of folate coenzymes (7), there is a direct relationship between the thymidine levels in culture media and SXT resistance (11). High thymidine content in agar provides an exogenous substrate which can be used by an organism to maintain folate metabolism and hence appear resistant to SXT. In early studies, most culture media contained sufficient thymidine to antagonize the inhibitory effects of sulfur drugs and hence produced resistant results when this class of antibacterials was tested (6).

Notably, lysed horse blood was found to contain the enzyme thymidine phosphorylase, which neutralized thymidine (46) and overcame this effect (21, 25). No other mammalian blood contains thymidine phosphorylase (21). However, the addition of lysed horse blood was not recommended for AST, despite several authors (5, 6, 21, 54) advising supplementation with lysed horse blood for any medium used to test sulfur antibacterial susceptibility if the thymidine concentration was above 0.03 μg/ml (5) (below which inhibition does not occur). In this context, the notion that S. pyogenes was resistant to sulfur antibacterials perpetuated.

SXT was introduced in 1968 (3, 28, 43) and has since become one of the most widely used antibacterials in the world. However, recommendations against the use of sulfur antibacterials, including SXT, for S. pyogenes infections continue in the belief that the organism is intrinsically resistant (33, 47, 51). Two studies (33, 51) have reported S. pyogenes uniformly resistant to SXT, but this was prior to thymidine content standardization in Mueller-Hinton agar (MHA) and was on agar containing sheep blood. There have also been reported clinical failures in the use of this agent in eradicating S. pyogenes from nasopharyngeal carriage (30). However, other centers have demonstrated full in vitro susceptibility of S. pyogenes to SXT.
pyogenes to SXT (14, 22, 36, 54). Since 2006, when the thymidine content of MHA became strictly regulated by the Clinical and Laboratory Standards Institute (CLSI) to maintain a low level of thymidine and hence avoid inhibition (M6-A2 protocol) (9), it has no longer been necessary to add lysed horse blood to the medium for AST. However, current methods do use agar supplemented with mammalian blood.

Given the prevailing view that caution should be exercised in using SXT for infections involving S. pyogenes and the paucity of clinical data of SXT efficacy against S. pyogenes, we sought to confirm or disprove the notion that S. pyogenes is resistant to SXT in vitro on various antibacterial susceptibility testing media. Co-infection of S. pyogenes with Staphylococcus aureus in skin and soft tissue infections (STTI) and the rising prevalence of methicillin-resistant S. aureus (MRSA) provide added stimulus to explore the utility of SXT in the treatment of these infections.

MATERIALS AND METHODS

Swab collection and identification of S. pyogenes. Skin, throat, or nose swabs were collected using a rayon tipped cotton swab (Copan, Interpath Services, Melbourne, Australia). In study 1, swabs were plated on horse blood agar (HBA) (Oxoid, Basingstoke, United Kingdom) and HBA containing colistin and nalidixic acid (HBA + CNA) (Oxoid) within 48 h of collection. In study 2, swabs were stored in skim milk-trypptone-glucose-glycerol broth (STGGB) at 70°C until plating on the above-described media. Incubation was at 37°C for 16 h in 5% CO₂. B-hemolytic colonies were identified morphologically, and confirmation of S. pyogenes was with the Lancefield streptococcal grouping test for group A (Oxoid). Isolates were stored in glycerol at −70°C until subsequent replating for AST.

Selection of isolates. (i) Study 1. We began by exploring the issue with a preliminary study of 100 skin and throat isolates of S. pyogenes collected from 3 remote Australian Aboriginal communities between 2003 and 2005 during surveillance studies (39). We used an SXT Etest strip (bioMérieux, France) on 3 different agars: MHA, MHA supplemented with horse blood (MHBA), and MHA supplemented with lysed horse blood (MHLHBA) (Oxoid). Interpretation was based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (20) released in 2010.

(ii) Study 2. The Skin Sore Trial is a randomized, controlled trial (RCT) comparing benzathine penicillin G (BPG) treatment of impetigo (the standard of care) with oral SXT. The first 100 S. pyogenes isolates from skin and nasal swabs from participants in the Skin Sore Trial were used in study 2. The participants were 3 months to 13 years old and were from 4 remote Aboriginal communities of the Top End of the Northern Territory, Australia, recruited in 2010. Swabs were collected from the anterior nares and at least 2 purulent or crusted sores from all children. Swabs were collected from skin sores on day 0 (pretreatment), day 2 (midtreatment), and day 7 (completion of treatment). Consent for participation and collection of specimens was obtained from the guardian or parent of each participant. The study was approved by the Northern Territory Territory Top End Human Research Ethics Committee (HREC 09/08) and has been registered with the Australian and New Zealand Clinical Trials Registry (ACTRN 12609008582941).

Antibacterial susceptibility testing methods and agar used. The two internationally accredited, standardized methods for AST (Table 1) are the CLSI (8) and EUCAST (19) methods. CLSI does not provide reference breakpoints for S. pyogenes susceptibility to SXT and recommends the use of Mueller-Hinton agar (MHA) supplemented with 5% sheep blood for testing the susceptibility of S. pyogenes to other antibacterials. In contrast, EUCAST has released breakpoints for the disk diffusion method and MIC on appropriate media. The medium recommended for Streptococcus groups A, B, C, and G is MHA supplemented with 5% defibrinated horse blood + 20 mg/liter β-NAD. This agar is commonly referred to as MHF (http://www.eucaast.org, accessed 25 July 2012).

### Table 1 Antibiotic susceptibility testing methods and agar used

<table>
<thead>
<tr>
<th>Method</th>
<th>Study</th>
<th>Agar used</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSI (8)</td>
<td>2</td>
<td>Mueller-Hinton agar supplemented with 5% sheep blood</td>
<td>MHS</td>
</tr>
<tr>
<td>EUCAST (19)</td>
<td>2</td>
<td>Mueller-Hinton agar supplemented with 5% defibrinated horse blood + β-NAD</td>
<td>MHF</td>
</tr>
<tr>
<td>Experimental</td>
<td>1 and 2</td>
<td>Mueller-Hinton agar</td>
<td>MHA</td>
</tr>
<tr>
<td>Experimental</td>
<td>1 and 2</td>
<td>Mueller-Hinton agar containing 5% lysed horse blood</td>
<td>MHLHBA</td>
</tr>
<tr>
<td>Experimental</td>
<td>1</td>
<td>Mueller-Hinton agar containing horse blood</td>
<td>MHBA</td>
</tr>
</tbody>
</table>

Two experimental agars were also used for susceptibility testing. MHA is routinely used in a number of other antibacterial susceptibility tests not involving β-hemolytic streptococci. MHLHBA was used to explore the hypothesis based on historical literature that the lysing of horse blood releases thymidine phosphorylase which breaks thymidine down to thymine. Using the EUCAST breakpoints (20), with an MIC of ≤1 mg/liter as sensitive and an MIC of >2 mg/liter as resistant, and an SXT Etest to perform antibacterial susceptibility, these studies compared the various media that have been recommended for AST. The Etest interpretation is based on the trimethoprim component of the trimethoprim-sulfamethoxazole combination, in a ratio of 1:19. The EUCAST methodology using the Etest was chosen due to the availability of published breakpoints; however, due to the widespread use of CLSI, the medium upon which this organism is tested was also used and results obtained were referenced to the EUCAST breakpoints.

Antibacterial susceptibility testing. Single colonies were isolated from frozen stocks following overnight incubation on HBA in 5% CO₂ at 37°C. Susceptibility testing for SXT was performed using a 0.5 McFarland suspension to create a confluent lawn inoculum and then applying an SXT Etest as per the manufacturer’s instructions. Plates were read by 2 readers following incubation in 5% CO₂ at 35°C for 16 to 20 h. The MIC was recorded where the inhibition ellipse intersected the scale. Where a difference in results of more than 2 gradations was noted between the 2 readers, a repeat test was performed with a fresh subculture of S. pyogenes. As SXT is a bacteriostatic antibacterial, this mode of action can alter the appearance of an MIC endpoint, resulting in hazy zones. Where haze was present, both the 80% and 100% points of ellipse intersection were recorded. A penicillin, erythromycin, and clindamycin disk diffusion test according to CLSI guidelines (8) was conducted concurrently on all 100 strains in study 2.

Quality control. In study 2, for every 20 S. pyogenes clinical isolates, a control strain of S. pyogenes (ATCC 19615) was also tested on the 4 agars and found to be susceptible. Quality control of the SXT Etests was performed throughout the study using Escherichia coli (ATCC 25922) on MHA and Streptococcus pneumoniae (ATCC 49619) on MHS. The reference ranges for each organism were achieved, namely, 0.064 to 0.25 μg/ml for E. coli and 0.125 to 1 μg/ml for S. pneumoniae. STATA version 12.0 (STATAcorp, College Station, TX) was used to determine the geometric means. The data were logarithmically transformed to a normal distribution, and paired t tests were used to determine the difference between agars for studies 1 and 2.
RESULTS

Study 1. On MHBA and MHLHBA, S. pyogenes isolates from the skin (n = 1100536) and throat (n = 1100564) were uniformly susceptible to SXT (Table 2). Ninety-nine isolates tested on MHA were susceptible to SXT (Table 2). The single isolate which appeared resistant on MHA was susceptible on both MHBA and MHLHBA.

There was no statistically significant difference between the geometric mean MIC measurements on MHA and MHBA. However, isolates tested on both MHA and MHBA had lower MICs than isolates tested on MHLHBA (Table 3).

Study 2. One hundred isolates of S. pyogenes from 43 children were included in this analysis. S. pyogenes isolates utilized were from sores (n = 98) and the anterior nares (n = 2). The majority of isolates (76%) were from day 0 before antibiotic treatment; 19% were from day 2, and 5% were from day 7. Sixty-four of the swabs from which S. pyogenes was identified also cultured S. aureus. Of these, 14% were methicillin-resistant S. aureus (MRSA) and 86% were methicillin-susceptible S. aureus (MSSA).

All 100 isolates of S. pyogenes were susceptible to SXT on all agars by both readers (Table 4). Interrater reliability was excellent, with 96% of all MIC readings within ±1 MIC gradation. In view of this, all analyses were done on results from reader 1. All 100 S. pyogenes isolates were also susceptible to penicillin, erythromycin, and clindamycin.

The geometric means were similar for MHA, MHBA, and MHLHBA (Table 4). MHS had a higher geometric mean MIC than the other media. This was statistically significant, with isolates tested on MHS having higher geometric mean MICs than the same isolates tested on all other agars (Table 5). Despite the higher MICs, all isolates tested on MHS were still susceptible to SXT. There was no difference in MIC between isolates tested on MHA and those tested on MHF. As in study 1, isolates tested on MHA had lower MICs than the same isolates tested on MHLHBA. This was also found for isolates tested on MHF (Table 5).

Ongoing surveillance with in vitro susceptibility testing is needed to monitor for changes in rates of SXT resistance with increased use of SXT. To date, we have tested 910 S. pyogenes isolates cultured from impetigo and anterior nares of children randomized in the Skin Sore Trial on MHF using an SXT Etest according to EUCAST guidelines. Only 8 (0.9%) have been found to be resistant, with MICs of >2 mg/liter (unpublished data). These results are consistent with those reported from the EUCAST group.

DISCUSSION

Although SXT is no longer commonly recommended for treatment of respiratory tract infections, it remains one of the most widely used and cheapest antibacterials in the world and is an important option for treatment of SSTI, where S. pyogenes and S. aureus are often copathogens (4, 12, 24, 34). In the era of rising MRSA prevalence, antibacterials that are active against both bacteria are highly valued.

Impetigo is a significant therapeutic problem in remote communities in the Northern Territory of Australia (37, 49, 50), with community-associated MRSA having become highly prevalent in this region (50). Impetigo is also an endemic problem in many less-developed countries (41, 45), and MRSA is likely to be on the rise in these contexts also (50). In patients with MRSA and S. pyogenes coinfection, finding a single oral agent that is effective, affordable, and easy to use would be a significant advance. Penicillins and cephalosporins are no longer an option for MRSA treatment. In the Northern Territory context, clindamycin is not an option, with up to 22% of MRSA isolates resistant (49), aside from its poor palatability in young children and the difficulties in maintaining adherence to a thrice-daily regimen. Tetracyclines are not recommended in children under 8 years of age (17), and linezolid is currently too expensive for the empirical treatment of such a common childhood condition. SXT, which is cheap, widely available, and well tolerated and requires only twice-daily dosing, is a potential single agent for treatment of both MRSA and S. pyogenes infections. Several studies have con-

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**TABLE 2** SXT Etest susceptibility results for study 1

<table>
<thead>
<tr>
<th>Medium</th>
<th>% of susceptible isolates (MIC ≤ 1 mg/liter)</th>
<th>Geometric mean MIC (mg/liter)</th>
<th>SD (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHBA</td>
<td>100</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>MHA</td>
<td>99</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>MHLHBA</td>
<td>100</td>
<td>0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**TABLE 4** SXT Etest susceptibility results for study 2

<table>
<thead>
<tr>
<th>Medium</th>
<th>% of susceptible isolates (MIC ≤ 1 mg/liter)</th>
<th>Geometric mean MIC (mg/liter)</th>
<th>SD (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHF</td>
<td>100</td>
<td>0.07</td>
<td>2.05</td>
</tr>
<tr>
<td>MHS</td>
<td>100</td>
<td>0.16</td>
<td>1.79</td>
</tr>
<tr>
<td>MHA</td>
<td>100</td>
<td>0.07</td>
<td>2.03</td>
</tr>
<tr>
<td>MHLHBA</td>
<td>100</td>
<td>0.09</td>
<td>2.3</td>
</tr>
</tbody>
</table>

---

**TABLE 3** Difference in MIC in study 1

<table>
<thead>
<tr>
<th>Medium</th>
<th>MHHA lower by 7% (−9% to 20%)</th>
<th>MHHLHBA lower by 25%* (13% to 35%)</th>
</tr>
</thead>
</table>

*Pairwise comparisons of geometric mean MICs of various media.

**TABLE 5** Difference in MIC in study 2

<table>
<thead>
<tr>
<th>Medium</th>
<th>MHF higher by 128%* (107% to 150%)</th>
<th>MHS higher by 132%* (113% to 153%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHLHBA</td>
<td>MHS lower by 16%* (3% to 27%)</td>
<td>MHS lower by 27%</td>
</tr>
</tbody>
</table>

*Pairwise comparisons of geometric mean MICs of various media.
firmed the ongoing susceptibility of S. aureus to SXT in this region of Australia (38, 49).

The breakpoints utilized for this study were defined by EUCAST using data collated from a wide range of sources on more than 2,500 isolates of S. pyogenes tested for susceptibility to SXT using a variety of methods (32). Of the 2,596 tests reported from multiple sources, geographical areas, and time periods, 2,559 were susceptible to SXT of \( \leq 1 \) mg/liter and 23 isolates were resistant (0.9%) (http://mic.eucast.org, last accessed 18 September 2012). This can be contrasted with results found in U.S.-based literature using the CLSI methods, where AST for S. pyogenes is performed on agar supplemented with defibrinated sheep blood and SXT is not routinely tested, as S. pyogenes strains are considered universally resistant (51). Defibrinated sheep blood is utilized, as the hemolytic reactions of \( \beta \)-hemolytic streptococci on blood agar containing sheep blood are deemed “true” (1).

<table>
<thead>
<tr>
<th>Publication</th>
<th>Yr</th>
<th>Country</th>
<th>Method and medium used</th>
<th>Findings</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yourassowsky et al. (54)</td>
<td>1974</td>
<td>Belgium</td>
<td>AST determined by the agar dilution method on Wellco test agar supplemented with 5% laked horse blood</td>
<td>59/59 strains of S. pyogenes susceptible to TMP, SMZ, and SXT</td>
<td>0%</td>
</tr>
<tr>
<td>Finland et al. (22)</td>
<td>1976</td>
<td>United States</td>
<td>Plate dilution method on a modified thymidine-deficient Mueller-Hinton medium containing 5% laked blood</td>
<td>35/35 strains of S. pyogenes susceptible to SXT; 32/35 strains of S. pyogenes susceptible to SMZ alone</td>
<td>0%</td>
</tr>
<tr>
<td>Darrell et al. (14)</td>
<td>1968</td>
<td>United Kingdom</td>
<td>MIC determined by the plate dilution method on diagnostic sensitivity agar containing 5% laked horse blood</td>
<td>14/14 strains of S. pyogenes susceptible to TMP with MIC ( \geq 1 ) ( \mu g/\text{ml} )</td>
<td>0%</td>
</tr>
<tr>
<td>Liebowitz et al. (36)</td>
<td>2003</td>
<td>South Africa</td>
<td>MICs determined by the broth microdilution method according NCCLS guidelines</td>
<td>66/66 S. pyogenes strains susceptible to SXT</td>
<td>0%</td>
</tr>
<tr>
<td>Hartman and Hoes (26)</td>
<td>1949</td>
<td>United States</td>
<td>Wilson’s method (53, 53a), a semi-solid medium with low sulfonamide antagonist content</td>
<td>94/96 strains of S. pyogenes susceptible to sulfadiazine</td>
<td>1.9%</td>
</tr>
<tr>
<td>Schultz and Frank (44)</td>
<td>1958</td>
<td>United States</td>
<td>Wilson’s method (53, 53a), a semi-solid medium with low sulfonamide antagonist content</td>
<td>84/86 S. pyogenes strains susceptible to sulfur antibiotics</td>
<td>2.3%</td>
</tr>
<tr>
<td>Eliopoulos and Wennersen (18)</td>
<td>1997</td>
<td>United States</td>
<td>MICs determined by agar dilution methods on Mueller-Hinton II agar + 5% laked horse blood or with thymidine phosphorylase at 0.2 IU/ml added</td>
<td>58/60 S. pyogenes susceptible to SXT</td>
<td>3.3%</td>
</tr>
<tr>
<td>Berger-Rabinowitz and Davies (2)</td>
<td>1970</td>
<td>Israel</td>
<td>Wilson’s method (53, 53a), a semi-solid medium with low sulfonamide antagonist content</td>
<td>849/890 S. pyogenes strains susceptible to sulfadiazine</td>
<td>4.6%</td>
</tr>
<tr>
<td>Dhande et al. (15a)</td>
<td>2011</td>
<td>India</td>
<td>Kirby-Bauer disk-diffusion test as per CLSI guidelines; agar not specified</td>
<td>24/26 S. pyogenes strains susceptible to SXT</td>
<td>6.7%</td>
</tr>
<tr>
<td>Bushby (6)</td>
<td>1973</td>
<td>United States</td>
<td>Disk diffusion using TMP/SMZ disks 1.25/23.75 ( \mu g ) on various media</td>
<td>699/757 S. pyogenes strains susceptible to SXT</td>
<td>7.7%</td>
</tr>
<tr>
<td>Lakshmy et al. (34a)</td>
<td>2011</td>
<td>India</td>
<td>Kirby-Bauer disk-diffusion test as per CLSI guidelines. Agar not specified</td>
<td>95/119 S. pyogenes strains susceptible to SXT</td>
<td>21.8%</td>
</tr>
<tr>
<td>Dumre et al. (16a)</td>
<td>2009</td>
<td>Nepal</td>
<td>Kirby-Bauer disk-diffusion test as per CLSI guidelines; agar not specified</td>
<td>11/38 S. pyogenes strains susceptible to SXT</td>
<td>71%</td>
</tr>
<tr>
<td>Traub and Leonhard (51)</td>
<td>1997</td>
<td>Germany</td>
<td>Agar disk diffusion using NCCLS criteria on sheep blood MHA</td>
<td>0/63 strains of S. pyogenes susceptible to SXT</td>
<td>100%</td>
</tr>
<tr>
<td>Kaplan et al. (33)</td>
<td>1999</td>
<td>United States</td>
<td>Etest performed on MHA containing 5% sheep blood</td>
<td>0/169 strains of S. pyogenes susceptible to SXT with MIC ( \geq 52 ) ( \mu g/\text{ml} )</td>
<td>100%</td>
</tr>
</tbody>
</table>

* TMP, trimethoprim, SMZ, sulfamethoxazole.

**TABLE 6** Summary of published results of S. pyogenes in vitro susceptibility to SXT*
The in vitro results reported in the current study confirming the susceptibility of S. pyogenes to SXT suggest that treatment of SSTI with SXT is worth considering. Our current RCT to assess the noninferiority of SXT to the standard treatment with benzathine penicillin G (BPG) for impetigo will provide the necessary clinical evidence to inform guidelines. It is based on a pilot study of 13 participants which indicated that both BPG and SXT were efficacious in healing impetigo (48). There is one study published comparing these agents for S. pyogenes infection in tonsillitis, which reported a 70% treatment efficacy for SXT compared to 88% for penicillin, a non-statistically significant difference (52).

The infrequent reports of susceptibility of S. pyogenes to SXT demonstrate resistance rates ranging from 0% to 100% depending on which medium and testing conditions are used (Table 6). Although the variation in results may relate to the particular strains included or the local prescribing patterns of SXT, it is most likely related to the methodology of testing. All of the studies reporting high resistance rates either used media known to have high concentrations of thymidine or did not provide details of the medium used. As standardization to ensure a low thymidine concentration of Mueller-Hinton medium was introduced only in 2006, it is likely that, unless low-thymidine media were specified (2, 14, 18, 22, 26, 44, 54), studies in publications prior to this may not have controlled for thymidine content.

Alongside this, S. pyogenes has remained 100% susceptible in vitro to penicillin. Hence there has been no pressing need to understand SXT susceptibility as an alternative antibacterial in the public health approach to treatment of S. pyogenes infections. However, this is changing in the context of rising MRSA rates for SSTI.

Our results show that testing of S. pyogenes for susceptibility to SXT on MHS gives a higher MIC than all of the other agars, although the organism remains in the susceptible range. This could possibly be due to the availability of thymidine or other inhibitory substances in this medium. However, thymidine concentrations of the various media utilized were not assessed. Alternatively, the absence of an enzyme to reduce the inhibition in sheep blood compared to horse blood may be the explanation.

The original paper describing the identification of the Harper-Cawston factor (25) as thymidine (21) reports an interesting observation that the study has partially demonstrated. Only lysed horse blood contains thymidine phosphorylase to convert thymidine to thymine and hence overcomes the inhibition of folate metabolism that occurs in the presence of thymidine. No other mammalian blood contains this enzyme, which is a possible reason for the higher MICs reported on MHS than on those agars containing horse blood. In the original paper, the presence of thymidine at concentrations of 1.6 μg/ml was sufficient to completely prevent inhibition by the drugs. The inclusion of lysed horse blood restored the inhibition. This has also been shown by Coll et al. (11). However, the MIC of S. pyogenes isolates tested on MHLHBA was higher than those of isolates tested on MHF (study 2), MHA (studies 1 and 2), or MHBA (study 1), which suggests other factors at play.

A limitation of this study is the reliance upon a single method for susceptibility testing, the Etest, which is a commercially derived method. Further work using broth or agar dilution methods would add to our understanding of the susceptibility of S. pyogenes to SXT. As shown in Table 6, when these additional methods have been assessed, the susceptibility of S. pyogenes ranges from 0% (54) to 3.2% (18) resistant, similarly low resistances to those reported in this study.

Reading MICs for SXT can be challenging due to haze. In particular, only faint growth of S. pyogenes was achieved on MHA (due to the absence of blood), and this made reading endpoints more difficult. MHLHBA and MHF had problems similar to those of MHA with respect to haze. Despite this in study 2, the MICs were reproducible between readers, with a high level of intersubject reliability within 1 MIC gradation.

Conclusions. The widespread belief that SXT is ineffective for S. pyogenes infections because of inherent antimicrobial resistance is a fallacy due to technical limitations in laboratory methodology: namely, the use of media containing high concentrations of thymidine, which inhibits the action of sulfur antibacterials. When media containing low concentrations of thymidine and/or high concentrations of the enzyme thymidine phosphorylase are used, resistance rates are low in most cases, although this must be monitored over time and may vary with local epidemiology and antibacterial prescribing patterns. This study provides justification to proceed to clinical trials of SXT for S. pyogenes infections. Corroboration with clinical trial data may convince clinicians that SXT can safely and appropriately be used for infections involving S. pyogenes. The Skin Sore Trial will answer the clinical applicability of this current in vitro study. In the era of rising MRSA prevalence, more clinical trials of SXT for treatment of SSTI, where S. pyogenes and S. aureus are frequently copathogens, are needed.

ACKNOWLEDGMENTS

The work of the research assistants in study 1 and the Skin Sore Trial team (Irene O’Meara, Jane Nelson, Tammy Fernades, Melita McKinnon, Diana Halliday, Colleen Mitchell, Valerie Coomber, and Christine Francis) in recruiting participants for study 2 has made this research possible. Study scientists who carried out this work include R.A.L., P.W., and Vanya Hampton. Mark Chatfield provided assistance with the statistical analysis. We also acknowledge all of the participants and their families who have participated in the Skin Sore Trial and Rheumatic Heart Disease studies.

This work was supported through a National Health and Medical Research Council (NHMRC) Project Grant (545234) on which A.C.B., S.Y.C.T., M.I.M., B.J.C., and J.R.C. are all investigators. A.C.B. is the recipient of a NHMRC scholarship for Ph.D. research (605845) as well as an Australian Academy of Sciences Douglas and Lola Douglas scholarship. S.Y.C.T. is the recipient of a NHMRC Early Career Fellowship (605829). We have no conflicts of interest to declare.

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December 2012 Volume 50 Number 12 jcm.asm.org 4071

S. pyogenes and Trimethoprim-Sulfamethoxazole
CHAPTER 4
THE CHALLENGES OF COLLECTING AND TRANSPORTING IMPETIGO SWABS IN A TROPICAL ENVIRONMENT

4.1 CHAPTER OVERVIEW

Being able to effectively collect and transport impetigo swabs in a tropical environment has been critical to the work undertaken in my thesis. This chapter describes the method developed and validated for the transportation of impetigo swabs from very remote contexts for processing in a central microbiology laboratory. It also succinctly describes the methods used to culture and identify *Staphylococcus aureus* and *Streptococcus pyogenes*. This supplements the methods for antibiotic susceptibility testing described in Chapter 3.

The primary objective of the Skin Sore Trial was to determine non-inferiority of trimethoprim/sulphamethoxazole to benzathine penicillin G for the treatment of impetigo. This objective was complemented by the secondary outcomes, which were to determine the relative abundance of *S. aureus* and *S. pyogenes* at days 0, 2, and 7 of the study. To be confident in these secondary outcomes, it was necessary to develop and test robust methods for collecting and transporting skin swab specimens.

The remote communities involved in the study were up to 1500 kilometres away from the laboratory and did not have daily flights connecting the community to the laboratory. As such, transportation of swabs was by either road or air. Previous research protocols for detecting *S. pyogenes* recommended either: 1) immediate plating of the collected swab in the community and transportation within 24 hours for incubation in the laboratory or; 2) plating and incubation of the swab within 48 hours of sampling. (Johnson, 1996, Kotloff, 2008) These recommendations were developed for contexts similar to the remote communities of the Northern Territory and had been previously utilised in
smaller studies. (McDonald et al., 2006, Carapetis et al., 1995) However, the size and complexity of recruiting 663 participants over three years from multiple remote communities with varying available transportation logistics resulted in the need to develop and test an alternative solution. Transport media have been recommended for this situation, although little data was available on freezing such specimens.

An additional benefit of the method reported in this chapter was that swabs could be processed in batches. This was important for the laboratory scientists working on the study to manage workflow as more than 4500 swabs of impetigo lesions and the anterior nares were collected and processed during the course of the RCT. This methodology also permitted freezing of the swabs for later molecular and metagenomic studies that have the potential to further our understanding of the mechanisms of transmission of bacterial skin pathogens within and between communities.

The transport medium validated in this study is cheap, easy to produce, effective in transporting skin pathogens, can be frozen and may be utilised in research in other remote contexts.

4.2 STATEMENT OF CONTRIBUTION TO JOINTLY AUTHORED WORK

Together with Steven Tong, I conceived this experiment, with oversight from Jonathan Carapetis. I wrote the SOP for this experiment and oversaw the data collection. I, Steven Tong and Mark Chatfield conducted the data analysis. I performed the literature review and wrote all drafts of the manuscript. All co-authors contributed to revisions of the manuscript and approved the final version for publication. Steven Tong agreed to be the corresponding author while I was on maternity leave.
Comparison of three methods for the recovery of skin pathogens from impetigo swabs collected in a remote community of the Northern Territory, Australia
CHAPTER 5
DEVELOPING A METHODOLOGY FOR CAPTURING AND SCORING STANDARDISED DIGITAL IMAGES OF IMPETIGO

5.1 CHAPTER OVERVIEW

This chapter describes the novel methods needed for assessing and blinding the primary end point within our RCT, which has the potential to be a standardised approach for similar studies in the future.

Prior impetigo studies synthesized in the Cochrane review of impetigo treatment (Koning et al., 2012) have predominantly been conducted in urban physician offices or hospital outpatient settings. This allows more ready access to experts who can immediately assess the participant for the primary outcome. Due to the remoteness of the research locations in our trial, it was not feasible to transport an expert to assess the outcome of every one of the 663 episodes of impetigo under investigation. As such, building on the work of tele-dermatology, digital images were determined to be the best method available to capture and evaluate the primary endpoint of this trial. However, no published protocols for capturing standardised digital images by amateur photographers were available.

As an open label RCT, we needed a blinded, robust, reproducible and defendable primary endpoint. This chapter describes in detail the development and evaluation of our approach. This was primarily work which I led. Expert photographic input was sought from a professional photographer to guide this process. Once standardised digital images of all enrolled sores from days 0, 2 and 7 were available, a method for scoring these images that minimised bias and provided readily analysable data was needed. This chapter will also describe the
method developed for scoring outcomes using assessors blinded to treatment allocation.

Having published our approach, these methods are now available for future impetigo treatment studies, and could be implemented in either remote or office-based research. It may be possible to compare the results from an expert who is immediately able to objectively assess the process of sore healing with results from other experts scoring the digital images distant from the participant. This translation will add value to future studies of impetigo, and facilitate high quality treatment studies where the burden of impetigo is the highest.

5.2 **STATEMENT OF CONTRIBUTION TO JOINTLY AUTHORED WORK**

I designed and wrote all the standard operating procedures for collecting digital images in the Skin Sore Trial. Assistance was sought from Kara Burns, a medical photographer, for expert advice. I developed the training packages for the research assistants and oversaw the quality checks of all digital images. Ross Andrews proposed the concept for scoring the digital images in a randomly allocated order, which I then revised and developed into a feasible protocol. I oversaw all aspects of digital image scoring. Robyn Liddle developed the databases within which digital images were scored. I cleaned and analysed the data, developed the quick lists and drafted all versions of the manuscript. All co-authors contributed to revisions of the manuscript and approved the final version for publication.

5.3 **JOURNAL ARTICLE (FOLLOWING PAGE)**

Standardising and assessing digital images for use in Clinical Trials: A practical, reproducible method that blinds the assessor to treatment allocation
Standardising and Assessing Digital Images for Use in Clinical Trials: A Practical, Reproducible Method That Blinds the Assessor to Treatment Allocation

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Abstract

With the increasing availability of high quality digital cameras that are easily operated by the non-professional photographer, the utility of using digital images to assess endpoints in clinical research of skin lesions has growing acceptance. However, rigorous protocols and description of experiences for digital image collection and assessment are not readily available, particularly for research conducted in remote settings. We describe the development and evaluation of a protocol for digital image collection by the non-professional photographer in a remote setting research trial, together with a novel methodology for assessment of clinical outcomes by an expert panel blinded to treatment allocation.

Introduction

Telemedicine is increasing in popularity, particularly for dermatologists and other specialties where a clinician is not always onsite for direct patient care [1]. With the increasing availability of cheap, simple, high quality digital cameras for the non-professional photographer, often a clinician or auxiliary [2,3], the appeal of utilising digital images to diagnose or monitor treatment progress is growing. An extension of this application is the use of digital images of cutaneous disease to assess outcomes for intervention studies including randomised controlled trials (RCTs) [4]. Advantages include: maintaining blinding of the outcome assessor to treatment allocation; scoring digital images in batches to improve work flow; and utilising expert outcome assessors remote from the site of data collection. These advantages have particular appeal for the conduct of research in remote regions or for multicentre studies where standardisation of clinical outcome measures is critical. However, published methodologies to guide such use of digital images are lacking. In addition, unlike hospital or clinic based health photography which is usually performed in a dedicated setting with instruments and lighting operated by a professional photographer [5,6], this ideal may not be achievable in remote, field-based research. Therefore we aimed to develop a method to facilitate the acquisition of consistent, high quality digital images of superficial skin lesions in the context of conducting a RCT in a remote setting. A medical photographer (KE) guided this process [4,7]. The digital images were taken by field research staff and allowed the assessment of outcomes in a blinded manner.

The four characteristics of an excellent clinical photograph are correct perspective, use of a scale aligned with the image frame, even lighting and a neutral background [8]. To achieve these characteristics, it is important to standardise the equipment, camera settings, participant positioning and photography technique so that reproducible images are captured [9,10]. Once images of satisfactory quality have been captured, consideration must be given to storing these confidential images for future use. Standardised protocols that address these priorities for collecting digital images are not available in the peer-reviewed literature.

Impetigo trials are an example of cutaneous disease research where digital images can improve the objectivity of outcome assessment. Impetigo is a common, non-benign cutaneous infection that mostly occurs in resource-limited contexts [11], affecting >2% of the global population at any one time [12]. Impetigo also regularly affects school-aged children in industrialised settings [13]. Treatment of impetigo is a public health priority to prevent severe sequelae including streptococcal and staphylococcal sepsis, focal invasive disease, post-streptococcal glomerulonephritis (which is in turn linked with chronic renal failure) [14], and a postulated causative link with rheumatic fever and rheumatic heart disease [15,16]. These sequelae mainly occur in
resource-limited settings and are responsible for hundreds of thousands of deaths each year globally [11].

The authors of a meta-analysis on interventions for impetigo recommended the use of clear and objective outcome measures for future impetigo research [17]. As the burden of impetigo is in resource-limited contexts where ready access to clinicians for immediate end-point assessment may not be feasible, digital image end-points are appealing and objective. The only RCT from a resource-limited context included in the meta-analysis reported the use of photographs for outcome assessment. In this RCT, successful treatment was defined as clinical cure or marked improvement with an additional measure using photographs, but they did not report the methodology of image collection or how they determined the outcome based on this end-point [18]. Well-defined, reproducible endpoints are needed and blinded outcomes are the cornerstone of good clinical trial design [19].

In conducting a RCT on impetigo treatment in a remote setting, we developed a standardised, reproducible method for collecting images of skin sores at different time points [6]. Reviewers blinded to treatment allocation assessed the images using a simple, reproducible, quick method that provided readily analysable data. The image capture protocol and novel methodology for blinded assessment may be useful for other trials in cutaneous disease research.

Method

Ethics

This study and all consent documentation were approved by the Human Research Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (HREC 09/08). Indigenous elders provided community consent before recruitment commenced. The parent or legal guardian for all participants provided written informed consent. The study was explained by a local interpreter or by using a talking book in the participant’s first language. Written consent was itemised for all study procedures including the collection of digital images.

Setting

We conducted a non-inferiority RCT in 7 remote Indigenous communities of the Northern Territory of Australia between 2009 and 2012 [20]. The research team was based in Darwin and travelled via plane or road to the remote communities up to 1500 kilometres away. There were 663 episodes of impetigo (in 508 children) enrolled in the trial and each had either one or two sores under investigation. Research assistants trained in the photography protocol captured digital images of all trial participants’ sores on days 0, 2 and 7. Images were stored electronically. A panel of paediatricians specialising in the care of Indigenous Australian children externally reviewed these digital images at a later date, according to a standardised scoring system as reported below.

Details of the protocol for capturing digital images of impetigo

Equipment. All images were captured using standardised equipment (table 1, figures 1–4).

Camera Settings. Due to data collection occurring simultaneously in more than one community, we purchased three identical cameras. All study camera settings were programmed by the study manager (figure 5) and checked against the standard operating procedure (SOP) by the research assistants before each use. Training in the programming of settings and operation of the camera was conducted with each new research assistant and image quality reviewed at the completion of most field work trips. A quick list to summarise the process for image capture in the field was developed (table 2).

Lighting Conditions. The main light source was the flash as force flash was always on. This provided a standard light source for all three cameras in all settings. In addition, preference was given to capturing digital images outdoors in the shade to improve the ability of the camera to focus on the subject and avoid distracting shadows [10]. Direct sunlight was avoided to minimise the potential of a stronger light source resulting in an over-exposed image. For uniformity of conditions, when it was not possible to photograph participants outdoors, maximal ambient light was achieved by turning on all lights and opening any curtains or doors. Night time photography was avoided by returning to photograph the participant first thing the following day.

Positioning of study participant. As we were working with children, prior to taking any photographs, the participants were reminded to remain still and the carer was engaged in reassuring the child. The participant was positioned comfortably in a chair or on the floor with a neutral grey background beneath the limb or site to be photographed (figures 4 and 6). Jewellery, clothing or hair that might obscure the area of interest were removed or tied back. Any dressings covering the lesion were also removed.

Once settings were rechecked, a 5 cm neutral grey scale was placed in a vertical position, in the same plane as the sore, as close as possible to the left of the sore without obscuring any edges of the lesion (figures 2 and 6). The upper limit (0) of the scale was positioned at the top of the frame and the lower limit (5) at the bottom. This ensured all images were captured at the same scale so that when paired images were reviewed the sores were comparable and any reduction in size could be assessed as a measure of sore healing.

Photography technique

To maximise sharpness by depth of field, the camera lens plane was positioned parallel to the sore plane with the photographer standing above the sore (figure 6). The sore was centred using the white square corners at the centre of the camera screen. The shutter was depressed halfway to focus the lesion prior to capturing the image.

A minimum of three images of each sore were taken at each time point, to ensure that at least one adequate image was available for outcome assessment, a technique known as bracketing [21]. The research assistant was instructed to check each image for SOP conformity and to take additional photographs if a clear, focused image showing all details of the sore had not been...
obtained. Each photograph number was recorded in the respective participant’s case report form (CRF).

Once all images had been captured, brief notes were made in the participant’s CRF to describe the positioning of the participant so that whenever possible the same position could be used for future images. Further follow up images of the same sore were required on day 2 and day 7 from enrolment. For consistency of orientation, previous images of the same sore were checked on the study camera before capturing the next image.

Image download and storage

Standardisation of image storage is critical to the meticulous utilisation of this method [9,22]. At the completion of each day, the image files were downloaded from the camera memory card to a password-protected laptop. The laptop files served as a data backup, which was important given that study visits lasted between two and three weeks and internet was not reliable enough in the remote context to upload numerous large files every day. Upon return of the team to the research centre in Darwin, all images were downloaded from the camera to the main computer server where daily backups occur. All images were taken and stored in high quality JPEG (Joint Photographic Experts Group) format for convenience as our chosen camera did not shoot in an uncompressed (‘raw’ or ‘loss-less’) format. There are limitations to using a compressed or ‘lossy’ format but the benefits of using a compact camera and storage of images for comparison outweighed these and is in line with other clinical studies [23]. Three copies of each unmodified image were saved: one in the participant’s folder labelled with participant number; one in the generic backup folder labelled with the camera-generated photograph number as recorded in the participant CRF; and one labelled with a randomly generated number between 1 and 15 000. Only once this had occurred were images deleted from the memory card. These re-identifiable images are to be stored on a secure server for up to 25 years, in keeping with ethical requirements for research in children [24]. From the three available digital images of each sore, the best quality image (key criteria were focus, exposure and magnification) was selected for outcome assessments.

Quality control process

As all images were collected by amateur photographers, images were regularly checked by the study doctor (AB) and feedback provided if the image did not conform to the SOP. In addition, prior to commencing primary outcome reviews, a quality control (QC) check of all available digital images was performed midway through the study (collected from the first 200 study participants) by a medical photographer (KB) experienced in capturing digital images of skin conditions. The QC was a priority in this study as the method described had not been previously used or evaluated and we were unsure whether digital image manipulation might be needed for image scoring. Digital images can be manipulated to overcome flaws in image capture [21], however this has limitations. Our a priori hypothesis was that digital image manipulation would not be needed. To confirm this after recruitment of 200 participants, 1 300 images were scored as either adequate or unable to be interpreted using the definitions in table 3. If “unable to be interpreted” was chosen, the reviewer was instructed to provide reasons (figure 7).

Figure 2. Use of scale to define the upper and lower boundaries of image in the landscape position. This series of images of the same sore on days 0 (A), 2 (B) and 7 (C) utilise the scale well with the 0 at the top of the image and the 5 at the bottom, are clear and focussed and demonstrate sore healing over time. Limitations include different availability of light as captured during different parts of the day using outdoor light. doi:10.1371/journal.pone.0110395.g002

Figure 3. An example of the participant identification card described in table 1. This card contains participant number, date of image, study day, and whether it is sore A or B as up to two-thirds of study participants had two sores enrolled in the study. doi:10.1371/journal.pone.0110395.g003

Figure 4. Capturing the image using the study camera, grey background, and grey scale in a remote context. The individuals in this image have given written informed consent to publish this image. doi:10.1371/journal.pone.0110395.g004
Methods for digital image assessment

We developed a method for scoring digital image pairs that was simple, limited bias, quick and afforded readily analysable data. As the methodology was novel, a paper-based pilot was conducted employing 13 clinicians and researchers to confirm usability prior to building an automated database for scoring. In the pilot, 22 paired digital images from 10 participants of either day 0 and 2 (5 participants) or day 0 and 7 (6 participants) were reviewed in random order (days 0/2 or 2/0 and 0/7 or 7/0) by the 13 reviewers. The initial definition of healing or improved included both a visual description of the sore pair and a clinical decision as to whether further antibiotic treatment was needed.

The primary outcome for the RCT was treatment success at day 7 according to paired digital image scoring. After the successful pilot, the digital image pairs were organised in random order in a purpose built database (figure 8) using the randomly assigned number between 0 and 15 000 as the only identifying information. Scoring of the digital image pairs was on non-standardised number between 0 and 15 000 as the only identifying information. In the pilot, the digital image pairs were organised in random order in a purpose built database (figure 8) using the randomly assigned number between 0 and 15 000 as the only identifying information.

Following un-blinding of the chronological order of sores and to produce readily analysable results from the blinded scoring system, if image B was the day 2 or 7 sore, treatment success was deemed to have occurred if image B was healed or improved compared to image A (day 0 sore). If image B was the day 0 sore, then success was determined using the definitions (table 4) and vice versa (i.e., image B compared to image A). To expedite this process, an auto-fill was used in the database. For example, when image A was scored as “worse”, auto-fill made available the options of “healed” or “improved” only for the comparison of image B to image A. The use of auto-fill made the scoring process as rapid as possible. Thus reviewers were blinded to both treatment allocation and the chronological order of sores. Every image pair was evaluated by two independent reviewers from the panel of eight. Where disagreements occurred, an expert panel of three determined the final result by consensus.

Table 1. Study equipment chosen including the required features, advantages and alternatives available or recommended in the literature.

<table>
<thead>
<tr>
<th>Item</th>
<th>Specific Choice</th>
<th>Features</th>
<th>Advantages</th>
<th>Alternatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digital Camera</td>
<td>Panasonic LUMIX DMC-TZ8 14.5 megapixel digital camera (figure 1)</td>
<td>Macro to 3 cm from a 12X zoom lens equivalent to a 25–300 mm lens on an SLR</td>
<td>Inexpensive</td>
<td>Digital single lens reflex (SLR) camera (32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Built in flash</td>
<td>Readily available</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Rugged metal casing</td>
<td>Automatic</td>
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<td></td>
<td></td>
<td>Lithium ion rechargeable battery</td>
<td>Pre-specified settings</td>
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<td></td>
<td>to achieve uniform, reproducible</td>
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<td></td>
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<td></td>
<td>images whilst using amateur</td>
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<td></td>
<td></td>
<td></td>
<td>photographers</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>4GB memory card</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>Grey background (figure 4)</td>
<td>A small grey board positioned behind the body part being assessed</td>
<td>Transportable and light weight</td>
<td>Green or grey surgical drapes [33]</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Scale [34–36]</td>
<td>5cm, non-reflective grey scale (figure 2)</td>
<td>Vertical scale</td>
<td>Defined the upper and lower boundaries of the photograph (figure 2)</td>
<td>Paper or commercially available scale e.g. the ABFO scale [34]</td>
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<tr>
<td></td>
<td></td>
<td>Single use sticker</td>
<td>Easily removed and discarded</td>
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<tr>
<td>Identification Card</td>
<td>Cardboard handwritten card (figure 3)</td>
<td>Pre-formatted to add individual randomisation number, study visit day, sore number, date and time of image capture</td>
<td>Maintained study blinding</td>
<td></td>
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<td>doi:10.1371/journal.pone.0110395.t001</td>
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</tbody>
</table>
## Table 2. Quick List for capturing standardised photographs.

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Confirm all camera settings are correct (Figure 5), participant, paperwork and previous image for orientation (if available)</td>
</tr>
<tr>
<td>2</td>
<td>Position participant in the shade: comfort, lesion exposed, neutral background, scale in the same plane as the lesion</td>
</tr>
<tr>
<td>3</td>
<td>Photograph participant ID (Table 1) prior to capturing series to preserve blinding.</td>
</tr>
<tr>
<td>4</td>
<td>Position camera in the same plane as sore (Figure 6). Centre the sore and focus camera. Take minimum of 3 photos. Take additional photographs if none are clear and focused.</td>
</tr>
<tr>
<td>5</td>
<td>Record photograph number and notes in participant’s file</td>
</tr>
<tr>
<td>6</td>
<td>Save digital images in secure location and delete from camera</td>
</tr>
</tbody>
</table>

This could be printed on a small card to be carried with the camera as a reminder to research assistants capturing images.

doi:10.1371/journal.pone.0110395.t002

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Figure 5. The camera settings and icons as used in the protocol (these icons and settings are standard across other popular camera models). In addition the rationale is provided on why these settings were adopted.

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doi:10.1371/journal.pone.0110395.g005

### Icon/Symbol | Setting | Rationale
---|---|---

| ![P](icon) | Program mode | Program mode facilitates the selection of the following settings outlined in the table below. |

| ![Macro Zoom](icon) | Macro Zoom | The digital macro zoom is for taking very close up photographs with a minimum distance of 3cm from the lesion. |

| ![Maximal Zoom](icon) | Maximal Zoom | Within macro zoom, the maximal 3X zoom could be utilised. |

| ![WB](icon) | White Balance | The white balance adjustment set to AUTO allows the camera to compensate for the colour of the light source. This avoids unnatural colour in the image |

| ![ISO](icon) | International Organisation Standardisation | The ISO standards specify the sensitivity to lighting for digital cameras and film. ISO was set to 100 for all images captured in this study. |

| ![Auto Focus](icon) | Auto Focus mode | The AF mode was set on high speed. |

| ![Metering](icon) | Metering mode | Setting the metering mode to centre weighted measures the brightness in the centre with the object of focus in the centre. This ensured a single, centred focus point. |
was deemed to have occurred if image B was worse compared to image A (day 2 or 7 sores) and image A was either healed or improved (table 4).

Results

Overall image collection

Over the 3-year study, almost 10 000 digital images were collected and stored by more than 20 research assistants who collected data in 7 remote communities covering an area of 1.35 million km². From all of the images collected, the best available image of the bracketed set was selected for outcome assessment. Approximately 3 300 digital images were required to determine the primary and secondary outcomes of the RCT by analysing the results of paired comparisons. The project manager (IO) reviewed all images and selected the best available image for the paired comparison. The best available image (determined by focus, exposure and magnification) was most often the second or third image captured. This was consistent by study visit day.

Of the 9 944 digital images collected, only 17 (0.17%) were not available for assessment due to staff error. These errors were the wrong site photographed on subsequent days to the original site (n = 6), the photograph not saved or filed (n = 4) and the photograph not taken due to staff error (n = 7).

Quality control check

For the QC check, 1 300 digital images from the first 200 participants that conformed to the described methodology were reviewed. 1 258 (96.8%) were deemed adequate using the definitions provided. Of the 42 images (3.2%) deemed unable to be interpreted there was some overlap in categorisation: 29 were due to incorrect exposure (8 too light, 21 too dark), 16 were due to lack of focus, 2 had incorrect magnification due to lack of focus and 1 had incorrect magnification (Table S1). Results of the QC review were reassuring and consequently digital image modification was not required.
Pilot for digital image scoring

Thirteen reviewers piloted the digital image scoring process. All pilot reviewers agreed the process was quick and manageable with image quality being adequate. Initially definitions for healing, improved, same or worse included a 2-armed definition with a description of both sore healing and a clinical judgement as to whether treatment with further antibiotics was indicated. The reviewers reported that the need for additional treatment was difficult based on images alone and as the decision had no timely clinical impact, we removed this decision from the definitions.

Digital image scoring results

Outcome scorers reported 98.3% of digital images as able to be interpreted using the quality codes shown in figure 8. Of these, 89.9% were adequate and 8.4% suboptimal but still able to be interpreted (Table S2). The inter-rater reliability of digital image scoring was moderate. When assessing for treatment success (pooled healed and improved, table 4) versus treatment failure (unchanged or worse), there was 86% agreement between reviewers with a kappa score of 0.4. When assessing using the 5

Table 3. Definitions used for the quality control assessment of digital images.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adequate</td>
<td>The entire sore was seen in enough detail to determine the margin and most of the interior; AND the scale was seen in sufficient detail to determine the approximate size.</td>
</tr>
<tr>
<td>Unable to be interpreted</td>
<td>The image of the sore could not be interpreted due to incorrect exposure (too light or too dark), focus (lack of focus or depth of field) or incorrect magnification.</td>
</tr>
</tbody>
</table>

doi:10.1371/journal.pone.0110395.t003

Figure 8. This shows the database format utilised for scoring digital image pairs. Image A was taken on day 0 and image B on day 7. Image B shows erythema and as such was scored as improved using the definitions in table 4. doi:10.1371/journal.pone.0110395.g008
available definitions (figure 8, table 4) there was 64% agreement between reviewers, with a kappa score of 0.3 (Table S3).

Discussion

This is the first description of a method for capturing and scoring comparative digital images of skin lesions in clinical research. The methods outlined were practical even in remote contexts, robust, reproducible and simple enough for non-professional photographers to consistently follow. Strengths of the described methodology include the quality control check and more than 98% of captured images being interpretable. The gold standard for needing to retake orthodontic photographs for poor quality was set at 90% [3] and our findings of adequacy were at this level, but when ‘suboptimal but still able to be interpreted’ was included exceeded this gold standard. In addition, the described method was followed by more than 20 study staff in remote contexts resulting in <0.2% of images being unavailable for assessment. Digital images were the only available form of documentary evidence for this blinded, clinical trial so it was essential to have a robust process. Our results support that the process outlined works.

The adoption of a standard set of image settings (figure 5) and a Quick List that guided training in the methodology (table 2) facilitated a uniform set of images that did not require any digital manipulation. Guidelines on the manipulation of digital images specify that while “it is acceptable practice to adjust the overall brightness and contrast of a whole image” [25] it is best practice if a group of images are to be compared to each other, that the processing of individual images should be identical. The question of “what constitutes a ‘reasonable’ adjustment of image settings such as brightness and contrast, etc.” has become important for publication in scientific journals and is now included in instructions to authors [26]. For example, the instructions to authors in the Journal of Cell Biology outline that, if manipulation of a digital image is undertaken, these manipulations must not obscure, eliminate, or misrepresent any information present in the original [25]. Forensic guidelines also emphasise this rigorous approach for reproducibility [27]. As the method for capturing digital images reported above had not been previously validated and the outcome was based on a comparison of image pairs, the QC check by a professional medical photographer was an important step in determining whether our images would meet this industry standard. Based on these results, we did not permit the use of photo-editing software to modify any of the images [27]. We recommend following a protocol such as ours that has been subjected to rigorous QC checks for future skin disease research which should largely obviate the need for any digital image manipulation.

High staff turnover when working in remote settings [28] resulted in frequent training and re-training sessions in capturing digital images using the methods described. Educational PowerPoint slides were developed for this purpose and supplemented by the quick reference guides developed (tables 1 and 2, figures 5 and 6). In addition, real time review of captured digital images with feedback to the research assistants was useful. Despite the use of a standard protocol and ongoing training, occasional human errors did occur as described above.

A limitation when using digital images for endpoint assessment is the inability of reviewers to make a clinical decision using the additional senses of hearing (patient feedback on pain and pruritus), touch (warmth, fluctuance) and smell, when provided only with the image. For cutaneous diseases where the appearance of a lesion is the primary determinant of outcome, this limitation can be partly addressed with a robust protocol for capturing reproducible, diagnostic images for outcome assessment. This known limitation impacted upon the inter-rater reliability agreement as physicians were asked as reviewers to use a novel diagnostic modality to score outcomes. To overcome this, we used a consensus panel of three to adjudicate any discrepant scoring.

The consensus panel discussed all image pairs until consensus was reached. The possibility that the use of the project manager to select the best available image introduced bias is a possible limitation. However, the QC check by a professional photographer confirms that the perceived bias was minimal with high quality images consistently being provided to reviewers.

We suggest that this protocol could also be adapted from the research setting for use in clinical care. In settings where specialised clinicians are not readily available, standardised digital photography of cutaneous lesions could be used in telemedicine to allow highly skilled clinicians to assist local health staff to manage patients in remote locations.

A unique feature of this protocol for standardising the comparison between image pairs of the same sore where the only detectable difference was changes in the appearance of the lesion [6], was the requirement for all research assistants to check the orientation of the image on the study camera before capturing the next image. Guidelines on doing this were provided. Previous expert advice has been for image capture to be performed consistently by the same photographer [21]. This was not possible within the remote research environment and overall <5% of participants had all 3 days of images collected by the same person. Nonetheless, >99% of image pairs were assessable for the primary outcome. This finding adds to the photography literature. Providing amateur photographers with simple instructions and guidance for collecting digital images using standardised camera settings results in digital images that are of a high quality and can

Table 4. Definitions used for final outcome scoring of digital images of impetigo.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Criteria</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healed</td>
<td>Lesions no longer evident or flat, with no evidence of crusting, erythema or purulence, but possibly with evidence of hyper- or hypo-pigmentation where the original sore was located</td>
<td>Success</td>
</tr>
<tr>
<td>Improved</td>
<td>Lesions reduced in sore diameter and erythema; AND progression from blister to crusting and flattening of the sore. Purulence not evident.</td>
<td></td>
</tr>
<tr>
<td>Same</td>
<td>No appreciable change in diameter, erythema or purulence of lesion.</td>
<td>Failure</td>
</tr>
</tbody>
</table>

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be assessed by blinded, independent reviewers for outcome determination.

A non-inferiority RCT comparing two treatments of a common condition such as impetigo requires rigorous, blinded, objective endpoints for assessment. Here, we have described the method developed based on the available evidence and expertise, for capturing digital images. We report these here for use in subsequent research trials. This method was simple, reproducible and from the QC check provided 97% of images that were adequate for assessment. Whilst other RCTs have used a digital image of the skin as a primary outcome, such as in pyoderma gangrenosum [29], wound healing [30], or pressure sores [31], this is the first report of a standardised, reproducible protocol that has been subjected to a rigorous QC assessment for an impetigo RCT. Our results confirm the reproducibility of the simple resources developed and published herein which will further enhance the rigour of trials using a photographic end-point.

Conclusions

This is the first report of a standardised, reproducible protocol that has been subjected to a rigorous QC assessment for research involving impetigo and could be adapted for other skin disease research. Our study confirms non-professional photographers are able to capture high quality digital images of skin for this purpose. We present a simple method for capturing high quality digital images of skin sores in a RCT and the methods used to score digital image pairs. Future trials for management of skin conditions, particularly in remote contexts, may benefit from adopting this protocol.

Supporting Information

Table S1 Results of quality control (QC) check. When the QC check was adequate, all other fields were automatically completed as not applicable. Where the QC check score was not interpretable, the subsequent fields of exposure, focus and magnification were provided for the professional photographer to give reasons for the decision. (XLSX)

Table S2 Results of the quality assessment conducted by the primary outcome reviewers of the trial. Results reported for simplicity are the combined quality result, as if either one of the images was suboptimal, the image pair decision was difficult. There were 8 reviewers in the study and quality assessments from all reviewers are included in this table. (XLSX)

Table S3 Dataset used to calculate inter-rater reliability and the kappa scores provided. Reviewers were numbered 5, 6, 7, 9, 10, 12, 14 and 16. When all reviewers scored all image pairs, the number of the reviewers selected for the calculation is listed in columns revA_num and revB_num. (XLSX)

Acknowledgments

We acknowledge the participants and families who participated in this trial in the hope of finding a better treatment option for impetigo. Jane Nelson, Deh Taylor-Thomson, Bart Currie, Malcolm McDonald and Mark Chatfield provided critical evaluation of the methodology as it was developed.

Author Contributions

Conceived and designed the experiments: AB ST RA JL. Performed the experiments: AB KB IO. Analyzed the data: AB ST. Wrote the paper: AB KB ST RL IO DW JC. Reviewed and approved the final version of the manuscript: AB KB ST RA JL IO DW JC. Built databases: RL.

References

Australian Aboriginal infants and barriers to health service delivery. BMC Health Serv Res 13: 250.


CHAPTER 6
THE RESULTS OF THE SKIN SORE TRIAL

6.1 CHAPTER OVERVIEW

This chapter presents the results of the Skin Sore Trial, one of the largest impetigo trials ever performed and the first to be conducted in remote Aboriginal communities, where the disease burden is the highest worldwide (Chapter 2). The trial results, now published in *The Lancet*, have already been incorporated into local treatment guidelines.

It was possible to conduct a high quality, randomised controlled trial in these remote communities due to a number of factors. These included the support from the staff at the government and non-government remote clinics where extensive consultation with health clinic managers and staff occurred. In all the communities, elders or traditional owners provided consent to conduct the trial. This facilitated participation. Finally, the commitment and dedication of the senior trial staff, who trained and led a diverse and often transient group of research assistants in the remote communities, was essential.

We initially proposed to recruit children presenting to the clinic for treatment of skin sores. However, it was quickly realised that the high activity and acuity of the clinics and the limited number of children presenting for treatment of sores, made this approach unlikely to be successful. Ultimately, only 5% of participants came from clinic referrals. Importantly, teachers at the local schools recognised the need for sores to be treated and invited us in. The interest, support and active participation of the school in each community made recruitment feasible. More than 65% of the study participants were recruited from school settings, with another 25% being siblings of those recruited in the school.
Each study trip had complicated logistics to arrange, including: coordination with the school (avoiding holiday intervals) and with the clinic; arranging access to accommodation and transportation within the community (both of which are in short supply); coordinating, training and employing study staff (who had limited longevity due to the demands of the trial) and; arranging transport schedules for microbiology specimens to arrive back for processing at the laboratory in Darwin. In total, 33 study trips were completed over the course of three years. The NT is vast and sparsely populated. More than 50,000 kilometres were travelled by road and air by the study team during the course of the study. Recruitment commenced on 26 November 2009 and concluded on 20 November 2012. The proportion of participants recruited per study year was: 2009 (1%), 2010 (11%), 2011 (40%) and 2012 (48%). Due to challenges of conducting research in these settings and previous experience, we allowed for up to 10% loss to follow up in calculating our sample size. Pleasingly, more than 96% of study participants completed all visits and of these, 97% received all doses of the study drug.

The key findings were that: treatment success was achieved in 85% of participants in both groups; SXT was non-inferior to BPG for the treatment of impetigo; impetigo was driven by *S. pyogenes* even in the presence of *S. aureus* and; BPG had a concerning rate of adverse events when administered intramuscularly. These findings are important for Indigenous children from remote communities. An alternative to the intramuscular BPG injection is now available that is evidence-based, palatable, simple for adherence purposes and pain-free. This study adds to the available evidence for treatment of extensive impetigo.

**6.2 STATEMENT OF CONTRIBUTION TO JOINTLY AUTHORED WORK**

The chief investigators designed and secured funding for the trial before I joined as a PhD student. I critically revised and oversaw the protocol, participated in data collection, oversaw the scientific direction of the study, wrote SOPs, and cleaned and analysed all the data. I was assisted in data cleaning by Irene.
O’Meara and Robyn Liddle. I led the statistical analysis with input from Mark Chatfield and Steven Tong. I conducted the literature review and wrote all drafts of the manuscript. All co-authors contributed to revisions of the manuscript and approved the final version for publication.

6.3 JOURNAL ARTICLE (FOLLOWING PAGE)

Short course oral cotrimoxazole versus intramuscular benzathine benzylpenicillin for impetigo in a highly endemic region: an open label, randomised, controlled, non-inferiority trial

APPENDIX 3

CHAPTER 7
THE MICROBIOLOGY OF IMPETIGO IN INDIGENOUS CHILDREN: ASSOCIATIONS BETWEEN STREPTOCOCCUS PYOGENES, STAPHYLOCOCCUS AUREUS, SCABIES, AND NASAL CARRIAGE

7.1 CHAPTER OVERVIEW

This chapter broadens our understanding of the microbiology of impetigo. It reinforces the ongoing dominance of *Streptococcus pyogenes* as a key pathogen in impetigo in tropical contexts, which differs to the microbiology reported from developed contexts. *Staphylococcus aureus*, and increasingly methicillin-resistant *S. aureus* (MRSA), are reported from these contexts where the majority of impetigo trials have been conducted. Few high-quality microbiology studies are conducted where the burden is the highest and it is important to know, in 2014, that treatment directed against *S. pyogenes* for impetigo remains a priority. This was assumed in the only other RCT conducted in a similar resource-limited context where severe disease was also prevalent, but no microbiology specimens were included in that study. (Faye et al 2009)

This chapter explores the associations of the microbiology with severe disease, the presence of scabies, age groups, sex and region. There was no association found between children who were in the severe strata of our study (≥ 2 purulent or crusted sores and > 5 overall body sores) and the detection of *S. pyogenes*, *S. aureus* or both. This was surprising, as our initial hypothesis was that severe sores might be more likely to be co-infected with both pathogens. The presence of scabies was associated with detection of *S. pyogenes*. Whilst this finding was not unexpected, this is the first study to confirm the association. Swabbing of the anterior nares was included in the study design to assess for *S. aureus* nasal
carriage. We found that there was no association between the presence of 
*S. aureus* in the anterior nares and detecting *S. aureus* in impetiginous lesions. In 
fact, participants without *S. aureus* in their skin sores were more likely to 
harbour *S. aureus* in the anterior nares. This suggests that nasal decolonisation 
strategies are unlikely to be effective in reducing the burden of impetigo in 
remote Indigenous communities.

The results of antibiotic susceptibility testing for both *S. pyogenes* and *S. aureus* 
are reported in more detail in this chapter. Susceptibility to trimethoprim-
sulphamethoxazole was above 99% for both skin pathogens in this study. 
However, increasing use of SXT may drive increasing resistance rates, and 
ongoing surveillance is a priority. Whilst performing antibiotic susceptibility 
testing of *S. pyogenes* to SXT is not routine in Australian microbiology 
laboratories, the methods to do so are available and interest is growing in 
incorporating this test.

### 7.2 Statement of Contribution to Jointly Authored Work

I wrote all of the standard operating procedures for the collection, transportation, 
storage, culture and antibiotic susceptibility testing of isolates from swabs 
collected during the trial. I oversaw the scientists working in the laboratories at 
the Menzies School of Health Research and Royal Darwin Hospital. I did all the 
statistical analysis with assistance from Steven Tong and Mark Chatfield. I 
drafted all versions of this manuscript. All co-authors contributed to revisions of 
the manuscript and approved the final version for publication.

### 7.3 Submitted Journal Article, Under Review (Following Page)

The microbiology of impetigo in Indigenous children: associations of 
*Streptococcus pyogenes*, *Staphylococcus aureus*, scabies and nasal carriage. 
BMC Microbiology, under review.
7.4 ABSTRACT

Background: Impetigo is caused by both *Streptococcus pyogenes* and *Staphylococcus aureus*; the relative contributions of each have been reported to fluctuate with time and region. While *S. aureus* is reportedly on the increase in most industrialised settings, *S. pyogenes* is still thought to drive impetigo in endemic, tropical regions. However, few studies have utilised high quality microbiological culture methods to confirm this assumption. We report the prevalence and antimicrobial resistance of impetigo pathogens recovered in a randomised, controlled trial of impetigo treatment conducted in remote Indigenous communities of northern Australia.

Results: From 508 children, we collected 872 swabs of sores and 504 swabs from the anterior nares prior to commencement of antibiotic therapy. *S. pyogenes* and *S. aureus* were identified together in 503/872 (58%) of sores; with an additional 207/872 (24%) sores having *S. pyogenes* and 81/872 (9%) *S. aureus*, in isolation. Skin sore swabs taken during episodes with a concurrent diagnosis of scabies were more likely to culture *S. pyogenes* (OR 2.2, 95% CI 1.1–4.4, p=0.03). Eighteen percent of children had nasal carriage of skin pathogens. There was no association between the presence of *S. aureus* in the nose and skin. Methicillin-resistance was detected in 15% of children who cultured *S. aureus* from either a sore or their nose. There was no association found between the severity of impetigo and the detection of a skin pathogen.

Conclusions: *S. pyogenes* remains the principal pathogen in tropical impetigo; the relatively high contribution of *S. aureus* as a co-pathogen has also been confirmed. Children with scabies were more likely to have *S. pyogenes* detected. While clearance of *S. pyogenes* is the key determinant of treatment efficacy, co-infection with *S. aureus* warrants consideration of treatment options that are effective against both pathogens where impetigo is severe and prevalent.

Trial Registration: This trial is registered; ACTRN12609000858291.
7.5 BACKGROUND

Impetigo is an epidermal infection caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. It is common in Indigenous children of northern Australia, with prevalence as high as 70% (Currie and Carapetis, 2000). The reported relative abundance of *S. aureus* and *S. pyogenes* has varied over time (Koning et al., 2012). In recent decades, *S. aureus* and increasingly, methicillin-resistant *S. aureus* (MRSA), has been the dominant reported pathogen in impetigo studies worldwide, most of which have taken place in temperate-climate regions, usually in affluent countries (Geria and Schwartz, 2010) where there is a low burden of disease. By contrast, in tropical regions, impetigo is far more common, and carries the greatest burden of sequelae (Carapetis et al., 2005). *S. pyogenes* is assumed to have remained the dominant pathogen (Parks et al., 2012) but some reports are emerging on skin and soft tissue infections caused by *S. aureus* (Abdel Fattah and Darwish, 2012, Alvarez-Uria and Reddy, 2012). There is limited microbiological surveillance of causative impetigo pathogens from high burden contexts and rates of antimicrobial resistance are often unknown (Parks et al., 2012). Impetigo is strongly associated with scabies infestation in tropical environments (Steer et al., 2009), but the influence of scabies on the microbiology of impetigo has not previously been described. We report here on the microbiology of impetigo in a high-burden setting, and explore the associations of this microbiology with age, sex, region, severity, presence of scabies and nasal carriage of skin pathogens. Our dataset derives from a large, non-inferiority randomised controlled trial (RCT) comparing trimethoprim-sulphamethoxazole (SXT) with benzathine penicillin G (BPG) for the treatment of impetigo in Indigenous children (Bowen et al., 2014).

7.6 METHODS

Study Design

Indigenous children aged 3 months to 13 years were participants in the RCT. Children were eligible to participate on more than one occasion if at least 90 days had elapsed since their last involvement. As such, 508 children from 12 remote
communities of the Northern Territory were enrolled for 663 episodes of impetigo; all analysis presented here has been restricted to a child’s first episode only. Six communities (comprising 463/508 children) were located in the tropical climatic region, commonly referred to as the ‘Top End’. The remaining communities were in Central Australia where a desert climate prevails. Children were stratified by impetigo severity. The severe stratum included children with \( \geq 2 \) purulent or crusted sores and \( \geq 5 \) overall body sores.

**Swabbing, transportation and culture methods**

Each child had swabs taken from one or two sores (according to whether the episode was classified as mild or severe) prior to commencing antibiotics. A swab of the anterior nares was obtained to determine carriage of impetigo pathogens in the context of infection. Swabs were collected between 26 November 2009 and 20 November 2012. Rayon tipped cotton swabs (Copan, Italy) were transported at 4°C in 1 mL of skim milk tryptone glucose glycogen broth (STGGB) and frozen at \(-70^\circ\)C within 5 days of collection. Swabs were defrosted, vortexed and an aliquot plated on horse blood agar and incubated for 48 hours at 37°C (Bowen et al., 2013). *S. aureus* and *S. pyogenes* were identified morphologically and confirmed with latex agglutination. All *S. aureus* isolates were staphytect (Oxoid, UK) and deoxyribonuclease (DNase, BD Diagnostics, USA) positive. *S. pyogenes* agglutinated with group A Lancefield antisera (Oxoid).

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing for *S. aureus* was determined on the Vitek2 platform using 22359 VITEK AST-P612 cards (bioMerieux, France) with Clinical and Laboratory and Standards Institute (CLSI, 2011) breakpoints utilised (CLSI, 2011). MRSA was defined as any *S. aureus* with a positive cefoxitin screen. Non-multidrug resistant MRSA (nmMRSA) was defined as MRSA resistant to <3 additional non beta-lactam antibiotics (Tong et al., 2009). Multidrug-resistant MRSA (mMRSA) was defined as MRSA that was resistant to \( \geq 3 \) non beta-lactam antibiotics (Tong et al., 2009).
We performed susceptibility testing for *S. pyogenes* with penicillin, erythromycin and clindamycin discs using CLSI disc diffusion standards. SXT susceptibility for *S. pyogenes* was determined with an E test® (bioMerieux) according to the European committee on antimicrobial susceptibility testing (EUCAST) standards (www.eucast.org, last accessed 15 November 2014). SXT susceptible strains had a MIC $\leq 1$mg/L and resistant isolates had a MIC $>2$mg/L.

**Ethics statement**

This study was approved by the Northern Territory Department of Health and Menzies School of Health Research human research ethics committee (09/08). Written informed consent was obtained from a child’s parent or guardian for all study procedures.

**Statistical analysis**

Mixed-effects logistic regression (random effects accounting for correlated data due to multiple sores for the children in the severe impetigo strata) using Stata 13 (Statacorp, Texas, USA) was performed to assess associations between the growth of skin pathogens and the presence of scabies, severe impetigo, age, sex, region and nasal carriage.

### 7.7 RESULTS

**Baseline Microbiology Results**

**Sores**

We obtained 872 swabs of sores from 508 children with untreated impetigo (median age 7 years, interquartile range [IQR] 5–9 years) at baseline. Seventy-two percent of children were in the severe impetigo stratum, all of whom had two sores swabbed. An impetigo pathogen was identified in 488/508 (96%) of children. From the 872 sores swabbed, *S. aureus* and *S. pyogenes* were identified together in 503/872 (58%), *S. pyogenes* alone in 207/872 (24%), and *S. aureus* alone in 81/872 (9%) of sores. Swabs from children in the severe impetigo stratum were not more likely to detect one or both skin pathogens compared with
swabs from children in the mild impetigo stratum (Table 1). *S. aureus* was less likely to be detected in older children (OR 0.6, 95% CI 0. –0.9 for children ≥ 5 years, Wald test on 2 degrees of freedom p = 0.04) or in children from Central Australia (OR 0.5, 95% CI 0.3–0.9, p=0.02). Children from Central Australia were less likely to have sores co-infected with both *S. aureus* and *S. pyogenes* than children from the Top End (OR 0.5, 95% CI 0.3–0.9, p=0.01)

**Table 1: Results from logistic regression models to assess associations between impetigo pathogens and age, sex, severity, presence of scabies and region.**

<table>
<thead>
<tr>
<th>Variable</th>
<th><em>S. pyogenes</em> in sores</th>
<th><em>S. aureus</em> in sores</th>
<th>Both <em>S. aureus</em> and <em>S. pyogenes</em> in sores</th>
<th>MRSA in sores positive for <em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Female</td>
<td>1.3</td>
<td>0.8–2.1</td>
<td>1.0</td>
<td>0.7–1.4</td>
</tr>
<tr>
<td>0–4 years</td>
<td>1</td>
<td>(ref)</td>
<td>1</td>
<td>(ref)</td>
</tr>
<tr>
<td>5–9 years</td>
<td>1.1</td>
<td>0.7–2.0</td>
<td>0.6</td>
<td>0.4–0.9</td>
</tr>
<tr>
<td>10–13 years</td>
<td>1.2</td>
<td>0.6–2.4</td>
<td>0.5</td>
<td>0.3–0.9</td>
</tr>
<tr>
<td>Severe strata</td>
<td>1.4</td>
<td>0.8–2.6</td>
<td>0.7</td>
<td>0.4–1.1</td>
</tr>
<tr>
<td>Scabies present</td>
<td>2.2</td>
<td>1.1–4.4</td>
<td>0.8</td>
<td>0.5–1.3</td>
</tr>
<tr>
<td>Central Australia</td>
<td>1.2</td>
<td>0.5–2.8</td>
<td>0.5</td>
<td>0.3–0.9</td>
</tr>
</tbody>
</table>

**Scabies**

Scabies was diagnosed at baseline in 84/508 (17%) of children: by age group: 0-4 years (28/136, 21%); 5–9 years (40/271, 15%) and 10–13 years (16/101, 16%). Those with scabies present were more likely to also have *S. pyogenes* detected from sores (OR 2.2, 95% CI 1.1–4.4, p = 0.03) (Table 1).
Other beta haemolytic streptococci

Seven hundred and fifty-four beta haemolytic streptococci were cultured from skin and nose swabs of 508 children with impetigo. Of these, 740/754 (98%) were *S. pyogenes* with 710 from skin swabs and 30 from the nose swabs. The remaining beta haemolytic streptococci were group C (2 skin, 2 nose) and group G (7 skin, 3 nose).

Antibiotic susceptibility

One isolate each of *S. pyogenes* and *S. aureus* was selected per child for reporting of the antibiotic susceptibility profile. Where possible, isolates cultured from skin sores were selected, with the remainder coming from swabs of the anterior nares.

*S. pyogenes*

There were 455 children with at least one *S. pyogenes* isolate available for antibiotic susceptibility assessment. All *S. pyogenes* isolates were susceptible to penicillin and erythromycin. Clindamycin resistance was detected in 9/455 (2%) *S. pyogenes* isolates. SXT resistance was detected in 4/455 (<1%) isolates at baseline with a MIC >2mg/L. (Bowen et al., 2012) The median SXT MIC for *S. pyogenes* was 0.094 (interquartile range: 0.094–0.125) mg/L.

*S. aureus*

There were 435 children with at least one *S. aureus* isolate available for antibiotic susceptibility assessment. Methicillin-resistance in *S. aureus* was detected in 65/435 (15%) isolates, all of which were nmMRSA. There was no detection of mMRSA. The respective resistance rates for other antibiotics by child were penicillin 413/435 (95%), SXT 3/435 (<1%), erythromycin 60/435 (14%) and fusidic acid 9/435 (2%). Inducible clindamycin resistance was reported in 60/435 (14%), 86% of which were MSSA. All 3 SXT-resistant isolates were nmMRSA. Other antibiotics included on the VITEK card for *S. aureus* susceptibility testing all had resistance rates below 0.02%. For those
with *S. aureus* detected, the presence of MRSA did not reach significance for sex, age group, severe strata, the presence of scabies or region (table 1).

**Nasal carriage of skin pathogens**

*S. aureus*

A nasal swab was taken at baseline for 504/508 (99%) of children. Before treatment, 91/504 (18%) children had confirmed carriage of a skin pathogen in the anterior nares. Both *S. aureus* and *S. pyogenes* were recovered from 16/91 (18%), *S. pyogenes* alone from 14/91 (15%) and *S. aureus* alone from 61/91 (67%). Of children with nasal carriage of *S. aureus*, 66/77 (86%) had MSSA and 11/77 (14%), MRSA. Nasal carriage of *S. aureus* and *S. pyogenes* was respectively 77/504 (15%) and 30/504 (6%).

We investigated the association of nasal carriage of *S. aureus* and the presence of *S. aureus* in impetigo lesions. There were 504 children with both skin and nose swabs available (Table 2). Surprisingly, of the 410 children culturing *S. aureus* on the skin, only 54 (13%) also harboured *S. aureus* in the nose. Based on the antibiogram, of the 54 with *S. aureus* in both the nose and skin sores, four had discordant *S. aureus* strains (i.e., MSSA at one site and MRSA at the other). Of the 94 children without *S. aureus* on the skin, there were 23 (24%) children who harboured *S. aureus* in the nose. Of 424 children harbouring *S. pyogenes* on the skin, 28 (7%) had concurrent growth of *S. pyogenes* from the anterior nares. There were 2 children with isolated growth of *S. pyogenes* from the anterior nares. Extending a model of Table 2, nasal colonisation with *S. aureus* was associated with less, not more, *S. aureus* on the skin (OR 0.6, 95% CI 0.4–1.0, \( p = 0.04 \)), suggesting a separate epidemiology of *S. aureus* at these two sites.
Table 2: Identification of *Staphylococcus aureus* from any impetigo lesion and the anterior nares for all children with at least one skin and nose swab available (n=504 children)

<table>
<thead>
<tr>
<th></th>
<th>Anterior Nares</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Impetigo At least one sore positive</td>
<td>54 (13%)</td>
<td>356 (87%)</td>
</tr>
<tr>
<td>Negative</td>
<td>23 (24%)</td>
<td>71 (76%)</td>
</tr>
<tr>
<td>Total</td>
<td>77 (15%)</td>
<td>427 (85%)</td>
</tr>
</tbody>
</table>

7.8 DISCUSSION

*S. pyogenes* remains the key impetigo pathogen in Indigenous children of remote Australia. Co-infection with *S. aureus* is also highly prevalent. In addition, our study extends understanding of the microbiology of impetigo where scabies is endemic. Where scabies is present, we found that *S. pyogenes* is more likely to be recovered from impetigo lesions. We also found no positive correlation between nasal carriage of *S. aureus* and recovery of *S. aureus* from impetigo lesions, and no association between the severity of impetigo and recovery of either *S. pyogenes*, *S. aureus* or both.

The stronger association of *S. pyogenes* (than *S. aureus*) with scabies presence concurs with earlier work that linked scabies outbreaks with subsequent epidemics of post-streptococcal glomerulonephritis (Svartman et al., 1972) and recommended treatment of scabies at a community level to reduce the complications of streptococcal pyoderma (Taplin et al., 1991, Carapetis et al., 1997). Scabies association with impetigo in urban Indigenous children was similarly high to that found in our study at a rate of 25/111 (23%) (Valery et al., 2008). No prior studies have reported on the association between scabies and MRSA detection. While the rates of both were high, we were unable to detect a significant association between these.
Both *S. pyogenes* and *S. aureus* have been reported as key impetigo pathogens, however the reported relative contributions of each have fluctuated over time and region. Previous microbiology studies of impetigo in both urban and remote Australian Indigenous children have shown high rates of co-infection (Valery et al., 2008, McDonald et al., 2006b). Valery *et al* detected co-infection with skin pathogens in 54% of urban Indigenous children. Of those children who had both impetigo and scabies, *S. pyogenes* was recovered from 82% and *S. aureus* from 77% (Valery et al., 2008). *S. pyogenes* remains an important skin pathogen in our region, as has also been reported from Fiji (Steer et al., 2009, Jenney et al., 2014) and supports the ongoing prescription of antibiotics active against *S. pyogenes* for the effective treatment of impetigo in tropical regions. Results of our clinical trial confirmed the importance of clearing *S. pyogenes* in order to achieve healing of impetigo lesions (Bowen et al., 2014).

The isolated dominance of *S. aureus* and rising rates of MRSA reported elsewhere in skin and soft tissue infections (Abdel Fattah and Darwish, 2012, Iovino et al., 2011, Phakade et al., 2012) were not found in our study. Methicillin-resistance was detected in 15% of *S. aureus* isolates. This is lower than previously reported for our remote tropical context (McDonald et al., 2006a) and lower than recent rates found in a 20 year survey of *S. aureus* in our region (Tong et al., 2014) but consistent with a study of impetigo in urban Indigenous children of northern Australia (Valery et al., 2008). We were uncertain whether MRSA varies by age, as this had not previously been explored, but we found no evidence that it does. The observation that MRSA rates were stable across age groups suggests early colonisation of children in remote communities with MRSA strains.

We found that children in the severe strata, who had more than five purulent or crusted sores, were not more likely than children in the mild strata to be infected with *S. pyogenes* and/or *S. aureus*. Although those stratified as mild, indicating fewer overall sores, may have had a less severe phenotype for each individual sore, the microbiology of these sores was no different. We thus provide robust evidence to refute earlier observational data in patients with impetigo where the
association of co-infection with a more severe phenotype was suspected but not confirmed (Barrow, 1955, Hughes and Wan, 1967).

Whilst throat carriage of *S. pyogenes* is commonly reported and is not thought to be the reservoir for skin infection, the anterior nares are thought to harbour *S. pyogenes* only rarely. (Masters et al., 1958) As such, throat swabs were not included in our study design as we focused on the characterisation of nasal carriage of *S. aureus*. However, our findings of nasal carriage of *S. pyogenes* in 7% of children provide external validation of results from military recruits where nasal carriage of *S. pyogenes* was found in 8% of those with ecthyma (Wasserzug et al., 2009). There was no correlation between *S. aureus* nasal carriage and the recovery of *S. aureus* from impetigo lesions. Previous studies have concluded that when impetigo predominantly affects the lower limbs, there is either an absence of *S. aureus* nasal carriage or a different genotype (Barth, 1987). Our data supports this observation in that 67% of impetigo episodes involved only the lower limbs (Bowen et al., 2014). As has previously been shown in military recruits (Ellis et al., 2007), nasal decolonisation is also unlikely to be a useful strategy in reducing the burden of impetigo in our tropical, endemic setting.

One study from Ghana in the 1970s with a similar tropical climate found impetigo to be dominated by Lancefield groups C and G streptococci (Belcher et al., 1975). These findings were not reproduced in our study and have not been confirmed in other published microbiology studies from our region (McDonald et al., 2006b) or other tropical contexts (Lawrence et al., 1979). Non-group A streptococci do not appear to play a significant role in the pathogenesis of impetigo.

A strength of this study is the standardisation of procedures for screening, swabbing and microbiological culture in the context of a clinical trial conducted according to International Conference on Harmonisation Good Clinical Practice guidelines. In addition, the large number of children recruited from 12 distinct communities from two regions of the Northern Territory suggests that the conclusions are rigorous. Genotypic assessment of the isolates was not conducted to determine the correlation of skin and nose isolates. The inability to correlate the molecular epidemiology with the phenotype is a limitation of this study.
While the cost of molecular typing overall has become more affordable, the large number of isolates in this study made further molecular analysis cost prohibitive.

### 7.9 CONCLUSIONS

*S. pyogenes* remains a key pathogen in impetigo in tropical contexts, despite the rise in *S. aureus* found in many industrialised and tropical settings. Our findings are in keeping with those reported from Fiji (Steer et al., 2009) and confirm that impetigo in tropical contexts continues to be driven by *S. pyogenes*. In the absence of local microbiology for impetigo, treatment algorithms should remain focused on the treatment of *S. pyogenes*. However, we have demonstrated that co-infection with both *S. pyogenes* and *S. aureus* is likely. As such, consideration of treatment of impetigo with an agent that is effective against both *S. pyogenes* and *S. aureus* is important. We have also concluded that in the context of impetigo, there is no association between nasal colonisation and skin infection with *S. aureus*.

### Competing Interests

The authors declare that they have no competing interests.

### Authors Contributions

AB, ST and JC participated in the design of the study. AB analysed the data and drafted the manuscript with assistance from ST and MC. All authors read and approved the final version of the manuscript.

### Acknowledgements

This work was supported through a National Health and Medical Research Council of Australia (NHMRC) project grant (545234) on which AB, ST, and JC are all investigators. The funder had no role in the design, collection, analysis or interpretation of the data, writing of the manuscript or decision to submit the manuscript for publication. AB is the recipient of a NHMRC scholarship for PhD research (605845) as well as an Australian Academy of Sciences Douglas and
Lola Douglas scholarship. ST is the recipient of an NHMRC Career Development Fellowship (1065736).

We acknowledge the children and families who contributed to this trial in the hope of finding a better treatment option for their sores, and the staff of the Menzies School of Health Research and Royal Darwin Hospital microbiology laboratory who contributed to data collection, culturing and antibiotic susceptibility testing of all isolates. We acknowledge the co-investigators on this study, Bart Currie, Ross Andrews and Malcolm McDonald. Malcolm McDonald and Rob Baird provided feedback on the manuscript in development.
CHAPTER 8
CONCLUSIONS

8.1 CHAPTER OVERVIEW

In this final chapter, the overall findings of the thesis are presented, conclusions drawn and recommendations for future research outlined. I have reviewed the global prevalence of impetigo and pyoderma using available population-based studies. This has extended our understanding of the prevalence of impetigo at a community level and, once published, should enhance the global understanding of this high burden, but relatively unstudied, problem in the world’s poor. The review also highlighted the ongoing problem that, despite living in a high-income, developed economy, Australia’s Indigenous children have the highest published rates of impetigo in the world.

There has been a long-standing dogma that trimethoprim-sulphamethoxazole (SXT) is not active or effective in the treatment of *Streptococcus pyogenes* infections. It was therefore important to re-look at the microbiology from which this dogma arose, and use standardised susceptibility testing to confirm *in vitro* susceptibility of *S. pyogenes* to (SXT). Chapter 3 describes this work and confirms that *S. pyogenes* isolates from the NT are susceptible to SXT. The accompanying literature review in Chapter 3 outlines the variability worldwide in susceptibility of *S. pyogenes* to SXT, ranging from 0 to 100%. Much of this variability can be attributed to the presence of thymidine, in media used for susceptibility testing, acting as an inhibitor to SXT and thus appearing resistant. The standardisation of thymidine content at a very low level, or addition of lysed horse blood to susceptibility testing media, are two strategies that have been used to overcome this appearance of resistance. This work was needed to better understand the results of our clinical trial, and to legitimise the use of SXT as an effective agent in the treatment of impetigo. Likewise, ongoing monitoring of *S. pyogenes* susceptibility to SXT is needed, as this antibiotic is more widely prescribed for impetigo.
In addition, we addressed methodological issues that were critical to the successful operation of the Skin Sore Trial in a very remote context. We compared a cheap, simple and effective transport medium that is likely to be cost-effective and efficient in resource-limited contexts for future impetigo research, to a known standard. Previous guidelines have recommended immediate plating of swabs. (Kotloff, 2008) This approach has microbiological merit, but is difficult to achieve in remote settings with long distances to the laboratory. As such, the microbiological description of impetigo where the burden is highest is under-reported. The ability to perform high-quality microbiology studies by transporting stored swabs back to a central laboratory for later processing builds our understanding of the local microbiology and antibiogram in endemic settings and, thus, makes uncovering the molecular epidemiology achievable.

Digital images are useful for capturing and storing an endpoint when it is not feasible to transport an expert to the point of care. These rigorous, standardised methods for scoring digital images will be useful in future impetigo and other skin disease trials.

Pleasingly, our in vivo results confirmed the in vitro finding of S. pyogenes being susceptible to SXT, with the demonstration of non-inferiority of SXT to BPG for the treatment of impetigo. In addition, clearance of S. pyogenes was equivalent between these two antibiotics, with penicillin having known excellent efficacy against S. pyogenes. No isolate of S. pyogenes has ever been reported that is resistant to penicillin. The demonstration of equivalent clearance with both antibiotics upholds the efficacy of SXT for S. pyogenes.

Surprisingly, in the MRSA era, our study confirmed the historical observations made over four decades ago that S. pyogenes is the dominant pathogen driving impetigo and that S. aureus is in fact a co-coloniser. We have shown that benzathine penicillin G (BPG), an antibiotic with limited S. aureus activity but very active against S. pyogenes, was effective in the treatment of impetigo, even in the presence of high rates of S. aureus including MRSA. As such, MRSA-targeted treatment for impetigo is not essential, but using an MRSA-active antibiotic such as SXT may have the additional benefit of reducing S. aureus and...
MRSA skin carriage in a region where the incidence of sequelae of both gram-positive pathogens is high. The increased use of SXT for treatment of impetigo may see a reduction in the consequences of both pathogens on the short- and long-term morbidity in Indigenous children.

Many questions remain and future studies, described below, are underway that will continue to illuminate our understanding of this ubiquitous problem of Indigenous childhood. The ultimate goal is that sores are not ignored, and are effectively treated, with the outcome of this being a dramatic decline in the prevalence of impetigo such that inter-personal transmission is no longer self-sustaining.

8.2 DISCUSSION OF MAIN FINDINGS

8.2.1 Trimethoprim/sulphamethoxazole is effective against *Streptococcus pyogenes* in vitro

The current recommendation by the Infectious Diseases Society of America (IDSA) when using SXT for treatment of skin and soft tissue infections involving both *S. pyogenes* and *S. aureus*, is to include a second agent that is active against *S. pyogenes* e.g. penicillin, cephalexin or amoxicillin. (Stevens et al., 2014) Our findings dispute the need for a second antibiotic and are important. The use of a single antibiotic when appropriate, rather than combination therapy, is a key principle in antimicrobial stewardship. (SHEA et al., 2012) It is also a critical factor in adherence to an oral treatment regimen, where the simplest, shortest regimen, that is directly associated with symptoms, has the best correlation with adherence. (Perez-Gorricho and Ripoll, 2003)

8.2.2 Trimethoprim/sulphamethoxazole is non-inferior to benzathine penicillin G for impetigo

The work in Chapter 3 paved the way for a complete understanding of the results of our trial outlined in Chapter 6. We concluded, using the robust digital image methodology described in Chapter 5, that SXT is non-inferior to BPG for the treatment of skin sores. In both treatment arms, treatment success was achieved in 85% of participants, absolute difference 0.5% (95% CI -6.2 to +7.3%).
We set the non-inferiority margin at 10%, to detect a clinically relevant difference, if one existed. There were two SXT dosing arms in the study, one treating children twice a day for three days (six doses) and the other once a day for five days (five doses). The regimens, pooled together and also analysed separately, both reached significance with respect to the non-inferiority margin and as such either can be recommended with confidence. This provides a palatable, effective, short course, non-painful alternative treatment to intramuscular BPG for children with impetigo in remote Indigenous communities. The challenge now is to see these findings implemented into practice. As discussed in Chapter 1, the CARPA manual is well utilised throughout our region as the standard treatment manual. The 6th edition has recently been published (October 2014) and the results of our study have been included. (CARPA, 2014) The results have also been included in the national antibiotic reference, Therapeutic Guidelines: Antibiotic, released in November 2014 (Antibiotic Expert Group, 2014) and in treatment guidelines in the Kimberley, the northern region of Western Australia with a large, remote, Indigenous population. SXT now sits in regional and national guidelines as an effective treatment for impetigo, based on the results of our trial.

Our study also confirmed, for the first time in our region, that BPG remains an effective treatment for impetigo. This is important, as there are circumstances where BPG will remain the preferred treatment for impetigo. Clinic nurses and clinicians in the region are familiar with prescribing and administering BPG, and the simplicity of a single dose without the need to address adherence, has appeal. This was the feedback received when our results were presented in health-care settings in remote communities. As such, BPG remains as first-line therapy in both the guidelines mentioned above, with the introduction of SXT as an alternate, evidence-based option. Future evaluation of the uptake of both antibiotics will be needed to understand the full impact of our study on community-based health care.
8.2.3 Trimethoprim/sulphamethoxazole is effective against *S. pyogenes in vivo*

Supporting our findings of *in vitro* efficacy outlined in Chapter 3, are the *in vivo* results reported in Chapter 6. As outlined above, SXT is an effective antibiotic in the treatment of impetigo caused by *S. pyogenes*. To be confident of any results reported from our trial (and allay concerns that adherence would be poor), all doses of antibiotics were directly observed. More than 96% of children had all doses of study drug observed. This strengthens our findings. Likewise, we have shown that *S. pyogenes* is cleared from infected sores with either BPG or SXT. Culture positive rates before treatment were above 85% for *S. pyogenes* and by day 7, *S. pyogenes* could be detected in less than 7% of sores in any of the three treatment arms. (Bowen et al., 2014) The clearance of *S. pyogenes* from impetigo lesions with BPG treatment is well known from earlier studies. (Ferrieri et al., 1973) The declining detection of *S. pyogenes* on days 2 and 7 with both antibiotics, with no difference in the rate of decline between BPG and SXT, provides supportive data to confirm the *in vivo* efficacy of SXT.

8.2.4 *S. pyogenes* remains the driver of impetigo even in the MRSA era

We have confirmed that impetigo is driven by *S. pyogenes*, with clearance of *S. pyogenes* being the only factor associated with sore healing in a step-wise backwards logistic regression (OR 5.2, 95% CI 1.8–14.1). However, *S. aureus* is frequently found in association with *S. pyogenes* as reported in Chapter 7. Whilst severe outcomes can ensue if *S. aureus* is untreated, the focus for the interruption of transmission of impetigo is on *S. pyogenes*. Our trial was designed mindful of the rising prevalence of MRSA in both the hospital and community in the NT. Our results suggest the significance of MRSA, as an impetigo driver, is lower than previously thought.

8.2.5 Trimethoprim/sulphamethoxazole has fewer side effects than benzathine penicillin G

Our study confirmed very few side effects from SXT. In the 343 children treated with SXT, we documented one case of urticarial rash without mucosal
involvement that resolved within 24 hours. Three older children were unable to
tolerate the large volumes of SXT syrup administered using weight-based dosing
(40ml per day). When switched to tablets, they continued in the trial without
further problems. One child had persistent vomiting and was discontinued. SXT
has been identified in the global literature as an antibiotic with a concerning side-
effect profile, (Goldman et al., 2013) and we were closely monitoring for these
events. We did not see any rash heralding the potentially life-threatening Stevens
Johnson syndrome. However the study was not powered to detect uncommon
side effects. Given the high prevalence of impetigo in remote communities in our
region, ongoing education about and monitoring for this rare, but life-
threatening, side effect will be needed.

The high rate of adverse events in the BPG arm of the trial was somewhat of a
surprise. Whilst anecdotal reports abound of the painful intramuscular injection
being a deterrent to treatment, the high rate of reported pain at 48 hours –
affecting one in three children randomised to the BPG arm – is concerning.
Likewise, treatment refusal by four children randomised to the BPG arm
confirms the impression that the painful needle is a deterrent to treatment. It is
possible that the 22 children who declined to participate in the trial did so for
similar reasons. Early experience of the painful intramuscular injection for sores
and a recurring need for treatment, with a median of three visits per year in
infants, (Kearns et al., 2013) in addition to the other childhood needles for
immunisation, might have conditioned children to these responses. By one year
of age, almost all infants in five remote communities had documented skin sores.
(Kearns et al., 2013) Having an oral regimen that circumvents the painful
injection is important to preserve intramuscular antibiotic injections for
circumstances where there is currently no alternative e.g. rheumatic heart disease
secondary prophylaxis. (Wyber, 2013)

The early studies that introduced BPG to the arena of treatment for impetigo
were in the context of acute post-streptococcal glomerulonephritis (APSGN)
outbreaks, where treatment that was easy to deliver and long acting was a
priority. (Ferrieri et al., 1974) APSGN epidemics are also problematic in the NT,
occurring every 5–7 years (Marshall et al., 2011). In the context of the high
burden of impetigo in the NT, and a limited evidence base for the treatment of extensive impetigo, treatment that was effective during and possibly effective between APSGN epidemics was adopted. Our study has confirmed that BPG is an effective antibiotic for the treatment of impetigo, and confirmed the anecdotal reports that the injection is a painful limitation to the use of this antibiotic. Where an alternative, effective antibiotic exists with fewer adverse events, we would recommend it in preference with antimicrobial resistance surveillance in place. However, there will remain circumstances where health care providers or parents prefer to administer BPG, and our study has confirmed that this remains an effective option.

8.2.6 Australian Indigenous children continue to have the highest reported rates of impetigo in the world

Chapter 2 summarises the prevalence of impetigo globally. We searched for population-based studies from anywhere in the world, however all available studies were conducted in resource-poor countries or contexts. This reinforces the connection between poverty and high rates of impetigo in children. In this context, it is unacceptable that Australian Indigenous children have the highest reported prevalence of impetigo in the world. Australia is a high-income country and must do more to reduce this burden of disease.

In the NT, epidemiological surveys of bacterial skin infection and scabies have been occurring for more than 20 years, with no real change in the prevalence. This imposes a considerable, ongoing burden on primary health care (Kearns et al., 2013, McMeniman et al., 2011) and we have estimated almost 16,000 children are in need of treatment in remote communities at any one time. Geopolitical borders do not restrict impetigo in remote Indigenous children; the burden of disease is equally high in Western Australia and Queensland. The next steps will involve developing a coordinated strategy for recognising and treating skin infections across these jurisdictions, with routine evaluations.

Clearly, effective treatment that is simple, palatable and has few adverse events is needed in our region. Communicating these findings is well underway and translation into regional guidelines has occurred. The responsibility for future
research lies in continuing to evaluate both the prevalence and utilisation of this
new regimen, with the hope that this population will not be world leaders for
impetigo prevalence in the future.

8.2.7 Methodologies to take impetigo research to high
burden contexts

Previous impetigo trials have mostly been conducted in physician offices or
hospital outpatient settings. (Koning et al., 2012) In order to conduct this trial in
the remote communities of Australia, several methods were developed and
published. This contribution to the global literature on the methodologies for
impetigo research reported in Chapters 4 and 5 will hopefully facilitate adoption
of treatment studies in similar contexts, where the burden of disease is the
highest. We intentionally published these papers in journals that are more likely
to come to the attention of researchers in these contexts; either open-access or
tropical medicine journals. In the digital era, experts are no longer needed at the
point of care for assessing end-points, and could easily be located in Darwin,
New York or Bamako to confirm findings of studies using digital images. We
have developed a method for collecting and scoring digital images. The next step
is to validate this method. As most impetigo trials to date have been conducted in
hospital outpatient or office-based settings where experts are readily available for
end-point assessment, it may be possible to validate this method in a future
impetigo trial. This would involve comparing the point-of-care expert assessment
with the remote review of digital images by a similarly qualified expert. All
reviewers would remain blinded to the prescribed treatment.

Likewise, the transport of skin swabs from remote contexts to a central
laboratory benefits from the use of a transport medium. We used one that was
familiar in our laboratory, and validated it against the commercially-available
Amies transport medium. The benefit of STGGB as a transport medium is the
ability to freeze for longer periods in order to facilitate batching of specimens
and preserve bacteria for future molecular work.
8.3 STUDY LIMITATIONS

8.3.1 Antiseptic or topical body washes?

In designing our trial, serious consideration was given to having a non-antibiotic arm, one that used only topical antiseptic. Partly because of concerns around the safety of not treating bacterial skin infection with antibiotics, but mainly to keep our sample size feasible and our findings simple and easily interpretable, this arm was not incorporated into the study design. Hygiene is very important in managing impetigo as was shown in Mozambique, where access to water influenced the burden of disease. (Cairncross and Cliff, 1987) In addition, hand-washing studies have shown a significant decline in impetigo using soap and water. (Luby et al., 2002, Luby et al., 2005) However, it is still unclear whether antiseptics or topical body washes would add considerable value as an adjunct to hygiene measures and treatment, (Koning et al., 2012) but these are unlikely to cause harm.

Neither were traditional, Indigenous methods for controlling skin disease evaluated in our trial. Bush medicine is still practised and many grandmothers and elders asked questions about these treatments when we visited their communities. Incorporating both worldviews into a regional treatment strategy to reduce the burden of skin disease will be an important step to consider in engaging the community.

8.3.2 Placebo control

Our trial lacked a placebo arm. It was not considered ethical with the high disease burden and complications of impetigo seen in the NT. Few placebo-controlled trials for impetigo exist, (Koning et al., 2012) and we deemed that the best design was a non-inferiority study, comparing the new treatment with the standard of care.

8.3.3 Adherence concerns

There has always been uncertainty about adherence to oral antibiotics and other prescribed medicines in Indigenous Australians. (Burns, 1992, Kemp K, 1994,
McConnel, 2003) This is a challenge when prescribing treatment for any patient, but has an added complexity for Indigenous patients, due to language and worldview differences between prescriber and patient. (McConnel, 2003) The known strategies for achieving treatment adherence such as prescribing a simple, short regimen, temporally linked to the disease process, are the most likely to be successful. (Perez-Gorricho and Ripoll, 2003) In childhood, palatability of syrup formulations is of equal importance. (Liu et al., 2014) These principles guided the selection of an oral regimen to compare with an intramuscular injection. As such, both oral regimens trialled were simple, palatable, short and easy to administer. In our study, directly observed treatment was incorporated to reinforce the message that adherence is important and to ensure our results were valid.

The once daily regimen has the benefit of being simple enough to administer via a directly observed approach at the school or health clinic in the remote community, if there are ongoing concerns about treatment adherence. This would be possible with a five-day course that coincides with the standard working week when both the clinic and school would be open, but may not be feasible or acceptable to families. This highlights an avenue for future qualitative research to understand whether uptake of these strategies is occurring and if not, what obstacles need to be overcome.

SXT is stable at room temperature (20–25ºC), but temperatures well above this occur in most remote communities of the NT on a daily basis and where access to a refrigerator is not universal, concerns abound about the efficacy of oral treatment. SXT was stored in air-conditioned environments monitored by the research assistants throughout the trial, and as such further work is needed to understand the impact this may have on treatment efficacy.

**8.3.4 Poverty and overcrowding**

Whilst poverty, racism, household overcrowding and social disadvantage exist, it is unclear whether a simple treatment regimen will on its own improve skin disease. These primordial factors need to be addressed in an ongoing program of
reducing the gap in health outcomes between Indigenous and non-Indigenous Australians.

8.3.5 Antibiotic resistance

Recent work in synthesizing the antibiotic resistance patterns of *S. aureus* from swabs collected in remote communities across the NT has shown a concerning trend. During the period under investigation (1993–2012), more than 20,000 *S. aureus* isolates were collected. The more recent years showed a doubling in the SXT resistance, albeit from a very low baseline value. (Tong et al., 2014b) This requires ongoing evaluation, and studies are being developed to validate both pharmaceutical prescriptions and changes in resistance phenotypes as guidelines incorporate the SXT regimen into the standard of care.

8.4 Future Work

8.4.1 Does the presence of scabies impact treatment efficacy?

In Chapter 7 we showed that the presence of scabies in children with impetigo was associated with an increased likelihood of detecting *S. pyogenes* from sores. As both regimens trialed are active against *S. pyogenes*, we hypothesised that treatment efficacy when analysed by the presence or absence of scabies would be similar. Preliminary analysis suggests the opposite, in that children with scabies co-infection had fewer treatment successes scored than children without scabies. Surprisingly, this also revealed that the absolute difference in treatment success for impetigo-scabies with BPG was 18.4% (95% CI -1 to 38%), whereas with SXT it was only 7% (-4 to 19%). The lower overall treatment success for impetigo-scabies and smaller difference between groups with and without scabies, treated with SXT (rather than BPG), warrants further investigation. It is possible that SXT might have added benefits in the treatment of impetigo when scabies is also present. Current animal models could be used to determine whether activity of SXT against scabies might explain some of these findings. (Holt and Fischer, 2013)
8.4.2 Whole genome sequencing to detect resistance mechanisms

Antibiotic resistance monitoring is an essential component of further research to understand the changes in microbiology that occur with increasing antibiotic pressure. However, we currently do not know the resistance mechanisms for either organism against SXT for isolates from our region. There are several possible molecular techniques that can be used to determine resistance mechanisms. Of these, we assessed whole genome sequencing (WGS) to be the most cost effective and efficient. In addition, the bioinformatics computer platform and collaborators with experience in detecting resistance mechanisms are also based at Menzies. We plan to use WGS to illuminate the possible genotypic mechanisms of SXT resistance in skin sore isolates that are phenotypically resistant. All SXT-resistant *S. pyogenes* and *S. aureus* isolates have been sub-cultured and DNA extracted, then submitted for WGS on the Illumina HiSeq2000 platform by Macrogen in Korea. Resulting data will be used to determine the mechanism of resistance in these isolates. This question has importance, as it will impact on the likely selective pressure of introducing SXT into routine treatment of skin sores in the NT, based on the results of our trial.

There were 8 (<0.5%) *S. pyogenes* isolates resistant to SXT detected in the overall study. The *S. pyogenes* resistance was identified using an SXT E test according to the EUCAST methodology reported in Chapter 3. Factors that cause trimethoprim resistance in *S. pyogenes* have recently been described in isolates from India and Germany. (Bergmann et al., 2014) We will explore whether these horizontally transferrable, trimethoprim-insensitive dihydrofolate reductase (dfr) genes are also found in our SXT resistant *S. pyogenes* isolates using WGS. Likewise, *S. aureus* resistance to SXT was at a low level in our study with only 24 (1%) *S. aureus* isolates resistant to SXT. The *S. aureus* resistance was identified using VITEK2 platform, with CLSI breakpoints as reported in Chapter 7. The correlation between the resistance phenotype and genotype will guide future assessments of antibiotic resistance.
8.4.3 Understanding the dynamics of skin sore transmission using WGS

The burden of impetigo suggests transmission of impetigo pathogens is common in Indigenous communities. However, little work has been conducted on defining the transmission dynamics in an endemic setting. We hypothesised that transmission within a household is more common than elsewhere in the community, in an endemic setting. If so, interventions to reduce household crowding are most likely to be effective in reducing the burden of impetigo in remote Indigenous communities. To explore this hypothesis, we have selected 54 isolates of *S. pyogenes* and *S. aureus* from 11 household clusters within the same community on the same recruitment trip in November 2011 to sequence. As such, we hope to understand how closely related these strains are and to build on this by developing methods for inferring transmission. These models are needed to determine the most appropriate interventions to reduce transmission, without conducting expensive interventional studies. This work on transmission dynamics can be built on the wealth of isolates, epidemiological data points and outcomes of studies conducted in remote NT communities by Menzies researchers over the past 20 years.

8.4.4 The niche for *Staphylococcus argenteus*?

Researchers at Menzies School of Health Research have recently described a new lineage of Staphylococcus in the Northern Territory of Australia. (Tong et al., 2013, Tong et al., 2014a) In light of this, we hypothesised that *S. argenteus* might be better adapted to skin infection rather than nasal colonisation. To explore this, multi-locus sequence typing (MLST) was performed on paired samples of *S. aureus* from the nose and skin of participants from the Skin Sore Trial. This hypothesis might explain why *S. argenteus* was more likely to be found in community rather than hospital samples in previous work. Preliminary work showed the rate of *S. argenteus* in our study samples to be very low, with no significant difference between skin and nasal specimens.
8.4.5 Evaluations of uptake

Our study was designed to find a better treatment option for impetigo, based on an assumption that pain may be a limitation in the acceptance of treatment for skin sores. However, health-care workers in the NT express a strong preference for the use of an injection for treatment of sores due to concerns about adherence. In a recent education session with remote doctors, comments about the injection ranged from “the mothers come wanting the needle as they don’t want to muck around with syrup or tablets” to “some mums won’t come to the clinic because they don’t want their kids to get the needle”. These assumptions have not been explored with parents or children in remote communities. There is a gap in our knowledge that affects the translation of this research into practice. To explore the uptake of our findings, qualitative interviews and monitoring of pharmacy dispensing records will be helpful. While children and parents do not decide the prescribed treatment when they seek care, qualitative research will guide the implementation of guidelines and development of policy from our results. Future studies are needed to evaluate the acceptance of this new regimen by both prescribers and families.

It was important for clinicians in the region to have data to confirm BPG is effective for the treatment of impetigo, despite high rates of \textit{S. aureus} and MRSA. Concerns about non-adherence to a short course antibiotic may drive the ongoing prescription of BPG. However, as was seen in our trial, children may vote with their feet, and run away if and when the needle is mentioned. Oral SXT now has a strong evidence base for its efficacy and can be recommended with confidence.

8.4.6 Ongoing surveillance

The increased use of any antibiotic causes increasing selection pressure and may drive resistance. Likewise, the introduction of a resistant clone could also compromise this regimen. (Nickerson et al., 2011) Ongoing surveillance is necessary, both to assess whether a more palatable treatment is able to reduce the burden of impetigo and what happens to antibiotic resistance profiles when this is widely prescribed. Surveillance for the development of antimicrobial resistance
in *S. aureus* and *S. pyogenes* to SXT will occur through routine microbiological channels, using both hospital and community pathology specimens throughout the region.

Our findings have highlighted the efficacy of SXT in *S. pyogenes* skin infections, have outlined the complex history that resulted in the myth being perpetuated and highlighted an available methodology with breakpoints for antibiotic susceptibility testing to facilitate ongoing surveillance for the emergence of SXT resistance.

Included in this will be ongoing surveillance of the incidence and prevalence of APSGN and RHD over the long term to see whether there is any impact on these severe complications of impetigo.

### 8.4.7 The ‘denormalisation’ of sores: A coordinated approach for recognition and treatment of skin disease in remote Indigenous children throughout Australia

Skin sores are common, rarely deemed serious enough to seek or obtain treatment, highly transmissible and hence found at epidemic levels in many remote Indigenous communities. The current paradigm is of skin infection being normal rather than the exception. A paradigm shift is needed to achieve the denormalisation of skin sores. The next steps will involve developing a coordinated strategy across jurisdictions for recognising and treating skin infections that is routinely evaluated.

### 8.5 FINAL CONCLUSIONS

The studies described in this thesis have contributed to our understanding of the burden, microbiology and efficacy of treatment of extensive impetigo. This fills a gap in the impetigo literature on evidence-based treatments for extensive impetigo. Our finding that impetigo is driven by *S. pyogenes* concurs with the only other trial on the treatment of extensive impetigo, which subjectively concluded that *S. pyogenes* was important. The methodology for conducting impetigo studies in remote circumstances where disease burden is often the highest has also been reported. This will provide a framework for future studies
on the treatment of impetigo for children who need it the most. As is evident in the systematic review (Koning et al., 2012), there is no generally agreed upon standard treatment for impetigo and as such, treatments that are appropriate for different contexts should continue to be trialled, perhaps using some of the methodologies we have reported.

Chapter 2 summarises the prevalence of impetigo in many resource-poor communities throughout the world, and highlights the ongoing burden suffered by remote Indigenous children of Australia. The association of impetigo with poverty make these rates unacceptable in a high-income country like Australia. Clearly, effective treatment that is simple, palatable and has few adverse events is needed in our region. These findings have been communicated throughout the region and translated into treatment guidelines. In support of this, ongoing health education and health promotion activities are needed to maintain a focus on skin disease. In conjunction, surveillance of changes in the prevalence, microbiology and antibiotic resistance profile as utilisation of a new regimen increases are needed. These studies are in development, with the hope that Indigenous children will not be world leaders for impetigo prevalence in the future.


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APPENDICES
**APPENDIX 1**

Table: Overall and childhood pyoderma and scabies prevalence from 89 studies synthesised in the Chapter 2 systematic review.

<table>
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<tr>
<th>Country</th>
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*Resource-poor populations within high-income OECD countries, ^reflects year of publication when year study was commenced is unknown.
APPENDIX 2

GELFAND MS, CLEVELAND KO, KETTERER DC. SUSCEPTIBILITY OF *STREPTOCOCCUS PYOGENES* TO TRIMETHOPRIM-SULFAMETHOXAZOLE. JOURNAL OF CLINICAL MICROBIOLOGY. 2013; 51(4): 1350.

BOWEN AC, TONG SY, CARAPETIS JR. REPLY TO “SUSCEPTIBILITY OF *STREPTOCOCCUS PYOGENES* TO TRIMETHOPRIM-SULFAMETHOXAZOLE.” JOURNAL OF CLINICAL MICROBIOLOGY. 2013; 51(4): 1351.
Susceptibility of *Streptococcus pyogenes* to Trimethoprim-Sulfamethoxazole

Michael S. Gelfand, Kerry O. Cleveland, Daniel C. Ketterer

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With interest we read the recent paper by Bowen et al. in which they propose that detection of in vitro susceptibility of *Streptococcus pyogenes* to trimethoprim-sulfamethoxazole (TMP-SMX) is enhanced by testing on media containing low concentrations of thymidine (1). Whether their data on in vitro susceptibility can be extrapolated to the clinical use of TMP-SMX in clinical infections due to this organism is unclear.

Traditional teaching in infectious diseases and microbiology has suggested that *S. pyogenes* is largely resistant to TMP-SMX, and, in fact, the drug has been incorporated into selective media for isolation of *S. pyogenes* from throat cultures (2–4).

Recently we observed two patients with skin and soft tissue infections due to *S. pyogenes* who were treated with oral TMP-SMX and suffered adverse outcomes.

The first patient, a 40-year-old previously healthy man, presented with a hand wound related to an occupational cut. After the wound was cleaned, he was discharged on TMP-SMX (160 mg of TMP component twice daily). He returned 48 h later with a hand infection that had progressed to necrotizing fasciitis. Wound and blood cultures grew *S. pyogenes*. Susceptibility testing with TMP-SMX was not done.

The second patient, a 38-year-old woman with multiple skin abscesses, was treated with surgical debridement and tigecycline (50 mg intravenous [i.v.] administration twice daily) for 3 days followed by oral TMP-SMX (160 mg of TMP component twice daily) for 6 days. A wound culture grew *S. pyogenes* (no susceptibility testing for TMP-SMX was performed) and methicillin-sensitive *Staphylococcus aureus* (MRSA) susceptible to TMP-SMX. She expired after presenting in septic shock 24 h after discharge.

In vitro susceptibility of *S. pyogenes* to TMP-SMX is less relevant than the clinical efficacy of this agent in treating infections due to that organism. Thymidine may become available to infecting microorganisms from damaged host tissues and bacteria, allowing microorganisms to overcome the metabolic block (5). Clinical failures in the treatment of MRSA infections have been attributed to this mechanism, and animal model data in MRSA infections support this explanation (5).

Human sera and urine contain detectable concentrations of thymidine and folates (6, 7). Even when an organism is demonstrated to have in vitro susceptibility to the drug, use of TMP-SMX has not proven to be an effective agent for treatment of streptococcal pharyngitis (1).

We urge caution in the use of TMP-SMX for *S. pyogenes* skin and soft tissue infections before the availability of human clinical studies. The study of impetigo being conducted by the authors may not resolve the question of clinical utility of TMP-SMX for these infections, as spontaneous resolution of impetigo is not uncommon (8). Data from animal models of infection would be desirable in helping to resolve this question.

**ACKNOWLEDGMENTS**

We declare that we have no conflicts of interest.

No financial support was received for this work.

**REFERENCES**


[For the author reply, see doi:10.1128/JCM.03329-12.]

[Address correspondence to Kerry O. Cleveland, kcleveland@uthsc.edu.] [Copyright © 2013, American Society for Microbiology. All Rights Reserved. doi:10.1128/JCM.03329-12]
Reply to “Susceptibility of *Streptococcus pyogenes* to Trimethoprim-Sulfamethoxazole”

Asha C. Bowen,* Steven Y. C. Tong,* Jonathan R. Carapetis*

Menzies School of Health Research, Darwin, Northern Territory, Australia; Telethon Institute for Child Health Research, Perth, Western Australia

We thank Gelfand et al. (1) for their interest in our article on the *in vitro* susceptibility of *Streptococcus pyogenes* to trimethoprim-sulfamethoxazole (SXT) (2). We agree that the two cases outlined by Gelfand et al. act as cautionary points for clinical practice, as there are currently no good clinical trial data to support the use of SXT for the treatment of *S. pyogenes* skin and soft tissue infections (SSTI). Our aim in publishing the *in vitro* data is to challenge the misconception that all *S. pyogenes* isolates are inherently resistant to SXT and hence open the way for clinical trials to more precisely determine the role for SXT in the treatment of *S. pyogenes* infections.

In the two clinical scenarios presented by Gelfand et al., the absence of SXT susceptibility data for the *S. pyogenes* isolates is concerning. Other reasons for treatment failure that are not elucidated include incomplete adherence, poor absorption, and the reality that the conditions of both of these patients might have deteriorated regardless of the antibacterial used. One wonders why the authors prescribed SXT for these SSTI despite their understanding of the traditional teaching that *S. pyogenes* is largely resistant to SXT. Perhaps it was due to the need for a simple, palatable, oral antibacterial regimen that would treat undifferentiated SSTI, in the era of highly methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence. We are in agreement with Gelfand et al. that human clinical studies are urgently needed to respond to this challenge.

The results of our clinical trial comparing oral SXT with intramuscular benzathine penicillin for impetigo treatment will inform treatment decisions and generate confidence in the use of SXT for impetigo treatment in our region and possibly beyond. To date, with recruitment of 660 participants completed, despite two-thirds of the participants presenting with severe impetigo, none have been hospitalized with invasive *S. pyogenes* infection due to presumed treatment failure. Although the numbers in our study are not large enough to detect a trend for bacteremia or other complications of inadequate treatment, we might have seen clinical outcomes similar to those Gelfand et al. have experienced if SXT really has no role in the treatment of impetigo. Spontaneous resolution of mild impetigo may occur; however, the natural history of impetigo, particularly when severe, is neither completely understood nor benign (3).

We agree that the role of thymidine and its availability in the context of damaged host tissues (4) are unknown with respect to the treatment of SSTI with SXT. There are now numerous trials under way that involve the use of SXT for SSTI (http://clinicaltrials.gov/), and the results are eagerly awaited to better define the clinical utility of SXT in such settings.

Rather than claiming *in vitro* data being less relevant than clinical efficacy, we would argue that *in vitro* data should be used to inform the design of appropriate clinical trials to determine the clinical role for SXT, particularly in an era with a truncated antimicrobial pipeline and high rates of mixed SSTI due to MRSA and *S. pyogenes*.

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APPENDIX 3

Treatment of impetigo in resource-limited settings

With an estimated worldwide prevalence of more than 140 million cases in 2010, impetigo is one of the seven skin disorders ranking in the 50 most common causes of disease. After dermatitis or eczema and viral warts, impetigo is the third most common skin disorder in children, and the most common skin infection in young children presenting in general practice in western Europe. In tropical areas, impetigo can be endemic and affect a large proportion of the population. Several Lancet articles have reported on the treatment of impetigo in endemic populations, notably in soldiers in World Wars 1 and 2, including two controlled (although not randomised) clinical trials.

Indigenous Australian people, like other resource-poor populations in tropical areas, have a raised risk of severe complications associated with impetigo, such as post-streptococcal glomerulonephritis, sepsis, and bone and joint infections. In The Lancet, Asha Bowen and colleagues present the results of their randomised, controlled non-inferiority trial in Indigenous children in remote communities in the Northern Territory of Australia, in which they compared a short course of oral co-trimoxazole to the standard treatment with intramuscular benzathine benzylpenicillin. Unlike most previous impetigo studies, more than 70% of the children had severe impetigo (five or more sores), and the lower limbs were the main affected area.

508 patients were randomly assigned to a study treatment: 165 to benzathine benzylpenicillin, 175 to twice-daily co-trimoxazole for 3 days, and 168 to once-daily co-trimoxazole for 5 days. The trial’s primary outcome was treatment success at day 7, objectively assessed by comparison of photographs of the sores at day 0 and day 7 by assessors who were masked to treatment and to the order of the photographs. Treatment success was achieved in 133 (85%) of 156 children who received benzathine benzylpenicillin, and in 283 (85%) of 334 who received co-trimoxazole (absolute difference 0·5% [95% CI –6·2 to 7·3]), falling within the prespecified margin of non-inferiority (±10%). Results for the two treatment schedules of co-trimoxazole were similar. The study also underlines the key pathogenic role of Staphylococcus pyogenes in impetigo in remote communities in Australia, instead of Staphylococcus aureus as seen over past decades in moderate climates. Adverse event profiles differed substantially between the treatment groups, with 30% of children in the benzylpenicillin group reporting injection-site pain, and one hospital admission due to an injection-site abscess, compared with vomiting and rash reported by less than 1% of children who received co-trimoxazole.

Bowen and colleagues’ study, with 508 randomised patients, is one of the largest impetigo studies done so far. Our Cochrane review included 68 randomised trials, with an average of only 82 patients per study, and only one of the included studies had randomised more than 500 patients with impetigo. Additionally, this new study is one of the few randomised impetigo trials done in a tropical setting for the benefit of an underprivileged population. Adherence to the prescribed treatment regimens, and follow-up to day 7 with very low attrition, are also commendable.

Cure rates were compared with those in the study done in Mali by Faye and colleagues, because Mali is a country where impetigo is also endemic and severe. However, the prevalence of disease might be less important for cure rates than for the risk of reinfection and development of complications.

A question not addressed by Bowen and colleagues’ study is whether successful treatment of severe impetigo reduces the risk of developing severe complications such as glomerulonephritis and sepsis, as is often believed to be the case, since this reduction in development of secondary complications is an important motive for treatment.

In conclusion, we believe that Bowen and colleagues’ new study in a resource-poor area makes a valuable contribution to the body of evidence about treatment of an important but under-investigated disease. The option of simple, palatable, pain-free, practical treatment of severe impetigo with co-trimoxazole is a welcome result for Indigenous children living in remote communities in Australia, and provides clinicians and patients with a simple regimen for treatment of impetigo in tropical regions where S pyogenes is the main causative agent.

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We declare no competing interests.


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