

Molecular Epidemiology of *Staphylococcus aureus* and Newer Treatment Strategies

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Declaration

To the best of my knowledge, the research presented in this thesis is original. The work of others and their ideas, if any, have been acknowledged. Manuscripts resulting from this thesis published in peer-reviewed scientific journals, or presented at scientific conferences, are described in the text. I have not shared or submitted this thesis to any other institution.

Author's contributions

The work presented in this thesis was carried out by the author under the supervision of Dr Matthew Vincent Neil O'Sullivan and Professor Gwendolyn. L. Gilbert, Sydney Medical School at The University of Sydney, and Professor Joshua S. Davis, School of Medicine and Public Health at The University of Newcastle, Australia.

The author conducted a comprehensive literature review, performed data collection and interpretation, served as the chief investigator for the study described in Chapter 8, and wrote all chapters included in this thesis. The author served as the primary contributor and corresponding author for all published manuscripts presented in this thesis, except for the manuscript in Chapter 4, where he was the second author but made substantial contributions to the work. A signed statement from the corresponding author of the Chapter 4 manuscript is included in the Appendix.

The binary typing described in this thesis was performed by a lab scientist employed at the faculty. The author was responsible for collecting data and analysing the results. Contributions of any other individuals, including co-authors in the manuscript, are outlined in each relevant section of this thesis.

Authorship attribution statement

Chapter 5 of this thesis is published as Dotel R, O'Sullivan MVN, Davis JS, Newton PJ, Gilbert GL. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates in New South Wales, Australia, 2012- 2017. Infect Dis Heal 2019; 24: 134–40.

I designed the study, analysed the data, and wrote the manuscript.

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I co-designed the study with JSD, SYCT and AB, performed the literature review, and wrote the study protocol.

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I designed the study, analysed the data, and wrote the manuscript.

In addition to the statements above, in Chapter 4 where I am not the corresponding author of a published item, permission to include the published material has been granted by the corresponding author (ACB). Campbell AJ, Dotel R, Blyth C, Davis JS, Tong SYC, Bowen

AC. Adjunctive protein synthesis inhibitor antibiotics for toxin suppression in *Staphylococcus aureus* infections: a systematic appraisal. *J Antimicrob Chemother.* 2019 Jan 1;74(1):1-5.

I performed an independent literature review and made significant contributions in writing the manuscript.

Ethical clearance

The research presented in this thesis had received prior ethics approval from the Western Sydney Local Health District Research and Ethics Committee, Westmead, Sydney. These include LNR/14/MEAD/117 (relevant Chapters 5, 6, and 7) and 2020/ETH0155 (Chapter 8). Ethics approval was not relevant for Chapters (1-4, and 9-10).

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Abstract

Staphylococcus aureus stands out as the most prevalent bacteria causing bloodstream infection (BSI). This formidable pathogen demonstrates its severity by claiming the lives of over one in four individuals within three months of a BSI. *S. aureus* includes penicillin-susceptible *S. aureus* (PSSA), methicillin-susceptible *S. aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA). These strains exhibit differences in geographic distribution, molecular epidemiology, and antibiotic susceptibility. Despite these distinctions, all three variants induce a comparable spectrum of illness and share a similar fatality rate in cases of BSI.

The introduction of penicillin in 1940s led to the emergence of penicillin-resistant *S. aureus*. Subsequently, with the introduction of methicillin, MRSA emerged. Bacterial typing of both MSSA and MRSA has revealed the presence of numerous sub-clones. Notably, MSSA exhibits a more diverse clonal population compared to MRSA. The predominant clones may vary from country to country, but successful clones have demonstrated the capacity to spread internationally.

Over the past 10-15 years, the epidemiology of *S. aureus* has undergone significant changes. MRSA rates have declined, while the proportion of *S. aureus* isolated from blood cultures identified as PSSA has increased. Despite these changes, the case fatality rates in *S. aureus* BSI have not altered. The disease continues to pose a significant threat and remains deadly.

We hypothesised that monitoring *S. aureus* in both sterile and non-sterile sites could offer insights into their population dynamics. Our aim was to identify potential hospital-acquired (HA) MSSA clones in the same way that there are particular clones of MRSA that are associated with hospital acquisition (HA-MRSA). By gaining a more comprehensive

understanding of *S. aureus* epidemiology, carriage patterns, and pathogenesis, our goal was to identify potential therapeutic gaps. These findings could then be explored further to improve treatment outcomes.

This thesis is presented as a combination of published papers (Chapters 4, 5, 9, and 10) and unpublished work (Chapters 1-3, and 6-8).

In chapters 1-4, a comprehensive literature review is presented. The first chapter provides a brief overview of the microbiology and clinical manifestations of *S. aureus* infection. Chapter 2 reviews bacterial typing methods and the molecular epidemiology of *S. aureus*. Chapter 3 provides an in-depth analysis of human *S. aureus* carriage with a view of understanding endogenous and exogenous sources of *S. aureus*. The application of this knowledge in preventing BSI is discussed. Chapter 4 delves into *S. aureus* toxins, discusses the limitations of the current therapy, and explores newer treatment strategies.

Chapters 5 and 6 provide an in-depth exploration of MRSA molecular epidemiology. Chapter 5 details a large-scale surveillance effort involving over 7,500 MRSA isolates collected from various regions across New South Wales, Australia. Meanwhile, Chapter 6 focuses on MRSA epidemiology in BSI. Noteworthy changes in MRSA clones are discussed, highlighting the decline of ST239-MRSA and the emergence of a novel Pantone-Valentine leukocidin-positive ST22-MRSA clone.

Chapter 7 provides detailed molecular epidemiological descriptions of MSSA blood culture isolates. The chapter also attempts to identify HA-MSSA clones by analysing strain distribution and diversity. In Chapter 8, the focus shifts to exploring *S. aureus* carriage in

patients with BSI and the role of whole-genome sequencing for surveillance in a facility with high rates of HA *S. aureus* BSI.

Chapters 9 and 10 delve into newer treatment strategies for *S. aureus* BSI, addressing research gaps identified from a comprehensive literature review, Chapter 9 investigates the potential role of clindamycin as an adjunctive anti-toxin therapy. Chapter 10 explores the use of rifampicin in preventing death and relapse in *S. aureus* BSI.

Introduction

S. aureus, a versatile pathogen, has perplexed clinicians since its first identification about 150 years ago. From nearly universal mortality due to staphylococcal sepsis in the pre-antibiotic era to the miraculous cures achieved with penicillin has been a wonder of modern medicine. However, the use of penicillin and subsequent antibiotics has applied evolutionary pressure, leading to penicillin-resistant *S. aureus* (PRSA), methicillin-resistant *S. aureus* (MRSA), and, more recently, vancomycin-resistant *S. aureus*.

In recent decades, MRSA has emerged as a pathogen of significant global concern. The emergence of community-acquired (CA) MRSA clones, coupled with an increase of hospital-acquired (HA) MRSA clones in 1990s, resulted in high MRSA rates that peaked around 2008-2013. Subsequently, in many regions, both the rates and the proportion of MRSA among *S. aureus* have declined.

While the clones associated with CA-MRSA and HA-MRSA are well documented, molecular studies on methicillin-susceptible *S. aureus* (MSSA) remains relatively scarce. Generally, MSSA strains have been considered polyclonal without defined CA or HA clones. Of note, MSSA bacteraemia rates have remained steady or slowly increased, as has the rate of penicillin-susceptibility in *S. aureus* isolates, while the MRSA rates have declined. This has led to renewed interest in susceptible *S. aureus*.

It is important to continue to evaluate the molecular epidemiology of MRSA to detect changes in predominant clones and to monitor newly emerging ones. Similarly, consideration should be given to routine monitoring of MSSA molecular epidemiology to detect clonal changes. Further research is necessary to identify possible HA-MSSA clones and the sources of *S.*

aureus implicated in nosocomial cross-transmissions. Finally, invasive *S. aureus* infection remains a disease with significant morbidity and mortality, highlighting the necessity for newer treatment approaches. This project will seek to address these research gaps.

Hypotheses:

1. Regular surveillance of MRSA isolates in both sterile and non-sterile sites can be used to observe the population dynamics of dominant clones.
2. MSSA clones exist that are associated with hospital acquisition, paralleling the known phenomenon of HA-MRSA clones.
3. Optimising treatment of *S. aureus* bacteraemia will lead to improved outcomes.

Research aims:

1. To evaluate the utility of routine molecular typing of MRSA isolated from sterile and non-sterile sites to describe clonal dynamics.
2. To describe the molecular epidemiology of MSSA bloodstream infection in an attempt to identify hospital adapted MSSA clones.
3. To define the utility of whole-genome sequencing to determine if *S. aureus* bacteraemia arises from endogenous (pre-existing colonisation) or exogenous sources.
4. To evaluate newer treatment strategies in managing *S. aureus* bacteraemia.

Publications arising from this thesis

This thesis is presented in a combined format with published and non-published chapters.

Publications:

1. Chapter 4*:

Campbell AJ, **Dotel R**, Blyth CC, Davis JS, Tong SYC, Bowen AC. Adjunctive protein synthesis inhibitor antibiotics for toxin suppression in *Staphylococcus aureus* infections: a systematic appraisal. J Antimicrob Chemother. 2019;74:1–5.

*Declaration from the corresponding author provided in the Appendix

2. Chapter 5:

Dotel R, O’Sullivan MVN, Davis JS, Newton PJ, Gilbert GL. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates in New South Wales, Australia, 2012-2017. Infect Dis Heal. 2019;24:134–40.

3. Chapter 9:

Dotel R, Tong SYC, Bowen A, Nelson JN, O’Sullivan MVN, Campbell AJ, et al. CASSETTE-clindamycin adjunctive therapy for severe *Staphylococcus aureus* treatment evaluation: study protocol for a randomised controlled trial. Trials. 2019;20:353.

4. Chapter 10:

Dotel R, Gilbert GL, Hutabarat SN, Davis JS and O’Sullivan MVN. Effectiveness of adjunctive rifampicin for treatment of *Staphylococcus aureus* bacteraemia: a systematic review and meta-analysis of randomised controlled trials. J Antimicrob Chemother. 2023;78:2419-2427

Abstract/Poster presentations:

1. **Dotel R**, Gilbert GL, Zhou F, O'Sullivan MVN. Utility of a 19-target binary typing method for large-scale surveillance of MRSA epidemiology (2012-2014). Presented at The Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), in collaboration with the International Congress of Chemotherapy, 15-17 September 2015, San Diego, USA.
2. **Dotel R**, Gilbert GL, Zhou F and O'Sullivan MVN. Nosocomial adaptation of methicillin-sensitive *Staphylococcus aureus* (MSSA) Clones. Presented at the 17th International Symposium on Staphylococci and Staphylococcal Infection, ISSSI 2016, Seoul, South Korea.
3. **Dotel R**, Gilbert GL, O'Sullivan MVN. Routine prospective molecular surveillance of MRSA isolates (2012-2017). Presented at the Australasian Society for Infectious Diseases Annual Scientific Meeting, 10-12 May 2018, Gold Coast, Queensland, Australia.
4. **Dotel R**, Nelson J, Tong SYC, Bowen A, O'Sullivan MVN, Davis JS. Protocol for clindamycin adjunctive therapy for severe *Staphylococcus aureus* treatment evaluation (CASSETTE STUDY). Presented at the 18th International Symposium on Staphylococci and Staphylococcal Infection, ISSSI 2018, Copenhagen, Denmark.
5. **Dotel R**, Gilbert GL, O'Sullivan MVN. Epidemiology of *Staphylococcus aureus* bloodstream isolates in Western Sydney, Australia, 2012 – 2017. Presented at the 18th International Symposium on Staphylococci and Staphylococcal Infection, ISSSI 2018, Copenhagen, Denmark

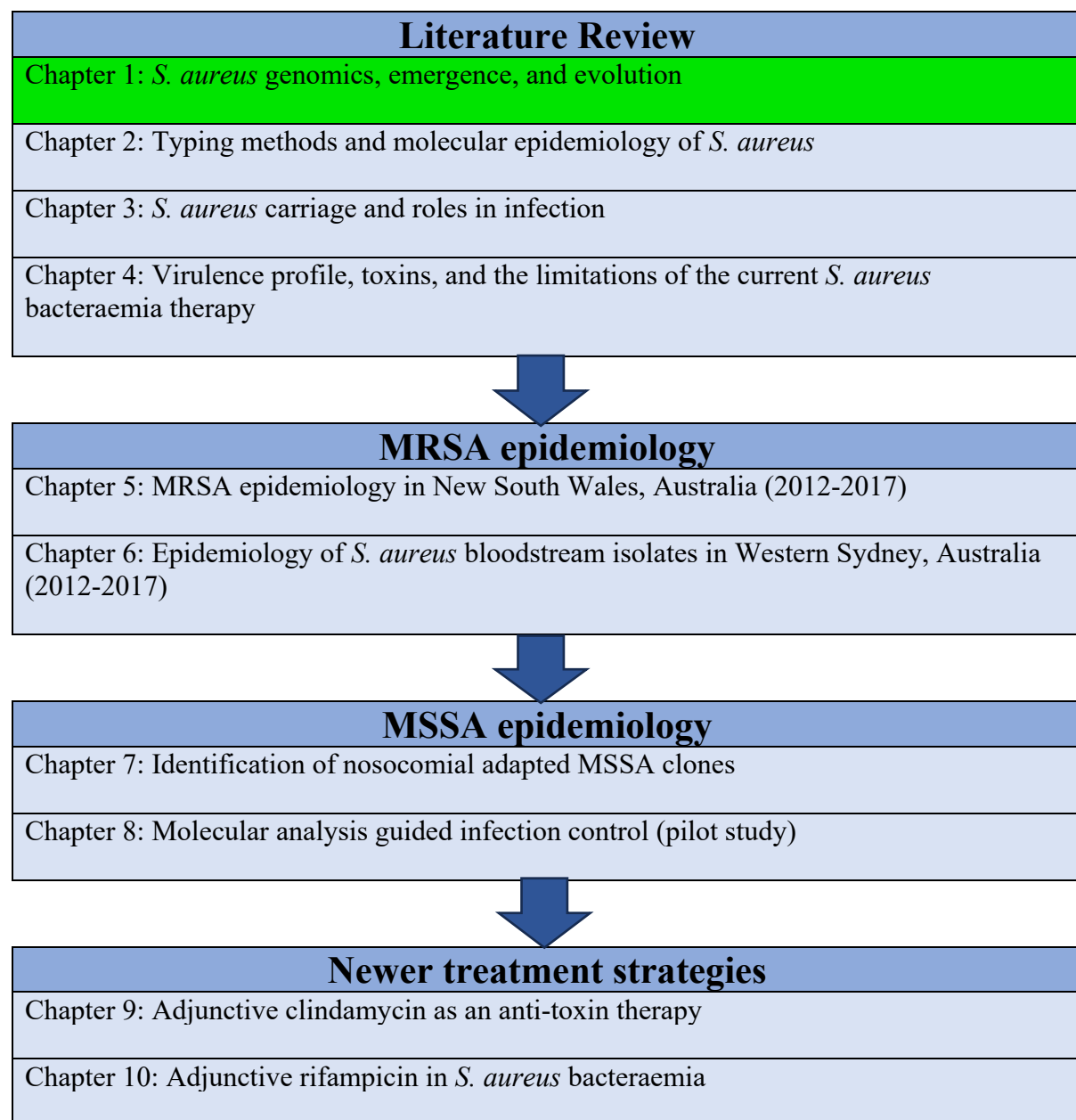
List of abbreviations

ACME	Arginine Catabolic Mobile Element
AGAR	Australian Group on Antimicrobial Resistance
AMS	Antimicrobial Stewardship
ASSOP	Australian <i>Staphylococcus aureus</i> Surveillance Outcome Program
BC	Blood Culture
BP	Base Pairs
BSI	Bloodstream Infection
BT	Binary Type
BURP	Based Upon Repeat Pattern
CA	Community-Acquired
CC	Clonal Complex
CEC	Clinical Excellence Commission
CI	Confidence Interval
CO	Community Onset
COVID-19	Coronavirus Disease
COW	Computer on Wheels
CVC	Central Venous Catheter
DI	Diversity Index
DNA	Deoxyribonucleic Acid
EMRSA	Epidemic Methicillin Resistant <i>S. aureus</i>
eTG	Web version of Therapeutic Guidelines Australia
HA	Hospital-Acquired
HACO	Healthcare-Associated Community Onset
HAI	Healthcare-Associated Infection

HCW	Healthcare Worker
HO	Hospital Onset
ICU	Intensive Care Unit
IE	Infective Endocarditis
IEC	Immune evasion Complex
IQR	Inter Quartile Range
IVDU	Intravenous Drug Use
IWG-SCC	International Working Group on the Staphylococcal Cassette Chromosome elements
LA-MRSA	Livestock Associated MRSA
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight
MGE	Mobile Genetic Elements
MIC	Minimum Inhibitory Concentration
MLEE	Multilocus Enzyme Electrophoresis
MLST	Multi Locus Sequence Typing
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-Susceptible <i>Staphylococcus aureus</i>
mPCR	Multiple Polymerase Chain Reaction
NSW	New South Wales
OR	Odds Ratio
PBP	Penicillin-Binding Proteins
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
PICC	Peripherally Inserted Central Venous Catheter
PIVC	Peripheral Intravenous Cannula

PRSA	Penicillin-Resistant <i>Staphylococcus aureus</i>
PSIs	Protein Synthesis Inhibitors
PSSA	Penicillin-Susceptible <i>Staphylococcus aureus</i>
PVL	Panton-Valentine leukocidin
RCT	Randomised Clinical Trial
RLB	Reverse Line Blot
SAB	<i>Staphylococcus aureus</i> bacteraemia
SAPIs	Staphylococcal Pathogenicity Islands
SCC	Staphylococcal Cassette Chromosome
SENTRY	SENTRY Antimicrobial Resistance Surveillance Program
SLV	Single Locus Variant
SNAP	<i>Staphylococcus aureus</i> Network Adaptive Platform
SNP	Single Nucleotide Polymorphism
SSTI	Skin and Skin Structure Infection
STs	Sequence Type
TSB	Tryptic Soy Broth
TSST-1	Toxic Shock Syndrome Toxin 1
UK	United Kingdom
US	United States
USA	United States of America
VRE	Vancomycin Resistant Enterococci
WA	Western Australia
WGS	Whole-Genome Sequencing
WSLHD	Western Sydney Local Health District

Figure: Schematic overview of the thesis structure



Chapter 1: Overview

A review of the basic microbiology of *S. aureus* and its genomics is essential to understand the nature of this bacterium, its evolution, and spread. These topics are discussed in Chapter 1.

The exchange of genetic materials, occurring both within and between species, has resulted in *S. aureus* becoming a successful human pathogen and shaping its evolution. Notably, the impact of antibiotic use has been significant. *S. aureus* has developed resistance mechanisms to overcome antimicrobial selective pressure, enhancing its ability to survive in the hospital environment. This evolutionary process is illustrated by the emergence and spread of penicillin-resistant *S. aureus* in the 1940-50s and the rise of methicillin-resistance *S. aureus* emergence since the 1960s – topics explored in detail in this chapter.

This chapter comprises unpublished work.

Chapter 1: *S. aureus* genomics, emergence, and evolution

1.1 Microbiology

Bacteria are the oldest forms of life on earth. They are primarily microscopic, unicellular, and are ubiquitously present, whether within a host or in the environment. The traditional classification of bacteria relies on their morphology and biochemical features, with Gram staining being a prominent method. Through this technique, most bacteria can be categorised into two distinct groups: gram-positive or gram-negative. Additionally, bacterial classification takes into account their shapes, distinguishing between the spherical (cocci) and rod-like (bacilli) forms (1).

Staphylococcus aureus is a gram-positive bacterium with spherical (coccus) shape and diameter of 0.5-1.5 μm . It is naturally abundant as a common coloniser of the skin and mucosa

of humans and other animals. It is non-motile, non-spore-forming, usually catalase positive, often unencapsulated or possesses a limited capsule, and is facultatively anaerobic (1). Historically, the genera *Streptococcus* and *Staphylococcus* were included in the *Micrococcus* genus. However, the recognition of their distinct forms - chains and clusters - led to the subsequent separation of the clustered form as *Staphylococcus* sp. (2).

The genus *Staphylococcus* encompasses more than 40 species, with *S. aureus* standing out as the most virulent, playing a pivotal role in infections worldwide. Phenotypic tests like coagulase and catalase aid in distinguishing *S. aureus* from other *Staphylococcus* species and other gram-positive cocci. However, in modern microbiology laboratories, molecular speciation methods are increasingly employed. Techniques such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry and testing specific genetic targets (e.g., *fem/nuc* genes) are utilised to differentiate *S. aureus* from coagulase-negative staphylococci (3). For clinical, infection control, and epidemiological purposes, a sub-classification of *S. aureus* is based on antimicrobial susceptibility. This classification includes methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).

S. aureus is a major contributor to both community-acquired (CA) and healthcare-associated (HA) infections, causing a diverse range of infections. These include skin and skin structure infections (SSTIs), organ specific infections (such as pneumonia, infective endocarditis, bone/joint infections, meningitis), bloodstream infections (BSI) that may disseminate to various body sites, and prosthesis infections involving cardiovascular devices, orthopaedic hardware, and vascular grafts (4). Typically, *S. aureus* infections manifest with suppuration (abscess formation) followed by dissemination through the lymphatics and bloodstream.

Beyond these, *S. aureus* is associated with toxin-mediated diseases such as food poisoning, scalded skin syndrome, and toxic shock syndrome (1).

1.2 Genomics

S. aureus has posed an enduring challenge to physicians and scientists. Sir Alexander Ogston first observed it in a microscopic examination of pus in the 1880s. Before the discovery of penicillin, BSIs with the bacterium were generally fatal (5). With the advent of penicillin, *S. aureus* adapted by acquiring resistance to penicillin, and subsequently to many newer antibiotics (6). The adaptability of *S. aureus* to selective pressures, including antimicrobial therapy, other pathogens, and to the human immune system, contributes to its status as a successful human pathogen of a great public health concern (1). Understanding its genomics is essential for comprehending its evolutionary dynamics and patterns of transmission.

1.2.1 *S. aureus* genome

The successful whole genome sequencing (WGS) of *S. aureus* was first reported in 2001 (7). Comprising around 2.8 million base pairs, its chromosome can be divided into the core and accessory genome. The core genome, constituting about 80% of the total, consists of genes present in all *S. aureus* isolates. These genes, originating from a common ancestor in the *Bacillus/Staphylococcus* group encode essential functions for *S. aureus* survival, including cellular metabolism and replication. In contrast, the accessory genome, making up the remaining 20% of the genome, introduces diversity within the *S. aureus* species. It contains genes responsible for adapting to various ecological niches, including virulence factors and antimicrobial resistance genes acquired horizontally from other species (1,7,8). Genetic material in the accessory genome is often located on mobile genetic elements (MGE) (8).

1.2.2 Mobile genetic elements (MGE)

MGEs facilitate horizontal transfer of genes. They are intra- or extra-chromosomal DNA segments that can be independently mobilised within and among bacterial species (9,10). Once acquired, they can transfer vertically from parent to progeny cell (9). MGEs constitute ~20% of the *S. aureus* genome and frequently harbour genes associated with virulence determinants and antimicrobial resistance (9). Examples of MGEs in *S. aureus* include bacteriophages, plasmids, staphylococcal pathogenicity islands (SAPIs), and staphylococcal cassette chromosomes (SCC) (9).

Bacteriophages are viruses that invade bacteria and integrate in the host chromosome. They carry antibiotic resistance genes and virulence factors, such as Panton-Valentine leucocidin toxin (PVL) and enterotoxins found in certain *S. aureus* clones (11,12).

Plasmids are generally extra-chromosomal DNA molecules, but they can also integrate into the chromosome. Plasmids introduced the gene responsible for penicillin resistance into *S. aureus* (*blaZ*) (8). From being 100% susceptible in the pre-penicillin era, only 20-25% *S. aureus* isolates are now susceptible to penicillin (13).

SAPIs are MGEs that rely on helper phages for their mobilisation (14). SAPIs encode genes for toxic shock syndrome toxin (TSST-1), enterotoxin B (SEB), and other superantigens, present in some *S. aureus* clones (14,15).

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) is another important MGE. It has been extensively studied, given its status as a defining feature of MRSA. Notably, there are also non-*mec* SCCs that have been detected in methicillin-susceptible *S. aureus* (16).

SCCmec

The *mec* gene complex is situated on a staphylococcal cassette chromosome (SCC), commonly referred to as SCCmec (18,19). The *mecA* gene is part of the *mec* gene complex, which includes regulatory genes and associated insertion sequences.

In MSSA, penicillin and other β -lactams bind to the natural penicillin-binding proteins (PBP) present in the cell wall, disrupting the synthesis of peptidoglycan layer - an essential component of the bacterial cell wall. This disruption leads to bacterial cell lysis and death. In MRSA, the *mecA* gene encodes a distinct PBP, namely PBP2a (also known as PCB2'). Most β -lactam antibiotics exhibit reduced affinity for this altered PBP, making them ineffective against MRSA (17).

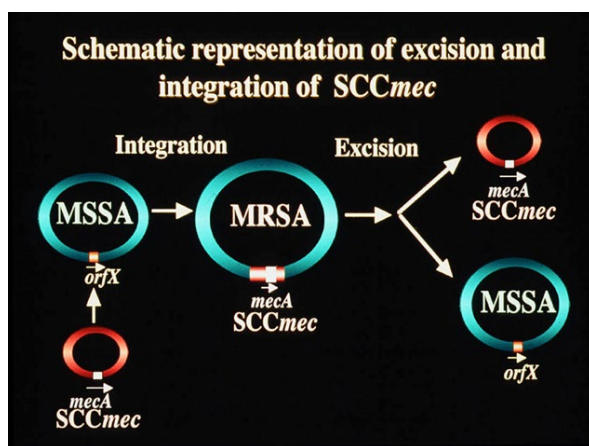


Figure 1: MRSA differs from MSSA by presence of an extra genome: SCCmec complex.

Source: International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC).

<https://www.sccmec.org/index.php/en/>

S. aureus likely acquired the SCCmec complex from coagulate-negative staphylococci (most likely from *S. sciuri*) (20–22). Initially, it was unclear whether the passage of SCCmec occurred to a single clone of MSSA (single-clone theory) or if it was acquired in several instances by various clones over time (multi-clone theory). Given the diversity of MRSA isolates and their

SCC*mec* complexes, it is now believed to have occurred through multiple acquisitions, each giving rise to new clones of MRSA and contributing to various MRSA epidemics (23,24). As of March 2024, 15 different SCC*mec* elements have been reported (<https://www.sccmec.org/index.php/en/>). It is noteworthy that MSSA clones may carry SCC*mec* without the *mecA* gene. These clones are often otherwise multi-resistant and are considered to be MRSA that have lost components of the SCC*mec* complex necessary for expressing methicillin resistance (25–27).

1.3 Emergence and spread of penicillin-resistant *S. aureus*

In 1928, Dr Alexander Fleming observed the inhibition of staphylococcus colonies in a culture plate surrounding a contaminating mould. He identified the “mould broth filtrate” (which he named “penicillin”) and noted its anti-bacterial properties (28). In 1940, Dr Howard Florey and biochemist Ernst Chain became the first to successfully extract and purify penicillin (29,30). Clinical use commenced in 1943 during World War II, followed shortly by the first report of naturally penicillin-resistant clones of *S. aureus* isolates in 1944 (31).

In 1946, following the war, there was a surge in penicillin use as it became widely available and inexpensive (30). Consequently, in the ensuing years, there was a steady increase in penicillin-resistant *S. aureus* (PRSA) (*S. aureus* was called *Staphylococcus pyogenes* in earlier reports) (32,33). Resistance was primarily noted in hospitalised patients and those with healthcare contact. By the early 1950s, three-quarters of hospital-acquired (HA) and one-fourth of community-acquired (CA) *S. aureus* isolates were penicillin-resistant (34).

The emergence and spread of PRSA are well-documented in a series of publications spanning 1946 to 1983 by Dr. Phyllis M Rountree, a bacteriologist at the Royal Prince Alfred Hospital

in Sydney, Australia. Starting in 1948, all *S. aureus* isolates in this institution underwent antibiotic susceptibility testing and phage typing. This comprehensive analysis not only helped illustrate the dissemination of PRSA but also traced the evolution of the notorious penicillin-resistant phage type 80/81 *S. aureus*.

1.3.1 PRSA: a hospital-acquired pathogen with a clonal distribution

PRSA was first noted at this hospital in August 1946. By the end of that year, it had already contributed to 50% (9 of 18) of post-operative surgical wound infections (35).

By 1949, PRSA had become a predominant HA pathogen with limited prevalence in the community. PRSA isolates displayed clonality, a distinct feature when compared to penicillin-susceptible *S. aureus* (PSSA), which belonged to a wide array of phage types (36,37). Out of 228 clinical *S. aureus* isolates, 53% (121) demonstrated penicillin-resistance, while 47% (107) remained susceptible. A restricted set of phage types predominated among PRSA clones, with five types accounting for 75% of the isolates, and 9% being non-typeable. Conversely, PSSA exhibited a broader variability in phage types, with no single type predominating – there were a total of 36 difference phage types, and 28% were non-typeable.

To further explore carriage patterns, nasal swabs were collected in 1949 from 200 healthcare workers (HCW), including 187 nurses and 13 doctors, along with 200 healthy blood donors in Sydney, representing the general community (36). Of the sampled population, 105 out of 200 HCW (53%) and 90 out of 200 blood donors (45%) were *S. aureus* carriers, revealing similar carriage rates ($P=0.13$). However, a significant difference emerged in the carriage of PRSA, with 60% (64 out of 105) of *S. aureus* carriers among HCW and 10% (8 out of 90) among blood donors demonstrating penicillin-resistance ($P<0.001$). Like the distribution of phage

types in clinical specimens (described earlier), phage typing of penicillin-susceptible carriage isolates revealed a diversity of types, with 31% being non-typeable. In contrast, among penicillin-resistant clones, the two most prevalent phage types accounted for 77% of the carriage isolates in HCWs. Phage-type distribution in blood donors was uninterpretable due to the low number of penicillin-resistant clones.

1.3.2 The hospital environment and HCW implicated in PRSA transmission

In 1949, the prevalence of PRSA carriage was investigated among neonates (aged 6-7 days), their mothers, and nurses, along with rates in neonatal clinical specimens and in the nursery environment (dust and air samples) (37). The findings revealed a notably higher carriage rate of both *S. aureus* and PRSA in neonates and HCWs compared to the mothers. Interestingly, the phage distribution differed between neonates and their mother, while it matched that observed in HCWs and the nursery environment.

A follow-up study conducted in 1951 provides additional support for the association between healthcare exposure and the acquisition of PRSA. The study examined the prevalence and duration of carriage in trainee nurses before and during their hospital rotation (38). In their first year of training, the trainee nurses underwent screening for *S. aureus* nasal carriage both before and during their hospital placement. The carriage rate was 52.6% at baseline, which increased to 71.4% within the first 5 weeks of hospital exposure. More concerning was the rise in the rate of penicillin-resistant clones, escalating from 4.3% at baseline to 32.1% within the same 5-week period. Notably, the trainee nurses who were non-carriers at baseline were more likely to acquire penicillin-resistant clones. Conversely, those who were already carriers appeared to be protected against acquisition of new clones.

1.3.3 Community spread of PRSA

Over the 10 years since the first recognition of PRSA as a HA pathogen, most isolates of *S. aureus* in hospitalised patients were resistant to penicillin (PRSA) (39). Concurrently, there was a notable increase in the community prevalence of PRSA, rising from 10% in 1949 (36) to 13% in 1954 (40) and further to 26% in 1955 (39). The detection of PRSA was more likely in clinical specimens than in carriage specimens during this period (39).

1.3.4 Emergence and spread of phage 80/81 *S. aureus*

Starting in July 1953, the emergence of a new clone of *S. aureus* was documented following an outbreak of pemphigus neonatorum and puerperal mastitis in Sydney (41). Subsequently, similar cases were identified in various hospitals across Australia. Phage typing was employed, and this strain was designated as number 80, belonging to the broad phage group I. This clone was notably associated with severe skin infections, toxæmia, and recurrent infections. Additionally, there were reports of severe pneumonia and an epidemic of furunculosis among HCW caused by this clone (6). In a specific investigation involving 50 HCWs with boils, 70% were found to have an infection with phage type 80 *S. aureus*, representing a higher predominance compared to previous epidemiological surveys (35). The increased infection from this specific phage type was observed alongside baseline infections with other *S. aureus* clones (42). It was noted that both phage 80 and page 81 typically lysed the same *S. aureus* clones, although there were occasional and rare instances of *S. aureus* clones being lysed by either 80 or 81 alone. Consequently, the nomenclature was combined, and the clones were collectively referred to as phage type 80/81 (42). By 1958, phage type 80/81 *S. aureus* had become prevalent in various parts of the world, including Canada, the USA, England, and New Zealand (43,44).

Phage type 80/81 initially emerged as predominantly a HA pathogen, gradually extending its presence into the community. A survey conducted on new blood donors (200 individuals) between March – April 1954, aimed at examining community prevalence, revealed that 47% were *S. aureus* carriers, but none had phage type 80/81 strain. However, by December 1957, 8% (7 out of 83) of carriers were found to have phage type 80/81, suggesting an increase in community rates (41,42). In 1958, a nationwide survey of *S. aureus* infections in general practice across Australia was conducted (45). It revealed that 47% of *S. aureus* infections were attributable to phage type 80/81. Within just five years, it had become a dominant community pathogen.

It is noteworthy that the epidemiology and clinical manifestations of phage 80/81 *S. aureus* differed from the commonly encountered phage group III clones, which remained largely confined to hospital settings and are considered precursors of the first MRSA. Clinically, phage type 80/81 tended to invade intact skin, leading to severe skin infections. These lesions often progressed rapidly, patients exhibited features of toxæmia, and infections were often recurrent. In contrast, phage group III primarily caused infections in damaged skin (such as surgical wounds, burns, and site of intravenous infusions) and diseased lungs (42).

The phage type 80/81 *S. aureus* pandemic persisted about a decade (1954-1964) (43,46). The emergence of this pandemic coincided with the widespread use of penicillin, leading to the replacement of susceptible clones with penicillin-resistant *S. aureus*. The exact reasons for their disappearance are not clear but coincides with the introduction of methicillin, an antibiotic effective against penicillin-resistant isolates (47).

1.3.5 PRSA in 2023

Newer DNA-based typing methods, such as pulsed-field gel electrophoresis (PFGE), Staphylococcal protein A (*spa*) typing, and multilocus sequence typing (MLST) have replaced traditional phenotypic methods such as phage typing. Whole genome sequencing (WGS) is being frequently used as the most discriminatory typing method. Further, many laboratories have moved away from phenotypic penicillin susceptibility testing due to its unreliability. Instead of reporting penicillin susceptibility, prescribers are now directed to use penicillinase-stable β -lactams, such as flu- or di-cloxacillin, cephalexin, and cefazolin.

Nevertheless, the prevalence of PSSA has shown an increase since around 2015, and it now accounts for 20-25% of all *S. aureus* in blood cultures (48,49). This trend has sparked a renewed interest in reporting of penicillin susceptibility, and ongoing clinical trials are actively assessing the most effective antibiotics for treating PSSA bacteraemia. One such trial is *Staphylococcus aureus* Network Adaptive Platform (SNAP) study where patients with PSSA bacteraemia are randomised to receive either benzylpenicillin or flu(cloxacillin) (50). Retrospective analyses suggest that treatment of penicillin-susceptible *Staphylococcus aureus* (PSSA) bacteremia with benzylpenicillin may be associated with lower 30-day mortality compared to treatment with flucloxacillin (51).

1.4 Emergence and spread of methicillin-resistant *S. aureus*

In the 1950s, penicillin ceased to be a reliable therapy for serious *S. aureus* infections, as the majority of isolates developed resistance to penicillin (52–54). As a result, mortality rates from staphylococcal sepsis began climbing toward levels seen in the pre-antibiotic era (Table 1).

Table 1: Reported case fatality in *S. aureus* sepsis (1940s-1960s)

Antibiotics (years)	Case fatality (<i>S. aureus</i> sepsis)	References
Before penicillin (pre-1945)	80-90%	5,59
With penicillin G (post-1945 and in 1950s)	≤30%	5,59
With erythromycin, chloramphenicol, tetracycline, or streptomycin (1950s)	40-50%	53,54
With vancomycin (1950s)	≤30%	52
With methicillin (1960s)	≤30%	60,61

In the 1950s, the chemical synthesis of 6-aminopenicillanic acid (6-APA), the basic building block of penicillin, was achieved. However, the process was complex with a low yield. Researchers noted that introducing substitutes to the β -lactam side chain provided acid-stability (for oral use) and broadened the antimicrobial spectrum (55). Despite these advancements, large quantities of 6-APA were still necessary.

A breakthrough came when it was discovered that certain microorganisms produced amidase enzymes capable of cleaving the side chain of penicillin, releasing 6-APA, and eliminating the need for complex chemical synthesis. This liberated 6-APA could then be used as the basis for creating new penicillin-based drugs. Beecham Laboratories in the UK utilised this discovery to synthesise methicillin in 1959 (labelled it “celbenin”). Methicillin exhibited stability against staphylococcal penicillinase and demonstrated high efficacy against PRSA. At the time, there was a theoretical belief that *S. aureus* would never develop resistance to this new antibiotic, as the only resistance mechanism in PRSA was related to penicillinase production, which methicillin successfully overcame. Furthermore, staphylococci were not rendered resistant to methicillin by *in vitro* passage (56,57).

Before the advent of methicillin, various antibiotics including erythromycin, chloramphenicol, tetracycline, and streptomycin, were used for treating PRSA infections. However, unlike

penicillin, these agents were bacteriostatic, inhibiting bacterial replication without inducing lethality. Vancomycin, which like penicillin was a bactericidal agent, became available in the mid-1950s. It received approval from the Federal Drug Administration (USA) in 1958 (58). Despite its efficacy against PRSA, vancomycin was not widely used due to the need for intravenous infusion and associated side effects such as phlebitis, chills, ototoxicity (tinnitus), and nephrotoxicity. Typically, vancomycin was reserved for patients allergic to β -lactams or when other antibiotics proved ineffective (52).

1.4.1 Emergence of MRSA

Methicillin was introduced for the treatment of PRSA in 1959. However, it was not long before the first “celbenin”- resistant *Staphylococci* was documented in July 1960 (62). In an analysis of 5,440 *S. aureus* samples sent to the *Staphylococcus* Reference Laboratory in London for phage typing, three samples, all originating from one hospital, were identified as methicillin-resistant. These samples were collected from a patient with a nephrectomy wound, an infected skin lesion in a nurse from the same ward, and the nasal swab of a patient two weeks later, also from the same ward. Importantly, these patients had not been exposed to methicillin, which was only introduced in that hospital four months later, in October 1960. MRSA was perceived as a “curious” phenomenon and was not initially considered a significant problem.

1.4.2 The spread of MRSA

In 1963, a comprehensive analysis was conducted on 27,749 out of 35,300 (78%) *S. aureus* isolates received at the *Staphylococcus* Reference Laboratory, London (63). Among these isolates, 101 cases of MRSA were identified. MRSA had become endemic in two British hospitals and was sporadically found in several others. Predominantly, MRSA was isolated from wounds (52% of cases). The analysis revealed nine phage-typing patterns among *S.*

aureus isolates, with almost all MRSA belonging to phage group III and demonstrating resistant to several other antibiotics, typically penicillin, streptomycin, and tetracycline. Interestingly, the phage pattern of MRSA isolates matched those of methicillin-sensitive clones frequently observed in outbreaks of hospital infection in Britain. Throughout the study, an increasing number of laboratories reported the detection of MRSA. The proportion of MRSA in all samples rose from 0.05% in October-December 1960 to 0.81% in July-September 1962.

1.4.3 The first reported nosocomial MRSA outbreak and its management

The first reported outbreak of MRSA occurred at Queen Mary's Hospital in Surrey, England. The hospital conducted monthly surveillance for *S. aureus*, involving nasal swabs from staff and patients. This led to the identification of the first MRSA case in the hospital (64). Methicillin was introduced in this hospital in the latter half of 1959, and until March 1961, no cases of MRSA had been detected. It was first identified in the nostril of an infant who had been admitted 6 weeks earlier for post-surgical PSSA umbilical infection. The child had been kept in for nutritional reasons. A routine nasal swab collected on March 13, 1961, isolated MRSA. Nasal decolonisation attempts were unsuccessful, and the child was eventually discharged on April 12. Upon returning for an outpatient visit in August 1961, the child still carried MRSA, which subsequently disappeared after additional applications of chlorhexidine, neomycin, and bacitracin.

Within a month of the initial MRSA isolation, three more infants were identified with MRSA; one through routine nasal screening, one from an infant with otitis externa, and one from an infant with mild eczema. In response, the ward was closed, and the affected infants were isolated. To contain the spread, all staff and patients underwent treatment with topical chlorhexidine applied to the nares and skin folds. Despite these rigorous efforts, including

routine screening, isolation, and decolonisation, MRSA persisted and spread within the hospital.

All MRSA clones identified during this period shared the same phage type (phage group III, phage type 75/77). By May 1962, 14 months after the initial outbreak, MRSA had been detected in a total of 37 children across eight of the 48 wards. Remarkably, none of these children initially exhibited serious infections until one child developed bacteraemia with multi-organ seeding, ultimately succumbing to sepsis despite antimicrobial therapy. Among the 37 children identified as MRSA carriers, 22 had previously received penicillin, seven had received methicillin/cloxacillin, and eight had no prior antibiotic exposure.

This underscores the complexity of MRSA infections and the challenges in managing its spread, even with early detection and intervention efforts.

1.4.4 Spread in Europe and beyond

By 1964-65, MRSA cases had been detected in various countries, including England, Denmark, the USA, Greece, Turkey, Poland, South Africa, Switzerland, and France (65,66). In 1964, approximately 15% *S. aureus* isolates in Paris hospitals were identified as methicillin-resistant (67). Subsequently, by 1967, 16% of *S. aureus* in Zurich, Switzerland, exhibited resistance to methicillin (68). In the USA, a Boston city hospital initially reported no MRSA until 1967 (69). However, within the next 12 months, 18 patients were diagnosed with MRSA in that hospital. Most of these cases were clinical samples, with only one patient identified as a carrier. Until this publication in 1969, only four clinical cases of MRSA had been reported in the USA (69). The contemporary epidemiology of a pathogen is most effectively determined by implementing regular surveillance. Denmark, in addition to England, initiated routine surveillance of *S.*

aureus samples in the 1950s. The first MRSA case in Denmark was isolated in August 1962 (66). Over the next 18 months, eight more MRSA cases were identified across six other Danish hospitals, all originating from clinical specimens. Methicillin resistance in Denmark displayed a consistent upward trend, with rates escalating each year. By 1966, the prevalence of methicillin resistance had reached 20% in hospital-onset infections and approximately 10% in community infections in Denmark.

MRSA was first documented in Turkey in 1962, followed by reports in Poland and India in 1964. Of interest, at the time of these initial reports, methicillin was not yet in use in these countries (70).

The dissemination of MRSA across Europe and beyond during this period was attributed to a new strain of MRSA, distinct from the first MRSA reported in England. The molecular epidemiology of MRSA is explored in the subsequent chapter, Chapter 2 of this thesis.

1.4.5 1970s and 1980s

There was a diverse prevalence rates and trends of MRSA in different regions during the specified period. Until the early 1970s, MRSA was infrequently reported in the USA, contrasting with its prevalence in Europe where it constituted up to 20% of *S. aureus* isolates (71). However, in the subsequent years, MRSA outbreaks were documented in some large U.S. teaching hospitals, resembling the earlier MRSA outbreaks in Europe during the 1960s (72). For instance, in a Cincinnati Hospital, within three years following the initial detection of MRSA in March 1977, MRSA accounted for 30% of all SAB cases (73). In contrast, England, the rest of the Europe, and Australia experienced a decline in methicillin-resistance during the 1970s, only to see a resurgence in the 1980s (74).

In the early 1980s, Italy reported a prevalence ranging from 6% to 44% with regional variations, while New Zealand recorded a rate of 0.05%, and the USA had a prevalence of 7% (83). Between 1975 and 1991, the MRSA prevalence in the U.S. hospitals increased markedly, rising from 2.4% in 1975 to 29% in 1991, with higher prevalence observed in larger hospitals (15% in hospitals with ≤ 200 beds and 38.3% with ≥ 500 beds) (84).

In the majority of hospitals where MRSA introduced, it became an endemic HA pathogen (81). However, there were notable exceptions. Denmark, for instance, had a low prevalence of MRSA in the 1980s through active interventions such as surveillance, isolation, and decolonisation of all MRSA cases. By the end of 1980, MRSA accounted for only 0.1% of SAB cases in Denmark (82). A national survey of teaching hospitals in Australia in 1986-87 revealed MRSA rates of 14%, with regional variations such as 25% in Queensland and 0.4% in Western Australia. Similar to Denmark, Western Australia had an active surveillance program to control MRSA (83).

1.4.6 1990's – Emergence of community-clones of MRSA

In the early 1980s, clinical trials showed that vancomycin was superior to other antimicrobial therapies for MRSA bacteraemia (73,75,76). The availability of purified commercial products and the implementation of vancomycin therapeutic drug monitoring helped mitigate its toxicity. Consequently, vancomycin became the drug of choice for MRSA infections (72). The increased use of vancomycin, probably contributed in the emergence of vancomycin resistant enterococci (VRE), first reported in Europe in 1986 and in the USA in 1987 (58). The first *S. aureus* strain with reduced susceptibility to vancomycin was reported a decade later in Japan in 1997 (77,78) and two years after that, in 1999, the first vancomycin-resistant *S. aureus* was

reported in the USA (79). The development of resistance to newer anti-MRSA antibiotics is a global concern (80).

In the 1990s, there was also a notable emergence of community-clones of MRSA. Prior to this period, MRSA was mainly considered a HA pathogen. The first reported case of true community-acquired (CA) MRSA surfaced in Western Australia (WA) in 1993 (85), although it was likely spreading as early as 1989 (86). Following a nosocomial MRSA outbreak in 1982, the state implemented a screening policy for all new patients and staff who had been in hospitals outside WA within the preceding 12 months. The objective was to control the importation of MRSA into the healthcare system, leading to a lower prevalence of MRSA in WA compared to the eastern states (83). However, starting in the early 1990s, there was a rising number of MRSA cases in patients who had not been outside WA (85). Notably, MRSA carriage and infections were detected in remote Indigenous communities without known healthcare contact, residing 2,000km away from the metropolitan tertiary centres in Perth. PFGE results indicated that these strains differed from the prevalent MRSA strains in the eastern states and other known MRSA clones (85).

Following the report of CA-MRSA cases in WA, similar occurrences were reported elsewhere, often affecting indigenous and/or underprivileged communities. Subsequently, there was a notable emergence and spread of USA100 and later USA300 CA-MRSA clones in the USA, along with the emergence of other MRSA clones in various countries (87,88). These CA infections differed from HA cases, as they affected individuals who were otherwise healthy with no usual predisposing risk factors (89–91). Unlike their HA counterparts, CA-MRSA clones were frequently susceptible to many non- β -lactam antibiotics, a notable contrast as HA clones were typically multidrug resistant.

1.4.7 2000-present day

2000-2010

During this period, MRSA prevalence peaked, as infections stemming from both CA and HA clones became commonplace, resulting in a blurring of the classifications. CA-MRSA was now frequently encountered in nosocomial infections, and vice versa (92–94). In certain countries and within specific hospital units, such as ICU, over 50% of *S. aureus* infections were attributed to MRSA (95). Regions and countries with active surveillance, isolation, and decolonisation measures throughout the 1990s managed to keep MRSA prevalence below 5% (96,97).

2010 – 2023

After 2008 - 2013, MRSA prevalence began to decline in many regions worldwide (48,98–100). This decline was primarily attributed to a reduction in HA-MRSA infections and decreased rates of MRSA in the elderly population (101,102). Factors such as infection control practices, hand-hygiene initiatives, and antimicrobial stewardship programs are believed to have played pivotal roles in controlling MRSA epidemic (103–106). The decrease in central-line associated *S. aureus* bacteraemia (SAB) has also contributed to fewer MRSA infections. The change could also be influenced by the dynamics of the predominant *S. aureus* clones (107–109). Interestingly, in the past decade following the decline in MRSA, MSSA bacteraemia rates have shown an increase (101,102).

1.4.8 Australian context

Australia's first reported MRSA case emerged in October 1965 in Sydney (70). The subsequent spread of MRSA within this hospital echoed the pattern observed during the earlier described MRSA outbreak at St. Mary's Hospital in Section 1.4.3. Antibiotic susceptibility and phage typing were routinely conducted on *S. aureus* isolates at Sydney's Royal Prince Alfred Hospital

since 1948 (6). Although methicillin was introduced in 1961, methicillin-resistant clones were not detected until October 1965 at which time MRSA was first isolated from a patient's sputum sample. Subsequent cases due to the same MRSA strain emerged in July 1966 in patients in surgical wards. In December 1966, two patients from New Caledonia, admitted with MRSA in sputum and pressure sores, inadvertently spread strains to other patients due to delayed isolation (70). In 1965, MRSA accounted for 0.1% *S. aureus* isolates at the Royal Prince Alfred Hospital, which increased to 5.7% by December 1967 (70). Throughout this period, the prevalence of penicillin-resistant isolates remained stable at 70-75%. The majority of multi-resistant MRSA clones belonged to phage type III.

The early methicillin-resistant clones at this hospital were often detected in surgical wounds (31% of isolates), sputum without pneumonia (25%), and nasal/throat carriage, the latter identified through an active surveillance (13%). None of the MRSA isolates were detected in blood cultures. Treatment for these MRSA infections involved the use of lincomycin or kanamycin. Nasal carriers received treatment with a topical cream containing neomycin or bacitracin and chlorhexidine. Once again, these MRSA strains predominantly belonged to phage group III. The evolution of new clones, replacing older prevalent clones, was noted. To reliably distinguish these emerging clones, new phages had to be found and added to the basic international set of typing phages, following the guidelines of the International Subcommittee of Phage Typing of Staphylococci.

A subsequent analysis of MRSA prevalence at the Royal Prince Alfred Hospital in Sydney was conducted in 1973 (110). Among inpatient and staff samples, 6.3% (40/631) isolates were identified as MRSA in the first half of 1968. This figure increased to 18.5% (111/601) by the second half of 1970. Group III phage types remained predominant, with the emergence of new

phage types within this group, such as phage type 88 *S. aureus*. During this period, MRSA isolates commonly exhibited resistance to penicillin-streptomycin-tetracycline-erythromycin “PSTE”, (246/581, 42%), or PSTE + chloramphenicol, (207/581, 36%). Unlike previous years, MRSA was now noted in blood cultures (1.7% of MRSA isolates). Additional sources included 41% from surgical wounds, 30% from sputum, and 10% from nose/throat carriage. Among the 11 cases with MRSA bacteraemia, all had underlying comorbidities, and six (56%) succumbed to the infection.

Australian Group on Antimicrobial Resistance (AGAR)

The continued emergence of MRSA in Australia is effectively documented through reports from the Australian Group on Antimicrobial Resistance (AGAR). Established in 1985 in response to the escalating prevalence of MRSA infections, AGAR has been reporting on the epidemiology of *S. aureus*, including MRSA, in Australia since 1986. The Australian Staphylococcal Surveillance Outcome Program (ASSOP) is one of AGAR’s three prime target areas of surveillance, alongside enterococcus surveillance and gram-negative surveillance. Since 2013, AGAR has shifted its focus to exclusively surveilling blood culture isolates.

In the inaugural AGAR report, a national survey of teaching hospital laboratories in Australia during 1986-87 revealed MRSA rates of 14%, showcasing regional variations (25% in Queensland and 0.4% in WA) (83). In 1996, AGAR reported resistance rates over a period from 1988 and 1994 (111). Each year, the first 100 clinical samples were collected from each of the 20 participating teaching hospitals within a fixed cross-sectional period. Over the six-year span, MRSA prevalence (all specimens) remained steady at around 25% in Sydney, Melbourne, and Brisbane. In the other state capitals (Perth, Hobart, Canberra, and Adelaide),

MRSA prevalence was lower, ranging between 5-10%, with yearly fluctuations. Separate data for blood isolates was not available from that time.

In 2003, AGAR reported findings from 15 years of MRSA surveillance in Australia (112). The report highlighted a gradual rise in MRSA prevalence among *S. aureus* clinical isolates, increasing from 14% in 1990 to almost 30% by 1999. Additionally, a gradual emergence of non-multi resistant CA-MRSA in Australia throughout the 1990s was reported.

In 2005, AGAR presented its findings based on prospectively collected blood culture isolates from 17 Australian hospitals between January 1, 1999, and December 31, 2002 (113). There were 12,771 bacteraemic episodes during the study, of which 3,192 were due to *S. aureus* (25%). Of the SAB cases, 50% (1,621/3,192) were categorised as hospital-onset (HO), with 60% (973/1,621) being MSSA and 40% (648/1,621) MRSA. HO was defined as occurrence >48 hours after hospital admission. The study combined community-acquired (CA) and healthcare-associated community onset (HACO) groups, labelled it as community-onset (1,571/3,192, 49%).

Overall, MRSA accounted for 26% of all SAB cases during 1999-2002, with 77% of these being HO. The predominant healthcare-associated strain was identified as ST239-MRSA, while the emergence of ST22-MRSA strain was noted, a strain already prevalent in the UK and known as EMRSA-15.

The AGAR/ASSOP survey of 2021 (13) reported all unique episodes of SAB collected between January 1 and December 31, 2021, from 30 participating public and private laboratories servicing 48 hospitals across all states and mainland territories in Australia. Each

new positive blood culture in a patient more than two weeks after an initial culture was considered a new episode. This report included 2,928 SAB episodes. The proportion of *S. aureus* that were MRSA had remained stable since 2013, accounting for 16.9% in 2021. However, since 2013, the proportion of CA-MRSA clones had significantly increased in New South Wales, Western Australia, and Northern Territory, In contrast, the proportion of HA-MRSA clones had significantly decreased in all states and territories except in Tasmania (although the Tasmanian trend might be unreliable due to the limited number of isolates available for comparison - only 10-15 isolates/year were available). Interestingly, resistance to clindamycin (10% in 2021), erythromycin (30% in 2021), and ciprofloxacin (35% in 2021) in MRSA had declined since 2017. Furthermore, 85% of nosocomial MRSA was found to be from community-clones.

The results of the 2021 AGAR/ASSOP survey were compared with data from 30 contributing European countries (excluding the UK) in the EARS-Net (European Antimicrobial Resistance Surveillance Network) program. In Australia, the rates of methicillin resistance in *S. aureus* were 16.9%, ranked 11th. Comparatively, in Europe, the countries with the highest MRSA prevalence were Cyprus (42.9%), Greece (41.9%) and Romania (41%), while the lowest rates were observed in Norway (0.9%), Iceland (1.1%), Estonia (1.5%), and Netherlands (1.5%) (13).

The molecular epidemiology of *S. aureus* is further discussed in Chapter 2 of this thesis.

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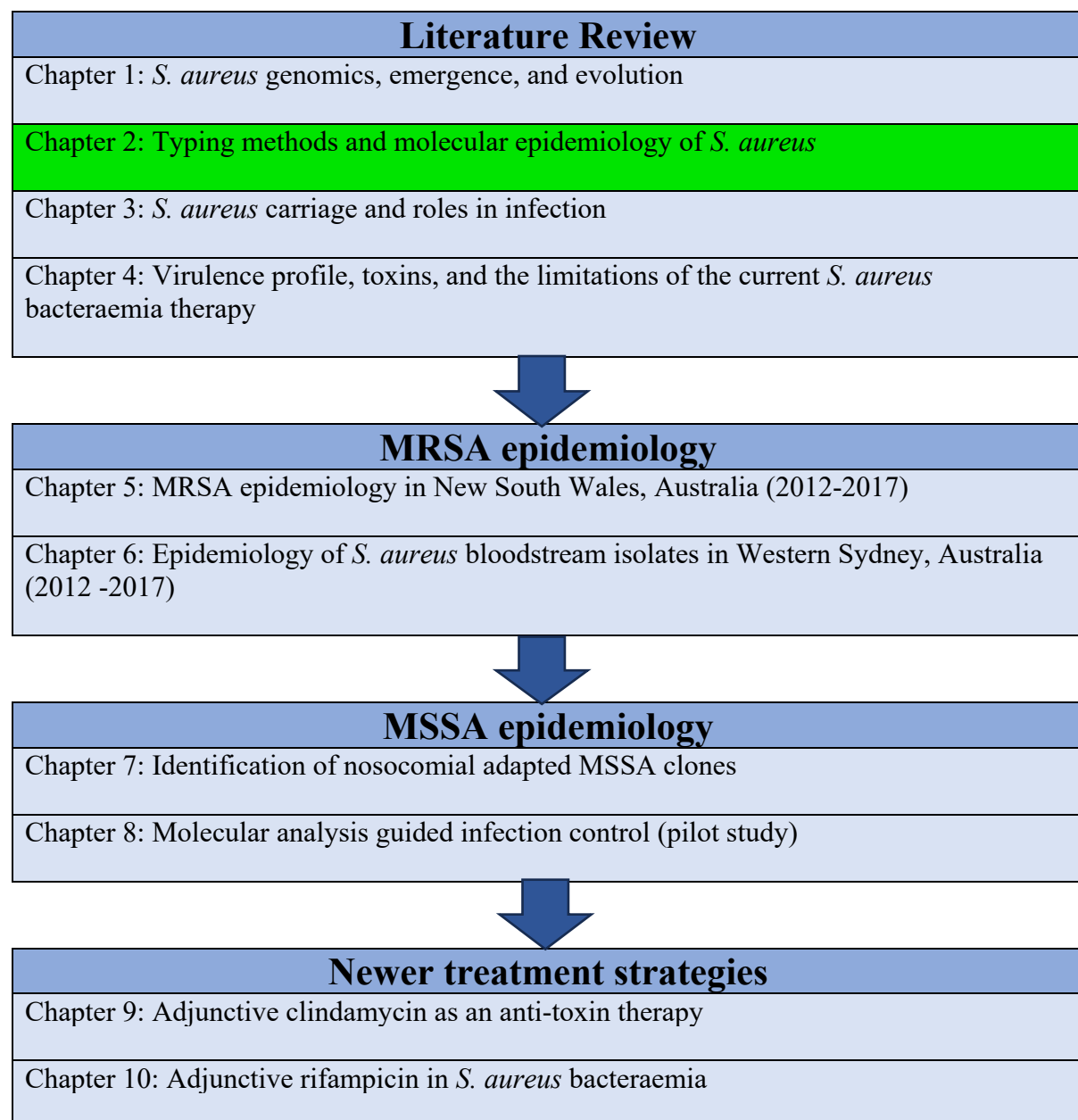
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Figure: Schematic overview of the thesis structure



Chapter 2: Overview

This chapter provides a comprehensive description of common bacterial typing methods employed in the molecular study of *S. aureus*. These analyses reveal a continuous evolution of the species, occasionally giving rise to successful clones that cause epidemics and pandemics. Initially confined to hospital environments, both methicillin-susceptible *S. aureus* (which encompasses penicillin-resistant *S. aureus*) and methicillin-resistant *S. aureus* later spread to the community.

Despite commencing with a limited number of clones, both MSSA and MRSA have evolved into polyclonal entities, particularly in the case of MSSA.

This chapter comprises unpublished work.

Chapter 2: Typing methods and molecular epidemiology of *S. aureus*

2.1 Typing methods

Molecular typing of pathogens can reveal clonal relationships among isolates, shedding light on their evolution and spread. This process is crucial for understanding the epidemiology of pathogens, especially in the context of outbreak investigations and for making informed public health decisions (1). In essence, molecular typing enables the determination of genetic similarities between two isolates of the same species.

Bacterial typing can be broadly categorised to phenotypic and molecular (or genotypic) typing methods (1). Phenotypic methods include various techniques such as observing colony characteristics on solid culture medium, assessing biochemical differences, studying variations in antibiogram, defining seroreactivity, and utilising phage typing. On the other hand,

molecular typing distinguishes isolates based on their genetic content, generally offering a higher resolution (2). Molecular typing can be further categorised into non-sequence-based methods, which include multiple locus variable-number tandem repeat analysis (MLVA) and pulsed field gel electrophoresis (PFGE), and sequence-based methods, such as multilocus sequence typing (MLST), *spa* typing, and SCC*mec* typing.

Molecular typing addresses two essential questions: i) short-term epidemiology, also known as local- or micro-epidemiology, involving the determination of whether two isolates identified during a disease outbreak are identical or distinct, and ii) long-term epidemiology, also referred to as international- or macro-epidemiology, aimed at examining geotemporal trends in phylogenetic relationships (3).

Prior to conducting strain typing, it is essential to establish both the purpose of typing and the subsequent actions that will be taken based on the results. Additionally, a thorough understanding of the limitations associated with different typing methods is important (4). Ideally, molecular typing methods should exhibit high levels of typeability (the ability to assign a result to any isolate), reproducibility (consistency in results across different occasions or laboratories), discrimination (the capacity to differentiate unrelated isolates), and ensuring ease of use and interpretation with minimal technical expertise required (5). These methods should be applicable to all relevant organisms, be portable (unambiguous results for interlaboratory comparisons), and economically feasible with low running costs (6).

The discriminatory power of a typing method is directly proportional to the number of identified types and inversely proportional to the frequency of the most common type. Simpson's Diversity Index (DI) is a commonly used metric for this purpose (7,8). A DI value

of 0.90 or higher is indicative of high discrimination, while values of 0.75 or lower are generally considered poorly discriminatory (8). A 95% confidence interval can be calculated for DI, and overlapping values suggest similar discriminatory power, assuming an adequate sample size (9–12).

Of the many molecular typing tests available for *S. aureus* (2,5), this review encompass phage typing (selected for its historical significance), PFGE (considered the “gold standard” for many years), *spa* typing, *SCCmec* typing, MLST, and whole genome sequencing (WGS). The binary typing, a locally developed test employed to investigate molecular epidemiology of MRSA in New South Wales, Australia, is discussed in Chapters 5 and 6 of this thesis.

2.1.1 Phage typing

Bacteriophage, or phage, typing stood as the primary tool for *S. aureus* epidemiological studies for nearly 50 years, from the 1940s to the early 1990s (13–15). In 1935, the ability to group *S. aureus* based on their sensitivity to various bacteriophages was demonstrated. Phage susceptibility was determined by observing the confluent lysis of *S. aureus* culture colonies on bacteria-coated agar plates exposed to a small drop of phage solution. Type-specific phages, capable of lysing only a single type of *S. aureus*, were uncommon. Instead, *S. aureus* strains were more likely to show susceptibility to various combinations of phages (13). The bacteriophages and their corresponding *S. aureus* strains were maintained by the National Collection of Type Cultures, Medical Research Council in London (16). Standardisation of typing sets, along with a protocolised testing process and interpretation, ensured that results were comparable across different laboratories (13).

With the emergence of new strains, the foundational phage sets required continuous expansion to ensure that every *S. aureus* could be lysed by at least one phage (14). In 1953, there were 19 basic phage sets, increasing to 21 in 1958, with several new ones added in subsequent years (17). This ongoing expansion was crucial to maintaining specificity and effectively distinguishing between different *S. aureus* strains (13).

Generally, human *S. aureus* isolates were classified into one of three phage groups. Phage types differing in susceptibility to two or more phages were considered unrelated, while those differing by only one phage were deemed related. A significant limitation of phage typing was the non-typeability of *S. aureus* isolates that were non-susceptible to any bacteriophage in the typing set, though this could sometimes be resolved by addition of new phages to the testing panel (13,17).

Phage typing played a critical role in identifying the emergence and spread of phage 80/81 *S. aureus* in the 1950s, as discussed in earlier sections, and laid the foundation for the molecular epidemiology of *S. aureus* (18–22). However, its applicability was limited to large reference laboratories due to the need for maintaining an extensive collection of phage stocks and propagating phages. The spread of the MRSA epidemic coincided with the development of newer typing tools that offered improved reproducibility and typeability. Consequently, this led to a discontinuation of *S. aureus* phage typing (5,15,17).

2.1.2 Pulsed field gel electrophoresis (PFGE)

Bacteriophage typing served as the method of choice at the U.S. Centre for Disease Control and Prevention for over 30 years until it was replaced by PFGE in 1994 (15). In an analysis involving 300 *S. aureus* isolates, comprising 96 isolates belonging from phage group 1, 19

from group II, 82 from group III, seven from group IV, and 96 (32%) classified as non-typeable isolates, PFGE typing demonstrated superior performance. When compared to the phage typing, PFGE exhibited better typeability (no nontypeable isolates), higher discriminatory power, and lower testing costs (US \$ 3 versus US \$10 per isolate in 1995) (15).

The PFGE test involves fragmenting *S. aureus* DNA using a restriction enzyme (*Sma*I) and embedding it in an agarose gel (23). The fragments are then separated through electrophoresis in an electric field with an alternating voltage gradient. The resulting band patterns are analysed using Tenover classification (1,24). A strain was defined by isolates yielding the same PFGE profile (having the same number and size of bands or $\geq 95\%$ band similarity). Those differing by a single genetic event, reflected in a difference of one to three bands, are considered closely related strains with usually $\geq 80\%$ band similarity. Isolates differing by four to six bands are classified as possibly related, while those with seven or more band differences are considered unrelated.

PFGE was once considered the gold standard for differentiating *S. aureus* strains in outbreak investigations and served as the benchmark for evaluating newer typing tests (24). Of note, *Sma*I occasionally fails to cleave *S. aureus* DNA in some isolates, especially livestock-associated MRSA, yielding a non-typeable result (25).

The primary limitation of PFGE was the difficulty in intra-laboratory comparison of results and its unsuitability for studying the long-term evolution of *S. aureus* (26,27). Similar band patterns were assigned different nomenclature in different countries. For instance, the epidemic CA-MRSA clone, USA300, and the hospital-acquired (HA) MRSA, USA100, in the USA were

known respectively as CMRSA-10 and CMRSA-2 in Canada, and more broadly as ST8-MRSA-IV and ST5-MRSA-IV (28).

The large chromosome fragments examined in PFGE are likely to have higher genetic variation than the smaller fragments (genes) used in MLST or *spa* typing. With an evolving pathogen such as *S. aureus*, this led to the emergence of multiple PFGE profiles over short periods, introducing complexity in interpretation and sometimes subjective bias. Additionally, clonal complex (CC) allocation based on PFGE patterns proved challenging (29). With the advent of sequence-based typing methods (MLST and *spa* typing), offering advantages such as speed, portability (data sharing), ease of use, and reproducibility, PFGE gradually became obsolete. In the USA, routine PFGE for typing *S. aureus* isolates was discontinued in 2008.

2.1.3 Staphylococcal protein A gene (*spa*) typing

Staphylococcal protein A is a surface protein of *S. aureus* known to carry polymorphic regions. It is encoded by the *spa* gene, consisting of approximately 2,100 base pairs. The X region of the *spa* gene contains a variable number, between 3 to 15, of 24-bp (21 to 30 bp) repeats (30). The *spa* typing method involves amplifying the DNA sequence of this X region. Each unique repeat is assigned a numerical code based on its sequence, and the *spa* type is determined from the order of these repeats (31). An online database, Ridom SpaServer (<http://www.spaServer.ridom.de>), automates the assignment of *spa* types based on previously curated isolates (12). The Based Upon Repeat Pattern (BURP) algorithm allocates *spa* types into *spa* clonal complexes (32). As of 9th March 2024, the Ridom SpaServer has identified 841 unique repeats, encompassing 21,648 different *spa* types from 463,674 *S. aureus* isolates. The four most frequent *spa* types recorded on the Ridom SpaServer are t032 (9.66%), t003 (8.33%), t002 (6.49%), and t008 (5.76%). These correspond to MLST ST22 (CC22), ST5/225 (CC5),

ST5/231 (CC5), and ST8/247/250/254 (CC8), respectively. Although several *spa* types may be associated with a single MLST, a given *spa* type typically aligns with one MLST CC (28,36).

In contrast to PFGE, *spa* typing is less labour-intensive, allows for inter-laboratory comparison, and provides unambiguous and easily interpretable results (29,33,34). Moreover, *spa* typing proves valuable for both *S. aureus* outbreak investigation and the study of its long-term evolution. When compared to MLST, *spa* typing is more user-friendly and cost-effective, involving the sequencing of a single gene target (35,36).

However, both genetic recombination and point mutations within the *spa* gene, can introduce inaccuracies in results. A recombination event might lead to two unrelated isolates having the same *spa* type or mismatched BURP and BURST clonal groups (37). Mutations within the *spa* gene can result in non-typeable isolates due to a failure of PCR amplification (38,39). While not universally adopted, *spa* typing can be combined with SCC*mec* typing (*spa*/SCC*mec*) to increase discriminatory power (11,40).

2.1.4 Multi locus sequence typing (MLST)

Multilocus enzyme electrophoresis (MLEE) served as a precursor to MLST (41). MLEE involved the analysis of the electrophoretic mobility of house-keeping gene products, whereas MLST is a direct sequencing of the house-keeping genes (42).

In MLST of *S. aureus*, 450-500 bp fragments from seven house-keeping genes on both strands of chromosomal DNA are amplified. The sequence of each gene fragment is then compared with known alleles at the locus. The analysis of the seven loci generates an allelic profile, which is matched on an internet database to generate a sequence type (ST) (42). Isolates with

matching STs have identical sequences at all seven MLST loci and are considered to belong to a single clone. Single locus variants (SLV) are isolates that differ at only one of the seven MLST loci. A clonal complex (CC) is assigned if a ST matches the founder ST of the CC (also called the central ST) at four or more loci, unless they more closely match another central genotype (<https://pubmlst.org>) (43). The e-BURST algorithm is used to automatically assign MLST CCs.

Due to a standardised nomenclature, MLST allelic profiles are unambiguous, a contrast to PFGE where *S. aureus* strains have received different names in various regions. For example, MRSA designated as Portuguese, Brazilian, Vienna, and EMRSA-1, -4 and -11 based on PFGE were all identified as the same clone through MLST (i.e., ST239-MRSA) (40).

Variations in house-keeping genes are anticipated to accumulate slowly, enabling epidemiological studies over extended time periods and broad geographical areas (42). In contrast, typing methods limited to the analysis of a single target (such as *spa* typing) may not be as suitable for global epidemiological studies, as over time, it can be significantly influenced by events like recombination (41).

MLST has played a crucial role in uncovering the evolution and spread of *S. aureus*. The emergence of MRSA has been traced to the horizontal transfer of the *SCCmec* element into different MSSA lineages. Consequently, there is minimal divergence in the core-genome between MRSA and MSSA, allowing them to cluster into similar MLST types and CC (42). MLST analysis has revealed that MRSA belongs to relatively limited clonal groups, suggesting not all MSSA lineages can accept *SCCmec* element (44). Additionally, MLST analysis has contributed to understanding the emergence of the first MRSA clone (Archaic clone), its evolution into the epidemic MRSA clone of 1960s/70s (Iberian clone), the development of

other successful MRSA clones, and the connection between the currently circulating CA-MRSA clone (ST30-MRSA) and the epidemic methicillin-susceptible phage type 80/81 *S. aureus* of the 1950s (40,44–46).

The primary drawback of MLST lies in its lower discriminatory power (12), which limits its effectiveness in outbreak investigations. Additionally, the need for sequencing multiple targets makes MLST a more costly method compared to *spa* typing.

2.1.5 SCC*mec* typing

The SCC*mec* attachment site is located at the 3' end of an open reading frame known as *orfX*. The integration and excision of SCC*mec* at this specific site in the *S. aureus* genome are regulated by cassette chromosome recombinase (*ccr*) genes present in all SCC*mec* variants. Different types of *ccr* genes (named A, B, and C) and *mec* complexes (referred to as *mec* Classes A to E) have been identified. The combination of specific *ccr* genes and *mec* complexes is used to name a given SCC*mec* type, with 15 types described as of March 2024 (type I to XV) (<https://www.sccmec.org/index.php/en/>) (47,48). SCC*mec* II and III have acquired additional drug resistance genes that confer resistance to multiple classes of antibiotics, in addition to β -lactams. The regions of SCC*mec* that are not part of the *ccr* genes or *mec* complexes are called J regions (previously known as “junkyard regions”), which may also harbour antibiotic resistance determinants (36,47).

The internationally recognised standard naming of *S. aureus* clones is “clonal type” based on its MLST type and SCC*mec* type (example: ST22-MRSA-IV) (40). This scheme allows efficient global tracking and surveillance. The clonal type of MSSA is written without an SCC*mec* type (example: ST22-MSSA).

2.1.6 Whole genome sequencing

WGS has emerged as the preferred method for characterising bacterial strains, offering valuable insights into population structure, evolution, spread, and virulence (2,49–54). It enables a comprehensive analysis of the entire *S. aureus* genome, comprising stable core regions (80-85%) and MGEs (15-20%) (55,56). WGS has substantiated that MRSA clones exhibit high similarity to MSSA clones of the same lineage (55).

WGS is a powerful tool that can infer the SCCmec type, *spa* type, MLST, and PFGE type, while characterising virulence determinants and toxin genes in a single test (43,49,50,57–59). WGS allows the comparison of strains with each other and with reference sequences to define evolutionary history (54). The current comparative analysis using WGS relies on single nucleotide polymorphisms (SNP) and the phylogenetic analysis based on all loci in a genome. Comparing strains at the level of SNP provides the highest DI of any available typing tool (54). SNP analysis is used for defining short term epidemiology (investigating outbreaks) (51). The rate of accumulation of SNPs, known as the molecular clock, is estimated as one SNP every 6-18 weeks in the *S. aureus* genome with variations recognised by colonisation versus infection status, lineages, and antibiotic selection pressure (50,53,60,61). Observing the stepwise accumulation of SNPs allows the identification of closely related strains, establishing the directionality of transmission, as well as working backwards to identify the most common recent ancestor (53,56,60–62). Generally, >40 SNP differences between two isolates are considered unrelated (63).

WGS offers higher resolution compared to other typing tools. For instance, clones that appear indistinguishable by PFGE can often be differentiated through WGS analysis (59) since many SNPs can accumulate before one occurs in a *smalI* cleavage site, necessary for an isolate to be

recognised by PFGE as non-identical. Recombination events may lead to substantial changes in the core genome, causing two closely related isolates to appear significantly different. However, bioinformatic analysis of WGS results can detect these recombination events, maintaining the utility of SNP-based analysis (50).

WGS is not without limitations. It requires bioinformatics expertise and infrastructure, limiting its widespread availability. Other drawbacks include occasional inaccuracies due to errors in library preparation, sequencing, or bioinformatic analysis (59). SNP calling may be subjective, limiting interlaboratory comparison. The throughput and turnaround time, while better than some previous methods, often do not lead to provision of results in an actionable timeframe. The relatively high cost for WGS will likely come down with further automation and wider use. At present, WGS of *S. aureus* is not necessary for day-to-day clinical practice and decision making (64). More data are generated than necessary since the function of many of these DNA loci is unknown. The presence of resistance or virulence genes does not always correlate with the antibiogram or disease phenotype; phenotypic susceptibility testing remains the gold standard for directing antimicrobial therapy (49,65).

2.1.7 Concordance between various typing methods

There is generally a good concordance between PFGE, *spa* type, and MLST ST results (11,12,29,66). For instance, PFGE based result USA300-MRSA is predictably t008 on *spa* typing and ST8-MRSA on MLST (Table 1). Given that PFGE is no longer in common use, there is now more data on the correlation between *spa* types and MLST STs. Due to the lower discriminatory index of MLST, it is possible for multiple *spa* types to belong to one MLST ST. In other words, some *spa* types may correspond to more than one MLST ST. This correlation

is well illustrated in the Ridom *spa* server, where *spa* types and their corresponding MLST are shown (<https://spa.ridom.de/spatypes.shtml>).

Furthermore, while the correlation between *spa* type and MLST CCs is generally very high (>90% probability), the agreement between *spa* CC (BURP) and MLST CC (eBURST) varies (12,29,66). Though they both provide insights into the phylogenetic structure of *S. aureus*, the analysis of a single hypervariable locus in *spa* typing means there is likely a faster evolutionary clock unlike MLST where analysis of several essential housekeeping genes means the evolutionary clock is slower. Recombination events that can occur frequently in the *spa* gene are unusual in the house-keeping genes utilised in MLST (12).

Extrapolating one molecular typing result with another was previously essential for global epidemiology and for consistent reporting as labs were preferentially doing one or the other test. This issue is less relevant given WGS has become widely adopted and can infer results of all molecular typing methods with a single test.

2.2 Molecular epidemiology of *S. aureus*

2.2.1 MRSA

The early MRSA clones of the 1960s can be traced to the then-circulating phage group III or a closely related 83A complex MSSA. They share similar phage types, PFGE patterns, *spa* types, and antibiograms (resistance to penicillin, streptomycin, and tetracycline), suggesting a common lineage (45,67).

Conducting an analysis of the housekeeping genes through MLST has been essential in comprehending the evolutionary dynamics of MRSA. The MSSA of phage group III or a

closely related 83A complex were ST8-MSSA and distinct from the pandemic clone of the 1950s (phage 80/81 *S. aureus*, which was ST30-MSSA) (68). ST250-MSSA was a single locus variant of ST8-MSSA, which acquired SCCmec I to become the first MRSA, named the “archaic clone” (ST250-MRSA-I) (40,45). Today, ST250-MSSA or ST250-MRSA are exceedingly rare. However, a single locus variant of ST250-MRSA is ST247-MRSA-I, the second reported MRSA clone (Iberian clone) (40,45). ST247-MRSA-I remains in circulation today.

ST8-MSSA is a successful clone and is the founder of MLST CC8 (40). It acquired SCCmec complexes II and III to become ST8-MRSA-II and ST8-MRSA-III. A SLV of the latter led to ST239-MRSA-III (also known as the Brazilian clone, EMRSA-1, or Aus-2/3), a common HA-MRSA clone in Asia and Australia in the 1990s to 2000s. All ST239 *S. aureus* are MRSA, suggesting that it evolved from ST8-MRSA-III (not ST8-MSSA). ST8-MSSA is also the precursor of the well-studied CA epidemic clone, USA300 MRSA (ST8-MRSA-IV). ST8-MSSA was likely carried from Europe to the USA in the 20th century, where it acquired further genetic material, including genes encoding Panton–Valentine leukocidin (PVL) and SCCmec IVa to become the USA300 clone (69).

The transformation of MSSA into MRSA through the acquisition of SCCmec is an infrequent occurrence, with only a limited number of *S. aureus* clones undergoing this process (40,70–72). This is evident in the diverse clonal pool of MSSA, while the majority of MRSA can be grouped to one of the five major CC, namely 5, 8, 22, 30, and 45, with CC5 and CC8 being the most prevalent (Table 1) (36,40,45).

Table 1: The five major MRSA clonal complexes and common MRSA clones with their corresponding MLST type and *spa* types[^] (28,36,73)

CC5

New York/Japan clone or USA100: ST5-MRSA-II (t001, t002, t003)
Paediatric clone: ST5-IV (t002, t214)

CC8

Archaic clone* (the first MRSA clone): ST250-MRSA-I (t008)
Iberian clone* (the second reported MRSA clone): ST247-MRSA-I (t051, t008)
USA300: ST8-MRSA-IV (t008)
USA500: ST8-MRSA-II (t064)
EMRSA-1 or Aus-2/3 or Hungarian/Brazilian clone: ST239-MRSA-III (t030)
Queensland clone: ST93-MRSA-IV (t202)

CC22

UK EMRSA-15: ST22-MRSA-IV (t022, t032)

CC30

Southwest Pacific Clone: ST30-MRSA-IV (t021)
EMRSA-16 or USA200: ST36-MRSA-II (t018)

CC45

Berlin clone: ST45-MRSA-IV (t004)
USA600: ST45-MRSA-II (clonally related to Berlin MRSA clone) (t004)

Notes:

A common CA-MRSA clone in Europe (European clone), ST80-MRSA-IV (t044), belongs to CC80.

[^]*spa* type list for each MLST type is not exhaustive

***Archaic MRSA**: ST250-MRSA-I derived from ST8-MSSA (differing by a point mutation at a single MLST locus)

***Iberian MRSA**: ST247-MRSA-I is closely related to the archaic clone differing only by a point mutation at a single MLST locus.

Table 2: The major community-acquired (CA) and hospital-acquired (HA) MRSA reported in various parts of the world in the past 20 years (28,73–76)

Country	CA MRSA	HA MRSA
Australia	ST93-MRSA-IV	ST239-MRSA-III and ST22-MRSA-IV
USA,	ST8-MRSA-IV	ST5-MRSA-II
Canada		
UK, Europe*	ST80-MRSA-IV	ST22-MRSA-IV, ST36-MRSA-II
Asia*	ST59-MRSA-IV/V ST772-MRSA-V	ST239-MRSA-III and ST5-MRSA-II
Africa	ST30-MRSA-IV, ST88-MRSA-IV	CC8 (ST239 subgroup), CC5 (ST5), and CC30 (ST36)
Latin America	ST8-MRSA-IV	CC5 (ST5), CC8 (ST239 subgroup), and CC30

* Molecular epidemiology of MRSA in Asia and Europe is more heterogenous than shown

The distribution of the common CA and HA-MRSA clones differs by geographic locations (Table 2). One of the major distinctions between them is their associated *SCCmec* type. HA-

MRSA clones mostly carry *SCCmec* I, II, or III with one exception, a dominant HA clone EMRSA15 (ST22-MRSA) possess *SCCmec* IV. *SCCmec* I was the first SCC element to transfer to *S. aureus* (28). Most CA-MRSA have *SCCmec* IV or V. *SCCmec* IV is the smallest *SCCmec* complex and usually lacks antibiotic resistance genes other than those encoding for methicillin resistance (77). Examples include USA300 (ST8-MRSA-IV), the Queensland clone (ST93- MRSA-IV), the Southwest Pacific clone (ST30-MRSA-IV), and the Paediatric clone (ST5-MRSA-IV).

Livestock associated MRSA

The prevalence of livestock-associated *S. aureus* colonisation is higher in individuals with close animal contact (78,79). The emergence of new human MRSA clones or additional genetic determinants from zoonotic exposure and subsequent global spread is an ongoing threat (80).

One well-recognised livestock-associated clone reported in humans is ST398-MRSA belonging to CC398. This clone is commonly reported in Europe and North America. Another LA-MRSA, ST9, along with ST239, is also commonly reported in Asia, usually in China (28,79). LA-MRSA is infrequent in Australia but has been noted in pig handlers. Equine veterinarians in Australia have been found to carry equine-associated MRSA, specifically ST612-MRSA-IV (81).

ST398 *S. aureus* is not exclusively MRSA or livestock associated. CC398 also includes human MSSA and PSSA isolates (82–84). LA-MRSA is predominantly found in humans as colonising isolates rather than causing infections or transmissions. They lack immune evasion complex (IEC) genes, which are typical markers of human-adapted *S. aureus* (82,84,85). LA-MRSA is non-typeable by PFGE using the standard restriction enzyme *Sma*I (25).

2.2.1.1 The Australian context (MRSA)

ST239-MRSA-III (Aus-2/3 MRSA) was the dominant HA-MRSA clone in Australia from the 1970s until it was displaced by ST22-MRSA-IV in the 2000s (81). This latter clone, known as EMRSA-15 in UK, was first isolated in 1991 in Southern England (86) and subsequently spread to become a successful nosocomial pathogen in the UK. It was likely introduced into the Australian Health System by overseas HCWs from the UK (87), though it is unclear why it remains uncommon in the USA (86). The intercontinental spread of *S. aureus* is common due to human movements, but only certain clones are able to replace the pre-existing ones (53,81,88,89).

The diversity of HA-MRSA clones in Australian hospitals is notably restricted when compared to CA-MRSA clones (90,102,108). One prominent CA-MRSA clone in Australia is ST93-MRSA-IV, also known as the Queensland clone, initially identified in the early 2000s following a survey in Ipswich, a town outside Brisbane in Queensland (91). This clone carries genes encoding PVL and is susceptible to most non- β -lactam antibiotics. By 2008, ST93-MRSA-IV had become the predominant CA-MRSA clone in Australia (90). It likely originated from PVL-positive ST93-MSSA, initially detected as far back as the 1970s in the north of Western Australia. Over time, it acquired SCCmec-IV, evolving into ST93-MRSA-IV (92).

2.2.2 MSSA

The phage type 80/81 *S. aureus* was penicillin-resistant but susceptible to methicillin. As discussed in the preceding sections, the bacteriophage typing played a crucial role in understanding the pandemic spread of this clone. Subsequent investigations indicated that phage 80/81 *S. aureus* was likely circulating in low numbers for many years before gaining prominence in the 1950s (93). The MLST analysis identified it as ST30-MSSA, belonging to

CC30 (46), and it carried the genes encoding PVL (94,95). Interestingly, the contemporary clones ST36-MRSA-II (EMRSA-16: a common HA clone in the UK) and ST30-MRSA-IV (Southwest Pacific clone: a common CA-MRSA in Australia and New Zealand) also share a lineage with phage 80/81 *S. aureus* (ST30-MSSA) (46,95,96).

Over the past 30 years, molecular epidemiology has predominantly focused on MRSA due to its rapid emergence and widespread dissemination. Comparatively, there have been only a few studies on the molecular epidemiology of MSSA, consistently revealing a more diverse (polyclonal) population (36,75,97–101). Most MSSA strains can be categorised into ten CCs (<https://pubmlst.org>) (43). These are CC1, CC5, CC8, CC15, CC 22, CC 30, CC45, CC97, CC93 and CC121. Among the CC shared by MSSA and MRSA, there is often a disproportionate distribution of these two phenotypes (102). For example, in a community prevalence study involving 2,146 participants from remote WA communities (102), within CC1, ST1-MRSA-IVa constituted 42.7% of all MRSA, while ST1-MSSA formed only 1.3% of all MSSA. Similarly, within CC5, ST5-MRSA-IVa formed 13.5% of all MRSA, whereas ST5-MSSA comprised only 0.6% of all MSSA.

MSSA have not been reported to exhibit predominant CA or HA clones (99,103). In the 1940s and 1950s, PRSA (a MSSA) was primarily a HA pathogen (104–106). Over time, it gradually spread to the community and today, MSSA clones associated with both hospital and community settings are indistinguishable (101,103,107). A similar trend has emerged with MRSA, where CA clones now account for the majority of nosocomial MRSA infections (28,108).

2.2.3 PSSA

As previously discussed, the proportion and the overall infections due to MRSA has decreased since reaching peak in the 2008-2009. In contrast, the proportion of infection due to PSSA has increased in the recent years (109,110). In a 20-year (1997–2016) analysis of *S. aureus* clinical isolates collected by the SENTRY surveillance program (110), the proportion of PSSA amongst *S. aureus* has gradually increased from 14% in 1997-2000 to 26% in 2013-2016. In Australia, it increased from 17.5% in 2013 to 23.7% in 2020 (109). In the analysis of 440 PSSA bacteraemic isolates from the 2020 AGAR surveillance, these isolates distributed across 84 distinct MLST types. Of these, 79 out of 84 STs were grouped to 22 different CCs. The most prevalent CCs included CC5 (31.9%), CC97 (10.2%), CC45 (10.0%), CC15 (8.7%), and CC188 (4.9%). This diversity underscores that the increase in PSSA is not attributable to the expansion of a single clone (109).

The distribution of dominant clonal complexes (CCs) among PSSA varies across geographic locations (83,111,112). In China, for instance, the most common PSSA CCs were CC188 (17.14%), CC398 (15.71%), and CC5 (15.71%). The t189 *spa* type was the most prevalent, followed by t571, with nine out of 22 t571 isolates belonging to CC398 (83).

Similarly, in Spain, CC398 PSSA isolates accounted for 25 out of 151 (16.6%) PSSA bloodstream isolates (111). The CC398 PSSA group exhibited diversity, encompassing eight different *spa* types, with t571 (13/25) being the most common. Although CC398 is commonly associated with livestock MRSA, as illustrated, this clonal complex also harbours human *S. aureus* isolates (82,84,85). Notably, only two of the 25 CC398 isolates were potentially of animal origin (111).

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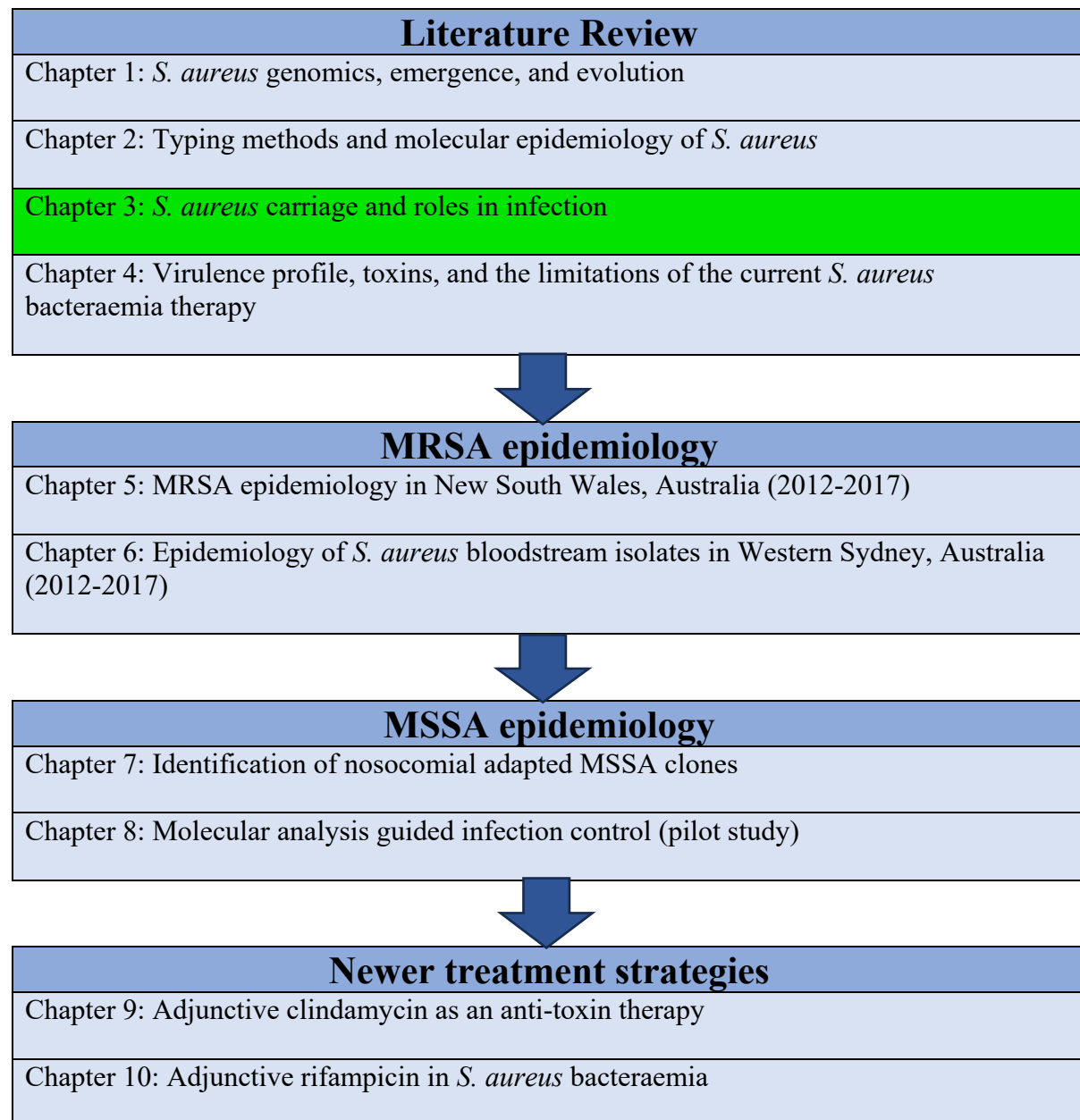
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Figure: Schematic overview of the thesis structure



Chapter 3: Overview

This chapter explores the phenomenon of human carriage of *S. aureus*. It discusses carriage frequency and patterns, explores the distinctions between MSSA versus MRSA carriage, and examines the factors influencing carriage and infection. Understanding these concepts is crucial for exploring the potential sources of *S. aureus* bloodstream infections, whether they originate from endogenous or exogenous sources. Application of this knowledge in preventing *S. aureus* bacteraemia in both healthcare and community settings is explored in this chapter.

This chapter comprises unpublished work.

Chapter 3: *S. aureus* carriage and roles in infection

3.1 Background

S. aureus is a leading cause of bacteraemia with a mortality of 16-20% at one month, and 22-33% at three months after onset (1). Mortality is higher in the elderly and in people with severe disease such as septic shock or infective endocarditis (2,3). *S. aureus* bacteraemia (SAB) is classified, for surveillance and management purposes, as either hospital-onset (HO), healthcare-associated community-onset (HACO), or community-acquired (CA). (4). HO SAB is defined as bacteraemia with onset >48 hours after hospital admission. HACO SAB is one with onset in the community or ≤48 hours after admission, in a patient who has had a recent significant healthcare contact (5). CA SAB is bacteraemia occurring ≤48 hours of hospital admission in the absence of recent healthcare contact.

HO infection could be exogenous when the source of the infectious agent is external to the patient, such as the hospital environment, equipment, a healthcare worker (HCW), another patient, or a visitor (4), or endogenous when the infectious agent arises from a body site

normally inhabited by the organism (4). However, HO SAB does not differentiate between exogenous or endogenous source of *S. aureus*. If a patient develops SAB >48 hours after hospital admission, it is assumed that healthcare interventions led to the SAB episode.

S. aureus carriage predisposes to infection, but most carriers do not develop bacteraemia (6–9). In adults, the average incidence of all types of SAB is 10-30 per 100000 person-years, whereas it is estimated that about a third of the world's population carry *S. aureus* (2,10–12). There is a disproportionate risk of SAB in MRSA compared to MSSA carriers (13). The incidence of SAB varies by age, geographic regions, and onset location (HO/HACO/CA) (10,11,14). Of all SABs, 20-40% are HO, 10-50% are HACO, and 30-70% are CA (2,3,15–21). In countries with a high prevalence of MRSA or where only MRSA SABs are reported, the majority are HO or HACO, while MSSA bacteraemias are more likely to be CA (19,22–24).

These data do not assist in identifying the sources of *S. aureus* responsible for individual cases of SAB, even in carriers. Therefore, a more detailed analysis is essential to comprehend the dynamics between carriage and infection. Ultimately, understanding the source of SAB is crucial for developing prevention strategies, which are discussed in this review.

3.2 *S. aureus* carriage

S. aureus colonisation is dynamic, with a complex interplay between the bacterium and the host (25,26). Carriage of *S. aureus* in healthy people has been recognised since the 1930s, after the coagulase test was adopted to discriminate *S. aureus* from other staphylococcal species. The nose, specifically the area 1-2cm inside the anterior nares (6), was identified as the principal site of colonisation. This conclusion was drawn based on the higher frequency of *S.*

aureus isolation and significantly higher colony counts from nasal culture compared to swabs of exposed skin (6). The composition of the nasal microbiome (26–30) is an important determinant of successful *S. aureus* carriage (31–34). Carriage studies involving monozygotic and dizygotic twins have demonstrated that age, gender, lifestyle, and skin conditions are stronger determinants of carriage than host genetics (26,31,35,36).

Common extra-nasal *S. aureus* colonisation sites include hands, rectum/perineum, pharynx, and skin of hair-bearing areas of the chest, abdomen and forearm (37). MRSA is more likely than MSSA to colonise extra-nasal sites (38–42). Multisite colonisation often indicates a higher bacterial load and an increased risk of infection (37,43). The throat (44) or intestine (screened with rectal/perineal swabs) (41,45) can also serve as the primary (or only) colonisation site in some carriers (46). Exclusive throat carriage is more prevalent in younger age-groups (aged <30 years) (44).

3.2.1 Carriage prevalence

In a population-based study, in the USA in 2001/02, involving 9,622 individuals aged ≥ 1 year, 30% had MSSA and 0.8% MRSA nasal carriage (47). The prevalence was higher in males than females, except for those aged ≥ 70 years. The highest prevalence in this study was observed in the 6–11-year age-group, reaching approximately 45%. In a similar prevalence study in Europe, involving 32,206 individuals aged ≥ 4 years in 2010/11, 21.3% had MSSA, and 0.3% had MRSA nasal carriage (48). These rates are lower than the 30-50% carriage rates reported in the 1930s-1970s (6,37,49,50), which could reflect differences in the age, sex, or socioeconomic status of the populations screened rather than true variations in carriage rates (47,48,51,52). On the other hand, improvements in screening methods, such as use of non-inhibitory swabs (e.g., flocked or charcoal), enrichment culture, or polymerase chain reaction

(PCR) to detect *S. aureus*, enhance carriage detection (53–57). Similar differences between studies could also contribute to the observed variations in carriage prevalence by country (48) and in specific population groups (58,59).

S. aureus carriage does not vary with seasons, temperature, or humidity (52), but repeated exposures to an environment with high *S. aureus* burden (known as “colonisation pressure”) increase the chance of colonisation (52,60). Patients with long-term comorbidities and regular healthcare contacts have higher carriage rates, and the prevalence is generally higher in HCWs (6,37) and the household contacts of carriers (49,61). In newborns, the peak carriage prevalence occurs at one month of age (50-60%), gradually decreasing to approximately 20% at one year (62,63).

There is an inverse correlation between *S. aureus* carriage and SAB mortality (12,47,64,65). While the prevalence of carriage decreases with age, SAB rates and mortality increase, suggesting contributing factors other than colonisation. Although *S. aureus* carriers have a threefold higher risk of SAB than non-carriers (37), colonisation does not affect long-term mortality when controlled for socio-economic status and comorbidities (66).

3.2.2 Carriage pattern

Longitudinal studies have established three categories of *S. aureus* nasal carriers: persistent, intermittent, and non-carriers (7,37,67,68). The proportions in these groups depend on age, sampling frequency, sampling methods, and the duration of follow-up (63,69,70). There is no consensus definition; while some authors suggest classification into two categories, namely persistent and ‘others’ (71), the three-pattern classification remains widely used (72). Generally, those who regularly test positive on nasal screening (e.g., $\geq 80\%$ of times on

consecutive nasal cultures) are labelled persistent carriers (73–75), those who are positive at a lower rate are intermittent carriers, and those that have repeatedly negative results are non-carriers (37,67,73,76). Combining quantitative (density of *S. aureus* in nasal culture) and qualitative (positive or negative) culture results of two nasal swabs taken one week apart, can predict persistent carriage with a high reliability (73). The carrier index, defined as the number of positive nasal swabs divided by the total number performed on that individual, has also been used to define carrier types. Persistent carriers have a carrier index of ≥ 0.8 , intermittent carriers have a range of 0.1 – 0.7, and non-carriers have an index of < 0.1 (69). However, even “persistent carriers” can become non-carriers when followed for longer durations (69,76).

On average, 20% of the adult population are persistent carriers, 30% are intermittent carriers, and 50% are non-carriers (37,72). Cross-sectional studies pick up persistent and some intermittent carriers and occasional non-carriers who are transiently carrying the bacteria. Colonisation of multiple sites is a significant risk factor for persistent colonisation (77). These distinctions are important because persistent carriers, characterised by higher bacterial loads, are more likely to acquire *S. aureus* infection (including SAB), and serve as a significant reservoir for transmission to others. In contrast, intermittent and non-carriers share a lower risk of infection and shorter duration of carriage (71).

3.2.3. Varied patterns of *S. aureus* colonisation

S. aureus nasal colonisation is not homogeneous. For instance, in a study involving 88 healthy carriers, *S. aureus* was isolated from only one nostril of 53 participants (60%), with no predominance of either nostril (78). Another study, involving Māori and Pacific school-aged children in New Zealand, found that 40% (57/142) had discordant *spa* types at the nares and oropharynx (46). Co-colonisation with MSSA and MRSA can occur, often in separate nostrils

(78,79). Strain switching is more common than was previously estimated (74), with approximately 5% of adult carriers co-colonised with multiple strains of *S. aureus* (80,81). These varied patterns of colonisation may also reflect within-host bacterial evolution (82).

In a prospective study (74), nasal swabs were collected weekly over a 13-week period from 122 healthy young carriers. Intermittent carriers tested positive for a median of 4 weeks, while persistent carriers rarely returned negative swabs throughout the study period. However, changes in *spa* type occurred in 32% (6/19) of persistent carriers, suggesting that strain replacement occurs about once a year in this group. A larger prospective study (69) conducted in Oxfordshire UK, followed 360 individuals, whose nasal swab cultures were positive for *S. aureus* (<1% with MRSA), and 211 non-carriers at recruitment, for a median of 2 years. Bimonthly screening swabs were collected, with a median of 14 swabs per participant. Acquisition and loss of *S. aureus spa* types were common in both groups; the median duration of carriage of a newly acquired *spa* type was 2 months. Changes in *spa* types were more frequent in younger subjects and those whose initial screening was negative, compared with persistent carriers (69).

3.3 *S. aureus* bacteraemia

3.3.1 Endogenous versus exogenous sources of *S. aureus*

The association between *S. aureus* carriage and invasive infection has been recognised since the 1940s (7). Unlike earlier studies, which were conducted in specific patient groups or used poorly discriminatory strain typing methods, the studies by von Eiff *et al* (8) and Wertheim *et al* (9) systematically evaluated this association in a general-patient population. Both studies were hospital-based and prospective. Single nasal screening swabs were performed for each

patient on admission (9), after the identification of SAB (8), or as a routine surveillance culture (8).

In the first part of the study by von Eiff *et al* (8), nasal swabs were obtained from the anterior nares of 219 patients with SAB. The nasal isolate matched blood culture isolates in 180 of 219 patients (82.2%). The likelihood of matching strain types did not vary significantly according to the time between the detection of bacteraemia and the collection of the nasal swabs. In the second part of the study (8), 1,640 patients with *S. aureus* identified on routine surveillance culture over five years were followed for the development of SAB, which occurred in 14 patients. The nasal isolate matched blood isolate in 12 of 14 patients (86%). Eleven of 14 SAB cases occurred within two weeks of collection of nasal swabs.

In the study by Wertheim *et al* (9), 24% of 14,008 non-surgical patients screened at admission were *S. aureus* carriers, with only 0.03% of isolates being MRSA. SAB (all due to MSSA) developed in 40 out of 3,380 carriers (1.2%) and 41 out of 10,547 non-carriers (0.4%). In *S. aureus* carriers who developed SAB, isolates from nasal swabs and blood were indistinguishable by pulsed-field gel electrophoresis (PFGE) in 80% (32/40) of cases, indicating an endogenous origin. More than half of all SABs (53% in carriers and 56% in non-carriers) were central venous catheter (CVC) related, with no significant difference in time to onset of SAB between the two groups (11 versus 16 days, $P=0.22$). However, among patients who developed SAB, non-carriers had a significantly longer mean duration of hospitalisation (50 versus 25 days, $P=0.01$), all-cause in-hospital mortality (46% versus 18%, $P=0.005$), and *S. aureus* related in-hospital mortality (32% versus 8%, $P=0.006$) than carriers. In a logistic regression analysis, only negative carrier status (OR 0.2) and presence the of a CVC (OR 4.7) were independently associated with all-cause mortality (9).

The observation of higher mortality in non-carriers may only apply to MSSA colonisation/infection. In a study similar to Wertheim's (9) but involving only MRSA and more frequent screening, the odds of SAB were much higher (OR 19.89) in patients colonised with MRSA than in non-carriers (83). Of the 29,370 individuals in the study, 3,261 (11%) were MRSA carriers. SAB occurred in 32 out of 3,261 (1%) MRSA carriers and 13 out of 26,109 (0.05%) non-carriers. All MRSA bacteraemias in this study were HO or HACO. Individuals identified as MRSA carriers and non-carriers, who developed bacteremia, showed no significant difference in 30-day or 1-year all-cause mortality (83).

Are the apparently non-endogenous *S. aureus* strains acquired during hospitalisation? In both the von Eiff and Wertheim studies, 15-20% of SAB in nasal carriers and all episodes in non-carriers were apparently of non-endogenous origin (8,9). However, the true proportion of exogenous sources of nosocomial SAB in carriers is probably much less. It is likely that colonisation of peripheral sites correlates better than nasal carriage as an endogenous source of invasive *S. aureus* isolates (84). In the von Eiff study, strain similarity between blood isolates and peripheral site isolates was higher than with nasal isolates (94.3% versus 82.2%) (8). Furthermore, negative nasal swab cultures at admission could be due to a false-negative result (53,54,57,85), intermittent carriage (37), or primary colonisation at other body sites (44,45) rather than true non-carriage (69).

3.3.2 MSSA versus MRSA: colonisation and infection

About 20-30% patients are colonised with *S. aureus* at hospital admission (9,37); the majority are colonised with MSSA rather than MRSA (47,48). MRSA colonisation is more likely in patients with recent hospitalisation, nursing home residence, previous exposure to healthcare-associated pathogens (MRSA, *C. difficile*, vancomycin-resistant *Enterococcus* sp.), and

comorbidities such as congestive cardiac failure, diabetes, pulmonary disease, immunosuppression, and renal failure (86).

Colonisation at admission largely determines whether any subsequent hospital-onset *S. aureus* infection will be due to MSSA or MRSA. In a retrospective review of hospitalised patients, MRSA caused 92% (552 out of 601) of *S. aureus* infections in MRSA carriers, compared to 8% (42 out of 516) of infections in MSSA carriers (OR 0.008, $P < 0.0001$) (87). The lower odds of MRSA infection in MSSA carriers were irrespective of age group, gender, length of stay, or whether the infection was associated with bacteraemia. In non-carriers (44% of the study population), 49% (430 out of 882) of *S. aureus* infections were due to MRSA, which reflects the ratio of MRSA (30%) and MSSA (26%) carriage prevalence among patients in the study hospital (87). In the Wertheim study, discussed above, all SABs in non-carriers were due to MSSA in a setting in which MRSA carriage was infrequent (9).

The risk of HO MSSA BSI among MSSA nasal carriers is threefold higher than in non-carriers (9). In contrast, the risk of HO MRSA BSI in MRSA nasal carriers is up to 20-fold higher than in non-carriers (83). In a head-to-head comparison, MRSA carriers have a four times higher risk of developing SAB than MSSA carriers (88,89). In a prospective study (13), which screened 758 patients at admission, 3.5% (26/758) were MRSA carriers and 18% (137/758) MSSA carriers. Clinical *S. aureus* infections occurred within one year in five (19%) MRSA carriers (one of whom had SAB), two (1.5%) MSSA carriers (one SAB) and 12 of 595 (2%) non-carriers (two SABs). SAB rates were too low to estimate the odds ratios, but overall, MRSA carriers had a 10-fold higher risk of developing a clinical infection than the MSSA carriers or non-carriers (13).

Increased virulence is unlikely to account for the higher infection rate in MRSA carriers compared with MSSA carriers. Instead, MRSA carriers are often older, sicker, have a longer hospital stay, and have more comorbidities (13,90). The majority of MRSA bacteraemias are healthcare-associated (2). Host differences also account for higher mortality in MRSA bacteraemia (65). After adjustment for major covariables (e.g., age and illness severity) there is no significant difference in all-cause or SAB-related mortality, or infection-related hospital readmission (91).

Except in countries where rates of MRSA colonisation and infection are very high, HO MSSA BSIs are more common than HO MRSA BSI. MRSA has added to, rather than replaced, the overall burden of *S. aureus* infection (92). However, MSSA and MRSA do compete for colonisation. For example, MRSA acquisition was 78% less likely in MSSA carriers than in noncarriers in one study (79), and MSSA carriers and non-carriers are significantly less likely to develop MRSA infection compared to those with known MRSA colonisation (87,93).

Antimicrobial use has a direct impact on *S. aureus* carriage. Selective pressure may promote MRSA rather than MSSA colonisation and infection. The proportion of HO *S. aureus* infections due to MRSA is disproportionally higher for MRSA versus MSSA colonisation prevalence in hospitalised patients (89). Most antibiotics used in clinical practice have some activity against MSSA but not MRSA, and can lower the bacterial load in MSSA carriers (94,95). β -lactam antibiotics and fluoroquinolones can significantly increase nasal MRSA bacterial load and, therefore, can increase environmental dispersal and spread to others (96,97). Fluoroquinolone use is also associated with persistent MRSA carriage, which may increase the risk of MRSA infection (93,98). In the absence of antibiotic exposure, the carriage burden of *S. aureus* is generally stable (97).

3.3.3 Does *S. aureus* genotype determine colonisation or infection?

It is generally recognised that colonisation with one strain of *S. aureus* protects against acquisition of another (6,7). This phenomenon has been labelled ‘bacterial interference’ (99,100). However, colonising strains can change, even in persistent carriers (74,101); this is more likely in MRSA than MSSA carriers (102).

Since most people are *S. aureus* non-carriers (~50% of the adult population) and the majority of carriers do not develop SAB (103), risk factors for invasive infection have been examined by comparing colonising and invasive strains of *S. aureus* (104, 105,106). The Wertheim *et al* study in 2004 (9) noted a higher mortality in non-carriers who developed SAB, which may be partly explained by higher levels of anti-staphylococcal antibodies in carriers (71). *S. aureus* virulence factors related to clonal complex (CC) could also contribute (107,108). In a follow-up study (106), the authors reported a similar distribution of CCs among invasive *S. aureus* strains in carriers and non-carriers, who developed SAB. Nonetheless, in both groups, *S. aureus* strains belonging to a CC had a higher mortality than those infected with singleton strains not belonging to a CC (20/69 (29%) versus 1/25 (4%), $P<0.05$).

The links between *S. aureus* CCs and the risk of an infection or death are conflicting, apart from an increased risk of invasive disease associated with CC 30 (101,105–107,109,110), to which the historically notorious, virulent phage type 80/81 *S. aureus* belonged (111). Overall, genetic analysis of carriage and invasive strains has usually found them to be indistinguishable (101, 112), suggesting that any colonising *S. aureus* strain has the potential to cause an invasive infection (113,114).

3.3.4 Role of hospital environment and HCW in HO SAB

Nosocomial infections caused by *S. aureus* are not only frequent (115,116) but also disproportionately more common and fatal among older age patients compared to their younger counterparts (117,118). Nosocomial bacteraemia accounts for 10-14% of all HO infections, and the proportion of HO bacteraemias that are due to *S. aureus*, has increased over time (117,119–121). A comprehensive 20-year analysis (1997-2016) from the global antimicrobial surveillance program found *S. aureus*, was responsible for 21.3% of nosocomial bacteraemia incidents (22).

Regional variations exist in the rates of HO SAB and the proportion caused by MRSA or MSSA (11). About a quarter of MSSA BSI (3,16,122–125) and 25-45% of MRSA BSI (16,122,123) are of nosocomial onset. HO SAB is particularly important as it is a potentially fatal, but often a preventable disease (118,126).

S. aureus demonstrates the ability to persist for days to weeks on dry inanimate surfaces and for months on plastic surfaces. Both MSSA and MRSA exhibit comparable survival times on various surfaces (127,128). The origins of nosocomial *S. aureus* transmission events are sometimes identifiable, yet in non-outbreak settings, these occurrences are typically multifactorial. Price *et al*, employed whole-genome sequencing (WGS) to investigate transmission dynamics in an intensive care unit (ICU) (129). During the 14-months study, 97 patients acquired *S. aureus*, with seven (7%) attributed to HCW carriers, two (2%) linked to the environment, and 16 (17%) with other patients. Sources of the remaining 74% of acquisitions remained unknown. This suggests that HCWs and the environment are relatively uncommon sources of cross-transmission. However, their roles may have been underestimated due to infrequent screening of HCWs and patients, as well as the use of only nasal swabs for

screening (130). Transiently contaminated hands or fomites are likely the usual, undetected sources of *S. aureus* transmission and vectors for patient-to-patient transmission (131).

In their study, Price *et al* (129) demonstrated the extensive genetic diversity of *S. aureus* isolates through WGS, indicating that multiple introductions from the community may play a more pivotal role in nosocomial infections than direct cross-transmission within hospital settings. Notably, even during outbreaks, HCWs are seldom identified as the source of transmission, especially when they are asymptomatic (132,133).

The occurrence of patient-patient intrahospital transmission of *S. aureus* is reported with variability (131). In a comprehensive study conducted in Houston, USA, 398 isolates (48% MRSA, 52% MSSA) obtained from 305 consecutive patients with *S. aureus* infection were analysed over a 6-month period using WGS (134). A noteworthy observation was that 60% of the isolates were multilocus sequence type (MLST) ST8 or ST5, with substantial diversity among the remaining isolates. Strains with an average pairwise distance of <40 single nucleotide polymorphisms (SNPs) were considered related. Intriguingly, the investigators did not find any evidence of patient-patient cross-transmission (134). However, the absence of evidence of patient-patient transmission may stem from the analysis solely focusing on isolates from clinical infections, excluding carriage sites. Some investigators have proposed a more conservative cut-off of 15 core genome SNPs to exclude cross-transmission within the previous 6-months (135).

Contrary to the above finding, two concurrent Irish studies, one focusing on MRSA (136) and the other on MSSA (137), revealed frequent transmission events involving HCWs and patients. *S. aureus* isolates were obtained from nasal swabs of both patients and HCWs, clinical isolates

(site not specified), and environmental samples within the hospital. The investigators employed WGS with core genome sequence based MLST (cgMLST) to delineate strain relatedness, which analyses 1821 core genes, in contrast to conventional MLST targeting seven housekeeping genes (138). Closely related isolates were defined as those with allelic differences of ≤ 24 cgMLST (136,137). While the study did not report single nucleotide polymorphism (SNP) analysis of the core genome, it is worth noting that genomic variations identified by cgMLST and SNP analysis occur at similar rates: 2.0–5.0 alleles per year for cgMLST analysis and 3.8–5.8 SNPs per genome per year for SNP-analysis (139,140). A potential limitation in these studies should be acknowledged, as the use of a conservative criterion to identify allelic differences (≤ 24 , instead of ≤ 15) and the two-year duration of the study may have led to the inadvertent clustering of some transmission events (135).

In hospitals with varying levels of endemicity, MRSA transmission typically involves the simultaneous circulation of multiple strains. Within this context, cycles of importation of community strains into hospitals and the exportation of nosocomial strains into the community, thereby contributes to *S. aureus* epidemics (141–143). The introduction of a new *S. aureus* strain into a hospital can be facilitated by a colonised or infected patient, HCW, or visitor. Once introduced, the strain has a potential to disseminate to other HCWs, patients, or the hospital environment. The constant movement of busy HCWs within the hospital can contribute to a wide circulation of these strains between environmental surfaces and patients (131,144,145). Although the source of nosocomial *S. aureus* outbreaks can sometimes be traced to a specific person, event, or object, it often remains elusive.

3.4 Interventions (Prevention of *S. aureus* bacteraemia)

3.4.1 Decolonisation

Since colonisation of *S. aureus* can lead to infections, efforts have been made to eliminate carriage as a means of reducing HO *S. aureus* infections. The preferred method involves the application of intra-nasal mupirocin, often concurrently with antiseptic (e.g., chlorhexidine) body wash (146). A typical 5-day course of mupirocin is highly effective in clearing nasal carriage, but its impact is often temporary (147–149). Unfortunately, many individuals are recolonised within two months (150–152). Selectively employing this approach in high-risk settings and for short durations has been advocated to minimise the risk of mupirocin resistance (153–155).

Mupirocin resistance rates in *S. aureus* exhibit geographical variations and are linked to the over-the-counter availability of mupirocin, along with its use in skin infections (156). A 2020 meta-analysis revealed global mupirocin resistance rates of 7.6% in MSSA and 13.8% in MRSA clinical isolates. Rates of high-level resistance (defined as minimum inhibitory concentration of $\geq 512 \mu\text{g /mL}$) were approximately 8.5% in both MSSA and MRSA (157). Decolonisation failure is associated with high-level resistance, while low-level resistance may only temporarily suppress growth, subsequently failing to prevent an infection (149,156). In Australia, mupirocin is available only on prescription and rarely used to treat skin infections. High-level mupirocin resistance is observed in only 1.1% of *S. aureus* blood culture isolates in this country (16).

In general, attempts to decolonise MSSA carriers are typically aimed at preventing infection in the colonised patients themselves, while in MRSA carriers, the objective is often twofold: benefiting the colonised patient and reducing cross-transmission. Decolonisation, with a 5-day mupirocin course, tends to be more successful in MSSA than MRSA carriage, with clearance ranging from 80-90% versus 50% (147,149,158,159). This discrepancy may be attributed to

the fact that MRSA more frequently colonises extra-nasal sites and exhibit a higher rate of mupirocin resistance (55,94,156–160).

Several factors may enhance the success of MRSA carriage clearance, including repeated decolonisation attempts (158), adherence to guidelines (159), testing and treating household contacts (159), or opting for a longer course of mupirocin (152). although unnecessarily prolonged courses may increase the risk of mupirocin resistance (161). As alternative short-term strategy to prevent HO SAB is daily antiseptic bathing of patients with 2% chlorhexidine. Clinical trials, mostly conducted in ICU settings, have yielded mixed results in preventing HO infections, including catheter-associated BSIs, and have contributed to higher rates of chlorhexidine resistance (162–165). Some recommendations propose using 4% chlorhexidine concentration and limiting use to ICUs with high rates of baseline HAIs (166).

Oral antibiotics such as rifampicin, cotrimoxazole, doxycycline or clindamycin, have been used to augment carriage clearance (159,160). A randomised controlled trial involved adding a 7-day course of oral rifampicin (300mg twice daily) and oral doxycycline (100mg twice daily) alongside 2% chlorhexidine gluconate body washes and intranasal application of 2% mupirocin ointment. This led to increased MRSA decolonisation, with 74% of participants maintaining negative MRSA cultures at three months post-decolonisation compared to 31% in the control group ($P=0.001$) (167). It is also suggested that rifampicin may be more effective in eliminating MSSA than MRSA carriage (168). Factors such as drug-toxicity, drug-interactions, and the risk of developing resistance are key reasons to avoid routine use of oral antibiotics. Additionally, individuals treated with clindamycin for skin and skin structure infections (SSTI) have shown both earlier and persistent clearance of *S. aureus* carriage (77,169).

Oral antibiotics for the purposes of decolonisation are not recommended in routine practice (146), rather, this approach is advised for complicated MRSA carriage cases, specifically those associated with active skin infection, failure of prior decolonisation, primary extra-nasal carriage, or mupirocin resistance (159).

There are no controlled trials evaluating oral care (e.g., chlorhexidine gargle) to clear throat carriage.

3.4.2 Preventing hospital-onset (HO) SAB

The factors contributing to HO SAB are multifactorial. In the presence of robust infection prevention and control measures, HO SAB is an infrequent event. Notably, single interventions often fail to demonstrate significant impact on reducing rates. In *S. aureus* carriers, interventions that increase the bacterial burden, such as prior antibiotic therapy or the presence of intravascular access devices, may lead to nosocomial infection with an endogenous strain (2). On the other hand, a non-carrier, susceptible due to predisposing risk factors, may temporarily acquire an exogenous strain in a high burden *S. aureus* hospital environment (“colonisation pressure”) leading to SAB. Crucial measures in reducing patient and hospital *S. aureus* load, and consequently preventing HO infections, including SAB, involve maintaining rigorous hand-hygiene, thorough disinfection and cleaning of the hospital environment, and judicious use of antimicrobials.

Between 2008 and 2015, there has been a reported decline in the rate of HO SAB in many regions, primarily attributed to a reduction in HO MRSA BSI, particularly notable in the age groups above 55 years (22,170–172). Factors contributing to this decline include enhanced MRSA surveillance, targeted intervention programs (involving screening, isolation, and

decolonisation), and improved compliance with hand-hygiene (171–173). Additionally, the decrease might be influenced by natural changes in the molecular epidemiology of MRSA, including the replacement of less fit clones (142,143,174,175). While the rates of HO MRSA BSI have stabilised since 2013 (170,172), the rates of HO MSSA BSI have not shown significant changes. Meanwhile there has been a notable increase in CA MSSA BSI in the past decade (170,172).

Many HO SABs are linked to infected vascular access devices. Strategies such as a bundled CVC management and accountability intervention (176–178), removal of unnecessary lines, and prompt initiation of antibiotic therapy or removal at the first sign of intravascular catheter site infection may have collectively contributed to the reduction of HO SAB rates (179,180).

Transmission-based precautions aimed at interrupting contact spread is commonly implemented to prevent HO infections, but the evidence supporting their benefits in non-outbreak settings is lacking. In an ICU, a cluster-randomised trial that involved the universal use of gloves and gown for all patient contact did not result in reduction of MRSA central line-associated bacteraemia or other nosocomial infections (181). Moreover, the discontinuation of contact precautions has not been associated with increased MRSA infection rates (182). Another randomised clinical trial using a stepped-wedge design with a bundled intervention to optimise hospital environmental cleaning also did not show a reduction in the incidence of SAB (183). These findings likely reflect the multifactorial nature of the drivers of HO SAB (184). Further, compliance with contact precautions by HCWs is often inconsistent (185), and, importantly, these precautions do not have an impact on endogenous carriage.

3.4.3 Preventing healthcare-associated community onset (HACO) SAB

HACO (also called “healthcare-associated outpatient”) infection is defined as a SAB of community-onset in a patient with recent significant healthcare contact. Often, HACO SAB and CA SAB are reported together as “community-onset”, or “community-acquired” or as “non-HAI” SAB (16,19,22). However, HACO and CA are distinct categories. HACO closely matches HO SAB in terms of patient group affected, source of infection, susceptibility patterns, and mortality rate (15).

HACO accounts for 10-50% of all SAB (15,17,18,24). The wide margin is due to differences in patient population examined, proportion of MSSA versus MRSA infections, and variations in the definition of “significant healthcare contact” in the various studies (Table 1).

The average inpatient length of stay in an acute care hospital is approximately 6 days (186,187). Consequently, new nosocomial acquisition and subsequent infections may only become apparent following discharge (188) with the majority of HACO infections becoming manifest within the first three to six months (189,190). Over time, the risk of infection decreases, as colonisation in people who are non-carriers on admission but acquire *S. aureus* during hospitalisation resolves. Interestingly, patients acquiring MRSA generally exhibit a quicker loss of colonisation than those acquiring MSSA (52). Various factors, such as antibiotic use, belonging to older age groups, residing in long-term care facilities, and the presence of wounds, ulcers, or skin conditions, are associated with delayed clearance of colonisation (191).

One study examining decolonisation of MRSA carriers identified at discharge showed a reduction in subsequent MRSA infections (192). The decolonisation protocol involved a five-day regimen consisting of twice-daily intranasal application of 2% mupirocin, daily bathing with 4% chlorhexidine, and mouthwash with 0.12% chlorhexidine. This regimen was repeated

Table 1: Healthcare-associated community-onset (HACO) infection definitions*

Culture was obtained in an outpatient setting or before the third calendar day of hospitalisation and had one or more of the following: 1. a history of hospitalisation, surgery, dialysis, or residence in a long-term care facility in the previous year, or 2. the presence of a central vascular catheter (CVC) within 2 days before culture	Dantes <i>et al</i> , 2013 (189)
Onset in the community (non-hospitalised patients or within 48 hours of hospital admission) associated with: 1. Hospitalisation in an acute care hospital for ≥ 2 days within the prior 90 days 2. Received dialysis or intravenous therapy (including chemotherapy) within the prior 30 days 3. Received wound care or specialised nursing care at home within the prior 30 days 4. Residence in a nursing home or other long term care facility	Friedman <i>et al</i> 2002, (15)
<i>S. aureus</i> bacteraemia detection within 2 days of admission and any of the following risk factors: 1. Transfer from another healthcare facility 2. Receiving chronic haemodialysis 3. Prior hospitalisation within 30 days 4. Currently on immunosuppressive medication and metastatic cancer	Shorr <i>et al</i> , 2006 (193)
<i>S. aureus</i> bacteraemia detection in blood culture collected within 48 hours after hospital admission and one or more of the following key clinical criteria are met: 1. It is a complication of the presence of an indwelling medical device (e.g., intravascular line, haemodialysis vascular access, cerebrospinal fluid shunt, urinary catheter) 2. Occurs within 30 days of a surgical procedure where the bacteraemia is related to the surgical site or 90 days for deep incisional/organ space infections related to a surgically implanted device 3. It was diagnosed within 48 hours of a related invasive instrumentation or incision 4. It is associated with neutropenia (neutrophils less $0.5 \times 10^9/L$) on two occasions contributed to by cytotoxic therapy	Clinical Excellence Commission, New South Wales, Australia, v3.3, 2021 (194)

*not an exhaustive list but to illustrate inconsistent definitions.

twice a month for six months, resulting in a 30% reduction in the rate of subsequent MRSA infections within one year (HR 0.70, 95% CI 0.52-0.96). The number needed to treat to prevent one MRSA infection was 30, and to prevent one hospitalisation was 34 (192). It is worth noting that a similar decolonisation approach for MSSA carriers has not been evaluated.

3.4.4 Preventing community-acquired SAB

Community-acquired SAB constitutes a significant portion of all SAB cases, ranging from, 30-70% (16–19). In CA SAB, MSSA accounts for the greater proportion of cases (24). Notably, there has been a gradual increase in the rates of CA MSSA bacteraemia over the past decade across various regions (170,172) coupled with a rise in the proportion of SAB due to penicillin-susceptible *S. aureus* (PSSA) (22,195).

Certain patient groups are identified as high-risk for CA SAB, including intravenous drug users (196), individuals with diabetes (2), those in low socioeconomic and specific ethnic groups (197,198), and individuals with eczema and other skin conditions, including wounds or ulcers (2,199). Mitigating predisposing conditions and improving community education and awareness may help limit CA SAB.

While CA SAB is relatively underexplored in research, further investigations should be prioritised. Community MSSA strains serve as a reservoir that can contribute to transmission in both the community and in healthcare settings. Interventions targeting individuals aged >55 years who are persistent carriers are likely to have the most significant impact in reducing SAB.

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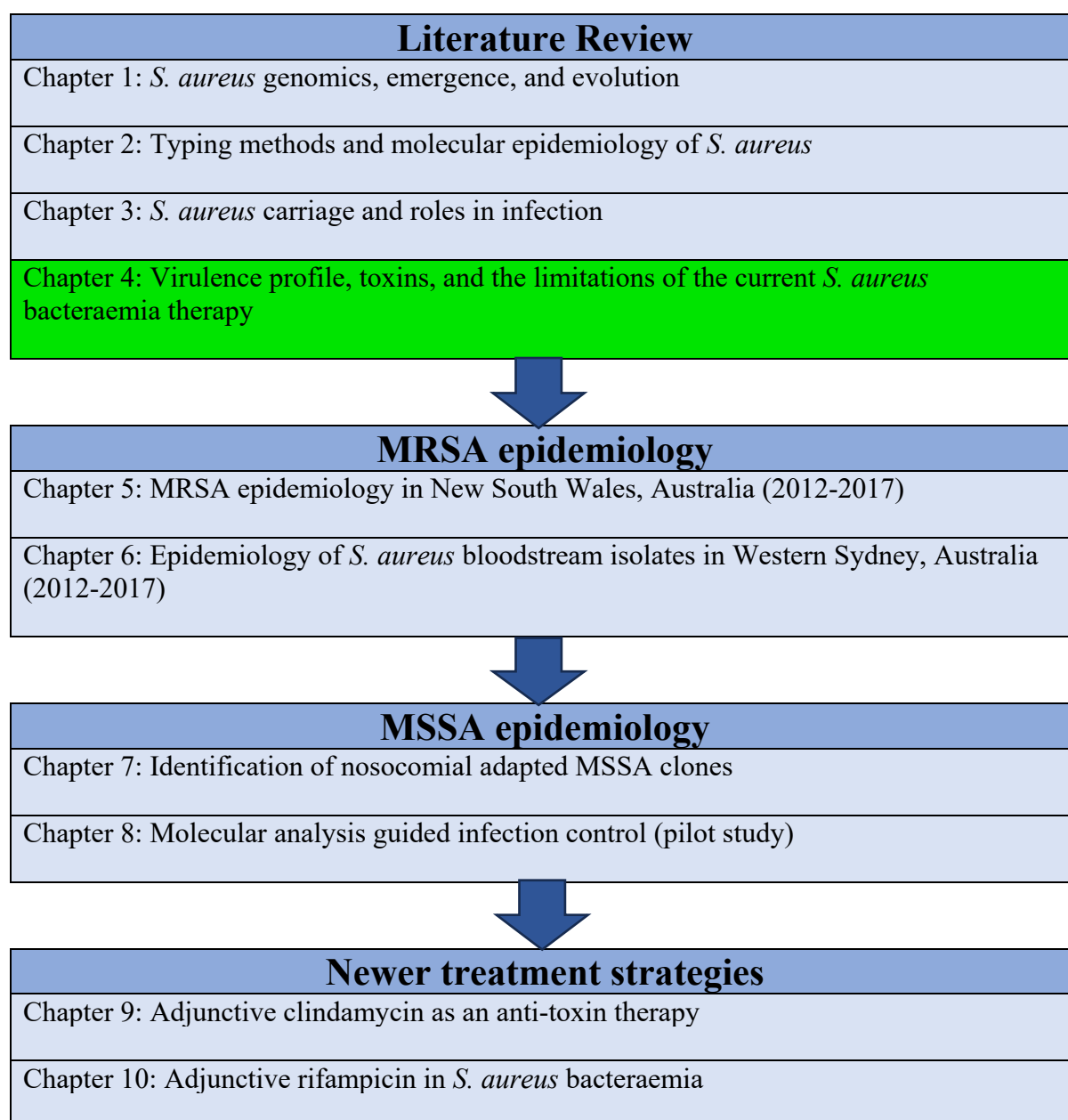
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Figure: Schematic overview of the thesis structure



Chapter 4: Overview

In this chapter, we explore novel approaches to the management of *S. aureus* bacteraemia while critically assessing the limitations of existing treatment approaches. We delve into the role of exotoxins in *S. aureus* bacteraemia and discuss the use of protein synthesis inhibitor antibiotics. This approach aims to counteract toxin production, thereby limiting pathogenesis in the context of SAB.

This chapter includes published work (included in Section 4.4).

The research titled “Protocol for Clindamycin Adjunctive Therapy for Severe *Staphylococcus aureus* Treatment Evaluation (CASSETTE STUDY)” was also presented as an abstract at the 18th International Symposium on Staphylococci and Staphylococcal Infection (ISSSI 2018). The resulting randomised controlled trial published in a peer-reviewed medical journal is included in the Appendix.

Chapter 4: Virulence profile, toxins, and limitations of the current *S. aureus* bacteraemia therapy

4.1 Virulence profile (including toxins)

4.1.1. *S. aureus* virulence

S. aureus stands out as a formidable pathogen. In the absence of antimicrobial therapy, bloodstream infection with this pathogen is almost invariably fatal (1). Even with antimicrobial therapy, mortality in SAB remains notably high, reaching up to 20% at one month and up to 33% at three months (2).

The Bundaberg tragedy of 1928 stands as an unfortunate event, shedding light on the virulence of *S. aureus* infection (3). On a single day in January 1928, 21 children received diphtheria

immunisation, resulting in a tragic death of 11 children within 24 hours, with another succumbing the following day. Additionally, six children were gravely ill but fortunately recovered, while three remained unharmed. Subsequent investigations revealed contamination of a vaccine vial with *S. aureus*, with use three days prior. The cause of death among the children was attributed to toxæmia resulting from staphylococcal exotoxins (3,4). During this period, no antibiotics had yet been discovered. Experiments with the toxins from ‘Bundaberg’ incident, and other *S. aureus* isolates, demonstrated their capacity to induce in vitro haemolysis, cause necrotic skin lesions upon intradermal injection, and lead to acute deaths in laboratory rabbits following intravenous injections (4).

4.1.2. *S. aureus* toxins

Most *S. aureus* isolates possess a variety of exotoxins, surface virulence factors, and exoenzymes (5,6). These include haemolysins, nucleases, proteases, lipases, hyaluronidase, and collagenase. Many isolates also harbour genes for toxic shock syndrome toxin-1 (TSST-1), exfoliative toxins, enterotoxins, and leucocidins (7–10).

S. aureus toxins can be categorised into two groups, namely, cytotoxins and superantigens (Table 1). *S. aureus* enzymes are additional virulence factors which cause tissue damage and assist the spread of infection (5). Cytotoxins are usually pore-forming toxins that disrupt host cells and help evade host immune response. Alpha-haemolysins (also called alpha-toxin) are cytolytic to human erythrocytes, whereas leucocidins are cytolytic to human neutrophils, macrophages, and monocytes. Exfoliative toxins are dermonecrotic. Superantigens are pyrogenic toxins that bypass a usual process of antigen presentation. They cause a clonal expansion of the T-cell population and a massive release of cytokines, contributing to the clinical picture of acute illness, fever, capillary leak, shock, and multiorgan failure (Table 2).

Table 1: Known *S. aureus* exotoxins (5,7,10,12)

Cytotoxins	• Haemolysins (alpha*, beta, gamma) • Delta haemolysin[^] • Leucocidins
Superantigens	• TSST-1 (enterotoxin F) • Enterotoxins (A-E, G) • Enterotoxin-like toxins (H, I, J-X) • Exfoliative toxins (epidermolytic toxins A and B)

*Alpha-haemolysin is also known as “alpha-toxin”.

[^]A representative exotoxin of phenol soluble modulins cytolytic

Table 2: Roles of *S. aureus* exotoxins

Toxin	Roles
<u>Haemolysins</u>	
Alpha (Alpha-toxin) (7,23–25)	Lyses erythrocytes; dermonecrotic; a key virulent factor in <i>S. aureus</i> infection
Beta (26,27)	Non-pore forming; hydrolyses sphingomyelin and lyses monocytes
Gamma (27)	Haemolytic to rabbit erythrocytes; cytolytic to leucocytes (neutrophils, monocytes, granulocytes, and macrophages)
Delta (Phenol soluble modulins) (28–30)	Haemolytic to erythrocytes, various organelles, bacterial protoplasts, and spheroplasts; lyse neutrophil post-phagocytosis; contributes to biofilm formation
Leucotoxins* (PVL) (31–33)	Lyses leucocytes; associated with skin and soft tissue infections, severe pneumonia in young adults and severe osteoarticular infections in children
Enterotoxins (34)	Food borne illness; vomiting and diarrhoea; SEB associated with non-menstrual Staphylococcal toxic shock syndrome
TSST-1 (34, 58)	Menstrual and non-menstrual Staphylococcal toxic shock syndrome
Exfoliative toxins (A and B) (28,35)	Causes staphylococcal scalded skin syndrome in neonates, infants, and adults with renal impairment and immunodeficiency

* Other leucotoxins includes lukDE and lukAB. PVL (LukSF) is much more potent than other leucotoxins.

S. aureus pathogenesis is due to a combination of virulence factors, including exotoxins (11).

For example, the highly successful common CA-MRSA clone in the USA, ST8-MRSA-IV (USA300), is believed to owe its virulence to an array of factors. These include increased expression of alpha- and delta- toxins, the presence of *S. aureus* pathogenicity island 5

containing *sek* and *seq* genes (these encode the superantigens enterotoxin K and enterotoxin Q, respectively), arginine catabolic mobile element (ACME) type I carrying the arginine deiminase cluster “*arc*” and *speG* gene, along with genes expressing Pantón-Valentine leukocidin (PVL) (13,14).

PVL is encoded by the *lukS-PV* and *lukF-PV* genes, which are transmitted through bacteriophage, and its presence tends to be lineage-specific (15,16). Although initially reported in 1932 (17), PVL gained prominence in the 1990s, especially when identified in CA-MRSA clones associated with severe infections in a younger population without prior healthcare exposure (18,19). Prominent PVL carrying strains include USA300 (in USA), ST80-MRSA-

Table 3: Prevalence of PVL in Australia (*S. aureus* blood culture isolates):

<i>S. aureus</i> (references)	PVL positive, % (n/total isolates tested)	Year	Notes
PSSA (36)	0.6% (3/470)	2020	
Adult MSSA*	4.4% (12/272)	2011- 2015	Results from Chapter 7 of this thesis
Paediatric MSSA (37)	15% (45/301)	2017- 2018	
MRSA (38) (Healthcare acquired) - Adults	0% (0/71)	2021	ST22-MRSA-IV was most common (64 isolates)
MRSA (38) (Community acquired) - Adults	38% (152/401)	2021	ST93-MRSA-IV was most common (95%, 94/99 were PVL-positive), whereas the second most common clone, ST45-MRSA-IV, were all PVL-negative (0/62)
Paediatric MRSA (37)	60% (31/52)	2017- 2018	23/52 MRSA BSI were from ST93- MRSA which is almost always PVL- positive. Most cases were community onset SAB (70/76).

*PVL-positivity in MSSA blood culture isolate is rarely reported in the published literature

IV (in Europe), ST772-MRSA-V (in India), and ST93-MRSA-IV and ST30-MRSA-IV in Australia (20,21).

PVL-positive MRSA is believed to have originated from PVL-positive MSSA (22). Interestingly, phage type 80/81 penicillin-resistant *S. aureus* also carried PVL, causing a disease spectrum comparable to that observed in PVL-positive CA-MRSA (3,39). For the prevalence of PVL-positive *S. aureus* in blood culture isolates in Australia, refer to Table 3.

The role of PVL in *S. aureus* is a subject of much debate. While not all community clones carry PVL (38), infection with PVL-carrying isolate is associated with chronic/recurrent skin and skin structure infections in young adults, necrotising pneumonia, and in children with osteoarticular infections (31–33). However, the presence of PVL does not determine treatment outcomes in MRSA or MSSA infections (40).

ACME is a phage-encoded mobile genetic element initially identified in the epidemic USA300 strain (ST8-MRSA-IV) (41). It appears to have been transferred to MRSA from *S. epidermidis* (41). ACME contributes to USA300 pathogenesis by enhancing its growth and survival in the human host (14,42). However, its exact role remains uncertain (44). ACME has also been detected in other MRSA clones (43).

4.2 Limitations of the current *S. aureus* bacteraemia therapy

S. aureus is the most frequent pathogen causing bacteraemia. In an analysis of over 264,000 bacteraemia cases collected over 20-year period (1997-2016) from 45 countries, *S. aureus* accounted for 20.7% of infections (45). It emerged as the most common pathogen causing bacteraemia across all age groups, except for those aged ≥ 65 years, where it ranked as the

second most frequent at 20.1%, trailing behind *E. coli* at 26.6%. It was also the most prevalent pathogen in hospital-onset bacteraemia, accounting for 21.3%, and was the second most frequent (22.4%) in community-onset bacteraemia, trailing behind *E. coli*.

It is estimated that more than 7,800 episodes of SAB occur annually in Australia, with a rate of 30 per 100,000 population, and approximately 1,500 of these episodes are healthcare-associated from Australian public hospitals (46). In the USA, around 120,000 cases of SAB occur each year (47). The proportion of MSSA or MRSA in SAB varies based on the local prevalence of MRSA. In Australia, as of 2021, 13% of SAB were MRSA, while 87% were MSSA (38). The proportion of penicillin-susceptible *S. aureus* (PSSA) within the MSSA group has increased in the recent years, both in Australia and globally (36,48). PSSA now accounts for approximately 25% of SAB cases (36,48).

SAB is almost invariably fatal without antimicrobial therapy (1,49). β -lactam antibiotics, such as flucloxacillin and cefazolin, serve as a first line therapy in MSSA bacteraemia (50), while vancomycin or daptomycin are the preferred agents for MRSA bacteraemia (50). However, the optimal therapy for PSSA bacteraemia remains uncertain. Observational reports suggest better outcomes when treated with benzylpenicillin (or Penicillin G) than with flucloxacillin or cefazolin (51). A randomised clinical trial is currently underway to address this question (52). Despite targeted antimicrobial therapy, SAB remains a serious infection. Mortality in SAB has declined in the past two decades, likely due to reduced rates of MRSA bacteraemia (2), increased clinician awareness (5,50,53), and infectious diseases consultation for SAB management (54). However, more than one in four patients still succumb within three months following SAB (2). In a systematic review of post-2011 studies, SAB related mortality at three months was higher at 31.4% (95% CI 24.9% - 38.8%) in MRSA bacteraemia compared to

MSSA bacteraemia, which stood at 26.3% (95% CI 21.8% - 31.3%), although the difference was not statistically significant (2). These rates are notably higher than in *E. coli* bacteraemia, the second most common pathogen causing bacteraemia, with a case fatality rate of 12.4% (55). Most studies included in the systematic review of mortality following *E. coli* bacteraemia reported death within 30 days or during the index hospitalisation, with only one study reporting 90-day mortality, at 8.5% (55).

In addition to high mortality, recurrence of SAB is also common. Recurrence typically occurs in the setting of inadequate duration of antimicrobial therapy, deep-seated infection, or a lack of adequate source control. It is observed in up to 10% of SAB cases (50,56,57).

A PubMed search using the terms “*Staphylococcus aureus*” and “bacteraemia”, filtered to display only randomised controlled trials (performed on 9 March, 2024) yielded 148 results with a total enrolment of less than 4,000 patients post-2000 (52), underscoring the need for prioritising research on improving the management of SAB and developing strategies to prevent it.

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4.4 Publication

Adjunctive protein synthesis inhibitor antibiotics for toxin suppression in *Staphylococcus aureus* infections: a systematic appraisal

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Adjunctive protein synthesis inhibitor antibiotics for toxin suppression in *Staphylococcus aureus* infections: a systematic appraisal

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Protein synthesis inhibitor antibiotics inhibit synthesis of new proteins, including exotoxins and other important virulence determinants in *Staphylococcus aureus*. A summary of the literature regarding the use of adjunctive protein synthesis inhibitors for toxin suppression in the setting of *S. aureus* infections is presented.

Introduction

Staphylococcus aureus is a Gram-positive bacterium that is among the most common pathogens in humans causing a broad spectrum of clinical manifestations.¹ Its ability to express multiple extracellular toxins, including haemolysins, leukotoxins, exfoliative toxins, enterotoxins and toxic shock syndrome toxin-1 (TSST-1), which destroy host cells and circumvent host immune defence mechanisms, contribute to its success as a pathogen.² Treatment of *S. aureus* infection routinely relies on cell-wall-active antibiotics, including β -lactams and glycopeptides, which do not attenuate toxin production. Indeed, *in vitro* and animal studies have demonstrated that β -lactams (at subinhibitory concentrations) can increase *S. aureus* toxin production^{3–5} and vancomycin has minimal effect (Figure 1).^{3,4}

The principal protein synthesis inhibitor (PSI) antibiotics that are active against *S. aureus* and reduce bacterial toxin production are clindamycin and linezolid. Other PSI antibiotics with antistaphylococcal activity include macrolides, chloramphenicol, fusidic acid and streptogramins (quinupristin/dalfopristin, pristinamycin), which all bind to the 50S ribosomal subunit, interfering with its functions of controlling guanosine triphosphate hydrolysis, the formation of peptide bonds and channelling the peptide through the subunit tunnel. Tetracyclines, tigecycline and aminoglycosides are PSI antibiotics that all bind to the 30S subunit and interfere with the principal function of decoding mRNA to peptides (Figure 1).^{3,6} Whilst the antibiotic mechanism of action has been established, evidence is conflicting regarding the mechanism of action (transcription, translation or both) PSIs have on toxin production.^{3,7} The resulting impact that antibiotic resistance has on *S. aureus* toxin production in laboratory *in vitro* studies is also inconsistent.^{8,9}

The addition of a PSI antibiotic to standard therapy (cell-wall-active antibiotic) may be used in the treatment of *S. aureus* infections to: broaden coverage during empirical therapy; achieve synergy; enhance tissue biofilm penetration; or limit toxin production.¹⁰ This review summarizes the current, available evidence on combination antibiotic therapy for suppression of toxin synthesis in the treatment of *S. aureus* infections, with a focus on the PSI antistaphylococcal antibiotics for which there is the most evidence, namely clindamycin, linezolid and gentamicin.¹⁰

Methods

Published and in press peer-reviewed journal articles in English were included from PubMed (1966–April 2017), the Cochrane Database of Systematic Reviews, Google Scholar and the ClinicalTrials.gov database. Search terms included 'adjunctive' OR 'combination' AND 'antibiotic' OR 'anti-bacterial' OR 'anti-infective' OR 'antimicrobial' OR 'protein synthesis inhibitor' AND '*Staphylococcus aureus*'. Two independent reviewers (A. J. C. and R. D.) assessed abstracts and titles for suitability. Full text articles were retrieved for further assessment (see Figure S1, available as [Supplementary data](#) at JAC Online). All clinical trials, observational studies, case series/reports and laboratory and animal studies were included if they involved *in vitro* or *in vivo* models of *S. aureus* infection, treated with adjunctive PSIs. Outcome measures examined included mortality, relapse, length of stay (LOS), adverse events, *S. aureus* toxin levels and/or genes responsible for *S. aureus* toxin production.

Results and discussion

PSI antibiotics

Clindamycin

Clindamycin is the most commonly used adjunctive PSI antibiotic.¹⁰ *In vitro* studies have demonstrated that subinhibitory

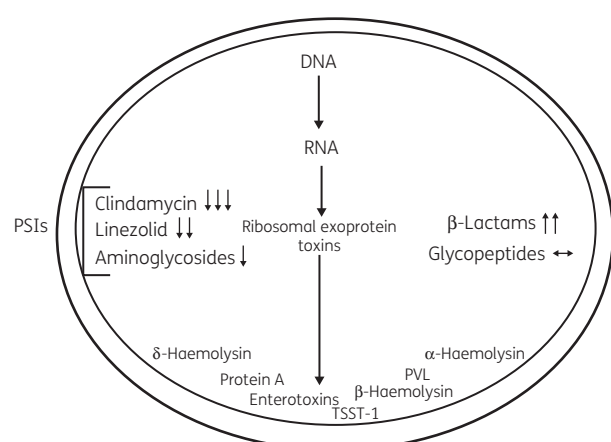


Figure 1. Proposed antibiotic effects on *S. aureus* exoprotoxin production based on *in vitro* and *in vivo* studies. Arrows denote antibiotic effects on *S. aureus* exoprotoxin levels: ↔ denotes a neutral effect, ↓↓↓ denotes a strongly inhibitory effect, ↓↓ denotes a moderately inhibitory effect and ↓ denotes a mildly inhibitory effect compared with controls. ↑↑=moderately increases toxin production.

concentrations (that do not inhibit bacterial growth) of clindamycin suppress exotoxins, including α -toxins (α -haemolysin), TSST-1, Pantone–Valentine leucocidin (PVL), β -haemolysin, δ -haemolysin and enterotoxins (Table 1).^{3,8,11–14} Clindamycin also down-regulates β -lactam-induced exotoxin production when given concurrently.^{12,15} Clindamycin is a derivative of the antibiotic lincomycin, which has also been shown to inhibit exotoxins in *S. aureus*.¹⁶ Clindamycin is preferred over lincomycin given its favourable pharmacokinetic profile and increased evidence to support its use as an antitoxin agent.^{17,18}

Clinical data on adjunctive clindamycin as antitoxin therapy in *S. aureus* infections is limited to case series and case reports.^{19–21} A single double-blind placebo randomized controlled trial (RCT) has been published, which targeted a clinical syndrome (cellulitis), but not specifically *S. aureus*.²¹ This trial assessed 48 h of adjunctive clindamycin in addition to standard therapy (flucloxacillin) versus standard therapy alone in adults with cellulitis.²¹ Importantly exclusion of patients with abscess was performed, indicating that *Streptococcus pyogenes* rather than *S. aureus* was the likely pathogen in most cases. Clinical improvement at day 5 was similar between combination and monotherapy groups (87% versus 81%, respectively, OR = 1.55, 95% CI = 0.81–3.01), with diarrhoea more common in clindamycin recipients (22% versus 9%, OR = 2.7, 95% CI = 1.41–5.07).²¹

Li *et al.*²⁰ retrospectively reviewed 92 cases of *S. aureus* necrotizing pneumonia and found that antibiotic therapy that included an antitoxin agent (clindamycin or linezolid) was associated with lower mortality ($P = 0.007$). Incomplete data, retrospective design and lack of severity-matched controls are limitations of this study. In another retrospective analysis of 269 patients with skin and skin-structure infections (146 culture positive: 70% MRSA, 15% MSSA), combination therapy with vancomycin and clindamycin was associated with a reduced LOS compared with vancomycin alone in a subgroup ($n = 134$) of patients with abscess (3.6 ± 1.5 versus 4.4 ± 2.3 days), OR = -0.82 (95% CI = -1.49 to -0.15), $P = 0.016$.¹⁷ In addition, the 90 day readmission rate was lower in

the combination group.¹⁷ Confounders, including age, diabetes, lack of vancomycin therapeutic monitoring data and a significantly higher proportion of those undergoing incision and drainage receiving combination therapy, limit the validity of the study. Conversely a retrospective case-control study examining 141 PVL-positive and 148 PVL-negative invasive *S. aureus* isolates found 30 day mortality was not influenced by adjunctive linezolid or lincosamide therapy [2.7% (PVL positive) versus 5.3% (PVL negative), $P = 0.534$].²² Selection bias and unmatched controls are limitations of this study.²²

Despite a lack of high-quality evidence with no clinical trials performed, some antibiotic guidelines support consideration of clindamycin in conjunction with standard therapy for severe toxin-mediated *S. aureus* infections, including staphylococcal toxic shock syndrome and necrotizing fasciitis.^{10,23} British and French guidelines addressing the management of PVL-positive *S. aureus* infection recommend the addition of an antitoxin agent, e.g. clindamycin, linezolid and/or rifampicin, when toxin-mediated staphylococcal infection is confirmed or suspected.^{23,24} Overall, there is a paucity of high-quality evidence to support adjunctive toxin suppression to improve clinical outcomes in *S. aureus* disease.^{22,25}

Summary The *in vitro* evidence indicates that adjunctive clindamycin can reduce toxin production. The current clinical evidence is weak and conflicting due to lack of high-quality clinical data. Well-designed clinical studies, including RCTs, are needed to further define its therapeutic role.

Linezolid

Linezolid is the first member of the antibacterial group oxazolidinones. *In vitro* and animal studies have shown that linezolid reduces the production of a range of *S. aureus* virulence factors, including enterotoxins A and B, PVL, TSST-1 and haemolysins (α , β and δ).^{26–30}

Linezolid for treatment of necrotizing staphylococcal pneumonia is appealing due to its enhanced tissue penetration and ability to inhibit *S. aureus* toxins. Small case series of PVL-positive necrotizing pneumonia detail a favourable clinical response and decreased toxin levels with the addition of antitoxin therapy (linezolid or clindamycin).^{19,31} However, these reports are confounded by the concurrent use of intravenous immunoglobulin, multiple other antibiotics and surgical interventions.^{19,31} Despite an RCT demonstrating that linezolid is as effective as vancomycin for the treatment of ventilator-associated pneumonia,³² there are no clinical trials assessing adjunctive linezolid therapy. Nonetheless, it is recommended for consideration as an alternative to clindamycin for severe *S. aureus* toxin-mediated disease.¹⁰

Summary The *in vitro* and animal model evidence indicates that adjunctive linezolid can reduce toxin production. High-quality clinical studies are needed to further define the therapeutic role of linezolid (and other newer agents in this class, e.g. tedizolid) as antitoxin agents for clinical infections.

Gentamicin

Gentamicin is a PSI that is biofilm-active and bactericidal.³³ Non-clinical and clinical experiments have therefore focused on these outcomes rather than *S. aureus* virulence factor suppression.³⁴

Table 1. Summary of exotoxin inhibition of PSI agents; clindamycin and linezolid in the treatment of *S. aureus* infection

	Type of study (first author, year)	Summary of outcomes measured
Antibiotic intervention	<i>In vitro</i> studies	Effect on SA toxin production or genes encoding toxin production
Subinhibitory clindamycin	(Otto, 2013)	Inhibition of SpA (encodes protein A) and concentration-dependent inhibition of protein A toxin production (clindamycin>linezolid). ¹³
	(Ohlsen, 1998); (Otto, 2013)	Inhibition of <i>hla</i> (encodes α -haemolysin) and α -haemolysin, ^{4,13} while methicillin increased expression of <i>hla</i> and α -haemolysin. ⁴
	(Schielvert, 1984); (Herbert, 2001); (Dickgiesser, 1987)	Inhibition of TSST-1. ^{5,8,16}
Subinhibitory clindamycin and subinhibitory linezolid	(Herbert, 2001)	Inhibition of SA exoproteins, including α - and δ -haemolysins and DNase. ⁸
	(Stevens, 2007); (Dumitrescu, 2007); (Otto, 2013)	Inhibition of mRNA PVL ¹³ and PVL toxin production compared with controls and β -lactam; ³ clindamycin>linezolid. ^{13,15} PVL production increased up to 2.5× by methicillin, which was suppressed when combined with clindamycin>linezolid. ¹⁵
Subinhibitory linezolid	(Bernardo, 2004) (Gemmell, 2002)	Reduced expression of α - and β -haemolysins, protein A ^{26,27} and coagulase. ²⁷
	Animal studies	Effect on SA toxin production or genes encoding toxin production and animal clinical outcomes
Linezolid versus vancomycin	rabbit MRSA necrotizing pneumonia model (Diep, 2013); murine PVL-positive pulmonary infection model (Yanagihara, 2009)	Reduced production of PVL and <i>hla</i> (encodes α -haemolysin) in the lungs of linezolid-treated rabbits compared with controls/vancomycin and improved survival outcome in linezolid- compared with vancomycin-treated rabbits ($P < 0.01$) and those not treated ($P < 0.001$). ²⁸ Reduced bacterial numbers and inflammation in lungs and improved survival rate at day 7 in the linezolid-treated group (100%) compared with the vancomycin-treated group (50%) or the control group (0%). ³⁰
Clindamycin versus ceftriaxone	murine SA sepsis model (Azeh, 2002)	Clindamycin group had higher survival than those treated with ceftriaxone after an intraperitoneal injection of 10^7 cfu of SA (58% versus 32%, $P = 0.015$). ¹⁸
Flucloxacillin versus clindamycin/flucloxacillin combination versus clindamycin/linezolid/flucloxacillin combination	murine abscess model (Turner, 2015)	Flucloxacillin/clindamycin combination was the most effective at decreasing leukotoxin activity, despite an increase in lukF-PV transcription, but the addition of linezolid conferred no additional benefit. ¹⁴
Vancomycin versus linezolid versus linezolid/vancomycin combination	rabbit MRSA endocarditis model (Chiang, 2003)	Vancomycin alone was more effective than linezolid alone or in combination with vancomycin resulting in a mean aortic valve vegetation bacterial count reduction of 6.93 log ₁₀ cfu/g compared with controls ($P < 0.05$). ⁴⁰
	Human studies	Effect on SA toxin production and human clinical outcomes
Clindamycin/vancomycin combination versus vancomycin	retrospective observational study ($n = 269$) (Wargo, 2015)	For the treatment of acute bacterial skin and skin-structure infections, combination therapy reduced the LOS in a subgroup of patients with abscesses (3.6 ± 1.5 versus 4.4 ± 2.3 days), OR = -0.82 (95% CI = -1.49 to -0.15), $P = 0.016$. 90 day readmission rate was lower in the combination group for acute bacterial skin and skin-structure infections overall. ¹⁷
Antibiotic therapy that included a PSI; lincosamide or linezolid	retrospective case-control study ($n = 141$) (Boan, 2015)	Invasive SA isolates (tissue/bone/joint/blood), 141 PVL positive/148 PVL negative: 30 day mortality was not influenced by adjunctive PSI therapy. ²²
Antibiotic therapy that included a PSI; clindamycin or linezolid	case series ($n = 93$) (Li, 2011)	For SA PVL-positive necrotizing pneumonia, antibiotic therapy that included an anti-toxin agent was associated with lower mortality ($P = 0.007$). ²⁰
Treatment included clindamycin/linezolid	case series ($n = 2$) (Pasquier, 2010)	Two cases of severe SA PVL-positive pneumonia received clindamycin/linezolid and intravenous immunoglobulin. Both cases survived; a decrease in PVL in sputum was shown. ³¹
Treatment changed to a PSI; linezolid then clindamycin	case report ($n = 1$) (Stevens, 2006)	Suppressed TSST-1 production <i>in vitro</i> demonstrated and favourable outcomes in a patient treated with linezolid then clindamycin with staphylococcal toxic shock syndrome. ²⁹

SpA, *S. aureus* protein A. SA, *S. aureus*.

The evidence for toxin suppression by gentamicin includes an *in vitro* model of combination gentamicin with flucloxacillin inhibiting *S. aureus* TSST-1 production by 75%; however, in comparison, clindamycin alone was more effective with a 95% decrease in TSST-1 production compared with controls.³⁵ Another *in vitro* model examining the production of exotoxins (α - and δ -haemolysins) in *S. aureus* showed suppression of these virulence factors by gentamicin.³⁶

Gentamicin's role as an adjunctive PSI remains limited, with a meta-analysis of three RCTs and a prospective study failing to demonstrate improved mortality or clinical cure (laboratory or imaging) when used in combination with β -lactams in the setting of *S. aureus* endocarditis.^{34,37} Increased risk of nephrotoxicity remains a concern^{34,37} and adjunctive gentamicin therapy is no longer recommended in the treatment of *S. aureus* bacteraemia or native valve infective endocarditis, due to these reasons.¹⁰

Summary Studies for the use of aminoglycosides in toxin-mediated *S. aureus* syndromes such as necrotizing pneumonia are lacking. It is unlikely that these will progress in the future, given the lack of demonstrated improvement in outcomes in RCTs for *S. aureus* endocarditis and adverse drug effects, such as nephrotoxicity, limiting their use.

Other PSIs

Other PSI agents that may be used for *S. aureus* disease include quinupristin/dalfopristin, pristinamycin, tetracyclines, tigecycline, fusidic acid, chloramphenicol and the macrolides. However, minimal clinical and experimental data, drug toxicity (quinupristin/dalfopristin, chloramphenicol),³³ antimicrobial resistance (macrolides),³⁸ treatment failure (tigecycline)³⁹ and inconsistent global availability (pristinamycin, fusidic acid) restrict their use as adjunctive PSIs in *S. aureus* infections. Rifampicin and quinolones are generally not considered true PSIs as they act at a DNA level to affect protein synthesis.⁶ Additionally, most studies examining adjunctive therapy with these agents focus on anti-biofilm activity, which remains outside the scope of this review. Clindamycin and linezolid are likely to remain preferred over other PSIs (Table 1).

Conclusions

Combination therapy involving standard treatment with an adjunctive PSI for toxin suppression may be considered in severe *S. aureus* infections, including toxic shock syndrome and necrotizing fasciitis.¹⁰ Supportive evidence for guidelines recommending the use of adjunctive PSIs for severe *S. aureus* toxin-mediated disease is currently limited to case reports, *in vitro* data, animal studies and analogies drawn from toxin-mediated streptococcal infections. Importantly, controlled trials are lacking. There are also potential disadvantages of combination therapy, including possible antagonism,⁴⁰ drug toxicity, unfavourable drug interactions and additional costs.³⁷ Observational studies are problematic given clinicians are more likely to prescribe combination therapy to sicker patients, impacting on the validity of reported outcomes. Equally, animal models and *in vitro* synergy testing have frequently failed to predict the results of subsequent clinical trials.⁴¹ Such discrepancy underscores the complexity and variety of different mechanisms involved in antibiotic modes of action.⁴¹ The transition of adjunctive PSI use into common clinical management of

S. aureus disease by some clinicians, supported by expert opinion,¹⁰ adds challenges for developing equipoise for clinical trials in this area. This issue was encountered when conducting trials for streptococcal toxic shock⁴² and may prevent trials for life-threatening diseases such as *S. aureus* toxic shock proceeding in the future. The need for further well-designed clinical studies to define the role of adjunctive PSIs, including the interaction with antibiotic resistance and how this impacts suppression of *S. aureus* toxin production, is critical to guide optimal treatment.

Transparency declarations

None to declare.

Supplementary data

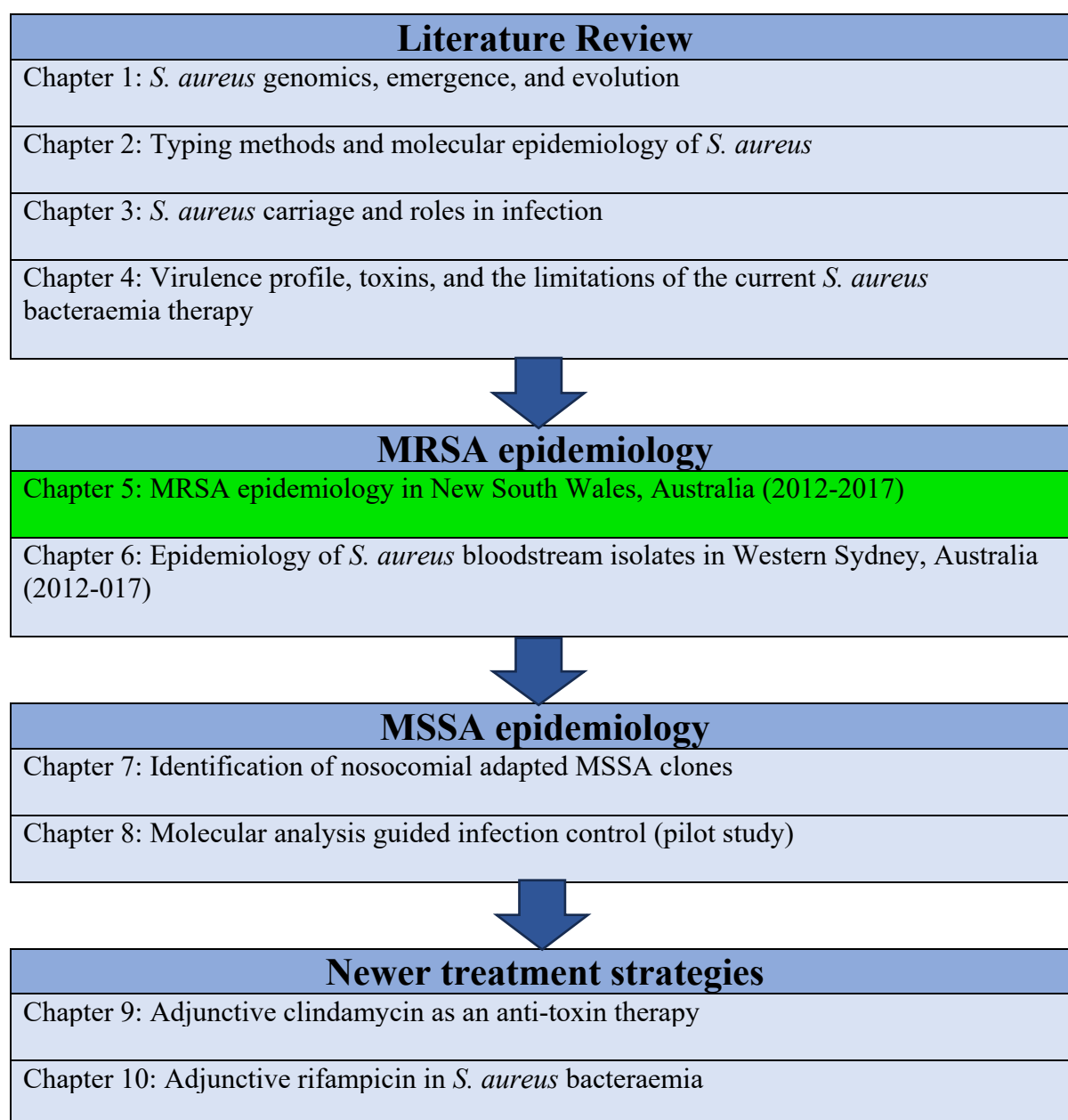
Figure S1 is available as [Supplementary data](#) at JAC Online.

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Figure: Schematic overview of the thesis structure



Chapter 5: Overview

The analysis of the molecular epidemiology of MRSA in Chapter 2 revealed that predominant clones varied based on geographic locations and it evolved over time. However, the employed approaches were not ideal, with gaps including delays in obtaining typing results, high costs associated with molecular typing of a large isolate collection, a general lack of data on the molecular epidemiology of MRSA beyond blood and skin infections, and limited micro-epidemiological applicability. To address these shortcomings, we conducted a large-scale epidemiological analysis, examining over 7,500 MRSA isolates collected from across New South Wales, Australia, over a six-year period (2012-2017).

Utilising an in-house molecular typing tool, specifically the 19-target MRSA binary typing, previously validated for its efficacy in MRSA outbreak investigations, we conducted simultaneous macro- and micro-epidemiological assessments of MRSA from blood, urine, sputum, screening swabs, and other invasive sites. The analysis not only investigates temporal changes in the distribution of predicted multilocus sequence types but also explores micro-epidemiological variations within these sequence types over time.

This chapter includes published work.

Chapter 5: MRSA epidemiology in New South Wales, Australia (2012-2017)

5.1 Introductory comments

The development of binary typing and its regular application in identifying transmission events during outbreak investigations has been previously outlined (1–4). In essence, it serves as a molecular typing tool utilising the multiplex polymerase chain reaction-based reverse line blot (mPCR/RLB) platform. The 19 binary typing targets consists of four toxic genes, nine phage-

derived open reading frames, and six *SCCmec* component genes. Among the targets detected in the binary typing system is a gene encoding Panton Valentine leukocidin (PVL) (1,4).

The primer selection is the most critical step for maximising discriminatory power of binary typing and to minimise competition between primer pairs during amplification (3). Two probes are allocated for each target on the membrane. A gene target is deemed present if either one target is strongly positive (in comparison to the positive control), or if both targets show weakly positive signal. In cases where no probe signal or only one weak signal is observed, the gene target is considered negative (1). The interpretation involves assigning a presence “1” or absence “0” status to each of the 19 genetic targets. Converting this 19-digit binary number to decimal yields the binary type (BT) of an isolate. For example, a binary number such as 0000011001110000000 translates to the binary type BT13184.

Binary typing of MRSA serves as an in-house tool with discriminatory power comparable to pulsed-field gel electrophoresis (PFGE). Binary typing can reliably predict multilocus sequence typing (MLST) sequence type (ST) and *spa* types, based on results obtained from isolates previously characterised by both methods (2,4). This facilitates inter-laboratory and international comparison of results. Additional advantages of binary typing include shorter turnaround time, cost-effectiveness, high throughput, and its applicability for both epidemiological and diagnosis purposes. Specifically, it enables detection of the presence of toxin genes, antibiotic resistant genes, and virulence genes (2,3).

5.2 Aims

Aims of the study were threefold:

1. To conduct a large-scale epidemiological surveillance using a molecular typing tool that is relatively cost-effective.
2. To describe potential changes in the molecular epidemiology of MRSA by analysing isolates from both sterile and non-sterile sites.
3. To analyse the micro-epidemiology of the major MRSA sequence types (ST) utilising the binary typing tool.

5.3 Publication

Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates in New South Wales, Australia, 2012-2017

Authors:

Dotel R, O’Sullivan MVN, Davis JS, Newton PJ, Gilbert GL.

Citation:

Dotel R, O’Sullivan MVN, Davis JS, Newton PJ, Gilbert GL. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates in New South Wales, Australia, 2012-2017. Infect Dis Heal 2019; 24: 134–40.

Contribution of authors:

I collected and cleaned up the data, conducted data analysis and interpretation, and prepared the manuscript.

MVNO and GLG provided data.

MVNO, GLG, JSD and PJN guided with data analysis and critical revision of the manuscript.

5.4 Remarks

Other outputs of this research (poster presentations) are included in Appendix. These include:

- i. R Dotel, GL Gilbert, F Zhou, MVN O’Sullivan. Utility of a 19-target binary typing method for large-scale surveillance of MRSA epidemiology (2012-2014). Presented at The Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), in collaboration with the International Congress of Chemotherapy, 15-17 September 2015, San Diego, USA (Abstract #C-1043)
- ii. R Dotel, GL Gilbert, MVN O’Sullivan. Routine prospective molecular surveillance of MRSA isolates (2012-2017). Presented at the Australasian Society for Infectious Diseases Annual Scientific Meeting, 10-12 May 2018, Gold Coast, Queensland, Australia (Abstract)

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Research paper

Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* isolates in New South Wales, Australia, 2012–2017

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Abstract *Background:* To better understand the molecular epidemiology of MRSA and to assess the utility of 19-target binary typing we undertook large-scale epidemiological surveillance of MRSA from invasive and non-invasive clinical specimens, and screening swabs.

Methods: Binary typing was performed on clinical MRSA isolates collected in New South Wales (NSW), Australia between 01/01/2012 - 31/12/2017. Binary type (BT) predicted multilocus sequence type (ST) and spa types based on results from isolates which had been characterised by both methods.

Results: 7624 MRSA isolates were analysed of which 3581 (47%) were wounds or skin & soft-tissue isolates (W/SSTI), 2436 (32%) screening swabs, 469 (6%) blood cultures (BC), 780 (10%) others, and 358 (5%) unknown. We identified 731 BTs, 54 spa types, and 31 STs. ST239 was the commonest MRSA clone in 2012 (30%), but it decreased to 7% in 2017 ($p < 0.001$). In contrast, $< 0.5\%$ of MRSA were ST45 in 2012 compared to 14% in 2017 ($p < 0.001$). An emergence of PVL-positive ST22 was also noted. Of all isolates, 28% (2122/7624) were lukS/PVL positive; the proportion, among prospectively collected isolates increased from 24% (1406/5858) to 33% (1933/5858) between 2012 and 2017 ($p < 0.0001$). 43% (1534/3581) W/SSTI, 20% (95/469) BC and 10% (239/2436) screening swabs were PVL-positive.

Conclusions: A major change in the epidemiology of MRSA was noted with a decline of ST239, an emergence of ST45 and PVL-positive ST22, and a significant increase in PVL-positive isolates. Binary typing can be a useful routine laboratory test for prospective molecular surveillance of MRSA colonisation and infection.

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Highlights

- Analysis of more than 7500 MRSA isolates collected over a period six years.
- Includes analysis of MRSA from screening swabs and non-invasive clinical specimens.
- Major changes in the molecular epidemiology of MRSA in NSW is described.
- A significant increase in PVL-positive MRSA isolates in NSW was noted.
- Supports the utility of binary typing as a routine laboratory tool.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common pathogen, which can cause asymptomatic colonisation or infections ranging from trivial to life-threatening [1]. It was first recognised in 1961 in the United Kingdom (UK) [2], and first isolated in Australia in 1966, in Sydney, from an overseas patient already known to be infected with MRSA [3]. However, it was not until the mid-1970s that MRSA became endemic in hospitals in the eastern States of Australia [4–6]. Western Australia noted its first case only in 1982 [7]. By 1987, MRSA accounted for 14.4% of *S. aureus* (SA) isolates from Australian teaching hospitals overall, but with notable variation between States [8].

In New South Wales (NSW) – the most populous State in Australia – MRSA accounted for 37% of nosocomial SA isolates in 2011 [9], 25% of all community SA isolates in 2012 [10], and 23% of SA blood culture isolates in 2015 compared to 18% of blood culture isolates nationally [11]. These rates are remarkably high compared to some European countries, where MRSA prevalence remains less than five percent [12] as a result of enhanced surveillance and control methods.

In this report, we describe large-scale epidemiological surveillance of MRSA isolates collected in NSW during the six-year period 2012–2017. Our aims were a) to analyse changing trends in the prevalence of MRSA multilocus sequence types (STs), through prospective surveillance of isolates collected from sterile and non-sterile sites, and b) to describe the micro-epidemiology of major MRSA STs, using binary typing.

Methods

MRSA isolates from clinical specimens and screening swabs were collected, prospectively, between 1st January 2012 and 31st December 2017 from patients whose specimens were referred to the Centre for Infectious Diseases and Microbiology (CIDM), Institute for Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, in Sydney. NSW has a population of 7.9 million (December 2017), and is divided into 15 local health districts (LHDs), each of which contains multiple hospitals. The majority of isolates were from Westmead and other hospitals in the Western Sydney

LHD (WSLHD), which were routinely strain-typed. We also included isolates referred, ad hoc, from 13 of the 14 other LHDs in NSW, and analysed separately.

Molecular typing, using a multiplex PCR and reverse line blot hybridisation (mPCR/RLB) assay, was performed at the CIDM. The method has been described in detail previously [13,14]. Briefly, strain types were determined by the presence or absence of 19 gene targets (oligonucleotide primers) – including toxin genes, phage derived open reading frames and *SCCmec* elements – expressed as a 19-digit binary number (0 = absent; 1 = present) converted to a decimal number e.g. binary number 000001100111000000 translates to binary type (BT) BT13184. The BT predicted multilocus sequence type (MLST) and *spa* type [13], based on results from isolates previously characterised by all three methods e.g. BT13184 corresponded to ST22 and *spa* type *t032*. MLST and *spa* typing (based on conventional typing or whole genome sequencing “WGS”) were performed on representative isolates of the common BTs (BTs with >10 isolates) and any isolates that were nontypeable by mPCR/RLB (Supplementary table A).

At Westmead Hospital, routine screening for MRSA was performed, as per the hospital’s screening policy, on admission and at least weekly using nasal and perianal/perineal swabs, in high risk settings, namely adult and neonatal intensive care, haematology/bone marrow transplant, renal transplant/haemodialysis units. The screening policy in Westmead has remained constant during the study period.

Patients’ age, specimen type, date of collection and body site were recorded. Clinical isolates (defined as isolates from all specimens other than screening swabs) collected more than 48 h after an admission to hospital were defined as healthcare onset (HO). Community onset, and healthcare-associated community onset information could not be distinguished. Repeat isolates from individual patients were only typed after an interval of at least six months, unless they were sterile site isolates, all of which were typed. If there were two or more isolates with the same BT from an individual patient within six months, the most clinically relevant was included for analysis (blood culture > other sterile site > non-sterile site > screening swabs). In a patient with multiple isolates from the same specimen type (e.g. multiple positive blood cultures), only the first isolate collected was recorded for analysis. Differences between categorical variables were analysed by

Table 1 Yearly distribution of 7624 MRSA isolates collected in New South Wales, Australia and included in this report.

LHDs	Total	2012	2013	2014	2015	2016	2017
WSLHD ^a (N, % of 5-year total)	5858	1081 (18)	1034 (18)	1019 (17)	827 (14)	910 (16)	987 (17)
ST239 ^b (N, % of WSLHD for year)	968 (17)	319 (30)	256 (25)	148 (15)	95 (11)	81 (9)	69 (7)
ST45 ^b (N, % of WSLHD for year)	456 (8)	4 (0.4)	25 (2)	87 (9)	103 (12)	105 (12)	132 (13)
Other LHDs ^c (N, % of 5-year total)	1766	291 (16)	257 (15)	672 (38)	123 (7)	385 (22)	38 (2)

Abbreviations - LHD: Local Health District; N: number of isolates.

^a Western Sydney LHD (WSLHD) is represented by two large acute hospitals – Westmead and Blacktown Hospitals. WSLHD isolates were prospectively collected. Only ST239 and ST45 shown.

^b Percentage for ST239 and ST45 indicates % of WSLHD isolates for the corresponding year.

^c Other LHDs (selectively referred): Nepean Blue Mountains LHD (NBMLDH: 424 isolates); Illawarra Shoalhaven LHD (ISLHD: 404 isolates); Sydney LHD (SLHD: 281 isolates); Hunter New England LHD (HNELHD: 233 isolates); Central Coast LHD (CCLHD: 190 isolates); Murrumbidgee LHD (MLHD: 119 isolates); Western NSW LHD (WNSWLHD: 40 isolates); South East Sydney LHD (SESLHD: 31 isolates); Northern Sydney LHD (NSLHD: 24 isolates); Southern NSW LHD (SNSWLHD: 9 isolates); South Western Sydney LHD (SWSLHD: 6 isolates); Northern NSW LHD (NNNSWLHD: 3 isolates) and Far West LHD (FWLHD: 2 isolates)]. No isolates were from available from Mid North Coast LHD (MNCLHD), which is a relatively small LHD with 3% of the state population (December 2017).

Table 2 Specimen type from which MRSA isolates were collected in NSW between 2012 and 2017.

Specimens	WSLHD ^a N (%) ^{c,a}	other LHDs ^b N (%) ^{c,b}	Total N (%) ^c	PVL-positive N (%) ^d
SSTI/wounds	2817 (48)	764 (43)	3581 (47)	1534 (43)
Screening swabs	2094 (36)	342 (20)	2436 (32)	239 (10)
Blood culture	303 (5)	166 (10)	469 (6)	95 (20)
Sputum	320 (5)	59 (3)	379 (5)	29 (8)
other invasive ^e	233 (4)	39 (2)	272 (3)	72 (26)
Urine	91 (2)	38 (2)	129 (2)	3 (2)
Unknown	—	358 (20)	358 (5)	150 (42)
TOTAL	5858	1766	7624	2122 (28)

Abbreviations - N: number of isolates; PVL: Pantone-Valentine leukocidin; SSTI: skin and soft tissue infections; LHD: Local Health District; WSLHD: Western Sydney LHD; NSW: New South Wales.

^a Prospectively collected isolates (Total = 5858 isolates).

^b Selectively referred isolates (Total = 1766 isolates).

^c Percentage of the column total shown.

^d Percentage of the total for each specimen type.

^e Other invasive includes tissue, aspirate, broncho-alveolar lavage, bone, line tip and drain samples.

Fisher's exact test or chi-squared tests as appropriate. P values of <0.05 were considered significant.

10 BTs accounted for 50% and 58% of isolates in WSLHD and in other LHDs respectively.

Results

We performed binary typing on 7624 MRSA isolates (Table 1). Of these, 5858 (77%) were from 5474 WSLHD patients, of whom 52% were aged >60 years and 6% were <15 years old. Eight hundred and twelve (22%) of the 3764 clinical isolates from WSLHD were HO (26% in 2012; 16% in 2017). There were 1766 (23%) referred isolates; patient ages were available for only 576 (33%) and, of these, 67% were from individuals aged >60 years. The majority of all isolates were from wounds or skin and soft tissue infections (W/SSTI) or screening swabs (Table 2).

Binary types (BTs)

731 BTs (623 from WSLHD and 308 from other LHDs) were identified amongst 7624 MRSA isolates. Twenty-six isolates (0.3%) had no target detected on mPCR/RLB (BT 0). The top

Predicted multilocus sequence types (STs)

Thirty-one STs were inferred from 6324 (83%) isolates. The remaining isolates (1300, 17%) belonged to 582 uncommon BTs for which a predicted ST was not available. ST22, ST93, ST239, ST45, ST30, ST1 and ST5 were the seven most common STs in both WSLHD and other LHDs, accounting for 75% of the isolates in WSLHD and 78% in other LHDs. Changes in major STs during the survey period (2012–2017) are shown in Fig. 1. ST distribution between different specimen types is shown in Table 3, and by age groups in Supplementary Table B.

Predicted *spa* types

Fifty-four *spa* types were identified from 6375 (84%) isolates. *Spa* type could not be inferred from BT for the remaining 1249 isolates, which belonged to 582 uncommon BTs for which *spa* typing had not been performed. Of the

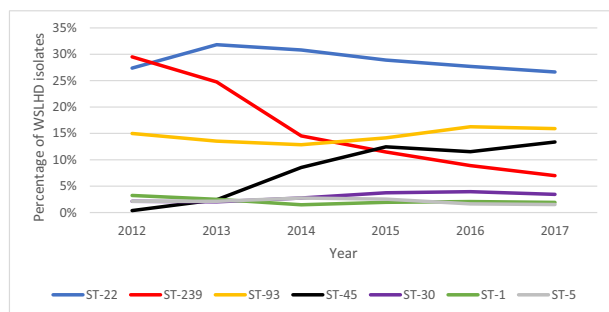


Figure 1 Changes in proportions of common MRSA multilocus sequence types (STs) between 2012 and 2017 at Western Sydney Local Health District. *Analysis of prospectively collected 5858 MRSA isolates (includes isolates from all body sites). The isolates from the other LHDs are not included as yearly trend would be unreliable given these are selectively referred. Note: Decrease in ST239 MRSA (red line), and emergence of ST45 MRSA (black line) (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.).

identified *spa* types, t032, t037, t202, t1081 and t515 were the five most common in both WSLHD and other LHDs, accounting for 62% of the isolates. The number of different BTs within each of these five commonest *spa* types ranged from 3 to 22. Distribution of the common BTs within these *spa* types, among WSLHD and other LHD isolates, were similar.

Panton-Valentine leukocidin (PVL)

lukS is one of two genes encoding PVL and one of the 19 targets used to determine a BT (other toxin targets were staphylococcal enterotoxins A [*sea*], C [*sec*] and D [*sed*]) [13]. Of the 7624 MRSA isolates, 2122 (28%) were *lukS*/PVL positive; the proportion, among prospectively collected

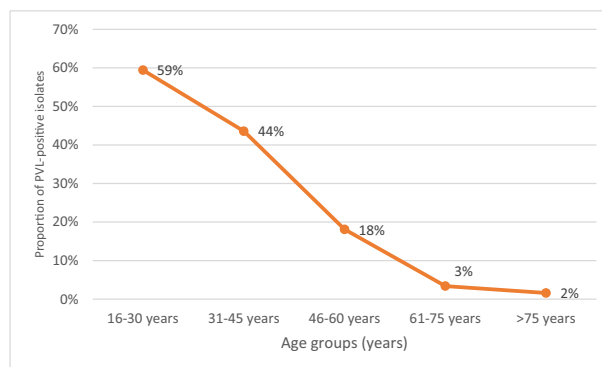


Figure 2 Distribution of *lukS*/PVL-positive MRSA isolates in adults, by age-group, among 6434 isolates collected in NSW between 2012 and 2017. *Age 15 or under excluded as it was not a true representative sample (43/48 isolates were from those aged <1 year). Age not available for 1190 isolates, 30% were PVL-positive. Abbreviations - PVL: Panton-Valentine leukocidin.

WSLHD isolates, increased from 24% to 33% between 2012 and 2017 ($p < 0.001$). Proportions of PVL-positive isolates differed between isolates from different age groups (Fig. 2) and in specimen types (Table 2). PVL-positive isolates were unevenly distributed between STs (Supplementary Table C – also includes results for *sea*, *sec* and *sed*).

Micro-epidemiological analysis of major STs in WSLHD

- i. **PVL-positive ST22:** Of 274 (of 2156; 13%) PVL-positive ST22 isolates, 223 (81%) were from WSLHD patients. In 2012, BT37248 (17; 52%) and BT45441 (13; 48%) were identified with similar frequency among 33 PVL-positive ST22 isolates in WSLHD. Since then BT37248 has become the predominant PVL-positive ST22 MRSA strain: 45 of 49

Table 3 Distribution of MRSA multilocus sequence type (ST) between 7266 isolates^a from different specimen types (all LHDs).

ST	Wounds/SSTI (N = 3581)	Screening swab (N = 2436)	Blood culture ^b (N = 469)	Sputum (N = 379)	other invasive ^c (N = 272)	Urine (N = 129)
N (%) for each column						
ST22	887 (25)	839 (34)	123 (26)	121 (32)	71 (26)	35 (27)
ST93	863 (24)	80 (3)	53 (11)	17 (4)	43 (16)	1 (1)
ST239	346 (10)	473 (19)	88 (19)	121 (32)	60 (22)	22 (17)
ST45	372 (10)	237 (10)	40 (9)	37 (10)	18 (7)	39 (30)
ST30	169 (5)	18 (1)	7 (1)	2 (1)	4 (1)	—
ST1	67 (2)	67 (3)	18 (4)	5 (1)	6 (2)	3 (2)
ST5	59 (2)	58 (2)	11 (2)	6 (2)	4 (1)	3 (2)
others	231 (6)	197 (8)	48 (10)	17 (4)	25 (9)	7 (5)
NA	587 (16)	467 (19)	81 (17)	53 (14)	41 (15)	19 (15)

Abbreviations - ST: multilocus sequence type; others: other STs; NA: ST undetermined; LHD: Local Health District; WSLHD: Western Sydney LHD.

^a Specimen type was not known in 358 isolates.

^b 65% (303) blood culture isolates were from WSLHD, of which 22% (68/303) were ST239. An annual decrease in the rate of healthcare onset MRSA bacteraemia was primarily due to a decrease in ST239 bacteraemia: 2012 (13/20); 2013 (14/18); 2014 (8/11); 2015 (7/11); 2016 (2/3); 2017 (2/5).

^c 'other invasive' includes tissues, aspirate, broncho-alveolar lavage, bone, line tip and drain samples.

(92%) isolates in 2017 vs three (6%) of BT45441. The majority of PVL-positive ST22 MRSA isolates were not HO (177/223; 79%).

Patients' ages were not available for 38 (14%) of 274 PVL-positive ST22 isolates. Of the remaining 236 isolates, 36 (15%) were from neonates (35 from screening swabs; one from a W/SSTI swab); 161 (68%) from patients aged 16–60 years and 27 (12%) from patients aged >60 years. Overall, 174 (64%) were from W/SSTI, 65 (24%) from screening swabs, nine (3%) from blood cultures and nine (3%) from other specimens. Specimen type was not available for 17 (6%) isolates.

A similar, but a less marked, change in proportions of PVL-negative ST22 strains was noted in WSLHD; of the two most common BTs, BT13184 increased from 45% (117/263) in 2012 to 58% (124/214) in 2017, while BT78720 fell from 16% (43/263) to 3% (6/214) in the same period.

ii. **Decline in ST239:** A total of 968 ST239 isolates were collected from WSLHD patients, in decreasing numbers, between 2012 and 2017 (Table 1) across all age groups. This was most notable in the predominant BT280845 which fell from 42% (134/319) in 2012 to 16% (11/69) in 2017 ($p < 0.001$). During the same period, the overall number of different BTs fell from 16 to seven. ST239 was isolated in similar proportions from screening swabs (437/968; 45%) and clinical isolates (W/SSTI 292; 30%; blood cultures 68; 7%). Overall, 46% (244/531) of ST239 clinical isolates were HO, but the proportion significantly decreased between 2012 and 2017 (80/160; 50% and 14/45; 31% $p = 0.024$) (Table 3).

iii. **Rise of ST45:** A total of 456 ST45 isolates were collected in WSLHD during the study period. In contrast to ST239, the numbers increased progressively (Table 1). ST45 included nine BTs, of which three (including BT1296 – see below) represented 86% of ST45 isolates. It was isolated particularly from patients >60 years (369/456; 81%) and from both screening swabs (170/456; 37%) and clinical isolates (W/SSTI 200; 44%; blood cultures 27; 6%). Overall, 25% (71/286) of ST45 clinical isolates were HO.

ST45 in Illawarra Shoalhaven (IS)LHD. Among 402 consecutive MRSA isolates referred from ISLHD over 17 months (November 2013 to March 2015), 225 (56%) were ST45. Of these, 92% (206) were BT1296; 24% (55) were from screening swabs, 60% (136) from W/SSTI and 16% from specimens including only 1% [3] from blood cultures.

Discussion

Among over 7000 MRSA isolates collected in NSW in 2012–2017, there was a significant decline in ST239 MRSA, an emergence of ST45 and PVL-positive ST22 MRSA isolates, and an increase in the overall proportion of PVL-positive isolates. The findings indicate a major change in the molecular epidemiology of MRSA in our region, with a shift to non-nosocomial acquisitions. A decline of ST239 MRSA, a typical nosocomial strain, was not associated with a decline

of another typical nosocomial MRSA (mainly PVL negative ST22; also known as EMRSA 15) (Fig. 1).

We included MRSA isolates from screening swabs and both invasive and non-invasive clinical specimens. Although the majority of isolates were from a single LHD, 14 of the 15 NSW LHDs were represented (Table 1) and covered the full range of age groups. We believe that such a large geographically diverse sample provides a comprehensive molecular epidemiological picture of MRSA in NSW, although proportions within various categories are reliable only for the prospectively collected isolates from WSLHD. The findings are consistent with other epidemiological studies in NSW [11,15], which have also shown a decrease in ST239 MRSA isolates overall, and frequent isolation of community strains from younger individuals. Furthermore, a prospective study like ours, with a consecutive and a comprehensive collection of MRSA isolates from all body sites, and a timely availability of results, augments findings of selective MRSA surveys such as those conducted by the Australian Group on Antimicrobial Resistance (AGAR) [11,16].

The high discriminatory power of this typing system [13], results in a large number of BTs. Moreover, binary typing can reliably identify MRSA clones, STs and *spa* types for most isolates [13,14], which allows comparison with other studies. Half of all isolates belonged to 10 BTs and two-thirds to four STs. MLST, *spa* typing, pulsed field gel electrophoresis (PFGE) and WGS are more commonly used methods to define MRSA clones. However, costs, availability and the time to results remain a limiting factor for these methods [17,18]. Binary typing is rapid, high-throughput and inexpensive (AU \$2.5 per isolate); its discriminatory power is similar to that of PFGE and much greater than MLST or *spa* typing [13]. Binary typing results are available within an actionable timeframe (usually less than a week). We have used it routinely at WSLHD since 2012 to identify MRSA transmission events, facilitate outbreak investigations and monitor the effectiveness of infection control interventions [13,19,20].

Our report of a decrease in ST239 MRSA corresponds to trends elsewhere [21,22]. Replacement of the common MRSA clones is well recognised [17,23,24], but the reasons remain unclear and is likely multifactorial including the natural MRSA cycles possibly due to selection pressure in healthcare environments [17,23], enhanced hospital infection control programs [21]), differential ability to persist in the environment as reservoirs or in humans as colonisers [25], or due to climate/environmental factors as noted by geographical differences in MRSA clones [22,25].

PVL-positive isolates increased in number and proportion between 2012 and 2017 due to both the decline in ST239, which is generally PVL-negative, and the emergence of PVL-positive ST22 MRSA.

PVL-positive ST22 MRSA is believed to have arisen, independently of PVL-negative ST22, from a PVL-positive ST22 MSSA precursor [17,26]. It is an uncommon pathogen outside India [27], but has caused nosocomial outbreaks in other countries [25,28]. WSLHD has a large population of residents born in India. In 2016, 6% of WSLHD residents were born in India compared to 2% in all of NSW [29]; 39% (55,706/143,459) lived in WSLHD. Thirteen percent of our ST22 isolates were PVL-positive - 9% in 2012 compared to

19% in 2017. In WSLHD, they were first noted in the neonatal intensive unit, in 2011, where two fatal cases of sepsis were followed by a prolonged outbreak of nosocomial transmission, due to a predominant BT (BT37248) [19]. Since then PVL-positive ST22 belonging to several BTs have been detected in 11 of the 14 LHDs.

Our study is not without limitations. 77% of isolates were from a single LHD and a significant proportion was from screening swabs from patients in high acuity wards where screening is done routinely. However, the screening policy in WSLHD was consistent during the study period and all clinical and screening isolates were collected prospectively and typed routinely. Only a few isolates were available from children aged over 1 month. There were also few isolates from private laboratories, and only six LHDs referred >100 isolates, which represented varied proportions of the total. Therefore, the distributions of STs and BTs in different categories may not be representative geographic areas outside Western Sydney and those results must be interpreted as such.

Despite these limitations, these comprehensive data from WSLHD - as one of the most populous LHDs in NSW - offers a representative epidemiological picture, and addition of isolates from other LHDs allowed us to compare results, which were broadly consistent, for a more complete view of the molecular epidemiology of MRSA in NSW. However, further research is necessary to explore the differential ability of the two nosocomial MRSA strains (ST239 and PVL-negative ST22) to persist in a hospital environment. We speculate that ST239 is more susceptible to infection control activities such as alcohol-based cleaning and hand-hygiene, and a loss of fitness has resulted in its decline. Long term care facilities remain a major reservoir of PVL-negative ST22 [8]. Also, an increase in non-nosocomial acquisitions of MRSA and an increase in PVL-positive isolates in younger individuals suggests a need to increase community awareness on MRSA.

In conclusion, we have described the large-scale molecular epidemiology of MRSA isolates in Western Sydney and throughout NSW between 2012 and 2017. The key identified trends were a decrease of ST239, and the emergence of ST45 and PVL-positive ST22 as clinically important strains. Binary typing can be a useful routine laboratory test for prospective molecular surveillance and detection and investigation of outbreaks of MRSA colonisation and infection.

Ethics

Ethics approval and a waiver for individual patient informed consent was obtained from the Western Sydney Local Health District Human Research Ethics Committee.

Authorship statement

R.D. analysed the data and prepared the manuscript. M.V.N.O'. and G.L.G. provided the data, and together with J.S.D. (Ph.D. supervisors for R.D.), guided data analysis and manuscript preparation. P.J.N. helped with data analysis and critically reviewed the manuscript.

Conflict of interest

Nil.

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Provenance and peer review

Not commissioned; externally peer reviewed.

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We thank Dr. Fei Zhou and Dr. Cecilia Li who performed binary typing. This project was presented as a poster (2012–2014 data) at ICAAC/ICC 2015, San Diego, California, U.S.A. And, 2012–2017 data was presented as a poster at the Australasian Society of Infectious Diseases (ASID) Conference 2018, Gold Coast, Australia.

Appendix A. Supplementary data

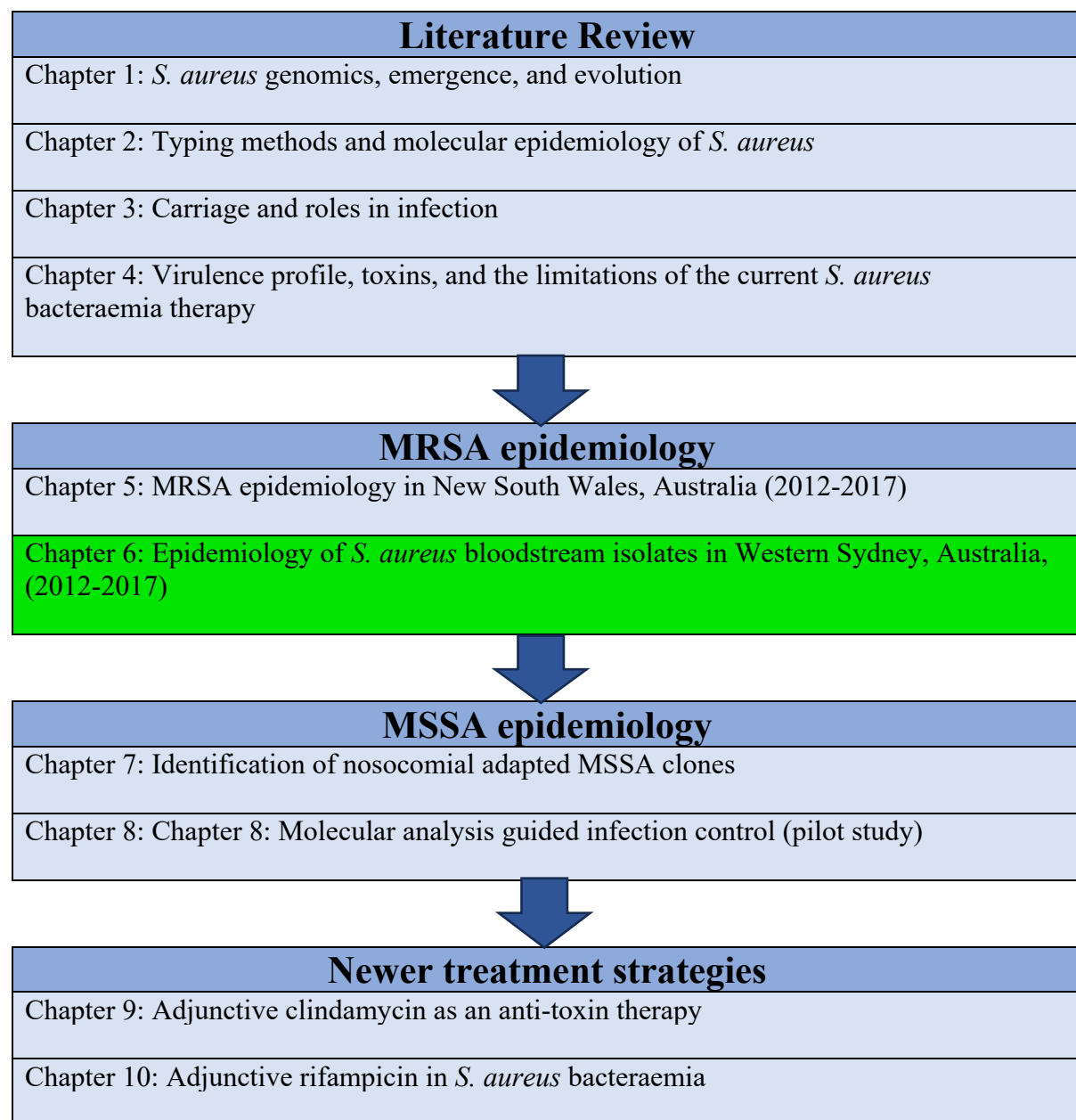
Supplementary data to this article can be found online at <https://doi.org/10.1016/j.idh.2019.04.002>.

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Figure: Schematic overview of the thesis structure



Chapter 6: Overview

This chapter expands on the molecular epidemiology of MRSA by detailing all blood culture isolates collected from 2012 to 2017 in the two major hospitals in Western Sydney. It compares the proportions and annual trends of MRSA bacteraemia with those of MSSA bacteraemia over the corresponding period. The study underscores the significance of local epidemiological analysis, revealing the emergence of MRSA clones that were seldom documented in national reports.

This chapter includes unpublished work.

The research discussed in this chapter was presented as an abstract titled “Epidemiology of *Staphylococcus aureus* Bloodstream Isolates in Western Sydney, Australia, 2012 – 2017” at the 18th International Symposium on Staphylococci and Staphylococcal Infection, Copenhagen, Denmark (ISSSI 2018). The presented poster is included in the Appendix.

Chapter 6: Epidemiology of *S. aureus* bloodstream isolates in Western Sydney, Australia (2012-2017)

6.1 Introduction

Most MRSA isolates belong to one of five multilocus sequence type (MLST) clonal complex (CC5, CC8, CC22, CC30 and CC45). This observation supports the notion that MRSA evolved from a limited pool of MSSA clones (1–3). Furthermore, the emergence of some new MRSA lineages have triggered waves of epidemics (4,5).

The late 1990s and early 2000s witnessed a surge in MRSA prevalence, attributed to the emergence of community strains and the spread of nosocomial strains (1,6). In many countries,

MRSA prevalence exceeded 25%, reaching over 50% in some instances (7–10). In New South Wales, the prevalence of MRSA among nosocomial *S. aureus* strains was 43.4% in 2005, often linked with healthcare-associated infections (11). Subsequently, community strains infiltrated healthcare systems, becoming common causative agents in nosocomial infections (12,13). To address the escalating MRSA prevalence, policy changes were implemented. These included routine screening for MRSA carriage in selected high-risk wards or in high-risk patients, mandatory reporting of hospital-onset (HO) *S. aureus* bacteraemia (SAB), enhanced management of venous access devices, improvements in infection control and hand-hygiene, and antimicrobial stewardship (AMS) initiatives (14–16). After 2008, the prevalence of MRSA began to decline, potentially attributed to these policy changes (17). However, as of the study period in 2016/17, there was a lack of prospective data to confirm the decrease in prevalence, ascertain changes in MRSA strain types, and determine whether the decline was sustained.

A study was undertaken to characterise changes in the molecular epidemiology of MRSA bacteraemia isolates from 2012 to 2017. This involved a comparative analysis with methicillin-susceptible *S. aureus* (MSSA) bacteraemia isolates.

6.2 Methods

S. aureus isolates (MSSA and MRSA) from bacteraemic episodes were routinely collected between 01/01/2012 – 31/12/2017 from patients aged ≥ 18 years attending two large acute hospitals in Western Sydney Local Health District, catering to nearly one million people or ~12% of the population of New South Wales (NSW) (18). Both hospitals maintained established infection control and AMS services, routinely conducting MRSA screening, and isolating high-risk patients. HO SAB cases were mandatorily notified and defined as the isolation of *S. aureus* from blood cultures obtained more than 48 hours after hospital admission.

HO is synonymous with terms like “healthcare-associated inpatient”, “healthcare-associated” (HA), “healthcare-associated hospital-onset”, or “nosocomial” infections (19,20). For this study, bacteraemia episodes not meeting HO criteria were categorised as non-HO, encompassing “community-acquired” and “healthcare-associated outpatient” SAB (19).

The molecular analysis of MRSA isolates was performed using 19-target binary typing approach (21–23). These targets included four toxin genes (including the gene encoding Panton-Valentine leukocidin, “PVL”), nine phage-derived open reading frames, and six SCC*mec* elements. Control targets included *nuc* and *mecA*. In MSSA, positive *nuc* and negative *mecA* results are anticipated. The assay determined the presence “1” or absence “0” of 19 genetic targets. The conversion of the 19-digit binary number into a decimal number yielded the binary type (BT) of the isolate. STs were predicted from the binary types based on results obtained from isolates previously characterised by both methods (21,23).

The p-value for categorical variables was calculated using a chi-squared test. The mean and standard deviation for age were calculated in Microsoft Excel, and the associated p-value was computed in MedCalc (24).

6.3 Results

A total of 1,065 patients aged ≥ 18 years with SAB were recorded in the two hospitals during the six-year study (Table 1). There were more cases of SAB in males, but the difference was not significant. Overall, the patients with MRSA bacteraemias were significantly older than those with MSSA bacteraemias (mean age 66 versus 61.8 years, $P=0.001$), however, there was no significant difference in the mean ages of those affected when the results were limited to HO bacteraemias (Table 1).

Table 1. Proportions of MRSA and MSSA bacteraemia isolates collected during the study period (2012-2017) in Western Sydney (All percentage values are of column total except for Year where row total is shown)

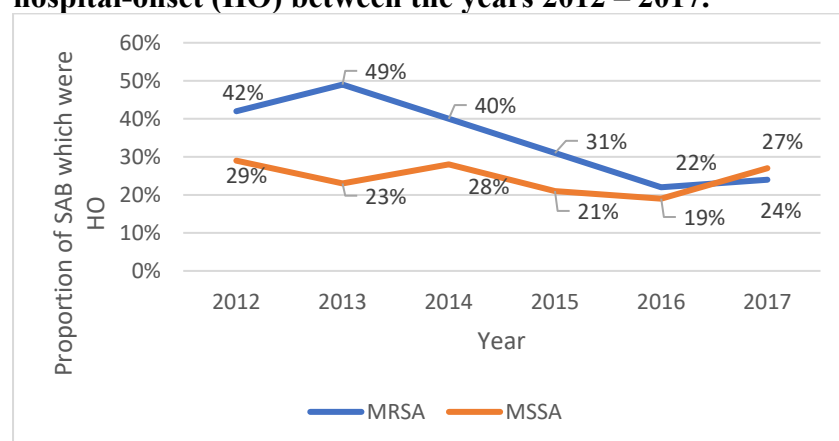
Variables	MRSA, n=298 (%)	MSSA, n=767 (%)	p-value
Westmead Hospital, n (%)	205 (69)	492 (64)	na
Blacktown Hospital, n (%)	93 (31)	275 (36)	na
Males, n (%)	190 (64)	501 (65)	baseline
Females, n (%)	108 (36)	266 (35)	0.6
Mean age, years (IQR), SD	66 (53-82), 19.3	61.8 (49-75), 18.2	0.001
HO bacteraemia	66.2 (57-80), 17.4	64 (52-79), 18.8	0.32
Non-HO bacteraemia	65.9 (50-82), 20.3	61.1 (48-73), 17.9	0.001
HO bacteraemia, n (%)	104 (35)	188 (25)	<0.001[^]
Westmead	76 (73)	117 (62)	baseline
Blacktown	28 (27)	71 (38)	0.06
PVL-positive, n (%)	53 (18)	-	na
Non-HO	43 (81)	-	baseline
HO	10 (19)	-	0.006
Year*, n (%)			
2012	59 (31)	133 (69)	baseline
2013	45 (31)	100 (69)	0.9
2014	50 (32)	106 (68)	0.8
2015	48 (28)	126 (72)	0.5
2016	59 (28)	155 (72)	0.5
2017	37 (20)	147 (80)	0.018

IQR: Inter-quartile range; HO: Hospital-onset; non-HO: include community-acquired and Healthcare-associated community onset; na: p-value calculation was not applicable.

[^]Denotes difference between HO bacteraemia due to MRSA and MSSA

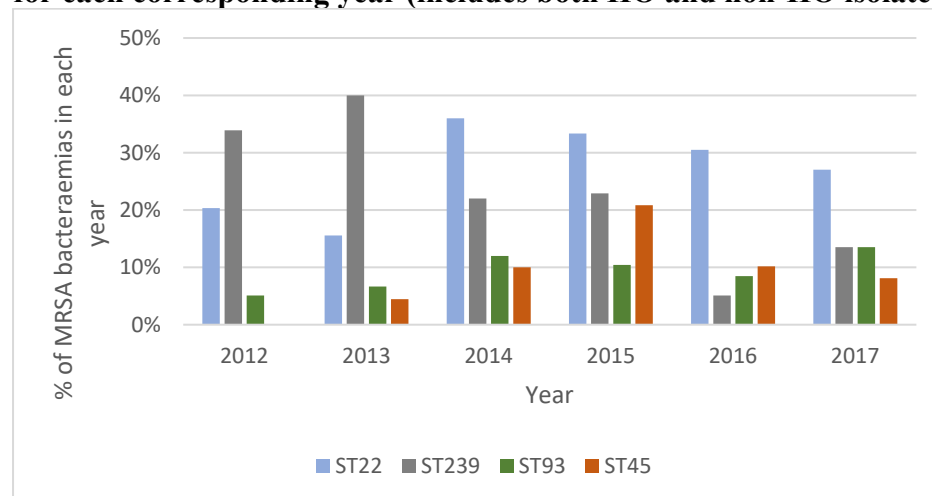
*% of row total shown

Figure 1: Changes in the proportion of MRSA and MSSA bacteraemias which were hospital-onset (HO) between the years 2012 – 2017.



SAB: *S. aureus* bacteraemia; HO: Hospital-onset

Figure 2: The proportion of common sequence types (ST) of total MRSA bacteraemias for each corresponding year (includes both HO and non-HO isolates).



There was a notable trend in MRSA prevalence during the study. In 2012, 31% of SAB were due to MRSA compared to 20% in 2017 ($P=0.018$) (Table 1). The change was due to a significant decrease in HO MRSA bacteraemias. . The proportion of MRSA SAB which were HO was 42% in 2012, peaked at 49% in 2013, but gradually decreased to 24% in 2017. A significant decrease occurred between 2013 and 2017 ($P=0.02$) (Figure 1). In contrast, the corresponding proportions for MSSA SAB were 29% in 2012 and 27% in 2017 ($P=0.6$) (Figure 1). Overall, MRSA bacteraemias were significantly more likely to be HO compared to MSSA bacteraemias, 35% (104/298) versus 25% (188/767), $P<0.001$.

MLST results were available for 253/298 (85%) of MRSA isolates based on predictions from previously characterised binary type-MLST pairs. ST could not be determined for the remaining 15% of MRSA isolates as they belonged to less common binary types for which a corresponding ST had not yet been determined.

The four most common STs were ST22, ST239, ST93 and ST45. They accounted for 68% of MRSA isolates (Table 2). Seven of 81 (7%) ST22-MRSA in our study were PVL-positive. Of these, three isolates were from HO and four from non-HO bacteraemias. All ST239-MRSA

Table 2: The identified STs of the MRSA bacteraemia isolates, proportion that were PVL positive, and the proportion of HO MRSA bacteraemia for each ST.

MRSA sequence type (ST)	Total number of isolates in each ST	PVL-positive, n (%)	HO MRSA, n (%)
22	81	7 (9)	26 (32)
239	68	0	46 (68)
93	27	27 (100)	3 (11)
45	26	0	8 (31)
1	13	1 (8)	2 (15)
5	9	0	3 (33)
30	5	4 (80)	0
97	5	0	0
72	3	0	1 (33)
78	3	0	0
188	3	0	2 (67)
59	2	0	1 (50)
88	2	0	0
8	1	1 (100)	1 (100)
25	1	0	0
73	1	0	1 (100)
338	1	1 (100)	0
772	1	1 (100)	0
923	1	1 (100)	0
Unknown	45	10 (22)	10 (22)

MLST: multilocus sequence typing; PVL: Panton-Valentine leukocidin

and ST45-MRSA were PVL-negative, and all ST93-MRSA were PVL-positive. Most nosocomial infections were due to ST22 or ST239-MRSA (Table 2). There was a gradual decrease in the rates and proportion of ST239 strain in both HO and non-HO groups, while ST22 and ST93 remained common. ST93 was primarily detected in non-HO BSI, whereas ST22 was commonly isolated in both HO and non-HO groups. We noted emergence of ST45 MRSA causing bacteraemia starting in 2013 (Figure 2).

6.4 Discussion

In our analysis of 1,065 SAB cases in Western Sydney spanning six years (2012-2017), several noteworthy findings emerged. The disease predominantly affected the elderly population, with the mean age of 65 years for bacteraemia. Individuals with non-HO MRSA bacteraemias were significantly older than those with non-HO MSSA bacteraemias (65.9 versus 61.1 years,

P=0.001), although no significant difference in age was observed between HO MRSA and HO MSSA bacteraemias. Over the study period, the proportion of SABs caused by MRSA significantly decreased from 31% in 2012 to 20% in 2017 (P=0.018). This decline was primarily attributed to a reduction in HO-MRSA bacteraemias, particularly driven by the waning prevalence of ST239 MRSA, despite the emergence of ST45 MRSA. In contrast, there was no notable change in the proportions of HO-MSSA or ST22-MRSA bacteraemia. Interestingly, seven percent of ST22 isolates in our cohort were PVL-positive and were detected in both HO and non-HO groups.

Our finding, that a decrease in HO MRSA without a concurrent change in MSSA bacteraemia frequency, aligns with reports from Europe, the UK, the USA, and other regions (25–29). There is an ongoing debate regarding whether this reduction in MRSA can be attributed to enhanced MRSA surveillance and infection control measures, a potential loss of fitness in MRSA, or the replacement by successful MSSA clones (30–33). The Australian Group on Antimicrobial Resistance (AGAR) regularly conducts the Australian *Staphylococcus aureus* Surveillance Outcomes Programme (ASSOP). Our findings are consistent with AGAR/ASSOP reports (34,35), except that PVL-positive ST22-MRSA was rarely reflected in the national SAB reports.

The observed reduction in MRSA prevalence since 2013 in our study is primarily attributed to a decline in ST239-MRSA, also known as Aus-2/3 MRSA. This strain belongs to MLST CC8 and has been a predominant nosocomial MRSA strain in Australia since the 1970s (36). ST239-MRSA was a major nosocomial pathogen in the UK during the 1980s (37) and subsequently became prevalent in Latin America, Eastern Europe, Portugal, and many parts of Asia (9,38–

40). It is referred to as EMRSA-1 in the UK and carries different names in various regions due to the previous lack of consensus nomenclature (1,2).

In the 1990s, ST239-MRSA experienced a global decline, with other clones gradually replacing it at various points over the past three decades (10,17,32,36,41,42). Interestingly, for reasons that remain unclear, ST239 MRSA has never been a prevalent strain in the USA (43). In China, the prevalence of MRSA in blood culture isolates decreased from 47.5% in 2010-11 to 36.6% in 2016 (32), showing variations between provinces. A significant reduction in ST239-MRSA (42.4% of MRSA in 2013 to 11.6% in 2016, $P < 0.001$), accompanied by an increase in ST59-MRSA (3.6% in 2010-11 to 15.1% in 2016), was reported. In Latin America, ST239-MRSA, also known as Brazilian clone, previously dominated, constituting 80% of MRSA isolate in 1996-1998 (40). However by 2011-2014, ST239-MRSA had become infrequent, replaced by ST5-MRSA-II (USA100), ST8-MRSA-IV (USA300), and ST5-MRSA-I (Chilean clone) (10).

The decline of ST239-MRSA is attributed to its high fitness cost, as it carries a large *SCCmec* complex, type III, and multiple antibiotic resistance genes. This strain was outcompeted by newer, more 'fit' clones (44).

ST22-MRSA-IV, also known as EMRSA-15, emerged in England during the 1990s (46). UK healthcare workers are believed to have unintentionally introduced it to Australia (47). Since the 2000s, it has become a prevalent nosocomial clone in Australian hospitals, and in recent years, it has also been frequently reported in community-onset infections (36,45). While it is typically PVL-negative, we detected PVL-positive ST22 in our cohort, first noted in our institution in 2012 during a neonatal intensive care unit outbreak investigation. This particular

strain caused two fatal neonatal infections and led to a prolonged outbreak lasting seven months (48). It is considered an emerging pathogen and has been reported to co-carry toxic shock syndrome toxin 1 genes, further enhancing its pathogenicity (49–51).

Community MRSA clones circulating in Australia exhibit much greater diversity compared to nosocomial MRSA clones (45,52,53). One predominant community MRSA clone in Australia is ST93-MRSA (36,52). This strain was first identified in Ipswich, Queensland, in the early 2000s (54). Notably, ST93-MRSA, carries PVL and is generally susceptible to most non- β -lactam antibiotics, categorising it as a non-multi-resistant MRSA.

ST45-MRSA was initially identified in hospitals in Berlin, Germany, carrying SCC*mec* IV and recognised as the Berlin epidemic MRSA (55,56). It is also prevalent in the USA, where it is known as USA600, but with a different SCC*mec*, specifically SCC*mec* II (ST45-MRSA-II) (56,57). In Australia, ST45-MRSA are ST45-MRSA-IV/V. While the US strain is considered a nosocomial strain, both the Berlin and Australian variants are community strains (2). In 2017, ST45-MRSA accounted for 10% (44/462) of MRSA bacteraemia cases in Australia, with a majority (66%) reported in NSW (35). Remarkably, some ST45-MRSA isolates in Australia are *spa* gene negative, leading to a laboratory misdiagnosis of MRSA, and this characteristic is unique to the Australian strains (56,58,59).

In 2021, ASSOP/AGAR conducted an analysis of SAB isolates recorded between January 1 and December 31. This analysis involved 30 participating public and private laboratories servicing 48 hospitals across all states and mainland territories in Australia (45). The dataset comprised 2,928 isolates. The proportion of *S. aureus* identified as MRSA had remained stable since 2013, with MRSA accounting for 17% of SAB in 2021. Community clones were

frequently associated with HO bacteraemia. Among nosocomial MRSA clones, ST22 was the most prevalent, constituting 90% (64/71), and none were PVL-positive. ST239 MRSA accounted for 8.5% (6/71) of nosocomial clones and was exclusively detected in NSW and South Australia. The two most frequent community MRSA clones were ST93 (99/401) and ST45 (62/401), comprising 8% (8/99) and 19.4% (12/62) of HO MRSA bacteraemia cases, respectively.

There are several limitations to our study. Firstly, the inclusion of MRSA-specific targets in the 19-target binary typing precluded us from comparing the molecular epidemiology of MRSA and MSSA isolates. Additionally, no prior correlation between MSSA binary typing and MLST was established. Secondly, the study involved sampling from a limited geographic area. However, our findings, which indicated a decline in HO MRSA without changes in MSSA epidemiology, were in line with national and international reports. Notably, our local epidemiological analysis identified PVL-positive ST22, a detail overlooked in national surveys. Thirdly, we combined community-acquired and 'healthcare-associated non-inpatients' bacteraemias into one category as non-HO due to a lack of data to separate the two categories. Lastly, our study focussed on understanding epidemiological changes and did not include data relating to sources of infection or clinical outcomes.

In conclusion, our analysis of a large prospective collection of SAB isolates spanning six years showed a decrease in the proportion of MRSA since 2013 in Western Sydney. This decline was predominantly attributed to a decrease in the prevalence of ST239-MRSA, despite the emergence of ST45-MRSA. Conversely, rates of MSSA bacteraemia rates remained unchanged during the study period.

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Contribution of authors:

I collated the isolates, performed data collection, analysis of the binary typing and the clinical data, and prepared the manuscript.

GLG and MVNO supervised me and helped with data analysis, interpretation, and manuscript writing.

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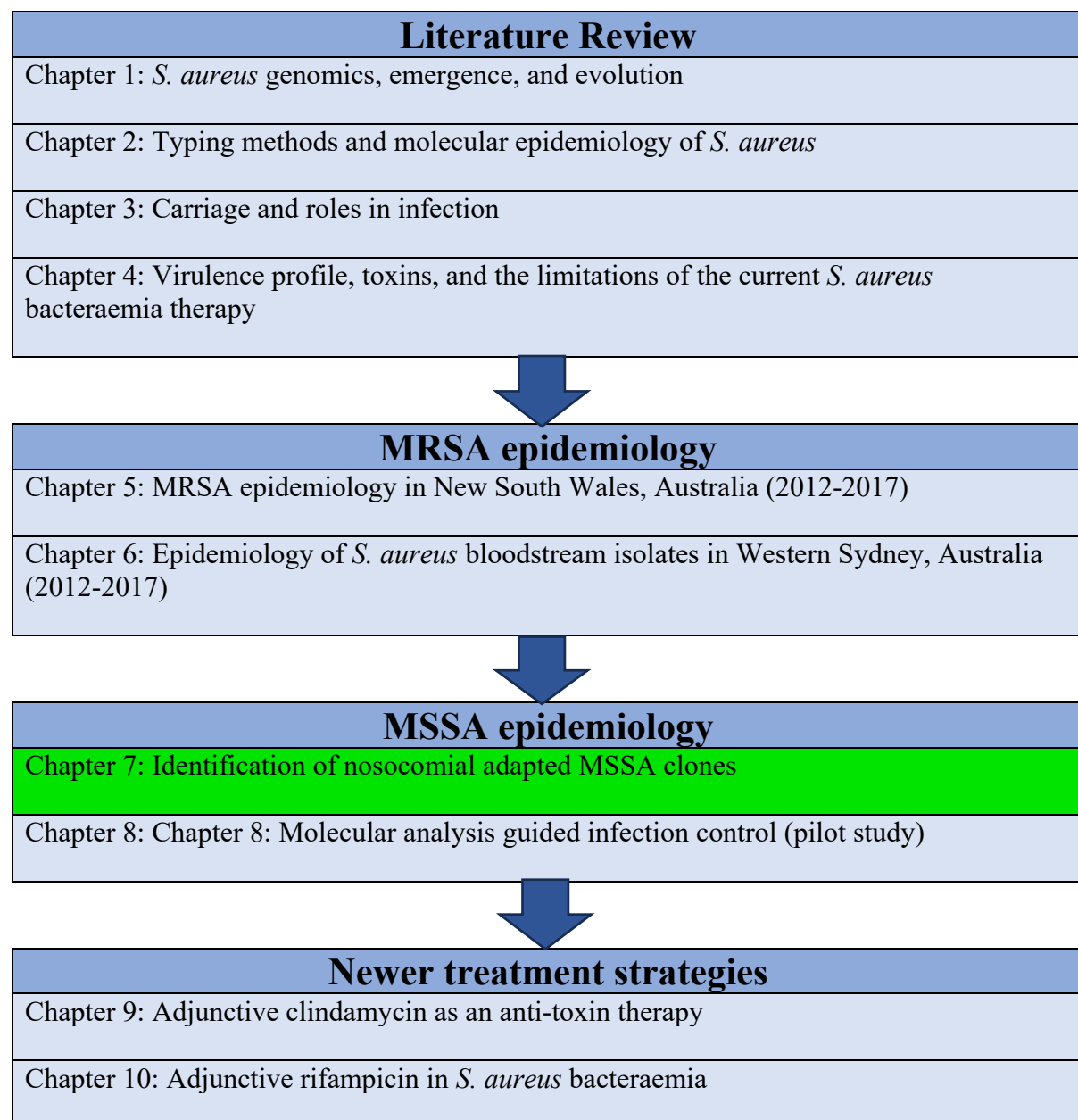
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Figure: Schematic overview of the thesis structure



Chapter 7: Overview

This chapter explores the molecular epidemiology of MSSA bacteraemia within a large tertiary hospital in Western Sydney from 2011-2015. Employing the 19-target MRSA binary typing test, the study aims to identify hospital adapted MSSA clones. This is achieved by examining the differences in strain distribution and diversity between hospital-onset and community-acquired infections. This study is among the few that thoroughly investigated the molecular epidemiology of MSSA isolates.

This chapter includes unpublished work.

The research discussed in this chapter was presented as an abstract titled “Nosocomial Adaptation of Methicillin-Sensitive *Staphylococcus aureus* (MSSA) Clones” at the 17th International Symposium on Staphylococci and Staphylococcal Infection, Seoul South Korea (ISSSI 2016). The corresponding poster is provided in the Appendix.

Chapter 7: Identification of nosocomial adapted methicillin-susceptible *Staphylococcus aureus* clones

7.1 Introduction

The introduction of penicillin in the 1940s marked the emergence of penicillin-resistant *Staphylococcus aureus* (PRSA), initially observed as a primary nosocomial pathogen. However, over time, its prevalence extended into the community (1–4). PRSA was associated with limited clonal types, in contrast to penicillin-susceptible clones, which exhibited diverse phage types (1). Strikingly, these early observations parallel the contemporary epidemiology of MSSA and MRSA. Following the re-emergence of MRSA in the 1980s, it was predominantly a nosocomial pathogen, while MSSA (penicillin-resistant *S. aureus*) was a

common community pathogen. MRSA was linked to a limited range of clonal types, whereas MSSA demonstrated a diversity of clones (5,6).

The MRSA epidemic peaked around 2008-2013 and has subsequently declined in many regions. This reduction is primarily linked to a decrease in hospital-onset (HO) MRSA bacteraemia (7–10). Meanwhile, HO MSSA bacteraemia persisted, while the rates of community-acquired (CA) MSSA bacteraemia showed a slow increase (9,11). In Australia, the decline of MRSA was mainly attributed to a reduction in nosocomial ST239-MRSA (10). In contrast, there is a lack of evidence regarding nosocomial adaptation or a comprehensive description of nosocomial clone dynamics for MSSA. Instead, the prevailing assumption is that nosocomial MSSA infection is endogenous, arising from the patient's own flora, rather than the acquisition of nosocomial adapted clones after hospitalisation (12,13).

In 2016, we conducted a study to investigate the genetic diversity of MSSA isolates causing bacteraemia. The primary objective of the study was to identify potential evidence of hospital adapted MSSA clones by analysing differences in strain distribution and diversity in CA and HO infections.

7.2 Methods

We included MSSA bacteraemia isolates, from cases spanning January 1, 2011, to December 31, 2015, at Westmead Hospital, a large adult tertiary hospital in Sydney, Australia. Data collected included age, sex, 30-day mortality, source of infection, and antibiogram. Each isolate represented a distinct episode of bacteraemia, excluding relapsed or recurrent occurrences. Episodes were categorised as community-acquired (CA), healthcare-associated inpatient (HCA inpatient), or healthcare-associated non-inpatient (HCA non-inpatient) based

on the definitions outlined by the Clinical Excellence Commission (CEC), New South Wales (NSW), Australia, version 2.0 (November 2008). These definitions have since been updated by the CEC (Box 1) (14). For clarity, HCA inpatient aligns with the internationally used terms healthcare-associated hospital-onset (HO), and HCA non-inpatient corresponds to healthcare-associated community-onset (HACO) infections. We have opted for the internationally recognised terms CA, HO, and HACO to describe acquisition contexts in this report.

Isolate strain typing was conducted using a previously described binary typing method (15–17), which employs a multiplex polymerase chain reaction and reverse line blot hybridisation assay (mPCR/RLB). Originally designed for MRSA, this test has demonstrated at least moderate discriminatory capability for MSSA - an aspect further explored in this thesis. The 19-target binary typing method includes four toxin genes (including the *lukS* gene encoding a subunit of Panton Valentine leukocidin, PVL), nine phage-derived open reading frames, and six *SCCmec* elements. Targets *nuc* and *mecA* are also included as controls. The assay determines the presence “1” or absence “0” of 19 genetic targets. The binary type (BT) of the isolate is determined through the decimal number conversion of the 19-digit binary number. While *SCCmec* targets are typically not detected in MSSA isolates, the remaining targets offer effective discrimination.

A high discriminatory power, which enables the differentiation of unrelated isolates, is an essential requirement for a molecular typing tool (18). To assess the discriminatory power of the binary typing test and describe population diversity within each of the three subgroups of *S. aureus* bacteraemia (SAB) isolates, we employed Simpson’s Diversity Index (DI) (19,20). This index considers both the number of clones and their relative abundance. The resulting values range from 0 (indicating no diversity) to 1 (representing infinite diversity). A DI value

of 0.9 or higher is considered highly discriminatory (20). DI with overlapping 95% confidence interval (CI) suggests similar population diversity (21).

Box 1: Definitions of healthcare association in *S. aureus* bacteraemia as defined by the Clinical Excellence Commission, New South Wales, Australia (Version 3.3) (14)

Healthcare associated infection
Criterion 1 (Equivalent to ‘healthcare associated inpatient’ in the older CEC definition, v2.0) The patient’s first positive <i>S. aureus</i> blood culture was collected more than 48 hours after hospital admission or less than 48 hours after discharge from hospital provided the event was NOT considered to be present or incubating on admission. Criterion 2 (Equivalent to ‘healthcare associated non-inpatient’ in the older CEC definition, v2.0) <i>S. aureus</i> detection in blood culture collected ≤ 48 hours after hospital admission and one or more of the following key clinical criteria are met: 1. It is a complication of the presence of an indwelling medical device (e.g., intravascular line, haemodialysis vascular access, CSF shunt, urinary catheter) 2. Occurs within 30 days of a surgical procedure where the bacteraemia is related to the surgical site or 90 days for deep incisional/organ space infections related to a surgically implanted device 3. It was diagnosed within 48 hours of a related invasive instrumentation or incision 4. It is associated with neutropenia (neutrophils less $0.5 \times 10^9/L$) on two occasions contributed to by cytotoxic chemotherapy
Community-acquired (CA)
<i>S. aureus</i> detection in blood culture collected within 48 hours after hospital admission and not meeting Criterion 1 or 2.

Antibiogram results were obtained from the microbiology department at Westmead Hospital. Susceptibility testing was conducted using either disc diffusion or BD Phoenix automated susceptibility testing device. As per the local policy, isolates collected in the first half of the study underwent disc diffusion testing and were subjected to BD Phoenix testing only if resistance to one or more antibiotics was detected. In the second half of the study, both disc diffusion and Phoenix testing were available for most isolates. When both sets of results were available, priority was given to the antibiogram reported on the BD Phoenix system.

The p-value for the continuous variables were calculated using unpaired t-test. For the categorical variables, a chi-squared test or Fisher's exact test was applied.

7.3 Results

During the study period, 333 unique MSSA bacteraemia episodes were documented. Strain typing was performed on isolates from 288 (86.5%) of these episodes. CA infection was more prevalent than the combined cases of HO and HACO infections. Participants' ages ranged from 16 to 95 years. The 30-day mortality rate was 14% (39/284), with mortality data missing for four participants. Although higher mortality was observed in CA bacteraemia, the difference did not reach statistical significance (Table 1).

Sixteen out of 288 (5.5%) isolates were deemed nontypeable by binary typing, indicating the absence of all 19 targets. Among these, eight were CA infections, while four each were linked to HO and HACO infections. The remaining 272 isolates displayed 128 different BTs. The Simpsons Index of diversity was 0.973, 0.974 and 0.985 for CA, HO and HACO categories, respectively. Overlapping 95% confidence intervals were observed (Table 2).

The distribution of the BTs in the three categories are shown in Table 2 and Table 3. Approximately a third of BTs were unique to each category (Table 2). Interestingly, the most prevalent binary type (bt256) in CA bacteraemia was also the most common binary type in both HO and HACO cases.

The gene encoding PVL was detected in 4.4% (12/272) of the isolates (Table 4). All patients with PVL-positive MSSA bacteraemia survived (12/12). In contrast, 14% (39/274) with PVL-negative MSSA bacteraemia died; however, this difference did not reach statistical significance ($P=0.38$). The mean age for patients with PVL-positive MSSA bacteraemia was 52 years,

compared to the overall mean age of 59 years ($P=0.19$) (Table 4). Notably, PVL-positive MSSA bacteraemia was significantly more likely from skin and skin structure infections (SSTI) than PVL-negative MSSA bacteraemia, with rates of 58% (7/12) versus 23% (63/276) ($P=0.01$).

Table 1: Basic demographics and 30-day outcomes of patients with MSSA bacteraemia (n = 288)

	Community Acquired (CA)	Healthcare- associated hospital- onset (HO)	Healthcare- associated community-onset (HACO)
Number of isolates	156 (54%)	73 (25%)	59 (21%)
Age (mean)*	58 years	60 years	62 years
Sex, male*	65%	70%	56%
30-day mortality^*	27/154 (18%)	8/72 (11%)	4/58 (7%)

*The differences were not significant ($P > 0.05$); $P=0.29$ (CA versus HO); $P=0.08$ (CA versus HACO)

^Data were not available for 4 cases

Table 2: Binary typing results of 288* episodes of MSSA bacteraemia

	CA n=148	HO n=69	HACO n=55
Number of binary types	83 (56%)	46 (67%)	42 (76%)
Simpson's index of diversity (95% CI)	0.973 (0.958 - 0.987)	0.974 (0.956 - 0.993)	0.985 (0.972 - 0.997)
Most common binary type (bt)	bt256 (20 isolates, 14%)	bt256 (nine isolates, 13%)	bt256 (five isolates, 9%)
Number of BTs unique to each group	56 (38%)	22 (32%)	19 (35%)

*Sixteen isolates non-typeable.

Combined Simpson's index of diversity: 0.974 (0.966 – 0.983)

CA: Community-acquired; HO: Healthcare-associated hospital-onset; HACO: Healthcare associated community-onset

While all isolates tested negative for *mecA*, 45 out of 288 (16%) MSSA isolates exhibited the presence of targets usually located on *SCCmec* complex. Among these, 32 were positive for *cadB*, 12 for *ccrAB1*, and one for ORF E007 of type I *SCCmec*. These 45 isolates were distributed across 28 different binary types, indicating a polyclonal distribution. Importantly,

none of the 45 isolates displayed multidrug resistance. Fusidic acid resistance was the most common, observed in 10 out of 45 isolates, while clindamycin/erythromycin resistance was present in six out of 45 isolates. Notably, one isolate demonstrated resistance to both clindamycin/erythromycin and fusidic acid.

Table 3. Binary types in common among the association groups.

	CA and HO (217 isolates)	CA and HACO (203 isolates)	HO and HACO (124 isolates)	CA, HO and HACO (272 isolates)
Number of binary types in common	20	19	15	12
Combined number of binary types in each group	109	106	72	128

CA: Community-acquired (148 isolates); HO: Healthcare-associated hospital-onset (69 isolates); HACO: Healthcare associated community-onset (55 isolates). Sixteen isolates were non-typeable and excluded.

Table 4. Analysis of PVL results in MSSA bacteraemia (n=272; PVL positive =12) ^

	<i>lukS</i> -detected (%)	p-value
<u>Acquisitions</u>		
CA	9/148 (6)	-
HO	1/69 (1)	0.17
HACO	2/55 (4)	0.7
<u>Mortality outcomes</u> (Death within 30 days)		
PVL-positive	0/12 (0)	-
PVL-negative	39/274*	0.38

CA: Community-acquired; HO: Healthcare-associated hospital-onset; HACO: Healthcare associated community-onset

*Two patients whose 30-day outcomes were not known are excluded.

^Of 288 MSSA bacteraemia, 16 were non-typeable whose PVL status was unknown

None of the remaining isolates exhibited resistance to more than one class of antibiotic (excluding penicillin). Of the 45 isolates positive for *SCCmec* elements, 25 (56%) were associated with CA infections, although this association did not reach statistical significance (P=0.32). Furthermore, there was no significant difference in 30-day mortality between MSSA isolates with or without the presence of *SCCmec* complex targets. The rates were 7% (3/45)

versus 17% (38/229), respectively ($P=0.13$). Four cases, where day 30 outcomes were unavailable or the isolates were non-typeable, were excluded from this analysis.

7.4 Discussion

The binary typing assay originally designed for MRSA demonstrated effective discrimination between MSSA clones (DI 0.974, 95% CI 0.966 – 0.983) (Table 2). Potential improvement in discriminatory power could be achieved by replacing some non-contributory targets. No significant difference in diversity between HO and CA MSSA clones was found, challenging the hypothesis of predominant healthcare-associated clones causing exogenous nosocomial infection (Table 2). Nevertheless, there were notable differences in strain distribution between the two groups (Table 2 and 3), suggesting the possibility that some clones may be more likely to cause healthcare associated infection, although no clustering or specific transmission events were detected. HACO clones displayed numerically greater diversity, potentially indicating a mix of community-acquired and hospital-acquired clones.

Among the HO MSSA isolates, 69 different BTs were identified. Interestingly, 22 (32%) of these BTs were not detected in CA or HACO infections. To date, a hypervirulent clone of MSSA or MRSA has not been identified, although there are suggestions that CC30 *S. aureus* is more likely to cause bloodstream infections (22,23). Similarly, nosocomially adapted MSSA clones have not been identified, with a possible exception of the historical phage 80/81 *S. aureus* in the mid-1950s. (24–26). Current evidence suggests that any colonising *S. aureus* strain has the potential to cause an invasive infection (27,28).

In contrast to MRSA, where limited clones cause HO infections (5,6), our analysis revealed a polyclonal distribution of MSSA isolates in both CA and HO categories. These findings align

with the molecular epidemiology of MSSA described by others using various typing methods such as PFGE, *spa* typing, or MLST typing (5,29,30).

The majority of MSSA bacteraemias in this study were CA, with only a quarter being of nosocomial onset. MSSA bacteraemia was more prevalent in males, but there was no significant difference in age or sex distribution between the CA, HO, or HACO categories. (Table 1). While there was a trend towards higher mortality in CA MSSA bacteraemia, the differences in mortality among the acquisition groups were not statistically significant.

Sixteen percent of MSSA bacteraemia isolates exhibited the presence of targets typically located on the *SCCmec* complex. This occurrence could be attributed to the presence of an *SCCmec*-variant without a functional *mec* complex or the existence of homologous sequences elsewhere in the MSSA genome (31). Such clones may emerge when MRSA loses the ability to express PBP2a due to the deletion or mutation of genes within *SCCmec* essential for methicillin resistance. These events potentially leads to multi-resistant MSSA if the original MRSA isolate was also multi-resistant (32–34). In this study, none of the MSSA isolates displayed resistance to more than one class of antibiotic, excluding penicillin.

PVL is an exotoxin frequently detected in community-clones of MRSA (5,6,10). PVL-status in MSSA isolates are poorly described in the literature. PVL has been associated with severe SSTIs, necrotising pneumonia, and musculoskeletal infections in children (35). The *lukS* gene, encoding one PVL subunit, was notably uncommon in our MSSA isolates, with only 12 out of 288 (4.4%) isolates being *lukS*-positive, and there was no significant difference between CA, HO, or HACO acquisitions. *lukS*-positive MSSA bacteraemia was not associated with any

difference in 30-day mortality but was significantly more likely to originate from an SSTI source.

Limitations of this study include the analysis being restricted to bacteraemic isolates from a single centre and the utilisation of a typing method optimised for MRSA. The 19-target MRSA binary typing has not been validated for MSSA typing; however, our analysis demonstrates its high discriminatory capacity, further optimisable by replacing some non-contributory targets. Presently, whole genome sequencing is more readily available for MSSA typing, rendering binary typing somewhat outdated. Additionally, our data are not amenable to comparison with other studies, except for the prevalence of PVL, as these isolates were not characterised by other typing methods such as PFGE, *spa* typing, or MLST (15,16).

In conclusion, this study did not identify a significant difference in diversity among MSSA isolates causing bacteraemia in hospital versus community settings, although differences in the distribution of strain types were observed. These findings do not support the hypothesis of predominant healthcare-associated MSSA clones causing exogenous nosocomial infection.

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Contribution of authors:

I collated the isolates, performed data collection, analysis of the binary typing and clinical results, performed data analysis, and prepared the manuscript.

GLG and MVNO supervised me and helped with data analysis, interpretation, and manuscript writing.

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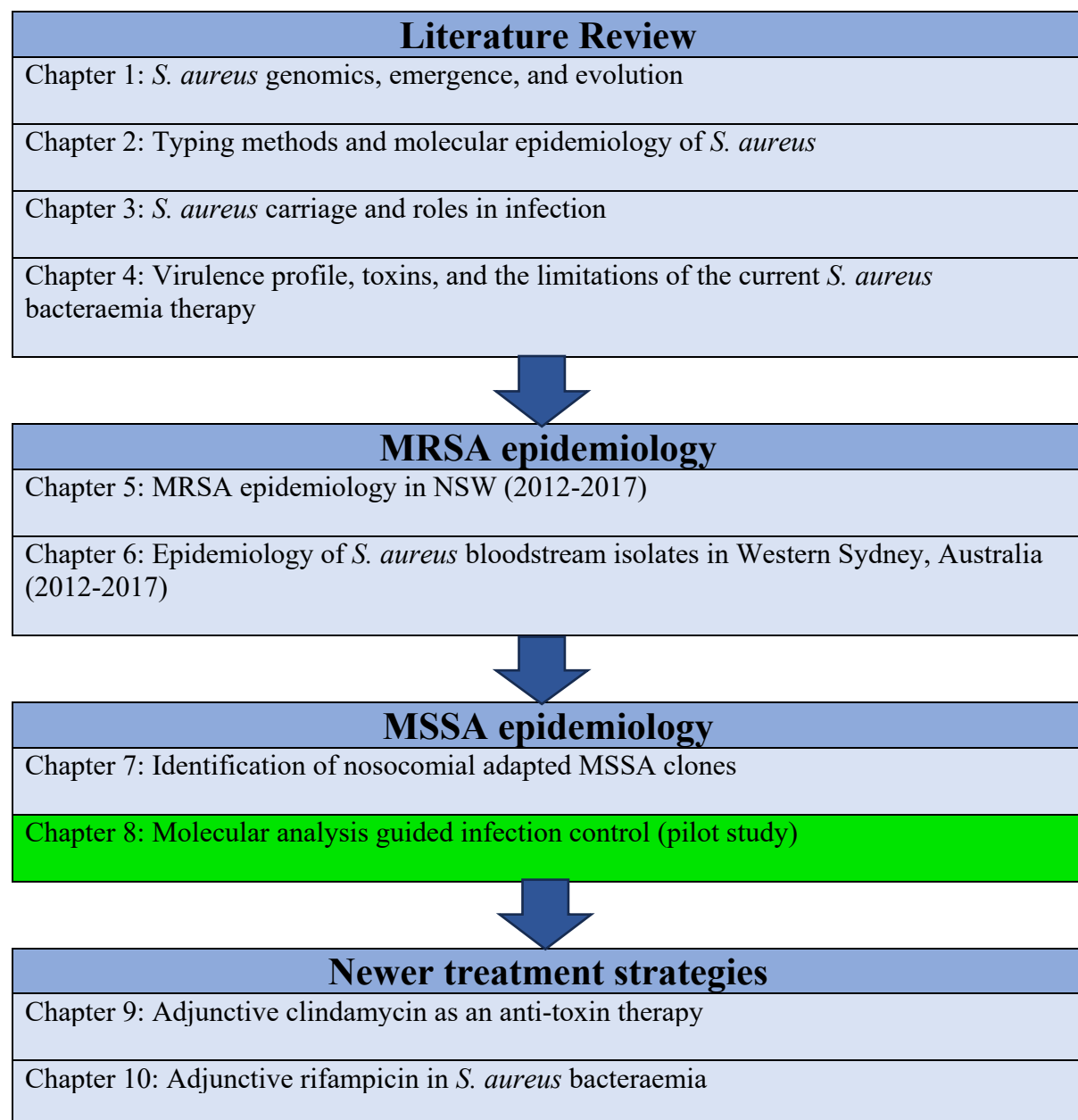
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Figure: Schematic overview of the thesis structure



Chapter 8: Overview

Chapter 3 of this thesis explored the distinction between exogenous versus endogenous sources of *S. aureus* isolates causing bloodstream infections. Traditionally, these infections in *S. aureus* carriers were thought to arise primarily from their own colonising bacteria. However, the origin of infections in non-carriers and in some carriers where the blood and the carriage isolates differ remains not fully understood. This chapter (Chapter 8) includes a prospective pilot study that aims to assess potential exogenous source or clustering through whole genome sequencing of *S. aureus* blood and screening isolates.

This chapter contains unpublished work, and funding for the study was obtained through a successful research grant from the Western Sydney Local Health District Research and Education Network (2020) with the author serving as the Chief Investigator.

A letter to the editor (Lancet Infectious Diseases) in relation to a work presented in this Chapter is listed in the Appendix.

Chapter 8: Molecular analysis guided infection control (pilot study)

8.1 Introduction

The available evidence suggests 80-90% of *S. aureus* bacteraemias (SABs) in *S. aureus* carriers are caused by the individuals' colonising strains (1,2). However, the source remains unexplained in non-carriers and in 10-20% of carriers, as the blood isolate differs from the carriage isolate. The likely source of these exogenous infections is a newly acquired *S. aureus* which, in a hospitalised patient, could be from a healthcare worker (HCW), another patient, or the hospital environment. Price *et al* (3) investigated this in an intensive care unit (ICU), in a non-outbreak setting. Based on whole-genome sequencing (WGS) of the *S. aureus* isolates,

HCWs or environmental surfaces were infrequent sources of cross-transmission to a patient. In contrast, others have reported patients and HCWs sharing similar clusters of *S. aureus* indicating co-transmissions (4–6). On the other hand, in an outbreak setting, the introduction of a new virulent strain via HCWs and/or the hospital environment (including contaminated equipment) is frequently implicated in *S. aureus* cross-transmission (7–9).

Hospital-onset (HO) and many healthcare-associated community onset (HACO) SABs are potentially avoidable (10,19). SAB rates are an important indicator for safety and quality in healthcare and have been reportable in New South Wales (NSW), Australia since 2009. HO SAB is notified as a critical incident as part of the quality improvement process. Each HO SAB is investigated to identify and manage preventable causes. The rate of HO SAB at our facility has been higher than in peer-group hospitals with an average of 1.11/10,000 patient days at Blacktown Hospital from 2017/18 – 2021/22, compared to 0.72/10,000 patient days in other “large hospitals” during the same period (11).

A study conducted between August 2008 and February 2010 in an affiliated hospital of ours reported 187 episodes of SAB. Of these, 123 episodes (66%) were healthcare-associated, with 45% classified as HO and 21% as HACO. Additionally, 64 episodes (34%) were classified as community-acquired (CA). MSSA accounted for 60% of the SABs, while MRSA accounted for the remaining 40% (19). Further analysis of the 123 episodes of healthcare-associated SAB revealed that 31 episodes were associated with peripheral intravenous cannula, 33 episodes with central intravenous catheter, and 11 with haemodialysis catheters. Preventable factors were identified in 50% of healthcare-acquired SABs and providing feedback to the treating clinicians resulted in a reduction in SAB occurrences (19).

A pilot study was designed to analyse the epidemiology of *S. aureus* in response to the higher burden of SAB in our facility despite the mandatory reporting and feedback. The aims of the study were:

1. To describe the local clinical and molecular epidemiology of SAB including preventable factors, potential sources, complications, and outcomes.
2. To investigate potential endogenous or environmental sources of SAB.

8.2 Methods

Study design and definitions

The study was conducted at Blacktown Hospital, one of two teaching hospitals in the Western Sydney Local Health District, NSW, Australia. SAB episodes were identified through an automated email notification system whenever *S. aureus* isolated from a blood culture was reported by the microbiology laboratory. Inpatients aged ≥ 18 years with SAB were screened for enrolment. Patients were excluded if they declined study participation or were unable to provide consent, if >96 hours had elapsed since identification of *S. aureus* in blood culture, if the attending primary clinician was unwilling for the patient to be enrolled, or if the patient had COVID-19 co-infection.

SAB episodes were classified as HO if *S. aureus* was first isolated from blood culture collected >48 hours after hospital admission; healthcare-associated community-onset (HACO) if the isolate was from a blood culture collected ≤ 48 hours after admission in a patient with recent healthcare contact; or CA if HO or HACO criteria were not met (12,13).

Patients who provided written informed consent had swabs collected for *S. aureus* screening from various sites, including the anterior nares, throat, hands, groin, and any wounds if present,

such as infected peripheral cannula entry sites. Additionally, swabs were collected from the environments of patients with HO SAB, including nurse call button, bed rails, computer on wheels, and desktop computers at ward nursing and medical stations. Swab samples were collected, using ESwab® (Copan Diagnostics) kits, within 96 hours of SAB diagnosis. The presence of *S. aureus* and whether MRSA or MSSA was detected, using BD Max StaphSR assay, directly from the ESwab® medium. Positive samples were subcultured on *S. aureus* chromogenic agar. All available *S. aureus* isolates from blood cultures, screening, and environmental swabs were to be stored at -80°C for whole genome sequencing (WGS), to define strain relatedness.

Frozen *S. aureus* cultures were thawed and grown overnight in 2ml tryptic soy broth (TSB) at room temperature. DNA was extracted from a single colony using QIAGEN DNeasy® UltraClean® Microbial Kit. Double-stranded DNA was quantitated using DNA Picogreen dsDNA quantification reagent. The library preparation was done using Illumina Nextera XT DNA Library Prep Kit with one nanogram of DNA. WGS was performed on the Illumina Nextseq 500 System. The Nullarbor bioinformatics pipeline (<https://github.com/tseemann/nullarbor>) was used for automated processing of the genomic samples. Multilocus sequence types (MLST) and clonal complexes (CC) were identified using PubMLST (<https://pubmlst.org>) (14). Comparative core genome SNP analysis was performed on isolates with the same MLST after removal of mobile genetic elements (MGEs, including phages and SCC*mec* elements if present) by manual curation. Isolates with >40 SNP differences in the core genome were considered ‘unrelated’ (3).

Sample size

All eligible SAB cases during the study period were to be enrolled. There was an average of 21 episodes of HO SAB per year at the Blacktown Hospital in the previous three years (2018-2020) and accounted for a third of all SAB. Hence, 60 SAB episodes were anticipated in 12 months study period (20 HO and 40 non-HO). We predicted that *S. aureus* would be isolated from up to 50% of participants' screening swabs and fewer than 10% of environmental samples.

Data collection and analysis

Patient demographics, the source of infection, disease manifestation, and outcomes were recorded from the hospital medical records.

Statistical analysis

Data entry and basic calculations were performed using Microsoft Excel. The chi-square test or Fisher's exact test was used as appropriate and reported the results as significant if the p-value was <0.05.

Ethics

The study was approved by the Western Sydney Local Health District Human Research and Ethics Committee (2020/ETH0155).

8.3 Results

A 12-month study was planned to start in late 2020. However, COVID-19 related restrictions in our facility led to a delayed start. The first enrolment was in June 2021, and the study terminated in December 2021 after a surge of COVID-19 cases.

Between June and December 2021, Blacktown Hospital recorded 40 unique episodes of SAB (30 MSSA and 10 MRSA). Twenty-five patients, 12 of whom (48%) were female, met the enrolment criteria and agreed to participate; their median age was 57 years (range 20-89 years). Cases were almost equally divided between community-acquired (13 cases, 52%) and healthcare-associated (12 cases, 48%; seven HO, six HACO). Four cases (one HO, three CA) were due to MRSA (Table 1).

Due to the impacts of COVID-19, on infection control staff and laboratory workflows, screening and environmental swabs were not collected from all participants, as planned, and isolates, from swabs that were collected, were not stored for genomic analysis. Therefore, strain relatedness between blood culture and carriage or environmental isolates could not be determined.

The findings of the study are summarised in Tables 1 and 2.

Hospital-onset (HO) SAB (cases 1-7)

Seven of 25 (28%) SABs were HO, including six due to MSSA. Five cases were associated with peripheral IV catheters (PIVC) and occurred 3-7 (median 4) days after admission. The source of one case, which occurred on day 6, was unknown. The only case of HO MRSA bacteraemia was linked to an infected peripherally inserted central (PICC) line and was diagnosed on day 16 (Table 1). All HO SAB occurred in non-ICU patients.

Genomic analysis of HO SAB isolates showed that all were distinct. They belonged to five MLSTs: ST45 (one MSSA, one MRSA); ST5 (two, with ~200 SNP differences, isolated two weeks apart from patients in different wards); one each of ST1, ST12, and ST144. Two isolates

were from patients in the same ward but had different STs (ST1 and ST5) and occurred three-weeks apart.

S. aureus was isolated from nine of 27 (33%) screening and/or wound swabs from six patients for whom results were available; all isolates were MSSA, except one MRSA from a wound (in a patient with MSSA SAB) (Tables 1 and 2). *S. aureus* was isolated from the environments of two of four patients for which results were available.

Healthcare-associated community-onset (HACO) SAB (cases 8-12)

Five of 25 (20%) SABs were HACO infections, all due to MSSA. Three were related to IV access lines and one to a post-operative MSSA urinary tract infection (UTI). The source was unknown, in one patient. All blood culture isolates belonged to different STs (ST5, ST8, ST188, an ST2276-single locus variant [SLV] and an ST5-SLV).

Community-acquired (CA) SAB (cases 13-25)

Thirteen of 25 (52%) SABs were CA, of which 10 were due to MSSA. Five were secondary to underlying skin disease or wounds, and three, including two due to MRSA, occurred in intravenous drug users (IVDUs). In five episodes the source was unknown.

Screening swabs were available from eight participants. Among them, *S. aureus* was found in the nares of five out of eight participants (63%). None of the participants had throat carriage.

Complications and outcomes

The most common complications of SAB were extravascular infections: infective endocarditis (IE) in four (one HO; three CA) and osteomyelitis/septic arthritis in four patients (one HACO,

three CA). IE cases were due to ST5, ST144, ST188, and ST953. Osteomyelitis/septic arthritis were due to ST 5-SLV, ST15, ST97, and ST188 belonging to different CCs. Six of eight extravascular infections occurred with CA SAB.

Prolonged bacteraemia (>72 hours) occurred in at least seven patients, all of whom had extravascular infections (six CA, one HO). There was no correlation between ST and prolonged bacteraemia (Table 1).

Day 90 outcomes were available for 22 of 25 participants, five of whom (two HO; three CA; all MSSA) had died. Numbers were too small to calculate differences between categories or strains.

At 12 months follow-up; 5/21 (71%) were alive and 6/21 (29%) had died, the outcome for the remaining four was unknown. Among the survivors, there were no relapses of SAB at 3 or 12 months, but two patients subsequently developed MRSA infections - a foot infection in a diabetic, and skin abscesses, one and four months, respectively, after MSSA SABs.

Strain relatedness

Of the 19 community-acquired SABs (CA+HACO), 16 were due to MSSA belonging to ST5 (3 cases), ST188 (3 cases), and 1 each of ST8, ST15, ST20, ST72, ST144, ST182, ST3902, ST5 SLV, ST12 SLV, and ST2276 SLV. There were >200 SNP differences between each of the ST5 and ST188 MSSA SAB isolates. The three ST5 SABs occurred with gaps of ~6 weeks between them and the three ST188 with gaps of 2 and 8 weeks. The three MRSA SAB isolates belonged to ST30, ST97 and ST953.

8.4 Discussion

Nearly half (48%) of the SABs identified in this study were healthcare-associated (HO or HACO) with infected venous access devices implicated in the majority and, therefore, potentially preventable. Compared with the study conducted 12 years ago at Westmead Hospital (Kok *et al*) (19), in the same LHD, there were lesser healthcare-associated SAB (48% versus 66%) and the proportion of MRSA causing SAB was smaller (16% versus 40%) in our study (19). A trend that has been noted in NSW and other regions over the past 10 years. Further, there was no evidence of epidemiological relatedness between SABs. Two STs were each represented by three isolates, but they differed by >200 SNPs and they were isolated 2-8 weeks apart. MSSA was the most frequent isolate in both hospital and community settings.

CA SABs were more likely than healthcare-associated SABs to be associated with complications, which may reflect longer intervals between onset and diagnosis. Most cases were associated with potential risk factors: IV drug use, skin infection and/or MSSA nasal carriage.

No recurrences of SAB occurred, but two patients developed MRSA infections, following MSSA SABs. This raises the possibility of MRSA acquisition in hospital, facilitated by antibiotic treatment for MSSA SAB.

SAB remains a serious illness; 22% of patients in this cohort died within 90 days of bacteraemia, which is consistent with the findings of a recent systematic review (16). Though a small size, the tendency towards higher mortality in SAB without CC affiliation is consistent with findings from Wertheim *et al* (2).

Throat carriage was not detected in any of our patients, which could be due to older age (median age 57 years) (17) Eradication and new colonisation during hospital (18) may also explain two cases who developed MRSA infections following MSSA bacteraemia therapy.

The limitation of this study includes a small sample size, incomplete carriage and environmental data, and lack of molecular typing on screening isolates. We did not review the impact of clinician feedback or if the PIVCs were retained for >72 hours, unlike the Kok *et al* study in 2010, but included molecular epidemiological analysis, and correlation between carriage/environmental samples and the bacteraemia. The study was conducted at the time of COVID-19 restrictions which affected specimen collection and study coordination. We found only two-third of participants with SAB had evidence of *S. aureus* carriage, which is less than reported in the literature, probably due to collection of screening swabs up to four days after blood culture identification. Antimicrobial therapy may have resulted in false-negative screening.

In conclusion, HO SABs in our facility are largely preventable as they were related to PIVC infections and occurred shortly after admission. Although the overall rates of healthcare-associated SABs and those due to MRSA have declined in the recent years, IV access devices remain a common and preventable source of infection. Furthermore, despite limitations imposed by sample size, analysis of carriage and environmental isolates indicated potential exogenous sources of health-care associated infections. A future study, similar in nature but larger in scale and with modifications, is warranted to demonstrate the effectiveness of practice improvement and identify possible additional risk factors and exogenous sources of SAB.

Table 1: Study findings

Cases	Age (yrs)	Sex	Source	MSSA/ MRSA	Onset	MLST	CC	Sites of infection	Days BC +ve	Skin/mucous membrane swabs	Environ-mental swabs	Status at 3 months
1	89	M	PIVC	MSSA	HO	144	na	IE, pacemaker lead infection	5	+ve: nose, hand, groin, wound (MSSA) -ve: throat	x	Died
2	76	M	Unknown	MSSA	HO	1	1	nil	1	-ve: nose, throat, hand, groin	-ve: computer mouse, keyboard, computer on wheels, bedrail and bed- remote	Unknown
3	79	F	PIVC	MSSA	HO	5	5	nil	1	+ve: wound - back (MRSA) -ve: nose, throat, hand, groin	+ve: computer on wheels (MSSA). -ve: computer mouse, keyboard, bed-rail, bed- remote	Alive

4	35	M	PIVC	MSSA	HO	5	5	nil	1	+ve: wound (MSSA) -ve: nose, throat, hand, groin	x	Died
5	29	M	PIVC	MSSA	HO	12	na	nil	1	+ve: hand (MSSA) -ve: nose, throat, groin	+ve: 2 sites (MSSA): location not specified	Alive
6	63	M	PIVC	MSSA	HO	45	45	nil	1	+ve: nose, groin (MSSA) -ve: throat, hand	-ve: bed rail, computer on wheels, keyboard, computer mouse, bed- remote	Alive
7	59	F	PICC	MRSA	HO	45	45	nil	2	x	x	Unknown
8	61	F	Vascath	MSSA	HACO	5	5	nil	2	x	na	Alive
9	57	F	PIVC	MSSA	HACO	8	8	nil	1	+ve: wound - foot (MSSA)	na	Alive

										-ve: nose, throat, hand, groin		
10	54	M	Urine	MSSA	HACO	188	1	Post operative UTI	1	-ve: nose, throat, hand, groin	na	Alive
11	76	F	Unclear	MSSA	HACO	2276	121	nil	1	na	na	Died
						SLV						
12	52	M	Vascath	MSSA	HACO	ST5	5	Calcaneal osteomyelitis (OM)	2	-ve: nose, throat, hand, groin, wound	na	Alive
						SLV						
13	48	F	IVDU	MRSA	CA	30	30	nil	1	na	na	Alive
14	48	M	Skin	MRSA	CA	97	97	Hip arthritis osteomyelitis	12	na	na	Alive
15	38	F	IVDU	MRSA	CA	953	97	IE, native tricuspid valve	5	+ve: nose (MRSA) -ve: throat, groin, hand	na	Alive

16	62	F	Unknown	MSSA	CA	5	5	IE, pacemaker	5		na	na	Alive
								lead infection,					
								empyema					
17	46	M	Skin	MSSA	CA	5	5	nil	1		+ve: nose	na	Alive
											(MSSA)		
											-ve: throat,		
											hand, groin,		
											wound		
18	77	F	Unknown	MSSA	CA	15	15	Shoulder	3		na	na	unknown
								septic arthritis					
19	69	M	Unknown	MSSA	CA	20	na	nil	1		+ve: nose	na	Alive
											(MSSA)		
											-ve: throat,		
											groin, hand		
20	20	F	Unknown	MSSA	CA	72	8	nil	2		-ve: nose,	na	Died
											throat, hand,		
											groin		
21	56	F	Skin	MSSA	CA	182	na	nil	1		+ve: nose	na	Alive
											(MSSA)		

										-ve: throat, groin, hand		
22	55	F	IVDU	MSSA	CA	188	1	IE, native mitral & tricuspid valves	4	na	na	Alive
23	52	M	Skin	MSSA	CA	188	1	Vertebral osteomyelitis	5	+ve: hand, groin (MSSA)	na	Alive
										-ve: nose, throat		
24	72	M	Unclear	MSSA	CA	3903	1	nil	1	-ve: nose, throat, hand, groin	na	Alive
25	84	M	Skin	MSSA	CA	ST12 SLV	na	nil	1	+ve: nose, hand, groin (MSSA)	na	Died
										-ve: throat		

Abbreviations: MLST: multilocus sequence typing; ST: sequence type; CC: MLST clonal complex; BC: blood culture; IVDU: intravenous drug use; PIVC: peripheral intravenous catheter; PICC: peripherally inserted central line; CA: community-acquired; HACO: healthcare-associated community-onset; HO: hospital-onset; IE: infective endocarditis; x: sample was either not collected or already discarded; na: not applicable; unknown: outcomes not known; +ve: positive; -ve: negative.

Table 2: *S. aureus* carriage/colonisation in patients with *S. aureus* bacteraemia

	SAB, n*	Nasal carriage detected n (%)	<i>S. aureus</i> isolated from skin or mucous membrane [#] n (%)
HO	6	2 (33)	4 (67)
HACO	3	0	1 (33)
CA	8	5 (63)	7 (88)

n: number of cases from whom swabs were collected; CA: community-acquired; HACO: healthcare-associated community-onset; HO: Healthcare-associated hospital-onset; SAB: *S. aureus* bacteraemia

* No specimens from skin/mucous membrane sites were collected from one HO, two HACO and five CA cases. (see Table 1 for details)

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Contribution of authors:

RD was the chief investigator and planned the study, obtained ethics, enrolled patients, organised screening swab collection, coordinate the study, data collection and interpretation, and drafted the manuscript. GLG and MVNO supervised RD and helped with data analysis and interpretation. All authors contributed to the study design and/or data collection, and critical review of the manuscript.

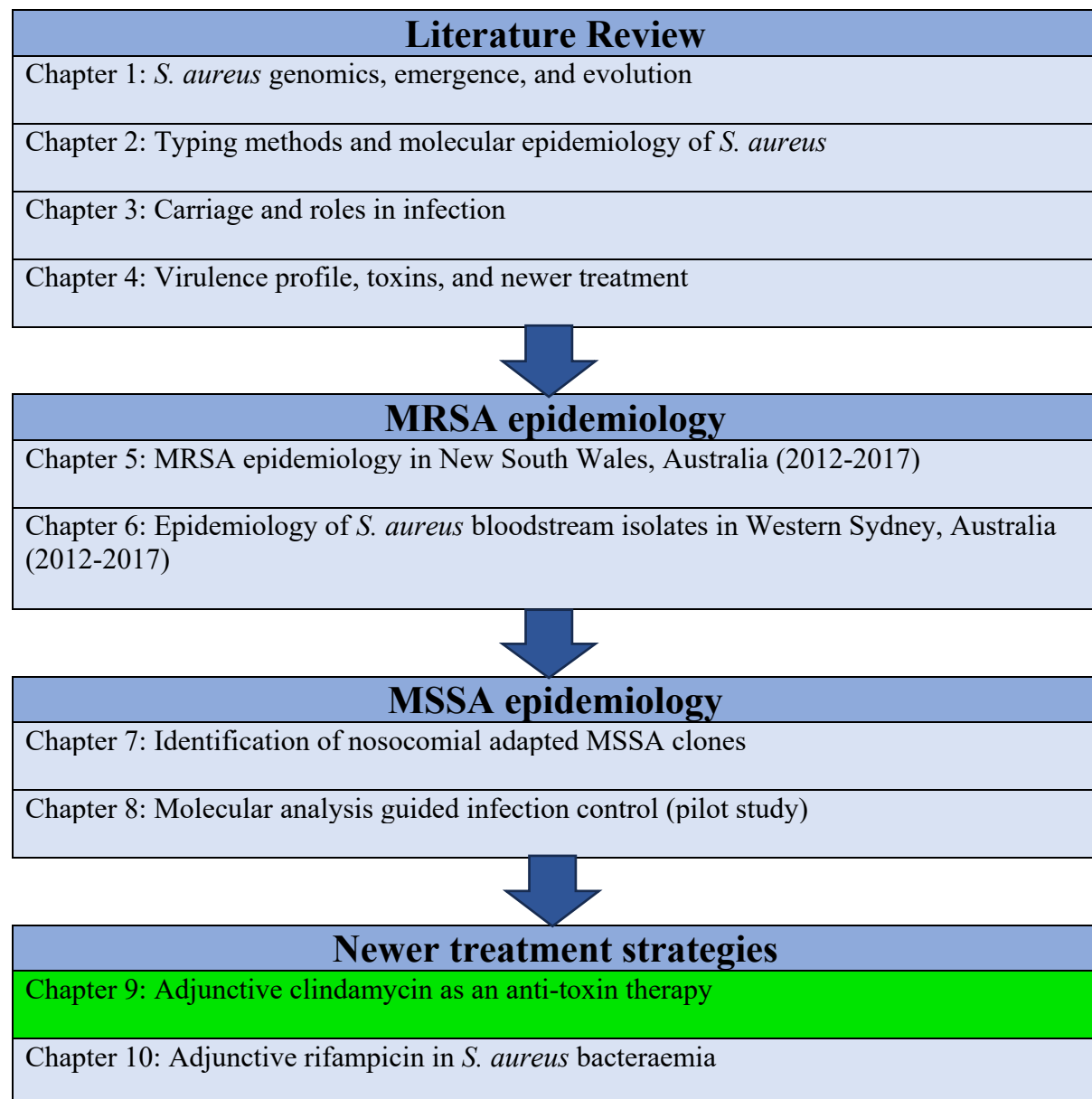
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Figure: Schematic overview of the thesis structure



Chapter 9: Overview

In Chapter 4 of this thesis, the roles of exotoxins in *S. aureus* infections were reviewed. Within that chapter, the potential value of adjunctive clindamycin therapy in limiting exotoxin production during serious *S. aureus* infections was explored as a novel therapeutic strategy.

The current chapter (Chapter 9) outlines a study protocol employing clindamycin as an anti-toxin therapy. A pilot randomised clinical trial based on this protocol was conducted between 2018 and 2020.

This chapter includes published work. The resulting randomised controlled trial is listed in the Appendix.

Chapter 9: Adjunctive clindamycin as an anti-toxin therapy

9.1 Introductory comments

S. aureus can cause a wide spectrum of clinical manifestations, ranging from milder conditions to severe diseases such as complicated skin and skin structure infections, necrotising pneumonia, and septic shock (1). The development of these severe manifestations is, at least in part, attributed to the organism's exotoxin production, including but not limited to Panton-Valentine leukocidin and toxic shock syndrome toxin. Exotoxins serve as crucial virulence factors, contributing to elevated morbidity and mortality rates (2). For example, *S. aureus* bacteraemia is associated with a mortality rate of up to 20% within 1 month (3). This risk becomes even more pronounced, reaching threefold higher levels (40-70%), in cases of staphylococcal septic shock (4).

S. aureus comprises methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). Standard treatment for MSSA and MRSA infections involve flucloxacillin (or cefazolin) and vancomycin (or daptomycin), respectively (1). While these antibiotics effectively prevent bacterial replication, they do not necessarily impede bacterial toxin synthesis. Whereas, clindamycin, an antibiotic that inhibits protein synthesis, targets the bacterial ribosome. Both in vitro and animal studies have demonstrated its effectiveness in inhibiting bacterial exotoxin production (5–7). Observational human studies also suggest a possible benefit of adjunctive clindamycin in the treatment in severe *S. aureus* infections (8). Despite a lack of definitive randomised controlled trials (RCTs) supporting this indication, international and Australian guidelines recommend its use (9,10).

A protocol for a pilot study has been proposed to assess the impact of adjunctive clindamycin in severe, toxin-mediated *S. aureus* infections in both adult and paediatric populations. This open-label, parallel-group, superiority, multicentre, RCT aims to compare outcomes in severe *S. aureus* infections between two groups: one receiving standard treatment for *S. aureus* infection (flucloxacillin, cefazolin, or vancomycin as indicated) and the other receiving the standard treatment plus additional clindamycin administered for seven days.

9.2 Aims

The study aims to analyse the effects of adjunctive clindamycin in both children and adults with severe MSSA and MRSA infections. The pilot study is designed to assess feasibility and refine the study design for a definitive RCT.

9.3 Publication

CASSETTE-clindamycin adjunctive therapy for severe *Staphylococcus aureus* treatment evaluation: study protocol for a randomised controlled trial.

Authors:

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Contribution of authors:

JD, ST, and AB conceptualised the idea and wrote the study synopsis.

I performed literature review, expanded the study design, and wrote the protocol. I was the corresponding author.

All authors contributed to the study design and critically reviewed the manuscript.

9.4 Remarks

The proposed pilot RCT was conducted between July 2018 and October 2020 (11). It enrolled 34 participants, including 11 children. Despite being terminated due to slow enrolment, the study demonstrated feasibility in enrolling both adults and children in a single RCT with harmonised endpoints. The published RCT is listed in the Appendix.

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STUDY PROTOCOL

Open Access



CASSETTE—clindamycin adjunctive therapy for severe *Staphylococcus aureus* treatment evaluation: study protocol for a randomised controlled trial

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Abstract

Background: Exotoxins are important virulence factors in *Staphylococcus aureus*. Clindamycin, a protein synthesis inhibitor antibiotic, is thought to limit exotoxin production and improve outcomes in severe *S. aureus* infections. However, randomised prospective data to support this are lacking.

Methods: An open-label, multicentre, randomised controlled trial (RCT) will compare outcome differences in severe *S. aureus* infection between standard treatment (flucloxacillin/cefazolin in methicillin-susceptible *S. aureus*; and vancomycin/daptomycin in methicillin-resistant *S. aureus*) and standard treatment plus an additional clindamycin given for 7 days. We will include a minimum of 60 participants (both adult and children) in the pilot study. Participants will be enrolled within 72 h of an index culture. Severe infections will include septic shock, necrotising pneumonia, or multifocal and non-contiguous skin and soft tissue/ostearticular infections. Individuals who are immunosuppressed, moribund, with current severe diarrhoea or *Clostridioides difficile* infection, pregnant, and those with anaphylaxis to β -lactams or lincosamides will be excluded.

The primary outcomes measure is the number of days alive and free (1 or 0) of systemic inflammatory response syndrome (SIRS) within the first 14 days post randomisation. The secondary outcomes measure will include all-cause mortality at 14, 42, and 90 days, time to resolution of SIRS, proportion with microbiological treatment failure, and rate of change of C-reactive protein over time. Impacts of inducible clindamycin resistance, strain types, methicillin susceptibility, and presence of various exotoxins will also be analysed.

Discussion: This study will assess the effect of adjunctive clindamycin on patient-centred outcomes in severe, toxin-mediated *S. aureus* infections. The pilot study will provide feasibility for a much larger RCT.

Trial registration: Australian New Zealand Clinical Trials Registry, [ACTRN12617001416381p](https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=361141). Registered on 6 October 2017.

Keywords: *Staphylococcus aureus*, Exotoxins, Prospective studies, Clindamycin, Leukocidins

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Background

Staphylococcus aureus is a ubiquitous bacterium, colonising at least 50% of the population (20% persistently and 30% intermittently) [1, 2] and causing a wide range of infections. *S. aureus* bacteraemia (SAB) is associated with a mortality rate of 20–30% in adults [3, 4] and approximately 5% in children [5]. Necrotising pneumonia and severe toxin-mediated infections with *S. aureus*, however, are associated with a much higher mortality [6–8]. This high mortality rate remains despite better understanding of *S. aureus* disease management in recent decades [8–13].

Anti-staphylococcal β -lactams are the drugs of choice for treatment of methicillin-susceptible *S. aureus* (MSSA) infections, whereas vancomycin (or daptomycin) is the drug of choice for methicillin-resistant *S. aureus* (MRSA) infections. Successful source control and early effective antibiotics are the crucial factors for treatment success. Use of a single antimicrobial agent is usually recommended, but two- or three-drug combination therapy is advised in selected cases [12, 13], for instance in prosthetic valve infective endocarditis and prosthetic joint infections. The rationale is either for synergy or biofilm penetration. Additionally, several guidelines [12, 14–16] recommend adding clindamycin to standard therapy in suspected toxin-mediated *S. aureus* infections. These recommendations are based on expert opinions with limited clinical evidence available. Clindamycin is a protein synthesis inhibitor (PSI) antibiotic. Although in vitro animal studies and observational human data suggest a possible benefit of adjunctive clindamycin [17], there are no published or registered randomised controlled trials (RCTs) testing this strategy in *S. aureus* infections.

Staphylococcus aureus toxins

S. aureus virulence is in part due to its exotoxins, surface virulence factors, and enzymes [18]. All *S. aureus* strains may produce haemolysins, nucleases, proteases, lipases, hyaluronidase, and collagenase. Many strains also harbour genes for toxic shock syndrome toxin-1 (TSST-1), exfoliative toxins, enterotoxins, and leucocidins [19–23]. In an analysis of 429 *S. aureus* isolates from Germany in 2003 [21] (219 blood isolates and 210 anterior nares isolates, 94% MSSA), 73% of the isolates harboured toxin-related genes. Enterotoxin-G (*seg*), enterotoxin-I (*sei*), and toxic shock syndrome toxin (*tst*) genes were the three most common. In a study by Peacock et al. in the UK in 2002 [23], α -haemolysin was near universal in *S. aureus* isolates (both carriage and invasive). Other common toxins were β -haemolysin, δ -haemolysin, γ -haemolysin, enterotoxin-G (*seg*), and enterotoxin-I (*sei*). Twenty-five per cent of carriage

isolates and 30% of bacteraemic isolates had the *tst* gene detected.

Panton-Valentine leucocidin (PVL) is a *S. aureus* leukotoxin. Its prevalence varies by population demographics, strain type, and source of infection [24, 25]. PVL-expressing strains have been associated with skin and soft tissue infections (SSTI), which often require surgical intervention [26]. Multi-locus sequence typing (MLST) ST93 *S. aureus* is a virulent strain, and is common in Australia. It carries *lukSF-PV* and hyper-expresses α -toxin [27]. This is true for ST93 MRSA, but MSSA of the same sequence type often co-exists [28].

S. aureus virulence is due to multiple determinants, and hence targeting a single factor is unlikely to prove successful in improving disease outcomes [23, 29, 30]. Clindamycin, through its role as a PSI, is expected to limit expression of multiple exotoxins and hence may improve clinical outcomes, but randomised prospective data to support this are lacking.

Antibiotics which act as protein synthesis inhibitors

Certain antibiotics exert their anti-bacterial activity by selectively blocking prokaryotic ribosomal protein synthesis [31, 32]. The antibiotics targeting the 30S ribosomal subunit (aminoglycosides, tetracyclines, and tigecycline) interfere with the principal function of translating mRNA to peptides. The 50S ribosomal subunit acting antibiotics (clindamycin, linezolid, macrolides, streptogramins, chloramphenicol, and fusidic acid) interfere with peptide bond formation [31]. Specifically, MLS_B antibiotics (macrolides, lincosamides, streptogramin B) bind to the narrowest portion of the 50S ribosomal subunit tunnel, which has a regulatory function in peptide synthesis [31].

Of the PSI antibiotics, clindamycin has well-recognised anti-toxin activity. It has shown more consistent toxin suppressive activity than other PSI antibiotics [17] and has been recommended in selected toxin-mediated staphylococcal and streptococcal infections. Linezolid has also been reviewed as an anti-toxin agent [33], but its use is limited by cost, potential for haematological toxicity, and preservation for treatment of resistant Gram-positive organisms and mycobacterial infections.

Clindamycin compared to β -lactam antibiotics for anti-toxin therapy

β -lactam antibiotics (penicillins, cephalosporins), the drugs of choice for MSSA infections, are less efficacious in high-inoculum infections. Penicillin has been demonstrated as ineffective against bacteria in a stationary phase of growth (i.e. not actively dividing) [34, 35]. Penicillin treatment (including anti-staphylococcal penicillins) may also stimulate toxin production [36–40]. On the other hand, clindamycin is not affected by the phase

of growth or inoculum size [29, 35]. It also represses penicillin-induced exotoxin production [40]. Vancomycin and daptomycin appear neither to induce nor inhibit exotoxin production [39, 41].

Objective

To determine the feasibility of and refine the study design for a future definitive RCT assessing the effect of adjunctive clindamycin on clinical outcomes in children and adults with severe *S. aureus* infections (both MSSA and MRSA).

Hypothesis

Clindamycin will lead to more rapid resolution of systemic inflammation due to blockade of exotoxin production by *S. aureus*.

Methods

Study setting

The CASSETTE study is an investigator-initiated, open-label, parallel-group, superiority, multicentre RCT (Fig. 1). This study will be conducted across 12 hospitals in

Australia (see Additional file 1: Table S7). Sites are selected based on: prevalence of *S. aureus* infection (an estimate of at least 10 potentially eligible cases per year for adults and five for children); availability of a committed site principal investigator (PI); and a second person available at each site to assist the PI, either a research nurse, registrar, or physician colleague.

Inclusion criteria

1. Age—both children and adults will be eligible. Infants must have a corrected age of ≥ 28 days.
2. *S. aureus* (MSSA or MRSA) identified in at least one clinically relevant specimen (including an isolation in a polymicrobial culture when the other isolates are thought to be non-significant).

A “clinically relevant specimen” refers to any specimen where the site PI judges the isolate to be contributing to the patient’s clinical syndrome. This does not have to be a sterile site. These specimens may include blood, fluids from other normally sterile sites (such as joint fluid or

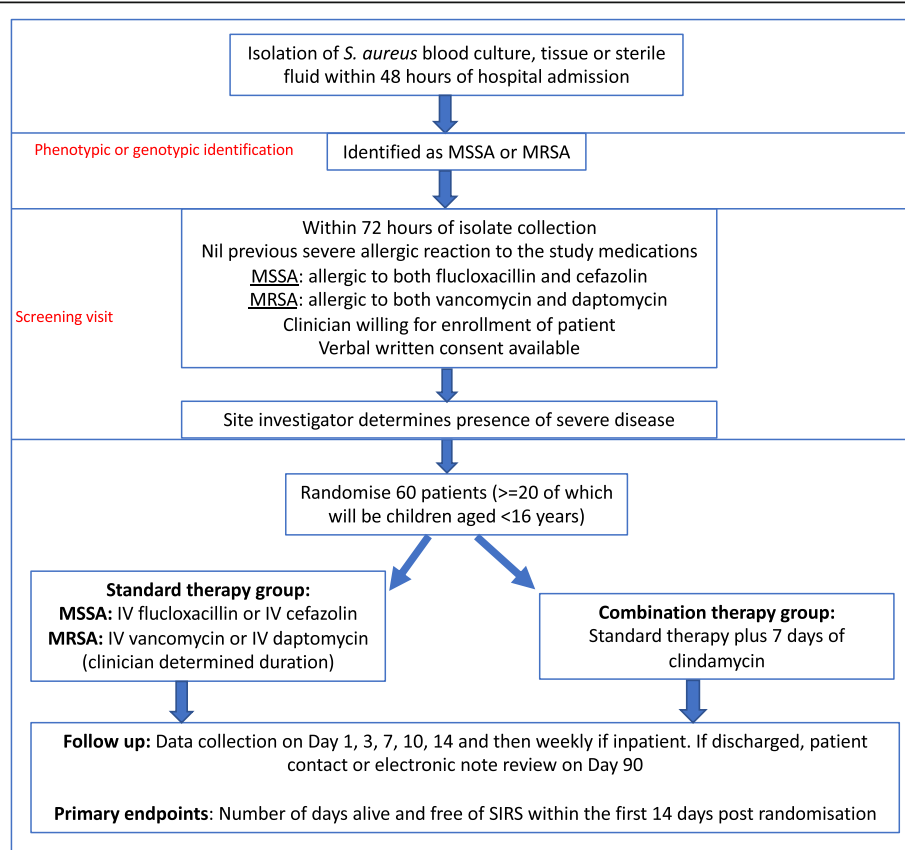


Fig. 1 Flowchart overview of the trial design. IV intravenous, MRSA methicillin-resistant *Staphylococcus aureus*, MSSA methicillin-susceptible *Staphylococcus aureus*, SIRS systemic inflammatory response syndrome. SPIRIT checklist (2013) provided in Additional file 2

cerebrospinal fluid), pus, tissue, or bone obtained surgically. The site PI may additionally make a clinical judgement on whether other specimens from non-sterile sample types yielding *S. aureus*, such as sputum and superficial swabs, are considered clinically relevant.

3. Ability to be randomised within 72 h of the collection of the index culture.

The index culture is the first clinically relevant specimen collected both after the participant has arrived at a hospital and after the onset of systemic symptoms. If more than one specimen is collected within the same 4-h period, the most clinically significant specimen will count as the index specimen (sterile site isolates are prioritised over others). Randomisation will be performed using a web-based system (REDCap).

Antimicrobial therapy will be clinician determined prior to randomisation. This may include clindamycin and a combination antibiotic that may include coverage for both MSSA and MRSA. The pre-randomisation treatments will be recorded and adjusted for in the analysis.

4. Probability of remaining as an inpatient of the study site hospital for at least 7 days following randomisation (or accessible for follow-up by the site PI, e.g. hospital in the home).
5. Index culture drawn no later than 48 h after hospital admission.
6. Severe disease: evidence of ≥ 2 systemic inflammatory response syndrome (SIRS) criteria within 48 h prior to randomisation and at least one of the following three conditions.
 - 6.1. Septic shock (including Staphylococcal toxic shock syndrome).

In adults, septic shock is defined as:

- i. Mean arterial pressure < 70 mmHg or systolic blood pressure < 90 mmHg despite at least 3 L of fluid administration; or
- ii. the need for intravenous vasopressors to maintain organ perfusion (i.e. any receipt of adrenaline, noradrenaline, dopamine, dobutamine, vasopressin, or terlipressin).

In children, septic shock is defined as sepsis with cardiovascular dysfunction. Sepsis is SIRS in a setting of suspected or proven infection. Cardiovascular dysfunction is defined by the International Paediatric Sepsis Consensus Conference [42] as follows—despite administration of isotonic intravenous fluid bolus ≥ 40 ml/kg in 1 h:

- i. decrease in blood pressure (BP) (hypotension) < 5 th percentile for age or systolic BP < 2 SD below normal for age (Table 1); or
- ii. need for vasoactive drug to maintain BP in normal range (dopamine > 5 μ g/kg/min or dobutamine, epinephrine, or norepinephrine at any dose); or
- iii. two of the following:
 - a. unexplained metabolic acidosis: base deficit > 5.0 mEq/L;
 - b. increased arterial lactate > 2 times upper limit of normal;
 - c. oliguria: urine output < 0.5 ml/kg/h;
 - d. prolonged capillary refill: > 5 s; and
 - e. core to peripheral temperature gap > 3 °C.

6.2. Necrotising lung/pleural space infection

Either:

- i. an exudative pleural fluid aspirate and has Gram-positive cocci in clusters on Gram stain (in the presence of MSSA or MRSA from another clinically relevant specimen) or has grown *S. aureus* from the pleural fluid; or
- ii. pneumonia which is:
 - a. multifocal (within the lung); and
 - b. *S. aureus* grown from any of sputum, endotracheal aspirate, bronchial lavage, blood, or pleural fluid.

The diagnosis of necrotising pneumonia may be aided by computerised tomography (CT) scan, if available.

- 6.3. Complicated skin/soft tissue/osteoarticular infection which is multifocal and non-contiguous
This includes pyomyositis, septic arthritis, osteomyelitis, skin or soft tissue abscess, or carbuncle.

Non-contiguous multifocal means it is at more than one anatomical site, which is likely to be due to systemic spread rather than direct extension. For example, osteomyelitis of the distal tibia with septic arthritis of the ankle joint does not qualify, as it is contiguous. Lumbar vertebral osteomyelitis with direct extension into the psoas muscle does not qualify. Humeral osteomyelitis with psoas abscess does qualify.

Exclusion criteria

1. Previous severe allergic reaction to both flucloxacillin and cefazolin (for MSSA), or to both

Table 1 Age-appropriate criteria for paediatric systemic inflammatory response syndrome

Age group	Heart rate (beats/min)		Respiratory rate (breaths/min)	Leucocyte count $\times 10^3/\text{mm}^3$	Systolic BP (mmHg)
	Tachycardia	Bradycardia			
1 month–1 year	> 180	< 90	> 60	> 17.5 or < 5	< 100
2–5 years	> 140	NA	> 40	> 15.5 or < 6	< 94
6–12 years	> 130	NA	> 20	> 13.5 or < 4.5	< 105
13–15 years	> 90	NA	> 20	> 11 or < 4.5	< 117

The values are the 5th or the 95th percentile for age

Modified from Goldstein et al.'s paediatric sepsis consensus report [42]

BP blood pressure, NA not applicable

vancomycin and daptomycin (for MRSA), or to lincosamides.

- Previous participation in the trial.
- Known pregnancy.
- Currently receiving a lincosamide or other potentially anti-toxin antibiotic which cannot be ceased or substituted (listed in the earlier section “Antibiotics which act as protein synthesis inhibitors”).
- Participant's primary clinician unwilling to enrol patient.
- Moribund (expected to die in next 24 h with or without treatment).
- Treatment limitations which preclude the use of antibiotics.
- Significant immunosuppression (prednisolone > 0.5 mg/kg/day for ≥ 14 days in the last 30 days, other immunosuppressive medication, known HIV with CD4 count < 200, congenital immunodeficiency).
- Necrotising fasciitis.
- Clostridioides difficile*-associated diarrhoea or severe diarrhoea (> 6 stools per day or clinician-determined severe diarrhoea in children) from any cause.

Interventions

Control group

The control group will receive “standard therapy”, also called “backbone therapy”. This will consist of IV flucloxacillin 50 mg/kg/dose up to 2 g every 4–6 h given intermittently or via 24-h infusion for MSSA infections. Flucloxacillin can be substituted with IV cefazolin 50 mg/kg/dose up to 2 g every 6–8 h if there is a history of minor allergic reaction to penicillin, defined as rash or unclear allergic reaction, but not anaphylaxis or angioedema, or at the site PI's discretion. The standard therapy for MRSA infection will consist of IV vancomycin with a loading dose of 25 mg/kg in adults (clinician discretion regarding loading dose in children < 18 years) followed by maintenance dose of 15–20 mg/kg every 12 h (for adults) or 15 mg/kg/dose every 6 h (for children) with subsequent adjustment to maintain trough levels at 15–20 mg/dl, which may require an infusion at clinician

discretion or IV daptomycin 6–10 mg/kg/day. Paediatric daptomycin dosing guidance [43]: for 1–6 years old, 12 mg/kg/dose every 24 h; for 7–11 years old, 9 mg/kg/dose every 24 h; and for 12–17 years old, 7 mg/kg/dose every 24 h. The dose of backbone therapy will be adjusted for renal function (Additional file 1: Tables S1–S5).

The duration of standard therapy is clinician determined but should be in line with recommendations in the current version of Therapeutic Guidelines (TG) Antibiotic [14] in adults and with expert consensus in children. The choice between vancomycin and daptomycin, and flucloxacillin and cefazolin is clinician determined, and may be based on local practice or the minimum inhibitory concentration (MIC) of the MRSA isolate against vancomycin.

The usual recommended duration of a standard treatment of *S. aureus* sepsis in adults is 14–42 days. In children, early switch (after 7–14 days of IV therapy) to oral therapy is practised commonly and is permitted in this trial.

Combination therapy group

All participants assigned to the combination group will receive standard therapy as above. In addition, they will also receive open-label clindamycin (10 mg/kg/dose up to 600 mg QID IV for both adult and children) for 7 days from randomisation (day 1 being the day of randomisation). The intravenous route for clindamycin is recommended but not mandated for the entire duration of therapy (7 days). If a switch to oral therapy is thought to be necessary and appropriate by the site PI, transition to oral clindamycin 450 mg TDS for adults or 10 mg/kg/dose PO TDS (maximum 450 mg) for children is recommended.

Criteria for discontinuing or modifying allocated interventions

- Adjustment for renal impairment.

Dosage adjustment for the standard therapy medication is as per the recommendations in TG [14] and

antimicrobial dosing in renal impairment guidelines of the Children's Hospital [44] at Westmead, Sydney, Australia (Additional file 1: Tables S1–S5; also includes vancomycin therapeutic drug monitoring). Continuous infusion of the backbone antibiotics (flucloxacillin/cefazolin/vancomycin) during inpatient admission is not routine practice in Australia; however, such use will not constitute a protocol violation. Clindamycin (IV or PO) does not need dose modification in renal impairment.

- b. Change of primary standard therapy after randomisation.

The change of primary standard therapy between flucloxacillin and cefazolin (for MSSA) or between vancomycin and daptomycin (for MRSA) is permitted at the discretion of treating clinicians, or if there are issues with supply or stock of these antimicrobials. Any unnecessary changes will be discouraged. Clinicians may change the backbone therapy if the patient develops an adverse drug reaction, such as rash with flucloxacillin or vancomycin, or raised creatinine kinase with daptomycin, or if the vancomycin MIC of the MRSA isolate is found to be ≥ 2 $\mu\text{g/ml}$, or in cases of persistent bacteraemia whilst receiving vancomycin.

The change within 7 days of randomisation will still be counted as β -lactam therapy and will not violate per-protocol analysis. However, it will affect the subgroup analysis (flucloxacillin vs cefazolin; vancomycin vs daptomycin). Participants who receive the majority of one or the other drugs in the 7 days will be analysed in the respective groups.

- c. Clindamycin use after day 7 post randomisation.

The use of clindamycin after the completion of 7 days of randomisation will be discouraged for up to 90 days post randomisation. The treating team will be advised to use an alternative to clindamycin if such treatment is necessary.

Strategies to improve adherence to the protocol, and any procedures for monitoring adherence

- a. Training of site investigators.

Each site will have a PI who will be trained in the study protocol, standard operating procedures, and their reporting requirements. The project manager will have regular contact (email or phone) with all enrolling site PIs.

- b. Documentation in patient's medical records.

On day 1 of randomisation, the patient's enrolment in the CASSETTE trial will be documented in the medical record, and the treating team will be made aware of the recruitment. Study information will be placed in the patient's folder. The PI or their associates (research nurse or registrar) will check drug charts (paper and/or electronic) daily (except weekends and public holidays) during the period of the intervention. Charts will be reviewed before weekends and public holidays to ensure appropriate doses are prescribed for the period, and to check when clindamycin requires ceasing (i.e. will complete 7 days). Follow-up will be done on the first working day.

- c. Monitoring.

As this pilot study is determining feasibility as well as refining assumptions and study design in preparation for a definitive RCT, we intend to utilise off-site risk-based monitoring to be completed by the central coordinating office. The web-based database will have logic checks included in the design to avoid data-entry errors. The central coordinator will also monitor data entry at each site for completeness and data checks will be regularly conducted to ensure protocol compliance.

Relevant concomitant care and interventions that are permitted or prohibited during the trial

Apart from the use of clindamycin, the patient's management will be at the discretion of the caring team in consultation with the site PI. Procedures undertaken and directly relevant to the study will be recorded (e.g. debridement of infected necrotic tissue, washout of an infected joint, or use of intravenous immunoglobulin). Actions that clearly violate the study protocol, such as early cessation of clindamycin or use of an additional PSI antibiotic, will be discouraged. Site investigators will actively recommend therapeutic drug monitoring or dose adjustment for renal failure per protocol as necessary.

Outcomes

Primary outcome

The primary outcome is the number of days alive and free of SIRS within the first 14 days post randomisation. "Free of SIRS" is defined as meeting < 2 SIRS (i.e. 1 or 0) criteria simultaneously. The day of randomisation is counted as day 1 (not day 0). We used SIRS criteria as they are routinely collected and are a more conservative measure of patient recovery. Also, this was thought to be a single measure of sepsis that could be used in both children and adults.

SIRS criteria in adults [45]

1. Abnormal body temperature (< 36 or 38 $^{\circ}\text{C}$).

2. Tachypnoea or mechanical ventilation (RR > 20 breaths/min in an adult, age dependent in children).
3. Tachycardia (HR > 90 beats/min in an adult, age dependent in children).
4. Abnormal leucocyte count, $> 12 \times 10^9/L$ or $< 4 \times 10^9/L$ (using last observation carried forward from any FBC).

SIRS criteria in children (Table 1) SIRS is defined as the presence of at least two of the following four criteria, one of which must be abnormal temperature or leucocyte count. The following are modified from the NSW Clinical Excellence Commission Sepsis Kills guidelines [46] and the International Paediatric Sepsis Consensus Conference [42] held in 2002. Heart rate, respiratory rate, and temperature will be recorded at least daily for the first 7 days, and after this time will be recorded at least daily as long as the patient remains in hospital or hospital in the home (HITH).

1. Abnormal body temperature of < 36 or $> 38^\circ C$.
2. Tachycardia or bradycardia (mean heart rate) that is otherwise unexplained and persists over at least 30 min.
3. Tachypnoea (see Table 1) or mechanical ventilation not related to underlying neuromuscular disease or the receipt of general anaesthesia.
4. Abnormal leucocyte count, or $> 10\%$ circulating immature neutrophils (“band forms”).

SIRS is considered present if ≥ 2 criteria were met at the same time on any given calendar day. For example: an adult had a heart rate of 95 beats/min at 9 a.m. but no other criteria at this time. She then had a heart rate < 90 beats/min for the rest of the day. At 3 p.m. she had a respiratory rate of 22 breaths/min for 1 h which also then normalised. Although she had two SIRS criteria on the same calendar day, they were not simultaneous and hence she was deemed to be free of SIRS on that day.

If the patient has been discharged from both hospital and HITH before day 14, then SIRS will be assumed to have resolved at the time of discharge if it had not already done so.

White blood counts will be measured on days 1, 3, 5, 7, and 14. There is a ± 1 -day window if the WBC is not collected on the stipulated days, with preference given to the preceding day's results. If more than one WBC is measured on the same day, then the most abnormal results will be recorded.

Secondary outcome measures

1. All-cause mortality at 14, 42, and 90 days.

2. Time to resolution of SIRS (number of days until the patient meets < 2 simultaneous SIRS criteria on a calendar day).
3. Proportion with microbiological relapse (positive blood culture for MSSA or MRSA at least 72 h after a preceding negative culture).
4. Proportion with microbiological treatment failure (positive sterile site culture for MSSA or MRSA at least 14 days after randomisation).
5. Number of surgical procedures performed for the purposes of *S. aureus* infection source control.
6. Duration of intravenous antibiotic treatment.
7. *C. difficile*-associated diarrhoea (3 or more loose stools per day along with a positive laboratory test for *C. difficile* toxin).
8. All cause diarrhoea (3 or more loose stools per day).
9. Slope of CRP curve days 1–14 (i.e. rate of change of CRP over time).

Participant timeline (Table 2)

Eligibility screening

We will identify potential patients as those who meet the two main inclusion criteria of: *S. aureus* (MSSA and MRSA) identified in a clinically relevant specimen; and clinically defined severe disease (septic shock, necrotising lung/pleural space infection, complicated multifocal skin or soft tissue, or osteoarticular infection). All such identified patients will be screened and assessed for potential enrolment and randomisation. The identification of such participants will typically be through notification by microbiology staff (e.g. when blood cultures are called through and clinical information obtained from the treating clinician) or other medical staff (e.g. infectious diseases, paediatric, ICU). Identification of *S. aureus* as MSSA or MRSA can be by any acceptable methods, such as nucleic acid amplification of *fem/nuc*, *mec* genes, or phenotypic susceptibility methods.

Informed consent

The PI or their delegate will approach an eligible patient or their next of kin (NOK) for a discussion on participation in the trial. Written information will also be provided. An interpreter will be used if necessary. Informed consent from a NOK will only be used in jurisdictions where there is legislative approval to do so, and where the site has research governance approval in place.

Paediatric-specific consent issues

All participants under the age of 18 years will have consent sought from a parent/guardian. Children and adolescents will have the study explained to them using appropriate language. Where deemed appropriate, the adolescent will be asked to co-sign the parent/guardian

Table 2 Schedule of visits, data collection, and follow-up

Timepoint	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 10	Day 14	Day 15–89	Day 90
Enrolment											
Eligibility screen	X										
Informed consent	X										
Demographic details	X										
Clinical details	X										
Randomisation	X										
Interventions											
Standard therapy (flucloxacillin/cefazolin or vancomycin/daptomycin)	X	X	X	X	X	X	X	X	X ^a	X ^a	
Clindamycin (if in combination group)	X	X	X	X	X	X	X				
Assessments											
Blood cultures			X		X ^b		X ^b				
FBC, EUC, LFTs, CRP	X		X		X		X		X		
Check SIRS score	X	X	X	X	X	X	X	X	X		
Check vital observations	X		X				X	X	X		
Clinical progress assessment	X		X				X	X	X	Weekly if inpatient	X
Vital status			X				X	X	X	X	X
Additional data											X

CRP C-reactive protein, EUC electrolytes, urea, and creatinine, FBC full blood count, IV intravenous, LFT liver function tests, SIRS systemic inflammatory response syndrome

^aThe usual duration of IV treatment for *Staphylococcus aureus* bloodstream infection is 14–42 days in adults and 7–14 days in children; 4–6 weeks of IV therapy may be necessary in children with infective endocarditis

^bIf day 3 blood culture is positive, repeat every 48–72 h until negative

consent form. If an adolescent does not want to participate in the study, this would be considered a refusal and they will be considered ineligible.

For Aboriginal and Torres Strait Islander children, informed consent can be given by a parent or caregiver who is recognised under Aboriginal customary law or Aboriginal tradition as having decision-making rights over the child's health care needs and who assumes day-to-day parental responsibilities.

If consenting parents are under the age of 18 years, consent will be sought from the child's grandparent with a parent signing as a witness.

Randomisation and blinding

Patients will be randomised using a module in the web-based study database. The randomisation list will be generated and held by an independent statistician. Randomisation will be stratified by age (child versus adult) in a 1:1 ratio, in permuted blocks of variable size. Randomisation will not be stratified by site, as an average site is only likely to randomise 5–10 patients and hence the strata will be too small. We will not stratify by MSSA/MRSA status for similar reasons—the strata will be too small, and the study is designed as a pilot study with the aim of assessing for feasibility.

Blinding is not relevant as this will be an open-label trial.

Days 2–90

The PI (or delegate) will visit the patient, review medical documentations, or contact the treating team in person or by phone daily for the first 7 days of randomisation to ensure compliance with the protocol and that the recommended tests are ordered. Case report forms (CRFs) with details on illness severity scores, clinical progress, and blood results will be completed daily. These details can be retrospectively collected up to 72 h after the intended day, allowing for weekends and public holidays. Standard operating procedures will contain step-by-step details on how to recruit patients and collect data.

Endpoint assessment

A panel blinded to treatment allocation will determine the primary endpoint. This will consist of three clinicians, each with expertise in infectious diseases, paediatrics, or critical care. The critical care clinician will chair to overcome discrepancies in the decision-making between the three decision-makers.

The panel will be given an extract of the database which contains all information needed to determine the

number of days alive and free of SIRS over the first 14 days but will not be given any information about the use of clindamycin.

If needed, the panel may request further information (e.g. medical record or observation chart extracts with identifying information redacted) for any particular patient.

Discontinuation/withdrawal of participants from trial treatment

Participants or NOK can voluntarily withdraw from study at any time if they wish to. Investigators may also discontinue a participant from the study if deemed appropriate at any time. Participants do not necessarily need to provide explanations for their withdrawal. If withdrawn by the site investigator, reasons for discontinuation will be recorded in CRFs. Participants will not be withdrawn from the study due to suspected adverse reactions of study treatment, unless the participant or their treating clinician requests discontinuation. If the participant or NOK withdraws consent to participate in the study and also withdraws consent for collection of future information, no further evaluations will be performed, and no additional data will be collected. The investigators may retain and continue to use any data collected before such withdrawal of consent. All withdrawal/discontinuation will be discussed with the coordinating investigators.

To avoid missing data, and for use in intention-to-treat analysis, all attempts will be made to collect information at day 90 for participants who discharge early, transfer to another facility, discharge against medical advice, abscond, or are lost to follow-up.

Sample size considerations

Sixty patients will be enrolled, at least 20 of whom are aged < 16 years. As this is a pilot study, the sample size is based on the number achievable, the number needed to determine feasibility, and to refine assumptions and study design.

Data management

Data collection

All initial data collection can either be in paper format (with subsequent entry into the electronic database) or directly entered into the electronic database. For database entry, each variable will have a validation range to minimise entry errors. The coordinating centre will check data for consistency and will seek any missing data with help of the PI.

During randomisation, each participant will be given a unique identifier number. This number and the hospital medical record number will be recorded in each CRF. These two numbers will be cross-checked each time data

entry is performed in CRFs to ensure correct documentation. The date, time, and name of the person performing data entry for each entry in the CRF will be mandatory. Any changes made in the CRF will be timed, dated, and signed and the reasons for changes documented. CRFs will not contain participant identifying details such as address, initials, or date of birth, but will contain age at recruitment.

Clinical details will be obtained from medical records (paper and/or electronic), bedside charts, medication charts, pathology results, correspondence notes, and telephone contact with the patient or their NOK. Clarification will be made with the treating team or the participants' general practitioners if necessary.

Data entry and storage

Data collected in paper CRFs will be entered onto a purpose-built secure web-based database. This will be done at each site by the PI or delegate. Paper CRFs will be securely stored at each site. Data will be retained for at least 10 years after study completion.

Statistical methods

Data reporting will follow CONSORT guidelines for reporting of RCTs.

Continuous variables will be analysed using the Mann–Whitney *U* test or Student's *t* test as appropriate. Proportions will be analysed by Fisher's exact test or chi-squared tests as appropriate. The 95% confidence interval will be reported for absolute difference in proportions. All-cause mortality will be shown with Kaplan–Meier graphs.

The primary analysis of both primary and secondary endpoints will be according to modified intention-to-treat principles (all participants with data available for the endpoint will be analysed according to the treatment allocation, regardless of what treatment they received).

Secondary per-protocol analysis of all endpoints will be conducted. The per-protocol population is defined as: the combination group that received at least 75% of clindamycin dose; the standard treatment group that received ≤ 1 defined daily dose of clindamycin in the week preceding randomisation; and those with data available for at least 90 days of follow-up.

There will be no planned interim efficacy analyses.

Pre-specified subgroup analyses

The number of subgroup analyses will be limited due to the small sample size:

- i) Main treatment was flucloxacillin vs cefazolin (for the MSSA cohort). Recent data suggest better outcomes for SAB with cefazolin versus nafcillin/

- oxacillin [47], although it is unclear whether this is true, and the effect size is likely to be small.
- ii) Those who received > 24 h of lincosamides or other anti-toxin antibiotics (as defined in the eligibility criteria) in the 7 days prior to randomisation, compared with those who did not. Recent receipt of lincosamides is likely to dilute the effect of the randomised intervention.
 - iii) Children vs adults. The distribution of outcomes is likely to be different in children from that in adults, although the effect of the intervention should not differ.
 - iv) MSSA vs MRSA infection. MRSA isolates may have higher rates of clindamycin resistance or may involve patients within certain risk groups (e.g. intravenous drug users, haemodialysis patients, or recent hospitalisation).

Data safety and monitoring board

An independent data safety and monitoring board (DSMB) will be established to review the progress of the study and monitor adherence to the protocol, participant recruitment, outcomes, complications, and other issues related to participant safety. They will also monitor the assumptions underlying sample size calculations for the study and alert the investigators if they see substantial departures from the study protocol as the data accumulate.

The DSMB will be composed of experts in infectious diseases (adult representative and paediatric representative) and biostatistics, and an intensivist. The DSMB members will all be independent of the investigators (none of them will be chief investigators or site PIs).

Safety aspects of the trial

Adverse events and adverse reaction (solicited and unsolicited)

Clindamycin is registered in Australia for therapeutic use. Clindamycin has a good safety profile but has some recognised adverse effects [48]. Antibiotic-associated diarrhoea and *C. difficile* diarrhoea are the most frequently encountered clinical adverse event with clindamycin. Rash, blood dyscrasias, and raised liver enzymes could be difficult to attribute to clindamycin, especially since the trial participants will also be receiving β -lactams and have a severe infection. Nonetheless, these and any other potential adverse events in both standard therapy and combination therapy groups will be monitored and recorded in CRFs. Clindamycin can be ceased if the patient develops *C. difficile* diarrhoea, severe non-*C. difficile* diarrhoea, or other serious adverse events.

Vancomycin adverse events include phlebitis, nephrotoxicity, rash, and red-man syndrome (if infused rapidly). Daptomycin adverse events include headache, rash,

infection site reactions, increased creatinine kinase, increased liver enzymes, and hypotension. Less common adverse effects of vancomycin include immune-mediated thrombocytopenia and ototoxicity, and those of daptomycin include eosinophilic pneumonia [48].

Adverse events will be graded as follows (Additional file 1: Table S6) [49]:

- Grade 1: mild symptoms. Causes nil or minimal interference with usual social and functional activities. No intervention indicated.
- Grade 2: moderate symptoms. Interferes with but does not limit usual social and functional activities. Intervention is indicated.
- Grade 3: severe symptoms. Inability to perform usual social and functional activities. Intervention or hospitalisation is needed.
- Grade 4: potentially life-threatening symptoms. Inability to perform basic self-care functions. Intervention is indicated to prevent permanent impairment, persistent disability, or death.
- Grade 5: death related to adverse event.

Serious adverse events

Serious adverse events (SAEs) are an undesirable experience as a result of the intervention that:

- results in death;
- is life-threatening;
- results in hospitalisation (initial or prolongation);
- results in disability or permanent damage; or
- is a medically important event or reaction.

All SAEs that are considered related (possible, probable, or definite) to any of the study drugs (backbone and combination therapy) which met one or more the above definitions require reporting on the SAE form. If the SAE is attributable to disease progression then this does not require expedited reporting in this trial, even when death is the outcome. The site PI is required to report any SAEs that occur at their site to the approving ethics committee in accordance with local guidelines; in addition, the site PI must adhere to any local institutional reporting requirements. Safety reporting will be in line with the National Health and Medical Research Council [50]. Guidance: Safety monitoring and reporting in clinical trials involving therapeutic goods. Canberra: National Health and Medical Research Council.

Suspected unexpected serious adverse reaction

If the SAE is considered unexpected (not listed in approved product information and not attributable to disease progression) and related (possible, probable, or definite) to the investigational drug (clindamycin), it

meets the definition of a suspected unexpected serious adverse reaction (SUSAR). All SUSARs must be entered on the SAE CRF and reported to the coordinating investigators (CIs) or delegate within 24 h from the time of the site study team becoming aware of it. If the SUSAR is fatal or life-threatening, the PI will report to the TGA within 7 calendar days of becoming aware and any follow-up reports will be submitted within a further 8 calendar days; all other SUSARs will be reported to the Therapeutic Goods Administration (TGA) within 15 calendar days of the PI becoming aware.

The site PI is required to report any SUSARs that occur at their site to the approving ethics committee in accordance with local guidelines; in addition, the site PI must adhere to any local institutional reporting requirements. Safety reporting will be in line with the NHMRC Guidance: Safety Monitoring and Reporting in Clinical Trials Involving Therapeutic Goods [50].

The relation of adverse event to the treatment will be defined as not related, unlikely, possible, probable, and definite [51].

Ethical considerations

The trial will be conducted in line with the Declaration of Helsinki. Written informed consent will be obtained from all study participants prior to any study procedure. Approval from a lead HREC and site-specific approval will be finalised at each site before the first patient is enrolled at that site. There will be two lead HRECs: one for the NT site (Menzies and NT Department of Health EC00153), and one for the remaining sites as per the National Mutual Acceptance scheme (Hunter New England EC00403).

Dissemination policy

The chief investigators (JSD, SYCT, AB) will have access to the study dataset. The results of this study will be submitted to peer-reviewed journals for publication regardless of the results. They will also be presented at national and/or international scientific meetings. Authorship of the main paper will be all PIs, “on behalf of the CASSETTE study group, the ASID CRN and Australia and New Zealand Paediatric Infectious Diseases Group (ANZPID)”. The CASSETTE Study Group will consist of all CIs and all site PIs, as well as up to two co-investigators per site if relevant.

Discussion

Although animal studies and observational reports support the concept of effective *S. aureus* exotoxin suppression with clindamycin [17], adequate supporting clinical evidence is lacking. The proposed study is an open-label, pilot, randomised controlled trial to determine whether 7 days of clindamycin in combination with standard therapy will lead to a faster resolution of systemic

inflammation than standard therapy alone in adults and children with severe *S. aureus* infection.

It is unclear whether the anti-toxin effect of clindamycin is retained in *S. aureus* strains that are resistant to it. This may be a limiting factor for our study design. Fortunately, most of the MSSA isolates in Australia have low rates of constitutive resistance against clindamycin (0.8–2.4% of blood culture isolates) [52–54]. Clindamycin resistance is, however, more common in MRSA isolates (16.5–21%) [52, 53]. Inducible resistance to clindamycin due to the presence of a mutation in the *erm* gene is much more commonly observed as a potential issue in isolates resistant to erythromycin (~50% of MRSA isolates from blood culture are erythromycin resistant) [55], although not all erythromycin-resistant strains are resistant to clindamycin. Resistance to macrolides due to efflux pumps does not create resistance to lincosamides (clindamycin and lincomycin). In the peptide tunnel of the 50S ribosome subunit, each of MLS_B antibiotics has its own interaction sites. Methylation at the common interaction site at nucleotide A2058/A2059 of 23S rRNA, mediated by an enzyme encoded by one or more *erm* genes, confers resistance to all MLS_B compounds [31, 32]. Mutations at other nucleotides create selective resistance to macrolides, and spares lincosamides [31]. Also, sub-inhibitory concentrations of clindamycin are consistently shown to inhibit *S. aureus* exotoxins [17]. A recent French study demonstrated that sub-inhibitory concentrations of clindamycin did lead to a dramatic decrease in toxin production in various *S. aureus* strains with inducible clindamycin resistance (positive D test) [56]. Hence, it is likely that clindamycin retains its efficacy as a toxin blocker in most *erm*-containing *S. aureus* strains, with the probable exception of those with constitutive resistance.

In conclusion, the proposed CASSETTE study will assess the effect of clindamycin in patients with severe *S. aureus* infections and will provide feasibility for a larger RCT. The study will provide further clinical evidence for such a use.

Trial status

This is protocol version 2.0 (18 August 2018). Recruitment started 10 July 2018. Expected completion 31 December 2020.

Additional files

Additional file 1: Renal dose adjustment, adverse events of interest and their grading, and proposed study sites (DOCX 21 kb)

Additional file 2: SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents (DOC 124 kb)

Abbreviations

ASID: Australasian Society for Infectious Diseases; CI: Coordinating investigator; CONSORT: Consolidated Standards of Reporting Trials; CRF: Case

report form; CRN: Clinical research network; CRP: C-reactive protein; DSMB: Data safety and monitoring board; FBC: Full blood count; HITH: Hospital in the home; HR: Heart rate; HREC: Human research and ethics committee; ICU: Intensive care unit; IV: Intravenous; Menzies: Menzies School of Health Research Global and Tropical Health Division; MIC: Minimum inhibitory concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*; MSSA: Methicillin-susceptible *Staphylococcus aureus*; NHMRC: National Health and Medical Research Council; NOK: Next of kin; PI: Principal Investigator; PO: Per oral; PSI: Protein synthesis inhibitor; PVL: Pantone-Valentine leucocidin; QID: Four times a day; RCT: Randomised controlled trials; RR: Respiratory rate; SAB: *Staphylococcus aureus* bacteraemia; SAE: Serious adverse event; SIRS: Systemic inflammatory response syndrome; SPIRIT: Standard Protocol Items Recommendations for Interventional Trials; SSTI: Skin and soft tissue infections; SUSAR: Suspected unexpected serious adverse reaction; TDS: Three times a day; TG: Therapeutic Guidelines (Australia) v15; TGA: Therapeutic goods administration; TSST: Toxic shock syndrome toxin

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Role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript
None

Authors' contributions

JSD, SYCT, and AB designed the study. RD performed the literature review and wrote the initial draft of the protocol. JNN, MVNOS, AJC, BJM, PNB, JRF, DPE, OR, and LM all contributed to the revised and final drafts of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable

Ethics approval and consent to participate

Informed consent will be obtained from all study participants. The following ethics board have approved the study:

1. Hunter New England Human Research Ethics Committee (HNEHREC); Hunter New England Local Health District, New South Wales, Australia (Reference No.: 17/09/20/3.02).
2. Human Research Ethics Committee of the Northern Territory Department of Health and Menzies Scholl of Health Research (Reference No.: 2017-2942).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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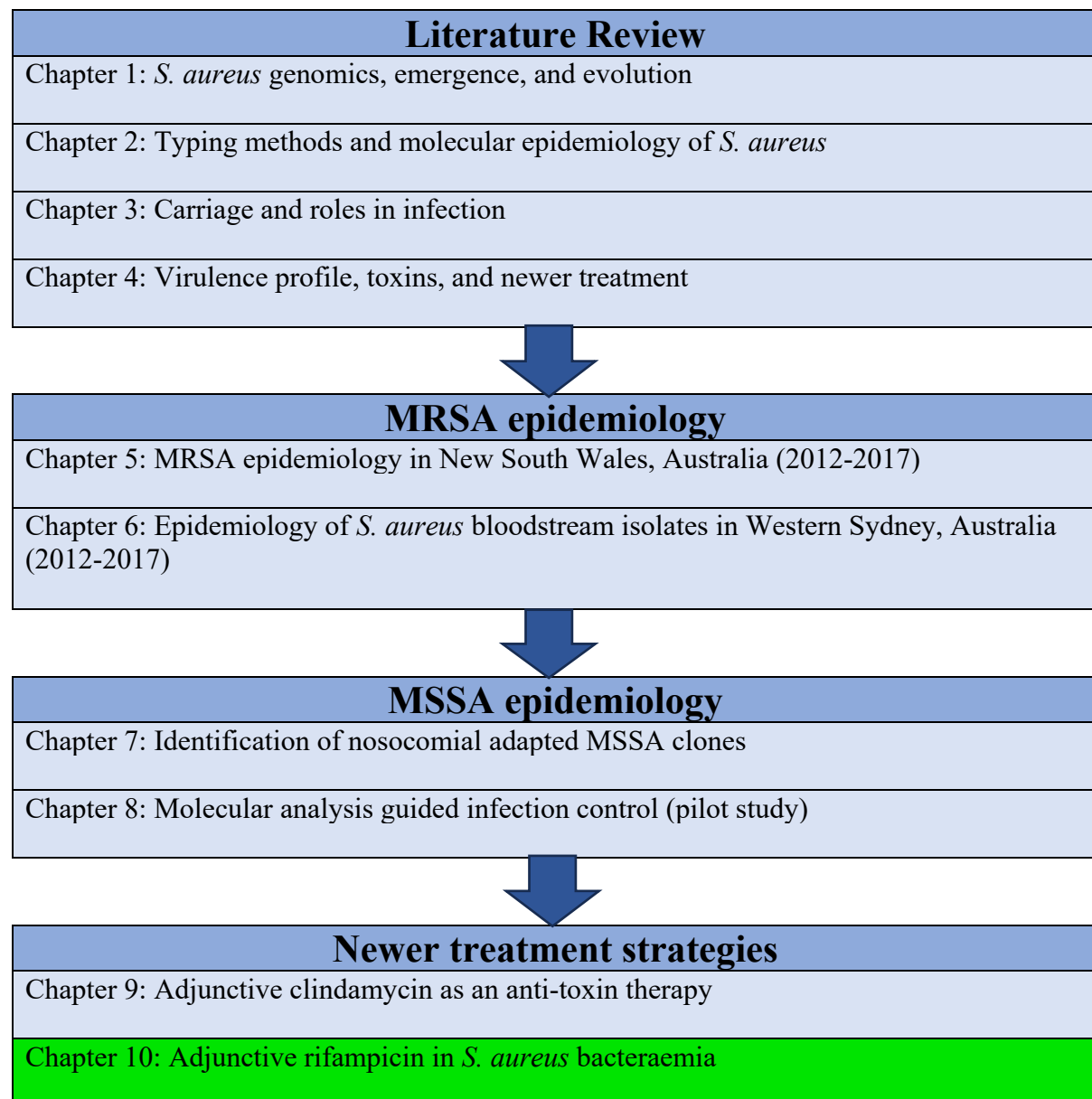
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Figure: Schematic overview of the thesis structure



Chapter 10: Overview

A review of the *S. aureus* bacteraemia management in Chapter 4 highlighted elevated mortality and relapse rates associated with current approaches. Consequently, this current chapter includes a systematic review of adjunctive rifampicin therapy in the management of *S. aureus* bacteraemia. During this investigation, a research gap was identified concerning adjunctive rifampicin in staphylococcus prosthetic valve endocarditis, which will be addressed through a randomised clinical trial within the *Staphylococcus aureus* Network Adaptive Platform (SNAP) Trial.

This chapter includes published work.

Chapter 10: Adjunctive rifampicin in *Staphylococcus aureus* bacteraemia

10.1 Introductory comments

Despite the use of appropriate first-line antimicrobial therapy with an anti-staphylococcal β -lactam or vancomycin, *S. aureus* bacteraemia (SAB) leads to significant morbidity, mortality, and disease recurrence (1,2). The dissemination of blood stream infection to distant sites, often facilitated by neutrophils and phagocytes, along with the potentially limited activity of first-line antibiotics at deep infection sites and their inability to effectively eliminate intracellular *S. aureus*, could contribute to the observed high mortality and recurrence rates (2,3). Additionally, factors such as inadequate therapeutic monitoring (e.g., vancomycin in all patients and flucloxacillin in overweight patients) may further contribute to these challenges. SAB is associated with a 90-day mortality of up to 33% (1) and a relapse rate of 4-8% (2,4,5).

Rifampicin is primarily used in *S. aureus* infections for its excellent tissue distribution and effectiveness against adherent and stationary-phase staphylococci in biofilms (6). This property

enhances the sterilisation of infection sites, potentially leading to improved survival and reduced rates of relapse (7).

To systematically assess the efficacy of adjunctive rifampicin in the context of SAB, a comprehensive study was conducted.

10.2 Aims

The study aims to assess whether supplementary rifampicin therapy in the treatment of *Staphylococcus aureus* bacteraemia (SAB) reduces rates of clinical failure, microbial failure, and death. Additionally, we will analyse for management gaps that may warrant further research.

10.3 Publication

Effectiveness of Adjunctive Rifampicin for Treatment of *Staphylococcus aureus* Bacteraemia: A Systematic Review and Meta-Analysis of Randomised Controlled Trials

Authors:

Ravindra Dotel, Gwendolyn Gilbert, Sonya Hutabarat, Joshua Davis, and Matthew O'Sullivan

Citation

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Contribution of Authors

I planned the study, conducted a systematic literature review, and wrote the manuscript, serving as the corresponding author.

SNH performed a second independent systematic literature review and provided valuable feedback on the manuscript.

GLG, JSD, and MVNO supervised my work, providing critical reviews of the manuscript.

10.4 Remarks

The supplementary materials, comprising the search strategies, risk of bias summary, and outcomes definitions, are included.

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Effectiveness of adjunctive rifampicin for treatment of *Staphylococcus aureus* bacteraemia: a systematic review and meta-analysis of randomized controlled trials

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Objectives: To assess whether the addition of rifampicin to conventional treatment of *Staphylococcus aureus* bacteraemia (SAB) reduces bacteriological or clinical failure or death.

Data Sources: PubMed, Embase and Cochrane CENTRAL databases were searched from inception to 31 December 2022. Reference lists and PubMed citations of eligible studies were checked.

Review methods: Two study authors independently identified randomized controlled trials (RCTs) involving adult participants with SAB, in which an intervention group received adjunctive rifampicin and the control group received usual care with or without a placebo. Dichotomous data (bacteriological and clinical failure and deaths) were analysed and pooled across studies using risk ratio (RR) with 95% confidence intervals (CI) using a Mantel-Haenszel random-effect model. The key variable of interest being whether rifampicin was used.

Results: Six RCTs including 894 participants—of which 758 (85%) were from one trial—met the inclusion criteria. The addition of rifampicin to conventional treatment of SAB significantly reduced bacteriological failure by 59% (RR 0.41, 95% CI 0.21–0.81, $I^2=0\%$, number need to treat 27). However, it did not reduce clinical failure (RR 0.70, 95% CI 0.47–1.03, $I^2=0\%$) or deaths (RR 0.96, 95% CI 0.70–1.32, $I^2=0\%$). Further, it did not reduce the duration of bacteraemia, or the length of hospital stay. Adjunctive rifampicin reduced SAB recurrences (1% versus 4%, $P=0.01$). Emergence of rifampicin resistance during treatment was uncommon ($<1\%$).

Conclusion: Although adjunctive rifampicin reduced the risk of bacteriological failure and recurrences, we found no mortality benefit to support its use in SAB.

Introduction

Staphylococcus aureus is a human pathogen, the effects of which range from asymptomatic colonization to life threatening invasive infections. *S. aureus* bacteraemia (SAB) is a serious common illness with a high morbidity and mortality.^{1,2} An anti-staphylococcal β lactam antibiotic (e.g. flucloxacillin) is the current first-line therapy for methicillin-susceptible *S. aureus* (MSSA) bacteraemia, and vancomycin or daptomycin are first-line agents for methicillin-resistance *S. aureus* (MRSA) bacteraemia.¹ Despite appropriate first-line therapy, SAB is associated with a 30-day mortality of 10%–30%^{1–3} and a relapse rate of 4%–8%.^{1,4}

Rifampicin is an effective anti-staphylococcal antibiotic active against both MSSA and MRSA.⁵ Potential advantages of rifampicin therapy include good oral bioavailability, excellent tissue penetration, biofilm activity and ability to kill *S. aureus* within neutrophils and phagocytes.^{5,6} Observational studies and controlled trials involving both bacteraemic and non-bacteraemic *S. aureus* infections have shown mixed outcomes with added rifampicin.^{7–10} Previous systematic reviews^{7,8} and meta-analysis^{10–12} have either not included all published randomized controlled trials (RCTs) evaluating rifampicin in SAB or included studies where rifampicin treatment was non-random thus creating selection bias. The pivotal adjunctive rifampicin for *Staphylococcus aureus* bacteraemia (ARREST) study by Thwaites *et al.* was an RCT published in 2018.⁴ It showed no added benefit of adjunctive

rifampicin therapy in SAB. However, a quantitative synthesis of all available RCTs is needed to determine whether the effect is truly not significant and if the results are applicable to certain sub-groups of SAB.

The aim of this study was to define a role, if any, for the addition of rifampicin to a backbone therapy of SAB, through a meta-analysis of participants enrolled in all published RCTs. A systematic review of the literature was conducted and a quantitative synthesis of the pre-defined outcomes was performed.

Review methods

This review was reported according to guidance provided by Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA).¹³

Selection criteria

RCTs that involved participants with SAB, treated with anti-staphylococcal therapy, where the intervention arm received adjunctive rifampicin and the control group received other anti-staphylococcal therapy (with or without a placebo) were included. The primary outcomes examined were bacteriological and clinical failure and death. Secondary outcomes examined were duration of bacteraemia, length of hospital stay, on-treatment development of rifampicin resistance, SAB recurrences, outcomes of MSSA versus MRSA bacteraemia, outcomes in infective endocarditis (IE) and rifampicin adverse events. The search was limited to *S aureus* (MSSA or MRSA) bacteraemia.

Studies that had enrolled both *S aureus* and coagulase-negative staphylococcal bacteraemia but had separately reported SAB-specific outcomes, were included in the meta-analysis. Only studies in which rifampicin was studied as part of the primary therapy were included. *S aureus* infections without bacteraemia, observational and non-human studies were excluded. There was no restriction based on language, age groups or year of publication.

Search methods

Database searches included PubMed, Embase and Cochrane CENTRAL from their inception until 31 December 2022. Search terms included staphylococcus, bacteraemia and rifampicin. A detailed search strategy is listed in the [Supplementary data](#) (available as [Supplementary data](#) at JAC Online). A second author (S.N.H.) performed an independent literature search. Any disagreement was resolved by mutual agreement.

Titles and abstracts of studies found in PubMed were screened to find publications that fulfilled selection criteria. Embase results were filtered to include only RCTs. Duplicate records were removed. No other records were found from Embase or Cochrane CENTRAL searches. Reference lists of eligible manuscripts and earlier systematic reviews/meta-analysis ('backward literature search') along with their 'cited by' records in PubMed ('forward literature search') was checked for any missed RCTs. Full texts of the qualifying records were reviewed. Studies meeting the selection criteria were included in quantitative synthesis (Figure 1).

Data analysis

Data extraction and bias analysis were performed by two authors (R.D. and S.N.H.), using a Cochrane Guide for Systematic Reviews of Interventions¹⁴; disagreements were resolved by mutual discussion. The certainty of the evidence for the three primary outcomes were analysed as per the GRADE recommendations.¹⁵ A funnel plot was not included as fewer than 10 studies were identified.¹⁴ An I^2 value of $\geq 50\%$ was considered high heterogeneity. Interventions with similar outcomes were analysed in RevMan (v.5.3). Dichotomous data (bacteriological and clinical failure and death) were analysed and pooled across studies using risk ratio (RR) with 95% confidence intervals (CI) using the Mantel-Haenszel random-effect model. The zero-cell correction was not applicable.¹⁴ Risk reduction and number needed to treat (NNT) from the meta-analysis were calculated by computing risk difference from RR.¹⁶ Transforming a pooled risk ratio into a risk difference is recommended by Cochrane Collaboration¹⁴ and Grade working group¹⁵ and the method most suitable for our meta-analysis¹⁶. Secondary outcomes (duration of bacteraemia, length of stay, on-treatment resistance, SAB recurrence, MSSA versus MRSA, IE and rifampicin adverse events) analyses were not pre-specified. These results are descriptive except for IE where a pooled analysis was performed with the methods described earlier.

Results

Fifteen records were assessed for eligibility and their full texts were reviewed. Of these, nine studies were excluded (Figure 1).^{7,8,10-12,17-20} The quantitative synthesis (meta-analysis) included six studies, with a total of 894 eligible participants.^{4,21-25} All three primary outcomes (bacteriological failure, clinical failure and death) were reported in three of the six studies.^{4,21,22} Characteristics of the included studies are shown in Table 1. A summary of findings¹⁵ of our meta-analysis is provided in Table 2.

There were slight variations in how each primary outcome was defined ([Supplementary data](#)), and treatment regimens in intervention and control groups. Heldman²⁴ and Schrenzel²⁵ combined rifampicin with a fluoroquinolone in the intervention group, whereas the control groups received oxacillin/flucloxacillin or vancomycin. We have reported primary outcomes with and without inclusion of these two studies. In the largest, pivotal study by Thwaites,⁴ 81% of participants in the rifampicin arm and 83% in the control arm received flucloxacillin as the primary active antibiotic; in 12% of participants, the main active antibiotic was a fluoroquinolone. In both groups, participants received a median of three other active antibiotics during the *S aureus* infection episode.

Risk of bias analysis

Except for the Thwaites⁴ study, the risk of bias ranged from unclear to high, across the included studies (see [Supplementary data](#)). Outcome assessors were not blinded, except in the Thwaites⁴ study, but in the remaining studies the measured outcomes were pre-specified. There were high risks of reporting bias in the Van Der Auwera,²² Heldman²⁴ and Schrenzel²⁵ studies. The findings did not include results based on intention-to-treat.

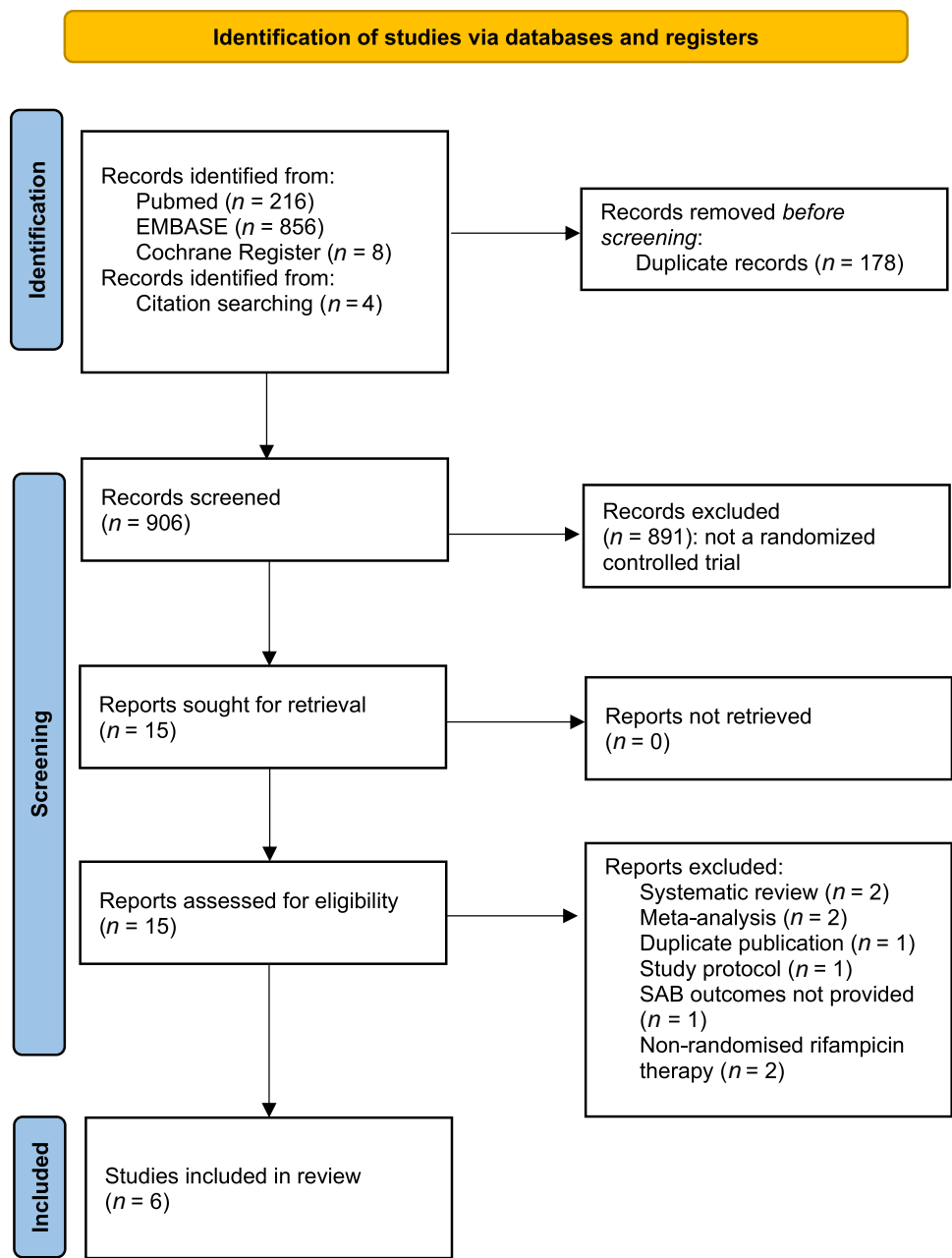


Figure 1. Flow diagram of trial identification for quantitative synthesis. (a) Bacteriological failure. (b) Clinical failure. (c) Death. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

The risk of performance bias was higher when oral therapy was used in the intervention group and intravenous (IV) therapy in the control group,^{24,25} or with open-label use of rifampicin.²²⁻²⁵ In the studies by Heldman²⁴ and Levine,²³ half of the participants dropped out or refused care according to trial requirements, which was a major cause of attrition bias.

The Thwaites,⁴ Levine²³ and Heldman studies²⁴ had other biases that were thought to be high risk. For example, Thwaites⁴ study excluded 232 of the 2896 (8%) patients screened, in whom rifampicin therapy was felt to be mandatory. This may have affected the results. Levine²³ terminated their trial earlier than expected due to changes in

admission policy; enrolment of a larger number of cases might have provided different results as they report a wide confidence interval. In the Heldman²⁴ study, the fact that IE was a clinical diagnosis, not requiring an echocardiogram, was another potential source of bias.

Reporting of primary outcomes

- (i) *Bacteriological failure* was reported in five studies (894 participants).^{4,21,22,24,25} Pooled analysis showed that addition of rifampicin significantly reduced bacteriological failure, RR 0.41 (95% CI 0.21–0.81, $I^2=0\%$, risk reduction 3.7%, NNT

Table 1. Characteristics of the included RCTs, analysing the role of adjunctive rifampicin therapy in *S. aureus* bacteraemia (SAB)—all six studies involved adult (≥ 18 years) participants

First author	RCT design	MSSA/MRSA	Intervention (treatment duration in days)	Control	Sample size	Rifampicin started	Notes
Thwaites ⁴	Double blind, placebo-controlled; multicentre study (UK)	MSSA 94%, MRSA 6%	Standard treatment (median 29 days) + added rifampicin 600 or 900 mg/day (14 days)	Standard treatment ¹ (median 30 days) + placebo (14 days)	758 (370 in intervention; 388 controls)	Within 96 h of BC collection	232/2896 (8%) were excluded from trial as rifampicin was felt mandatory
Schrenzel ¹⁶	Open label; multicentre (Switzerland and Belgium)	MSSA 80%, MRSA 2%, CoNS 18% ^a	Oral fleroxacin 400 mg OD + rifampicin 600 mg OD. Mean treatment duration 19 days	Standard treatment (Flucloxacillin 2 g QID or vancomycin 1 g BD). Mean treatment duration 21 days	127 (68 in intervention and 59 controls).	Once <i>S aureus</i> was confirmed	Sample size includes non-bacteraemic infections and CoNS bacteraemia. Only SAB included in the meta-analysis
Heldman ¹⁵	Open label; multicentre (USA)	MSSA 89%, MRSA 5%, CoNS 6% ^a Native valve right sided IE (100%)	Oral ciprofloxacin 750 mg BD + oral rifampicin 300 mg BD for 28 days	Standard treatment (oxacillin 2 g q4 hourly or vancomycin 1 g BD) for 28 days + initial 5 days gentamicin	85 (40 cases in intervention and 45 in control)	Treatment started prior to confirmation of BC isolate	HIV positive (2/3 cases in each group). All participants were intravenous drug users. 21/40 in the intervention and 20/45 in the control arm did not complete treatment. ^b
Levine ²²	Open label; single centre (USA)	MRSA (100%) Native valve IE (81% right sided)	Vancomycin 1 g BD + rifampicin 600 mg daily (28 days)	Vancomycin 1 g BD (28 days)	42 (20 in intervention and 22 in control)	Once MRSA was confirmed	6/22 (27%) participants in the control groups received additional gentamicin for IE.
Van Der Auwera ²⁰	Single blind placebo controlled; multicentre (Belgium)	45% cases had SAB: MSSA (92%) MRSA (8%)	Oxacillin 3 g QID (or vancomycin 1 g BD) + rifampicin 600 mg BD oral. Mean treatment duration 18.9 days	Oxacillin 3 g QID (or vancomycin 1 g BD) + pyridium (placebo) 150 mg BD oral. Mean treatment duration 21.7 days	29 SAB (13 in intervention and 16 on control)	Once <i>S aureus</i> was confirmed	Pyridium used as it discolours body fluid like rifampicin
Van Der Auwera ²¹	Open label; single centre (Belgium)	34% cases had SAB: MSSA (70%), MRSA (30%)	Oxacillin 3 g QID (or vancomycin 1 g BD) + rifampicin 300 mg BD; Mean treatment 18 days	Oxacillin 3 g QID (or vancomycin 1 g BD). Mean treatment 10 days	19 SAB (10 in rifampicin group and 9 in control group)	Once <i>S aureus</i> was confirmed	

BC, blood culture; MSSA, methicillin-susceptible *S aureus*; MRSA, methicillin-resistant *S aureus*; CoNS, coagulase-negative *S aureus*; PO, per oral; IV, intravenous; IVDU, intravenous drug users; IE, infective endocarditis; OD, once daily; BD, twice daily, QID, four times daily.

^aNot included in the meta-analysis.

^bThe most common withdrawal reasons included exclusion criteria after entry and elopement or discharge against medical advice with a similar distribution between the intervention and control arms.

Table 2. Summary of findingsPatient or population: adults with *Staphylococcus aureus* bacteraemia

Setting: inpatient

Intervention: usual therapy plus rifampicin

Comparison: usual therapy

Outcomes	Number of participants (studies) follow-up	Certainty of the evidence (GRADE) ^a	Relative effect (95% CI)	Anticipated absolute effects ^b	
				Risk with usual therapy	Risk difference with usual therapy plus rifampicin
Bacteriological failure	894 (5 RCTs)	⊕⊕⊕○ Moderate	RR 0.41 (0.21 to 0.81)	75 per 1000	44 fewer per 1000 (59 fewer to 14 fewer)
Clinical failure	894 (5 RCTs)	⊕○○○ Very low	RR 0.70 (0.47 to 1.03)	149 per 1000	45 fewer per 1000 (79 fewer to 4 more)
Death	848 (4 RCTs)	⊕○○○ Very low	RR 0.96 (0.70 to 1.32)	163 per 1000	7 fewer per 1000 (49 fewer to 52 more)

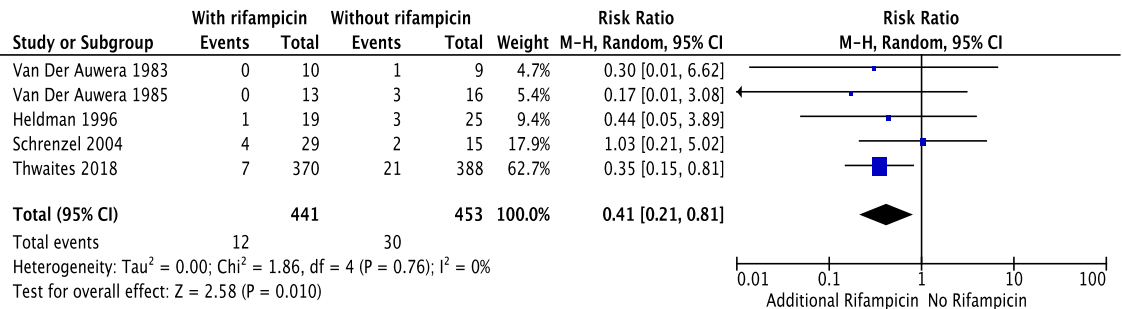
^a**GRADE Working Group grades of evidence****High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect.**Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.**Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.**Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.^b**The risk in the intervention group** (and its 95% CI) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

- 27). Certainty of evidence was moderate (Table 2). RR remained significant, 0.32 (95% CI 0.15–0.72) even after excluding the Heldman²⁴ and Schrenzel²⁵ studies in which oral fluoroquinolone was combined with rifampicin in the intervention arms (Figure 2a).
- (ii) *Clinical failure* was reported in five studies (894 participants).^{4,21–23,25} While the point estimate of the clinical failure rate with adjunctive rifampicin of 9.2% was lower than with for standard therapy (13.1%), this difference was not significant (RR 0.70, 95% CI 0.47–1.03, $I^2=0\%$). Certainty of evidence was very low (Table 2). The result remained non-significant even with the exclusion of the Schrenzel²⁵ study, RR 0.67 (95% CI 0.42–1.06) (Figure 2b).
- (iii) *Death (all-cause mortality)* was reported in four studies (848 participants).^{4,21–23} In a pooled analysis, adjunctive rifampicin was not associated with a significantly reduced mortality, RR 0.96 (95% CI 0.70–1.32, $I^2=0\%$). Certainty of evidence was very low (Table 2). The result remained non-significant after exclusion of the Van Der Auwera^{21,22} and Levine²³ studies, which only reported deaths occurring during therapy. Thwaites⁴ reported death at 12 weeks (Figure 2c).
- (ii) *Length of stay:* Thwaites⁴ reported no difference in the median duration of initial hospital stay or in readmission (within 12 weeks) with added rifampicin. Schrenzel²⁵ showed a shorter median length of stay (12 versus 23 days, $P=0.006$) in the added rifampicin group where the intervention group had received oral and the control group received intravenous backbone therapy.
- (iii) *On-treatment resistance:* Van Der Auwera,²¹ Schrenzel²⁵ and Thwaites⁴ reported none of their bacteriological failures were due to development of rifampicin-resistant infection. Only Thwaites⁴ systematically reported on-treatment resistance to rifampicin. It occurred in 2/370 (0.5%) SAB. It is worth noting that on-treatment resistance was checked only in clinically indicated blood cultures, and there was no surveillance or colonization cultures collected.
- (iv) *Recurrence of SAB:* Levine²³ reported no recurrence in either group, but it was not systematically measured. Thwaites⁴ reported bacteriological recurrences in 3/370 (1%) and clinical recurrences in 8/370 (2%) in the intervention group compared to 16/388 (4%) and 23/388 (6%), respectively ($P=0.01$ for both). The reported NNT to prevent one bacteriologically confirmed and one clinical recurrence were 29 and 26, respectively. A *post hoc* analysis of the Thwaites study suggested scoring criteria based on diabetes, liver disease, kidney disease, immunosuppression and BMI >30 kg/m² to predict *S aureus* infection recurrence.²⁶

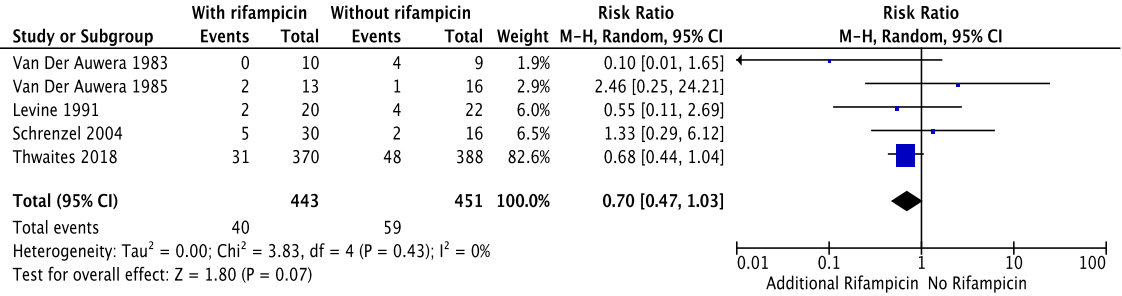
Secondary outcomes

- (i) *Duration of bacteraemia:* there was no significant difference in the median duration of bacteraemia with added rifampicin in the Levine²³ study (9 versus 7 days, $P>0.05$) or the Thwaites⁴ study (no difference at 3 or 7 days, $P=0.66$).

(a) Bacteriological failure



(b) Clinical failure



(c) Death

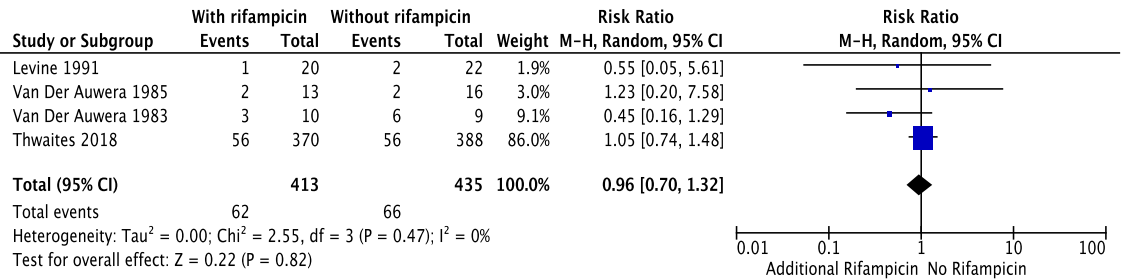


Figure 2. Forest plot of RR comparing (a) bacteriological failure, (b) clinical failure and (c) death in patients with SAB treated with versus without added rifampicin. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

- (v) *MSSA versus MRSA*: Thwaites⁴ reported no significant difference in composite outcomes (bacteriological treatment failure, recurrence or death) at 12 weeks between MSSA [hazard ratio (HR) 0.87, 95% CI 0.60–1.24] and MRSA (HR 2.74, 95% CI 0.74–10.15) ($P=0.07$) subgroups. Separate analysis by MSSA or MRSA were not provided in the other studies.

(vi) *Infective endocarditis*: All participants in the Levine²³ and Heldman²⁴ studies, and 40/758 (5%) in the Thwaites⁴ study had IE with a total of 126 participants across the three studies. All were native valve disease except 7/40 cases with prosthetic valve IE in Thwaites⁴ study. Pooled results of these three studies showed no significant reduction in bacteriological failure, recurrence or death in patients with *S aureus* IE, 4/61(7%) with versus 10/65(15%) without rifampicin (RR 0.40, 95% CI 0.13–1.19).
- (vii) *Rifampicin adverse events*: Thwaites⁴ reported no significant difference in serious adverse events (grade 3–4) between the rifampicin and placebo groups, including hepatobiliary disorders. However, significantly more participants in the rifampicin group developed gastrointestinal disorders (6.5% versus 2.1%), $P=0.003$, and drug interactions (6% versus 2%), $P<0.001$ (despite those with predicted drug interactions having been excluded before randomization).⁴ Van Der Auwera^{21,22} reported no difference in adverse events between the two groups. Rifampicin adverse events could not be found or were not reported in the other studies.

Discussion

We conducted a systematic review and meta-analysis of RCTs to assess the effects of adjunctive rifampicin on clinical outcomes in SAB. We noted significantly reduced bacteriological failure and SAB recurrence rates, with added rifampicin. However, there were no reductions in clinical failure or all-cause mortality, or mean length of admission or duration of bacteraemia. We also found no evidence to support adjunctive rifampicin in IE (94% cases had native valve IE). On-treatment rifampicin resistance occurred in <1% of SAB episodes. Rifampicin was well tolerated but there were small but increased rates of drug interactions and gastrointestinal intolerance.

Various strategies for optimization of SAB therapy have been explored, including combinations of β lactam and vancomycin,²⁷ daptomycin monotherapy or as an adjunctive treatment,^{28,29} adjunctive fosfomycin³⁰ and anti-toxin therapy in severe *S aureus* infection.³¹ A large platform trial is currently underway to optimize SAB treatment strategies.³² This trial also plans to assess the role of adjunctive rifampicin in prosthetic valve IE (personal communication). Meanwhile, a previously well accepted therapy—the addition of gentamicin for *S aureus* IE—is no longer used due to a lack of evidence of benefit and a high incidence of nephrotoxicity.³³ On the other hand, rifampicin use in *S aureus* infection remains common.^{10,34} It is particularly used in deep infections and those involving prosthetic materials.^{9,35} An RCT is currently underway where rifampicin is being considered as a step-down therapy in left-sided IE in combination with levofloxacin.³⁶ Heldman²⁴ and Schrenzel²⁵ study included in our review used fluoroquinolone and rifampicin for an entire treatment duration in the intervention arm whereas in the planned RCT it is started after at least 10 days of standard intravenous therapy. Rifampicin provides good tissue penetration, biofilm activity and has good oral bioavailability.⁵ However, it can cause drug interactions (as a cytochrome P450 enzyme inducer), hepatotoxicity and on-treatment resistance can occur, which can lead to spread of rifampicin-resistant *S aureus* strains.¹⁷ Unnecessary rifampicin use may inadvertently contribute to the prevalence of rifampicin-resistant *Mycobacterium tuberculosis* in high endemic countries.

The strength of our review is that it is more comprehensive than previous systematic reviews^{7,8} and meta-analyses.^{10–12} We have analysed the three primary outcomes and several secondary outcomes. Although we have included Heldman²⁴ and Schrenzel²⁵ in which the first-line therapy differed between intervention and control arms, we have analysed the primary outcomes with and without these trial results. The systematic review conducted by Ma¹² included the study by Ruotsalainen¹⁹ in which added levofloxacin was compared with a usual care, and outcomes associated with added rifampicin were analysed *post hoc*. The review by Grillo¹¹ excluded MRSA bacteraemia and reported a pooled analysis of six RCTs, of which only one study involved added rifampicin.

Our analysis, which excluded non-SAB infections and observational studies and involved quantitative synthesis through a meta-analysis, supports rifampicin use in SAB to reduce bacteriological failure and SAB recurrence. However, the significance of these outcomes is unclear, as adjunctive rifampicin did not lead to survival benefits or reduction in the duration of bacteraemia.

This review is not without limitations. It was not pre-recorded in a prospective systematic review registry. Although all included studies involved SAB, the foci of infection were often different. For instance, all patients in the Levine²³ and Heldman²⁴ studies had IE compared with only 5% of participants in Thwaites.⁴ Schrenzel²⁵ and Heldman²⁴ used an oral fluoroquinolone with rifampicin in the intervention arm but a standard first-line antibiotic in control arm (without rifampicin). Although no heterogeneity was detected across the included studies (I^2 score 0%), all except that of Thwaites⁴ had a high or unclear risks of bias. The dose and duration of rifampicin, as well as the duration of standard therapy differed across the included studies, though all measured similar outcomes. Thwaites⁴ study is the largest of the included studies, therefore it has greatest weight in determining the point estimates.

Conclusion

Implications for practice: although our data showed a significant benefit of adding rifampicin to therapy in SAB for reducing bacteriological failures, RR 0.41 (95% CI 0.21–0.81), no significant benefit was noted for other meaningful outcomes such as deaths, duration of bacteraemia or length of stay.

Implications for research: prosthetic valve endocarditis is the main SAB-related complication for which uncertainty about the value of adjunctive rifampicin remains and could be resolved by a future RCT.

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Transparency declarations

No conflict of interest to declare.

Author contributions

R.D. planned the study, performed systematic literature review and wrote the manuscript. S.N.H. performed systematic literature review and provided feedback on the manuscript. G.L.G., J.S.D. and M.V.N.O. supervised R.D. and critically reviewed the manuscript.

Supplementary data

Supplementary data are available as [Supplementary data](#) at JAC Online.

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- 35** Ryder JH, Tong SYC, Gallagher JC *et al*. Deconstructing the Dogma: systematic literature review and meta-analysis of adjunctive gentamicin and rifampin in staphylococcal prosthetic valve endocarditis. *Open forum Infect Dis* 2022; **9**: ofac583. <https://doi.org/10.1093/ofid/ofac583>
- 36** Lemaigen A, Bernard L, Tattevin P *et al*. Oral switch versus standard intravenous antibiotic therapy in left-sided endocarditis due to susceptible staphylococci, streptococci or enterococci (RODEO): a protocol for two open-label randomized controlled trials. *BMJ Open* 2020; **10**: e033540. <https://doi.org/10.1136/bmjopen-2019-033540>

SUPPLEMENTARY DATA

A: Search Strategy (Dates searched: Inception – 31st December 2022)

1. Pubmed

Staphylococcus AND bacteraemia AND rifampicin (216).

2. Embase

#	Search Statement	Results
1	Staphylococcus aureus/	162440
2	bacteremia/	50208
3	staphylococcal bacteremia/	3002
4	mssa.mp.	6685
5	mrna.mp.	40715
6	bsi.mp.	8978
7	bloodstream infection/	17297
8	rifampicin/	97920
9	(rifampicin or rifampin).mp.	104557
10	1 or 4 or 5	193313
11	2 or 3 or 6 or 7	72600
12	8 or 9	104557
13	10 and 11 and 12	856
14	(random\$ or placebo\$ or single blind\$ or double blind\$ or triple blind\$).ti,ab.	2034459
15	retracted article/	12980
16	or/14-15	2046913
17	(animal\$ not human\$).sh,hw.	4750979

18	(book or conference paper or editorial or letter or review).pt. not exp randomized controlled trial/	5720386
19	(random sampl\$ or random digit\$ or random effect\$ or random survey or random regression).ti,ab. not exp randomized controlled trial/	152405
20	16 not (17 or 18 or 19)	1561369
21	13 and 20	20

3. Cochrane CENTRAL

ID	Search	Hits
1	MeSH descriptor: [Staphylococcus aureus] explode all trees	872
2	(s aureus):ti,ab,kw	1396
3	(mssa):ti,ab,kw	138
4	(mrsa):ti,ab,kw	1079
5	#1 OR #2 OR #3 OR #4	2583
6	MeSH descriptor: [Bacteremia] explode all trees	1050
7	(bacteraemia):ti,ab,kw	2701
8	(bloodstream):ti,ab,kw	1833
9	(BSI):ti,ab,kw	738
10	(SAB):ti,ab,kw	312
11	#6 OR #7 OR #8 OR #9 OR #10	5309
12	#5 AND #11	362
13	MeSH descriptor: [Rifampin] explode all trees	1263
14	(rifampin):ti,ab,kw	1703
15	#13 AND #14	1263
16	#12 AND #15	8

B: Risk of bias summary

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Heldman 1996	High risk of bias	Unclear risk of bias	High risk of bias	Unclear risk of bias	High risk of bias	High risk of bias	High risk of bias
Levine 1991	Unclear risk of bias	High risk of bias	High risk of bias	Unclear risk of bias	High risk of bias	Low risk of bias	High risk of bias
Schrenzel 2004	High risk of bias	Unclear risk of bias	High risk of bias	Unclear risk of bias	Unclear risk of bias	High risk of bias	Unclear risk of bias
Thwaites 2018	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	High risk of bias
Van Der Auwera 1983	Unclear risk of bias	High risk of bias	High risk of bias	Unclear risk of bias	High risk of bias	High risk of bias	Unclear risk of bias
Van Der Auwera 1985	Unclear risk of bias	Low risk of bias	Unclear risk of bias	Unclear risk of bias	Low risk of bias	Low risk of bias	Unclear risk of bias

 Low risk of bias
  Unclear risk of bias
  High risk of bias

Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

C: Outcomes definitions (as reported in the primary studies):

1. **Bacteriological failure:** Van Der Auwera (1983, 1985) defined it as persistence of the primary pathogen at the primary site of infection or a metastatic focus. Heldman (1996) defined it as positive blood culture (BC) one week after ceasing antibiotic therapy.

Schrenzel (2004) defined it as microbiologically documented persistence or relapse of the original pathogen. Thwaites (2018) described ‘bacteriologically confirmed treatment failure’ as, (i) symptoms and signs of infection ongoing for more than 14 days from randomisation, with *S aureus* isolated from blood or another sterile site or (ii) ‘bacteriologically confirmed disease recurrence’ with *S aureus* isolated from a sterile site more than 7 days of apparent clinical improvement.

2. **Clinical failure:** Van Der Auwera (1983 and 1985) defined clinical failure as death during the first four days of therapy, or lack of clinical improvement or deterioration, requiring a change in antimicrobial therapy, after treatment for at least three days. Levine (1991) defined it as death or a requirement for surgery. Schrenzel (2004) defined it as no clinical improvement or deterioration that required change from the per protocol randomised treatment regimen. Thwaites (2018) did not elaborate on what was meant by ‘clinically defined treatment failure or disease recurrence from randomisation to 12 weeks’ except that it was assessed by an independent endpoint review committee blind to treatment allocation.
3. **Death:** Van Der Auwera (1983 and 1985) used all deaths from any cause at end of therapy. Levine (1991) also used all-cause deaths before the completion of treatment. Thwaites (2018) reported death at 12 weeks.
4. **Repeat blood cultures:** In Levine’s (1991) study BCs were performed daily, until negative. A BC was also collected if a participant developed fever, and at the end of therapy. Thwaites (2018) study required BC collections on day 0-, 3- and 7-days post randomisation, and at any other times as clinically necessary. In Levine (1991) study the duration of bacteraemia was determined in 17/42 participants (40%), 7 in rifampicin group (median duration 9 days) and 10 in the control group (median duration 7 days ($p > 0.05$)). The remaining 25 patients included patients who declined BC, self-discharged

before therapy was complete or whose BCs remained positive at completion of treatment. All bacteraemia were from MRSA. Both intervention and control groups had lower median vancomycin trough levels (10.4 ug/mL and 11.4 ug/mL respectively) possibly contributing to prolonged bacteraemia. Thwaites (2018) reported “the global test of difference between the two groups across days 3, 7 showed no significant difference in the duration of bacteraemia (p value = 0.66)”.

Chapter 11: Concluding remarks

11.1 Conclusions

This thesis presents research on the emergence and epidemiological trends of *S. aureus*, molecular analysis of both MRSA and MSSA isolates, and optimisation of treatment for *S. aureus* bacteraemia. The study includes: (i) an in-depth literature review; (ii) an analysis of over 7,500 MRSA isolates collected from sterile and non-sterile sites in New South Wales, Australia, between 2012 and 2017; (iii) an analysis of MSSA blood culture isolates from Western Sydney (2011-2015); and (iv) an analysis of *S. aureus* (MSSA and MRSA) blood culture, carriage, and environmental isolates from Blacktown Hospital (New South Wales, Australia) collected in 2021 .

We have demonstrated a reduction in the burden of MRSA, attributed to a decrease in ST239-MRSA, despite an increase in ST45-MRSA and PVL-positive ST22-MRSA. Our report features a micro-epidemiological analysis of ST239-MRSA and ST22-MRSA, explores the emergence of PLV-positive ST-22-MRSA in our district, and provides detailed epidemiological data on the frequency of Panton-Valentine leukocidin in *S. aureus* isolates (Chapters 5, 6 and 7).

The molecular epidemiology of MSSA has been a relatively understudied area . In our analysis conducted and reported in 2015/2016, we sought to identify hospital-acquired MSSA clones but found no evidence of such (Chapter 7). Additionally, we present a whole-genome sequencing (WGS)-based analysis of both MSSA and MRSA isolates to investigate the sources of *S. aureus* bacteraemia. While the small size limited the scope of the study, the pilot trial demonstrated feasibility for a larger project (Chapter 8).

Our overarching objective was to identify treatment gaps in the management of *S. aureus* infections for further exploration in future clinical trials. Chapter 9 details a study protocol for adjunctive clindamycin in severe *S. aureus* infections, leading to a pilot randomised clinical trial (RCT) included in the Appendix. Additionally, we conducted a systematic review and meta-analysis of added rifampicin therapy in *S. aureus* bacteraemia, revealing no evidence of benefit except for an identified gap in its role in Staphylococcal prosthetic valve infective endocarditis. A large RCT is currently in the planning phase to provide a definitive answer to this question.

This thesis has successfully achieved the aims outlined in the Introduction.

11.2 Limitations

While our research yields valuable insights, it is essential to acknowledge several limitations. The use of an in-house molecular typing tool to delineate the molecular epidemiology of MRSA and MSSA, though advantageous in terms of lower costs, timely results, micro-epidemiological analysis, and additional information on the presence or absence of Pantone-Valentine leukocidin in *S. aureus*, it also had its limitations. While we could confidently infer the multilocus sequence type of MRSA isolates from binary typing result, similar data was lacking for MSSA.

Many of the described epidemiological studies were conducted before 2017, potentially diminishing their current relevance. Nevertheless, our results underscore the advantages of local epidemiological studies. Local surveillance can identify and monitor clones not reflected in the national reports, as demonstrated by the example of PVL-positive ST22-MRSA.

Lastly, we had planned a study to investigate the exogenous and endogenous sources of *S. aureus* in bloodstream infections (Chapter 8). Unfortunately, due to the impact of Covid-19, we had to terminate the study prematurely. The resulting small sample size led to inconclusive finding, except that the study did help determine the timing and the sources of nosocomial *S. aureus* bloodstream infections in our facility.

11.3 Future research directions

The research presented in Chapter 8 has provided feasibility for a larger prospective study on healthcare-associated *S. aureus* bacteraemia. Building on the detailed analysis of *S. aureus* carriage and infection in Chapter 3 and drawing inspiration from the works of Phyllis Roundtree discussed in Chapter 1, further investigation is warranted. Collaborative efforts will be sought to conduct regular community surveillance, similar to the studies by Rountree, aimed at monitoring *S. aureus* epidemiology in a healthy population. Correlating these results with those reported in Australia by the AGAR/ASSOP group, particularly their analysis of *S. aureus* blood culture isolates, is likely the most effective method for monitoring *S. aureus* epidemiology.

In Chapter 3, my review has illuminated a concerning trend – individuals aged ≥ 60 years, identified as “persistent carriers” with underlying comorbidities, face the highest risk of *S. aureus* bloodstream infection. Despite advancements, mortality from this infection has remained persistently high, showing little change over the past 50 years. This underscores the necessity of shifting our focus towards studies aimed at preventing *S. aureus* bacteraemia, rather than merely searching for alternative antibiotics.

Appendix

Chapter 4:

1. Declaration from the corresponding author



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Proudly supported by the
people of Western Australia
through Channel 7's Telethon

29th June 2023

Dear Colleagues

As the corresponding author on the manuscript, "Adjunctive Protein Synthesis inhibitor antibiotics for toxin suppression in *Staphylococcus aureus* infections: a systematic appraisal" published in the Journal of Antimicrobial Chemotherapy in 2019, I confirm that Dr Ravi Dotel provided substantial contribution. Dr Dotel completed an independent literature review for this and as second author made significant intellectual contributions to the final published paper. This contribution meets the criteria for inclusion in his PhD thesis.

Sincerely

[REDACTION]

A/Prof Asha Bowen | BA MBBS DCH FRACP PhD

Head of Department | Department of Infectious Diseases | Perth Children's Hospital

Program Head, END RHD | Telethon Kids Institute

Head, Healthy Skin and ARF Prevention Team | Wesfarmers Centre for Vaccines and Infectious Diseases | Telethon Kids Institute

Associate Professor | Division of Paediatrics, School of Medicine | University of Western Australia

Honorary Fellow | Menzies School of Health Research | Charles Darwin University

Honorary Fellow | University of Notre Dame

Honorary Fellow | University of Cape Town

Discover. Prevent. Cure.



Chapter 5:

1. Posters (ICAAC 2015 and ASID 2018)

Utility of a 19-target Binary Typing Method for Large-Scale Surveillance of MRSA Epidemiology

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Abstract

Background: MRSA is a leading cause of healthcare-associated infection. Binary typing (BT) methods for prospective surveillance of MRSA to detect and track transmission events are used to identify and control outbreaks. This method has the advantage of being able to identify the multilocus sequence type (MLST) of an isolate, as well as being rapid, high resolution, and cost-effective. The aim of this study was to evaluate the utility of BT for large-scale epidemiological surveillance.

Methods: New South Wales (NSW) is the largest state in Australia with a population of 7.4 million people, and is divided into 15 Local Health Districts (LHDs). The Centre for Infectious Diseases and Microbiology for molecular typing using a multiplex PCR and binary typing (BT) was developed. The BT method was used to identify and track transmission events. The BT method was used to identify and track transmission events. The BT method was used to identify and track transmission events.

Results: 5373 isolates were identified from 573 LHDs across NSW. Ten of 100 (10%) of the isolates were identified as ST22 (50%), and ST22 (50%) and ST22 (50%) were the most common community clones. The most common nosocomial clones were ST22 (50%) and ST22 (50%). The most common community clones were ST22 (50%) and ST22 (50%). The most common community clones were ST22 (50%) and ST22 (50%).

Conclusions: The BT method can be used for large-scale surveillance of MRSA and has the advantage of being able to identify the MLST of an isolate, as well as being rapid, high resolution, and cost-effective. The aim of this study was to evaluate the utility of BT for large-scale epidemiological surveillance.

Introduction

We have previously described the routine use of a novel 19-target binary typing in outbreak investigation and to identify nosocomial transmission events (1,2). The test has a similar discriminatory power as Pulsed-Field Gel Electrophoresis (PFGE) and reliably predicts MLST and spa types (2). Its utility in a large-scale surveillance of MRSA epidemiology will be described.

Methods

Isolates: All MRSA isolates (screening and clinical).
Study sites: State of New South Wales, Australia. All prospectively collected isolates from public hospital laboratories in three LHDs (WSLHD, NBMLHD, ISLHD) and selectively referred isolates from 10 other LHDs, representing 96% of the state population of 7.4 million people and 31% of the national population of 2.3 million people (2013).

Study period: Jan 2012 – Dec 2014.
Typing method: 19-target binary typing, based on multiplex-PCR/reverse line blot hybridization platform as described elsewhere (2).
Targets: 4 Toxic genes (including PVL), 9 Phage derived open reading frames and 6 SCCmec elements = total 19 targets. Two probes for each target on the membrane.
MLST and spa-types: Predicted from binary types based on results from isolates that had been previously characterized by both methods.

Results

A. Statewide MRSA molecular epidemiology snapshot

Of 5373 isolates:
→ 66% isolates were from WSLHD, 7% from NBMLHD, and 20% were selectively referred isolates.
→ 598 different BT's were identified of which ten BT's accounted for 52% of the isolates
→ There were 47 different predicted spa types; t032 (25%), t037 (23%) and t202 (15%) were most common.

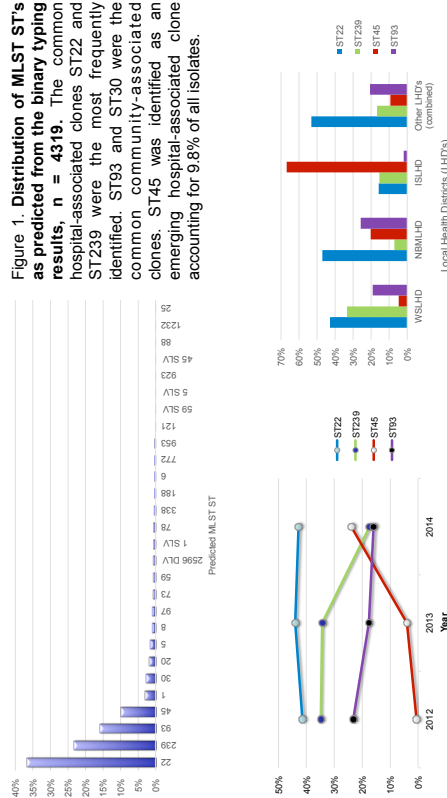


Figure 1. Distribution of MLST STs as predicted from the binary typing results, n = 4319. The common hospital-associated clones ST22 and ST239 were the most frequently identified. ST93 and ST30 were the common community-associated clones. ST45 was identified as an emerging hospital-associated clone accounting for 9.8% of all isolates.

Figure 2. Trend analysis of common ST's, n = 3678. The proportion of MRSA identified as ST239 has decreased by half over the years of the study, while there has been a simultaneous emergence of ST45



Figure 3. Common ST Distribution by local health districts (LHDs), n = 3672. The frequency of STs varied geographically, particularly of ST45. Western Sydney (WS), Nepean Blue Mountains (NBM) and Illawarra Shoalhaven (IS).

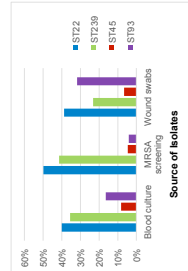


Figure 4. Common ST distribution by decades of life, n = 889. PVL positivity decreases with increasing age after the third decade of life

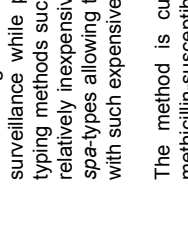


Figure 5. PVL + distribution by source of isolates, n = 2223. ST93 was more commonly found in non-invasive clinical specimens, but was an uncommonly isolated from screening specimens.

B. Microepidemiologic analysis by binary type

MLST ST	Blood culture isolates	Non-invasive isolates
ST22	13	24
ST239	11	20
ST93	2	5
ST45	2	7

Table: Number of binary types found within each predicted MLST ST. More diversity was found in noninvasive isolates than blood culture isolates, and more molecular diversity was found in ST22 and ST239 isolates than ST93 and ST45.

PVL+ ST22 MRSA:

- Distinct clones of MLST ST22 MRSA which carried PVL characterized by binary typing (figure 7).
- Associated with outbreaks in neonatal wards and community acquired infections.
- Was detected from 9 of 13 LHD's.
- Possesses spa t005 similar to other reports (4) (in comparison PVL-negative ST22 generally possesses spa t032.)
- Differs from PVL- ST22 by a number of binary targets (figure 8).
- Unlike PVL- ST22, PVL+ ST22 commonly resistant to gentamicin and cotrimoxazole.

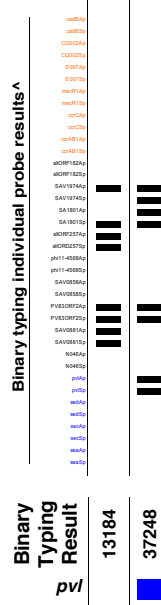


Figure 7. Number of isolates of ST22 carrying PVL by year and binary type (BT). Increasing frequency of BT 37248 has been noted.

Discussion

This 19-target binary MRSA typing system can be used for large-scale epidemiological surveillance while providing valuable micro-epidemiological information (unlike other typing methods such as MLST). It is rapid, high throughput, highly discriminatory and relatively inexpensive method (around US\$2 per isolate). It reliably predicts MLST and spa-types allowing the global comparison of results and obviating the costs associated with such expensive and labor-intensive tests.

The method is currently being adapted to study the molecular epidemiology of methicillin-susceptible *S. aureus* (MSSA).

References

- Emergence and spread of a outbreak of infections due to *Staphylococcus aureus* in a neonatal intensive care unit. *Clinical microbiology and infection*. 2012;12(1):18-9.
- Prospective genotyping of hospital-acquired methicillin-resistant *Staphylococcus aureus* isolates by use of a novel, highly discriminatory binary typing system. *Journal of clinical microbiology*. 2012;50(11):3615-9.
- Genomic diversity of *Staphylococcus aureus* isolates from a neonatal intensive care unit. *Journal of clinical microbiology*. 2012;50(11):3615-9.
- Superficial skin infections caused by *Staphylococcus aureus* in Japan. *Journal of infection and chemotherapy*. 2012;18(2):187-98.

ROUTINE PROSPECTIVE MOLECULAR SURVEILLANCE OF MRSA ISOLATES (2012-2017)

Introduction:

The proportion of *Staphylococcus aureus* isolates with methicillin-resistance has decreased in many jurisdictions in recent years¹. The change could be better understood by studying the molecular epidemiology of a large collection of prospectively collected methicillin resistant *S. aureus* (MRSA) isolates.

Aims:

1. To define changes in common multilocus sequence types (MLST) over the past six years.
2. Analyse the molecular epidemiology of MRSA isolates from both sterile and non-sterile sites.

Methods:

MRSA isolates were routinely collected from individuals attending two large acute hospitals in Western Sydney Local Health District (Westmead and Blacktown) between 01/01/2012 – 31/12/2017.

Molecular analysis was performed using 19-target binary typing². Typing of repeat isolates from an individual patient was only performed after a gap of six months, with the exception of blood culture isolates; if there were two or more isolates with same binary type within six months, the most clinically relevant was included for analysis (e.g. blood culture preferred over a screening isolate). MLSTs were predicted from the binary types³. MRSA isolates identified >48 hours after hospital admission were labelled as hospital acquired (HA).

Results:

5858 MRSA isolates from 5476 individuals were analysed (36% screening swabs, 48% swabs from wounds and skin & soft-tissue infections, 5% blood cultures, 11% others). 52% of samples were from people aged >60 years, while 6% were from children aged <15 years. Five MLSTs (ST-22, ST-239, ST-93, ST-45 and ST-30) accounted for 71% of the isolates.

ST-239 was the commonest MRSA clone in 2012 (30%), but it decreased to 7% in 2017 ($p < 0.001$). In contrast, <0.5% of MRSA were ST-45 in 2012 compared to 13% in 2017 ($p < 0.001$). The changes were gradual over six years (Figure).

The decrease in ST-239 MRSA was noted in all specimen types and in all age groups and was primarily due to a decrease in a single binary type (bt280845: 134 isolates in 2012 and 11 isolates in 2017; >90% decrease). The emergence of ST-45 was most notable in people aged >70 years (311 of 456 isolates = 68%); it was isolated from all types of specimens including blood cultures.

There was minimal change in either the rates (Table 1) or the proportions (Figure) of ST-22 and ST-93 MRSA, the other common MRSA clones. The proportion of hospital acquired ST-22 MRSA isolates has also decreased (Table 1).

Distribution of the MLSTs varied between different specimen types (Table 2). ST-22 MRSA was the commonest clone in all groups. ST-93 MRSA was more commonly isolated from SSTI/wound swabs than from screening or blood culture specimens.

Discussion:

1. A large number of MRSA isolates collected over six years from both the screening swabs and clinical isolates were analysed.
2. The recent overall decrease in both the MRSA rates and proportions in our setting is primarily due to a decline in nosocomial ST-239 MRSA.
3. ST-45 MRSA is an emerging clone, 2/3rd of isolates of which were from people aged >70 years.
4. ST-22 MRSA and ST-93 MRSA remain common.
5. The distribution of MRSA clones generally varies between different specimen classes. Studies limiting to the blood culture isolates may fail to provide a true epidemiological information.

References: (1) <https://www.ecdc.europa.eu/sites/portal/files/documents/AMR-surveillance-Europe-2016.pdf> (accessed 4th Apr 2018) (2) Kong F, Gilbert GL. Multiplex PCR-based reverse line blot hybridization assay (mPCR/RLB)→a practical epidemiological and diagnostic tool. Nature protocols. 2006;1:2668-80 (3) O'Sullivan MV, Zhou F, Sintchenko V, Gilbert GL. Prospective genotyping of hospital-acquired methicillin-resistant *Staphylococcus aureus* isolates by use of a novel, highly discriminatory binary typing system. Journal of clinical microbiology. 2012;50:3513-9.

Figure: Changes in the proportion of common MLSTs between 2012 – 2017 (Note: Decrease in ST-239 MRSA, and emergence of ST-45 MRSA)

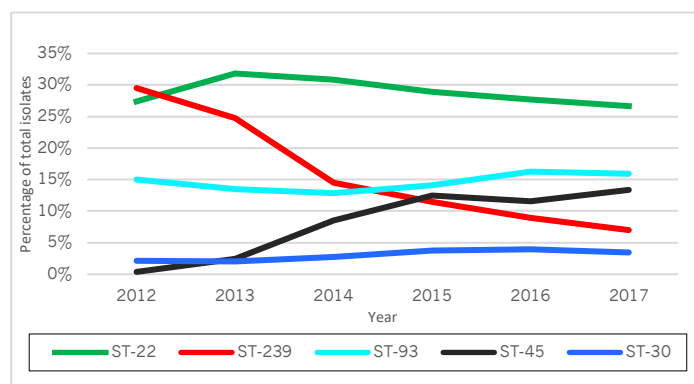


Table 1. Changes in the proportion of hospital acquired (HA) MRSA isolates between 2012-2017. Both rates and proportion of HA ST-239 MRSA have decreased, contributing to the overall decrease in HA MRSA. ST-93 MRSA remains a common community strain, whereas ST-45 MRSA is an emerging nosocomial strain.

YEAR	All strains	ST-22	ST-239	ST-45	ST-93
HA % (all MRSA isolates)					
2012	32% (1081)	31% (296)	57% (319)	- (4)	2% (162)
2013	30% (1034)	33% (329)	50% (256)	12% (25)	7% (140)
2014	30% (1019)	32% (314)	52% (148)	32% (87)	10% (131)
2015	26% (827)	33% (239)	41% (95)	30% (103)	6% (117)
2016	21% (910)	26% (252)	41% (81)	25% (105)	7% (148)
2017	21% (987)	26% (263)	45% (69)	23% (132)	10% (157)
TOTAL	27% (5858)	30% (1693)	51% (968)	26% (456)	7% (855)

Table 2. Distribution of the common MLST ST in different specimen classes

MLST ST	Blood culture (303)	SSTI (2817)	Screening swab (2094)	Sputum (320)	Other * (324)
ST-22	27%	25%	34%	33%	28%
ST-239	22%	10%	21%	33%	21%
ST-93	10%	25%	3%	4%	13%
ST-45	9%	7%	8%	9%	10%
ST-30	2%	5%	1%	1%	1%
Other STs	16%	11%	13%	7%	12%
NA	15%	16%	20%	14%	15%

*Other includes tissue, aspirate, broncho-alveolar lavage, bone, line tip and drain samples; the number within the brackets indicates sample size; NA: MLST ST could not be determined from binary typing.

Chapter 6:

1. Poster (ISSSI 2018)

Introduction:

Recent reports suggest a decrease in methicillin-resistant *S. aureus* (MRSA) infections at many centres¹. This could be attributed to mandatory reporting of healthcare onset (HO) *S. aureus* (SA) bacteraemia (SAB), infection control practices such as hand-hygiene, better management of venous access devices, and antimicrobial stewardship (AMS). However, prospective data to support a decrease in MRSA prevalence are lacking.

The aim of this study was to describe changes in the epidemiology of MRSA and methicillin-susceptible *S. aureus* (MSSA) bloodstream isolates (BSI) in the last six years.

Methods:

SA isolates from bacteraemic episodes were routinely collected between 01/01/2012 – 31/12/2017 from patients attending two large acute hospitals in Western Sydney Local Health District, which serves a population of almost 1 million (12% of the State population)². Both hospitals have established infection control and AMS services, and HO SAB are mandatorily notified. MRSA screening and isolation of high-risk patients are routinely performed.

SAB occurrence more than 48 hours after hospital admission was labelled as healthcare onset (HO)³. Molecular analysis of MRSA isolates was performed using 19-target binary typing^{4,5}, which also predicted multilocus sequence type (MLST)⁵. The *p*-value for categorical variables was calculated using a chi-squared test.

Results:

A total of 1130 unique patients had SAB over the study period, of whom 302 (27%) had MRSA and 828 (73%) had MSSA isolated (Table).

The proportion of isolates that were MRSA peaked to 31% in 2014 but gradually decreased to 20% in 2017. The change was due to a significant decrease in HO MRSA BSI (Figure 1). At its peak in 2013, 49% (22/45) of MRSA BSI were HO compared to 22% (13/60) in 2016 and 24% (9/38) in 2017 (*p*=0.018). On the other hand, the proportion of HO MSSA BSI varied each year with no trend (16-26%). Overall, 35% of MRSA BSI were HO compared with 21% of MSSA BSI.

The four most common MLSTs (ST22, ST239, ST93 and ST45) accounted for 69% of MRSA isolates. ST22 and ST239 alone accounted for 50% of the isolates. ST could not be determined for 15% (46/302) isolates as they belonged to less common binary types for which a corresponding ST had not yet been determined.

A gradual decrease in the rates (data not shown) and proportion (Figure 2) of ST239-MRSA in both HO and non-HO groups was noted. In contrast, HO ST22-MRSA remained common. ST93 was primarily detected in non-HO MRSA BSI (Figure 3).

ST45-MRSA was previously never isolated from BSI, however, it comprised 21% of MRSA BSI isolates in 2015 but since has decreased to 8% in 2017 (Figure 2). It caused both HO and non-HO BSI (Figure 3).

Conclusions:

1. A significant decrease in HO-MRSA BSI was noted, which was primarily due to a decline in HO ST239 MRSA. In contrast, there was no change in the rates of HO-MSSA or ST22 MRSA BSI.
2. The proportion of MRSA in SAB has decreased, due to a decrease in ST239 MRSA and despite the emergence of ST45 MRSA. ST22 MRSA and ST93 MRSA remain common.
3. MSSA BSI rates have remained unchanged but the proportion of SA that are MSSA has increased.

References:

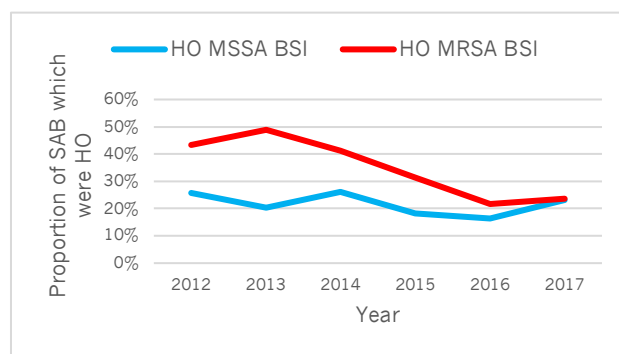
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Table. Proportions of MSSA and MRSA bloodstream isolates collected during the study period (2012-2017) in Western Sydney (includes both HO and non-HO)

Year	Total=1130	MSSA, n (%)	MRSA, n (%)
2012	212	152 (72)	60 (28)
2013	155	110 (71)	45 (29)
2014	166	115 (69)	51 (31)
2015	179	131 (73)	48 (27)
2016	225	165 (73)	60 (27)
2017	193	155 (80)	38 (20)

N = number of bloodstream isolates; HO = healthcare onset

Figure 1: Changes in the proportion of MRSA and MSSA blood stream isolates (BSI) which were healthcare onset (HO) between the years 2012 - 2017



SAB = *S. aureus* bacteraemia; BSI = bloodstream isolates

Figure 2: The proportion of common sequence types (ST) of total MRSA blood stream isolates (BSI) for each corresponding year (includes both HO and non-HO isolates).

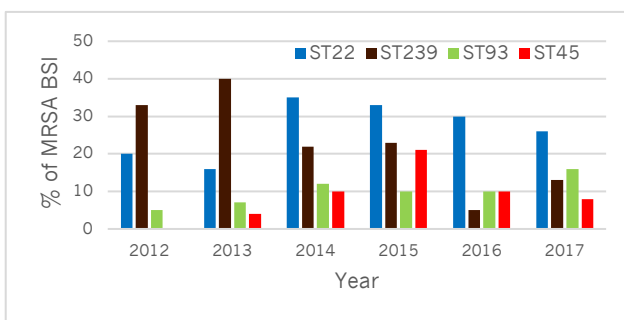
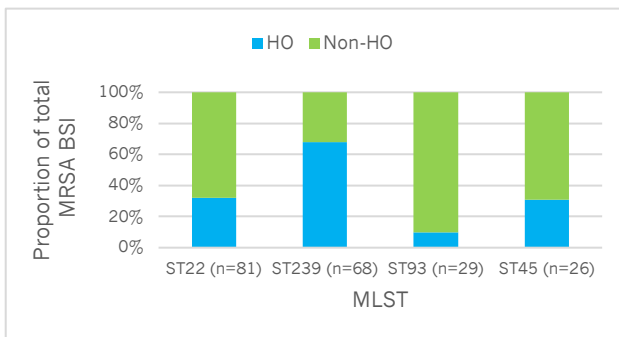


Figure 3: The proportion of healthcare-onset (HO) isolates among the four most common MRSA sequence types (ST).



n = number of MRSA blood stream isolates for each ST collected in Western Sydney between 2012-2017
MLST = multilocus sequence type

#P170

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Chapter 7:

1. Poster (ISSSI 2016)

Nosocomial Adaptation of Methicillin-Sensitive *Staphylococcus aureus* (MSSA) Clones



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INTRODUCTION

Strains of MRSA that are adapted to the hospital environment are well described. This adaptation has not been recognised for methicillin-susceptible *Staphylococcus aureus* (MSSA).

It is frequently assumed that nosocomial MSSA infection is of endogenous origin (arising from the patient's own flora, rather than acquisition of a new strain). However, studies indicate twenty percent or more of MSSA infections may be of exogenous origin¹. These exogenous strains may represent hospital adapted clones causing unrecognised nosocomial acquisition and/or transmission of MSSA.

Aims of the study

1. To examine the genetic diversity of MSSA bloodstream infection (BSI) isolates using binary typing.
2. To look for evidence of hospital transmission of MSSA clones based on differences in strain distribution or diversity in community versus nosocomial onset infections.

METHODS

Isolates from MSSA BSIs diagnosed at a large, adult tertiary hospital in Sydney, Australia between January 1, 2011, and December 31, 2015 were used in this study. Each isolate represented an unique episode of BSI. Relapsed or recurrent MSSA BSIs were excluded.

Each BSI episode prospectively recorded as community acquired (CA), healthcare associated inpatient (HAI) or healthcare associated non-inpatient (HANI) according to standard criteria². HANI is equivalent to healthcare associated community onset infection.

Strain typing of isolates was performed using a previously described novel binary typing method, which utilises a multiplex polymerase chain reaction and reverse line blot hybridisation assay (mPCR/RLB)³. The test was designed for MRSA⁴, but which has been shown to be at least moderately discriminatory for MSSA (*unpublished*).

The 19-target binary typing included four toxin genes (including the gene encoding PVL), nine phage-derived open reading frames and six SCCmec elements. *nuc* and *mecA* are also included as controls. The assay determines the presence "1" or absence "0" of 19 genetic targets. Decimal number conversion of the 19-digit binary number was the binary type (BT) of a isolate.

The *p*-value for the continuous variables were calculated using unpaired *t*-test. For the categorical variables, a chi-squared test was used.

RESULTS

Three hundred and thirty-three unique MSSA BSI were recorded during the study period. Isolates from 288 (86.5%) of these were available for strain typing. Participants' ages ranged from 16 to 95 years. There was no significant difference in age or sex distribution in MSSA BSI by healthcare association (Table 1).

16/288 (5.5%) isolates were nontypeable by this method (0/19 targets present). Eight of these were CA, and four each were HAI and HANI. One-hundred twenty-eight different BTs were detected among the remaining 272 isolates. Simpsons Index of diversity was 0.973, 0.974 and 0.985 for CA, HAI and HANI respectively (Table 2). Distribution of the BTs in the three healthcare association groups are shown in Table 2 and 3.

The gene encoding PVL was detected in 4% of the isolates. All patients with PVL positive isolates survived. Mean age for patients with PVL positive MSSA BSI was 52 years (overall mean age 59 years), *p*=0.19 (Table 3 and 4).

While all isolates were negative for *mecA*, 45/288 (16%) MSSA isolates showed presence of targets usually located on SCCmec. Thirty-two of these were positive for *cadB*, 12 for *ccrAB1* and 1 for ORF E007 of type I SCCmec. The detection of these targets may be due to the presence of an SCCmec-variant without a functional *mec* complex⁵, or due to the presence of homologous sequences elsewhere in the MSSA genome. 25/45 SCCmec element positive isolates were CA, however the association was not significant (*p*=0.32).

Table 1. Basic demographics

	Community Acquired (CA)	Healthcare associated inpatient (HAI)	Healthcare associated non-inpatient (HANI)
Number of isolates	156 (54%)	73 (25%)	59 (21%)
Age (mean)*	58 years	60 years	62 years
Sex, male*	65%	70%	56%

*The differences were not significant (*p*-value > 0.05)

Table 2. Binary typing results of 288* episodes of MSSA BSI

	Community acquired (CA), n=148	Healthcare associated inpatient (HAI), n=69	Healthcare associated non-inpatient (HANI), n=55
Number of binary types	83	46	42
Simpson's index of diversity (95% CI)	0.973 (0.958 - 0.987)	0.974 (0.956 - 0.993)	0.985 (0.972 - 0.997)
Most common binary type (bt), (number)	bt256 (20)	bt256 (9)	bt256 (5)
Number of BTs unique to each group	56	22	19

*Sixteen isolates non-typeable.

Table 3. Binary types in common among the healthcare association groups

	CA and HAI	CA and HANI	HA and HANI	CA, HAI and HANI
Number of binary types in common	20	19	15	12
Combined number of binary types in each group	109	106	72	128

CA: Community-acquired; HAI: Healthcare associated inpatient; HANI: Healthcare associated non-inpatient

Table 4. Analysis of PVL results in MSSA BSI

	PVL present	P-value
Healthcare association		
- Community acquired (CA)	9/158 (6%)	-
- Healthcare associated inpatient (HAI)	1/78 (1%)	0.11
- Healthcare associated non-inpatient (HANI)	2/59 (3%)	0.49
Mortality outcomes		
- Death within 30 days	0/12 (0%)*^	0.15

*39/272 (14%) cases with PVL negative MSSA BSI died within Day 30.

^Day 30 outcomes was not available for 4 cases with PVL negative MSSA BSI.

DISCUSSION

- The binary typing assay designed for MRSA was also reasonably discriminatory for MSSA (Table 2), although the replacement of some non-contributory targets could increase the discriminatory power even further.
- This study did not find a significant difference in diversity between HAI and CA MSSA strains to support the hypothesis of predominant healthcare associated strains causing exogenous nosocomial infection (Table 2). However, the HANI group, which is likely to have a mixture of true community acquired strains and nosocomial strains, had a non-significantly higher level of diversity than either the HAI and CA groups.
- Furthermore, there were differences in strain distribution between the groups (Table 2 and 3), suggesting some strains may be more likely to cause healthcare associated infection (though no clustering or specific transmission events were detected).
 - 22/46 (48%) BTs among HAI MSSA BSI were not detected in CA or HANI infections.
- The majority of MSSA BSI in this study were community acquired, while a quarter of MSSA BSI were of nosocomial onset. In contrast to other reports⁶, HAI MSSA BSI was more common among males.

Limitations of this study include restriction of the analysis to BSI isolates and the use of a typing method optimised for MRSA. To further explore the possibility of nosocomial transmission of MSSA, prospective typing-based surveillance of MSSA infection and colonisation in hospital setting, using a method designed specifically for MSSA, is now underway.

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Chapter 8:

1. Letter to the Editor (Lancet Infectious Diseases)
2. Study Protocol
3. PICF form
4. Laboratory form

programmes to combat antimicrobial resistance and their efforts are undermined by the questionable practices of the pharmaceutical manufacturing units.³

Public availability of information about the origin of APIs and evidence of good manufacturing practice compliance of the manufactured APIs imported by the European Union and the USA can improve transparency. WHO good manufacturing practice guidelines for workplace safety, environmental protection, pollution prevention, and adoption of cleaner production technology should be adopted and enforced consistently for all the countries that are responsible for antimicrobial resistance.²

We declare no competing interests.

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e-learning for global antimicrobial stewardship

In their Correspondence, Elizabeth Robilotti and colleagues¹ highlighted the demand and need for open access, globally accessible educational resources for health-care professionals across all health economies, and discussed the resources they offer in response to this need. To support this demand, the British Society for Antimicrobial Chemotherapy (BSAC) developed a massive open online course (MOOC) on antimicrobial stewardship in partnership with the University of Dundee, Scotland, UK. Free at the point of access, it has been accessed by almost 33 000 learners in 163 countries since October, 2015. Assessment of its use and impact is highlighted in the appendix. The course empowers health-care professionals to provide safe, high-quality antibiotic use and to assess the effectiveness of using strategies, tools, and interventions in antimicrobial stewardship to drive improvement in their locality and practice. Adding to its global value, the open access course is available in English² and Mandarin,³ with Spanish and Russian versions planned.

Future Learn, the platform host, has partnered directly with BSAC, providing a global learning platform for concise, open access courses for all health-care communities and a market reach of 5.5 million learners worldwide. The first two courses, “Challenges in antibiotic resistance: point prevalence surveys” and “Challenges in antibiotic resistance: Gram-negative bacteria”, opened for registration on March 13, 2017. Early 2018 will see the launch of an e-book on antimicrobial stewardship. The remit is broader and deeper than the MOOC—eg, it encompasses stewardship in the non-hospital setting and in special populations. The value and preference of a range of e-learning tools to augment traditional face-to-face and practice-based learning, termed hybrid learning, has been identified in three large surveys of the stewardship educational needs and

existing activity of Indian, African, and Russian health-care professionals (D Nathwani, unpublished). These surveys were supported by the UK Foreign and Commonwealth Office Science and Innovation Fund.

Lastly, we are members of the Conscience of Antimicrobial Resistance Alliance,⁴ established to support implementation of the UN Resolution on Antimicrobial Resistance.⁵ BSAC is proposed to lead the education sub-branch to develop indicators against which to measure the availability, implementation, and success of educational initiatives across the world. We look forward to hearing from other potential partners who would like to work with us in this important venture.

We declare no competing interests.

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Staphylococcus aureus in critical care

The Article by James Price and colleagues¹ describes an important study that explores the role of health-



See Online for appendix

For Future Learn see
www.futurelearn.com

care workers and the environment in patient acquisition of *Staphylococcus aureus*. We congratulate the team on the study, which, impressively, enrolled 95% of eligible health-care workers who, along with patients and the environment, were screened for *S aureus*; the large number of isolates were then analysed using whole-genome sequencing.

However, we feel the authors' conclusion that "health-care workers were infrequently sources of transmission to patients" is not warranted, in light of the study's limitations. Several of these limitations, including the fact that the study was done at a single hospital with a low prevalence of methicillin-resistant *S aureus*, were cited by the authors, and in the accompanying Comment.² However, in addition, large numbers of health-care workers (eg, visiting medical teams, radiographers, allied health staff other than physiotherapists, food handlers, cleaners, porters) and visitors were not screened and the intervals between samplings of those who were screened were long. Members of staff were only considered to be sources of acquisition for patient colonisation if the staff member's isolate was obtained before, or at the same time as, the patient's isolate. However, the order of sampling does not necessarily reflect the direction of transmission, and staff might only be found to be colonised after transmission has occurred.

Considering the high proportion of health-care workers who were transient *S aureus* carriers, the median staff screening interval of 4 weeks, and the relatively poor sensitivity of nasal swabs alone (70.3%), the conclusion that health-care workers were only infrequent sources of acquisition seem unjustified. Almost 40% (727 of 1854) of patients admitted to the units were screened only once and none were screened after discharge, so a large number of patient acquisitions were likely to be unrecognised. Potential sources of *S aureus* were not identified

in 72 (76%) of 97 recognised patient acquisitions. Although the authors suggest that these might not be true acquisitions, but rather explained by false-negative admission screens, it also raises an alternative possibility that nosocomial acquisition of *S aureus* is much more frequent than suggested by this study.

Identifying sources of *S aureus* transmission is difficult, but can be facilitated by more frequent sampling and rapid screening of isolates, using a high-throughput, inexpensive strain-typing tool, supplemented by whole-genome sequencing, to detect transmissions events.³

RD's PhD is supported by Australian Postgraduate Awards (2016). MOS and GG declare no competing interests.

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Authors' reply

Ravindra Dotel and colleagues highlight several limitations of our study¹ and argue that, taken together, these limitations undermine our conclusion that health-care workers are infrequently sources of *Staphylococcus aureus* transmission. Although we acknowledge the criticisms made, we do not agree with this analysis.

The key findings of our study are the very high rate of *S aureus* carriage measured among health-care workers with the greatest patient contact and how few instances of transmission

were detected from these carriers. Dotel and colleagues are concerned that by considering directionality we have underestimated transmission, but figure 4 of our Article shows that the same subtype of *S aureus* was only found in patients and health-care workers on 17 occasions over the whole study, irrespective of directionality. Staff with less contact are unlikely to account for substantially more transmission. Dotel and colleagues are correct that some staff carried *S aureus* intermittently but not that we identified transient carriage. In fact, in the sub-study in which nurses underwent swabbing before and after shifts, we did not identify any transient carriage.

The comprehensive prospective sampling that underpinned our study cannot be achieved across multiple centres and would not add substantial value. The issue with generalisability is primarily that the study was done in the presence of robust infection control practice and low rates of methicillin-resistant *S aureus*. We believe our results are relevant to similar settings elsewhere, but not in settings where robust infection control measures are not sustained.²

We acknowledge that some patients left the critical care unit quickly and so were only screened once. These patients could have acquired *S aureus* without detection. However, this scenario would increase both the acquisition rate and potentially the number of transmissions, rather than be expected to increase the proportion of acquisitions with identified transmissions.

We agree that identification of sources of transmission is difficult, but not only because it requires intensive sampling and follow up. Nosocomial *S aureus* strains are often closely related. We have previously shown that insufficiently discriminatory typing techniques are unreliable tools to measure *S aureus* transmission.³ Researchers should hesitate before applying potentially lower-resolution,

Study Protocol

Study Title: Molecular analysis guided infection control to determine the source of healthcare-associated *Staphylococcus aureus* bloodstream infections

Short Title: MAGIC SABSI Study

Investigators:

1. Dr Ravindra Dotel - Principal Investigator (Infectious Diseases Staff Specialist, Blacktown Hospital)
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6. Rola Tawbe (Assistant Director of Nursing and Standard 3 Nursing Lead, Blacktown Hospital)
7. Lesly Mathew (Clinical Nurse Consultant, Infection Prevention and Control, Blacktown Hospital)
8. Carolyn Lambeth (Registered Nurse, Infection Prevention and Control, Blacktown Hospital)

Background:

Healthcare-associated (HA) *S. aureus* bloodstream infection (SABSI) is an avoidable infection¹. It is a reportable illness for hospital accreditation as it indicates safety and quality in healthcare. This mandatory reporting and investigation to identify and manage preventable causes have reduced these infections, however, both Westmead and Blacktown Hospitals have higher rates of HA SABSI than the peer-group hospitals².

The available evidence suggests that more than 80% of SABSI are caused by an individual's colonising strain³, however, it is unclear if this is also true for an HA infection. These HA infections may occur from an acquisition of a new strain of *S. aureus* acquired during an admission. Healthcare staff or the environment are repeatedly shown to be a reservoir for a prolonged nosocomial outbreak of *S. aureus* infection, and that treatment of implicated staff leading to termination of the outbreak⁴. On the other hand, the roles of these external factors in non-outbreak settings are poorly understood. Price *et al*⁵ performed screening of *S. aureus* in healthcare staff and the environment in an intensive care unit (ICU) using whole-genome sequencing and reported that staff members/environment were infrequent sources of transmission. Contrary to the findings of Price *et al*, others have reported patients and staff sharing similar clusters of *S. aureus* indicating co-transmissions⁶.

Our research will explore the distribution of *S. aureus* strains in the patient's carriage sites and their external environment following episodes of HA SABSI to identify the source of the nosocomial strain. This will augment an investigation of HA SABSI, which is already notified as a critical incident in the Western Sydney Local Health District (WSLHD) incident management system (ims+) as part of the quality improvement process.

Aims: The study aims to identify the source of *S. aureus* causing HA SABSI to aid infection control interventions. The research will quantify the benefits of screening the patient's environment and other sources in the investigation of preventable causes of HA SABSI.

Hypothesis: It is unclear if the source of the strain causing HA SABSI is patient's colonising bacteria or an external strain shared with the hospital environment, and other patients. We hypothesize that the comprehensive screening of the environment and the endogenous flora of patients for *S. aureus* carriage will help identify the source or reservoir of the bacterial strain causing HA SABSI.

Predictions: Our study findings will strengthen infection control programs at WSLHD. This will facilitate a reduction in HA SABSI and other nosocomial *S. aureus* infections.

Methods:

This is a prospective observational study with a cross-sectional design conducted at Blacktown Hospital. SABSI is defined as the identification of methicillin-susceptible *S. aureus* (MSSA) or methicillin-resistant *S. aureus* (MRSA) in a blood culture. HA SABSI will be defined as the onset of infection after >48 hours of hospital admission and reflects occurrence as a consequence of inpatient management. SABSI episodes within 48 hours of admission will be classified as community-associated (CA) or healthcare-onset community-associated (HCA) as per the standard definitions. If the symptoms (e.g. fever) onset were within 48 hours of admission but the blood culture was only collected after 48 hours, this will be labelled as non-HA (i.e. CA or HCA) after assessment by the investigators.

Inclusion criteria:

1. Age ≥18 years of age
2. *S. aureus* identified in blood culture
3. Admitted to the hospital

Exclusion criteria:

1. Patients declining study participation
2. >96 hours elapsed since the identification of SABSI
3. Primary clinician unwilling for patient enrolment

Intervention and follow up:

The cases of SABSI will be identified through the automated email reporting system already in use. Two investigators (RD or AO) will screen SABSI cases and classify them as HA or non-HA. All SABSI cases (after a written informed consent) will undergo *S. aureus* carriage screening involving nasal, pharyngeal, hands, groin and/or wound swabs. Those with HA SABSI will have the screening swabs collected from the call-button (or bed-side remote), the side-rails, computer on wheels (COW) and desktop's keyboards/mouse at the nursing and medical stations in that ward. We will also seek a consent from other patients sharing the room for *S. aureus* carriage screening with a nasal, pharyngeal, hands and/or wound swabs. There may be one to three other patients sharing a room. The swabs from the patients and the environment will be collected within 96 hours from laboratory identification of SABSI. Patients with HA SABSI (index cases) will also be asked to fill in a questionnaire on HAIs. All participants microbiology results will be monitored for 12 months following the enrolment for SABSI episodes. No other patient contact will be required after the enrolment.

The index participant's (those with HA SABSI) *S. aureus* strain will be checked against his/her screening isolate, the environment, and other patients' strains to define strain relatedness and to identify the source or reservoir of HA SABSI. The isolates obtained from non-HA SABSI cases will serve as a comparator and provide a contemporary community *S. aureus* strains during the study period. Any positive blood cultures in the 12 months following the enrolment will also be analysed to define strain relatedness to the initial screening or the environmental isolates.

The molecular analysis will be performed by binary typing⁷ using multiplex-PCR/reverse line blot assay available at Westmead Hospital microbiology laboratory. It is a high throughput and inexpensive typing method (\$5 per isolate) which helps differentiate unrelated strains of *S. aureus* with high certainty (Simpson's diversity index 0.97). Unlike other molecular typing methods such as multilocus sequence typing and pulsed-field gel electrophoresis, binary typing has a rapid turnaround time⁷. It was developed and validated at the microbiology department, Westmead Hospital.

Patient survey questionnaire:

This is based on a questionnaire developed by Madeo *et al*⁸ in the UK and previously used in Australian settings to study a patient's perception of HAI⁹. It consists of 10 questions¹² including (1) were you aware of the risk of HAI before coming to a hospital; (2) adequate knowledge on HAI prior to the admission; (3) feel that you have enough understanding of HAI; (4) does media portray an accurate picture of HAI; (5) what can be done to decrease the risk of patients acquiring HAI; (6) are all HAI preventable; (7) name any bacteria that contribute to HAI; (8) understanding of microorganism spread

in hospitals; (9) satisfaction with infection control measures taken during the admission; and (10) if measures to control HAI are improving. All questions have yes/no answers except questions 5 and 7. Question 5 has a drop-down list. (*see supplementary files: questionnaire*)

Primary outcomes:

1. The proportions of SABSI caused by patient's own colonising strain and that due to an external strain (surrounding environment or healthcare contacts).

Secondary outcomes:

1. The prevalence of *S. aureus* carriage in patients with HA SABSI, their immediate and healthcare environment, and in patients sharing the room.
2. Distribution of *S. aureus* strain types.
3. Understand the knowledge and opinion of patients on HA infections.

Sample size:

We expect 15-25 cases of HA SABSI (and an additional 30-40 non-HA SABSI) in Blacktown Hospital during the 12-month study period. There were 21 HA SABSI in Blacktown in 2019, 12 in 2018 and 12 in 2017. Excluding the blood culture isolate, at least four additional body swabs and up to 14 environmental swabs (2 index patient's immediate environment, up to 6 COWs, and up to 6 desktops), and nine or more swabs will be collected from other patients sharing the room. In total, there may be 27 or more screening swabs collected after each episode of HA SABSI. For non-HA SABSI cases, there will be four or more screening swabs collected (environmental swabs and swabs from other patients not required). We expect up to 50% of screening swabs to isolate *S. aureus*¹⁰. Strain typing will be performed on these and the blood culture isolates.

Data Collection:

Master log form will include a unique study code linked to participant identifying details, including name, initials, medical record number (MRN) and date of birth. Case report forms will include details such as hospital admission date to correctly classify cases as HA or non-HCA, body sites swabbed, and the markers that correlate with severity of the infection (these include the duration of bacteraemia, age, source of the bacteraemia and survival status at discharge). No blood tests or imaging are required for the study. Off-site access to medical records will not be necessary. Cases with HA SABSI will be asked to fill a questionnaire to understand their perception of HAI. (*See supplementary files: case report forms 1 and 2, and questionnaire*)

Statistical Analysis:

Data entry will be performed in Excel and the statistical analysis will be performed using SPSS (Statistical Service Product Solutions). We will use the chi-square test or Fisher's exact test as appropriate and report the result as significant if the p-value is <0.05. The open-ended patient questionnaire provides quantitative data not amenable for direct statistical analysis. Key themes and categories will be analysed from the questionnaire.

Ethical considerations:

The wound swabs and the screening swabs are routinely performed in hospitalised patients to identify pathogens implicated in wound infections and to check the carriage of multi-resistant organisms. The study augments the current investigation of HA SABSI which is a 'ims+' as part of the quality improvement process. We will seek a written informed consent from the participants for collecting *S. aureus* swabs and to conduct a questionnaire. Next of kin may be approached if the participant is unable to provide consent (e.g. sedated or intubated or delirious). We will not collect pharyngeal screening swabs in those who are not alert (i.e. not responding appropriately or able to engage in a conversation). All other swabs are non-invasive. Those refusing to participate or unable to provide consent will be excluded from enrolment. Interpreter service will be used if necessary.

Infection Prevention and Control (IPC) nurses and/or all co-investigators will be involved in screening and questionnaire collection. This expanded screening will be incorporated into the current infection control program already in existence. The researchers have access to the clinical data (as available in the electronic medical records) as part of their usual role.

Each case of SABSI will be given a unique study code and the environmental samples and the samples from other patients sharing the room will be identified through this study code. Master log from will be kept in the PI's office in a locked filing cabinet and separate from the case report forms which are kept in the IPC office. Only authorised staff are allowed to enter the IPC offices and require swipe card access. The electronic data will not contain any personal identifying information, which will be stored in the WSLHD computer. These computers are protected by the WSLHD firewall and require employee number and password to log in.

Conclusion/Outcomes:

The research will quantify the benefits of screening a patient's environment and other sources in an investigation of HA SABSI. Incorporation of molecular typing will help identify the source of the *S. aureus* strain and help understand its transmission within the hospital¹¹.

Our study findings will aid the infection control program leading to a reduction in HA SABSI and other HA *S. aureus* infections. If the infection is found to be from an external source, this will reinforce the use of hand hygiene, environmental cleaning and the use of contact precautions, as applicable. On the other hand, if the infecting strain is found to be patient's own and different to the external strain, this will prioritise decolonisation strategies and efficient management of lines and devices in preventing HA SABSI. Further, the incorporation of molecular analysis in infection control will help identify strains commonly associated with HA *S. aureus* infections. This will enact early interventions to limit further spread.

The study will be submitted for a publication in a peer-reviewed journal and presentation at a national infection control/infectious disease conference. Binary typing of *S. aureus* is also being rolled out across other public health laboratories in New South Wales. This strain-typing aided infection control surveillance will ultimately be available for use in other public hospitals in NSW if shown to be of benefit.

References:

1. Mitchell BG, Shaban RZ, MacBeth D, Wood CJ, Russo PL. The burden of healthcare- associated infection in Australian hospitals: a systematic review of the literature. *Infection, Disease & Health*. 2017;22(3):117-28
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11. Singh A, Goering R V, Simjee S, Foley SL, Zervos MJ. Application of molecular techniques to the study of hospital infection. *Clin Microbiol Rev*. 2006;19:512–30.

Participant Information Sheet/Consent Form
PARTICIPANTS (ADULTS WITH S. AUREUS BLOODSTREAM INFECTION)

Interventional Study - Adult providing own consent

Title	Molecular analysis guided infection control to determine the source of healthcare-associated <i>Staphylococcus aureus</i> bloodstream infections
Short Title	MAGIC SABSI Study
Principal Investigator	Dr. Ravindra Dotel MBBS, FRACP, MMed (ClinEpi)
Associate Investigator(s)	Dr. M. O'Sullivan, Dr A. Ong, Dr. C. Bahn, Ms. N. Tolhurst, Ms. R. Tawbe, Ms. L. Mathew, and Ms. C. Lambeth

Part 1: What does my participation involve?

1. Introduction

You are invited to take part in this research project because you have *Staphylococcus aureus* (also commonly known as “Staph” or “Staphylococcus” or “golden Staph”) bloodstream infection.

Before you decide whether or not you wish to participate in this study, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others such as your family if you wish. If English is not your preferred language, please let us know and we will arrange the use of an interpreter if needed.

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part. You will be given a copy of this Participant Information Sheet and the Consent Form to keep.

2. What is the purpose of this research?

“Staph” is a common bacterium that usually resides on body sites such as nostrils, hands, throat, groin, and/or wounds. It can also be found in the hospital environment passed on by patients, visitors or the healthcare workers. The proposed research will investigate the source of “Staph” causing the bloodstream infection.

3. What does participation in this research involve?

Our research involves taking “Staph” screening swabs on these body sites and the hospital environment that includes areas commonly touched by patients, visitors and the hospital staff. However, if your infection had occurred within 48 hours of your admission, we will only collect swabs from your body sites as the current evidence indicates that these infections are from your own colonising bacteria. For all infections acquired after 48 hours of hospital admission, we will also request you to complete a questionnaire (includes 10 questions) to understand your perception of healthcare-associated infections.

We will also seek consent from other patients that may be sharing a room with you and screen their body sites for “Staph” carriage.

4. What do I have to do?

The study is aiming to enrol patients found to have a “Staph” bloodstream infection at Blacktown Hospital. There may be 50 or more such infections during the 12 months study.

5. Other relevant information about the research project

Adults admitted to this hospital with a “Staph” bloodstream infection can participate. A participant must be enrolled in the study within 96 hours of the initial blood culture collection. Your doctor currently treating you has advised that this study may be suitable for you to participate in.

6. Do I have to take part in this research project?

Participation in this study is voluntary. It is completely up to you whether or not you participate. If you decide not to participate, it will not affect the treatment you receive now or in the future. Whatever your decision, it will not affect your relationship with the staff caring for you.

7. What are the alternatives to participation?

You do not have to take part in this research project if you don't want to. This will not affect the treatment you are receiving for your illness.

8. What if I withdraw from this research project?

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional information from you, although information already collected will be retained to ensure that the results of the research project can be measured properly. You should be aware that data collected up to the time you withdraw will form part of the research project results.

9. What are risks of participating?

Side Effect	How often is it likely to occur?	How severe might it be?	How long might it last?
Uncomfortable feeling at nose and throat following a swab	Common	Mild	Temporary
Painful at swab site following a wound and nasal swab	Uncommon	Mild	Temporary
Nosebleed following nasal swab	Rare	Mild-Severe	Temporary
Nausea and vomiting (for throat swab)	Uncommon	Mild	Temporary

10. What are the benefits of participating?

You may or may not benefit from participating in this study. The results of this study could help prevent future episodes of “Staph” infections.

11. What will happen to my test samples?

“Staph” screening swabs obtained for the purpose of this research project will be sent to the microbiology laboratory at Westmead Hospital for processing. The samples that does not

isolate “Staph” will be discarded. When “Staph” is isolated, we will perform tests to identify sub-types of “Staph” (molecular analysis). When the test is completed, the samples are sterilised and discarded securely by the laboratory.

12. Will the study cost me anything?

There are no additional costs associated with participating in this research project, nor will you be paid.

13. What if new information arises during this research project?

Sometimes during the course of a research project, new information becomes available about the intervention that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

14. Can I have other treatments during this research project?

Your participation in this research does not alter any of your treatment. You can continue all your usual treatment, or any new treatment provided by your treating doctors during or after this research.

15. What happens when the research project ends?

We plan to discuss/publish the results in peer-reviewed journals, presentation at conferences or other professional forums. In any publication, information will be provided in such a way that you cannot be identified. Results of the study will be provided to you, if you wish.

Part 2: How is the research project being conducted?

16. What will happen to information about me?

The doctors and nurses involved in your care will know whether or not you are participating in this study. This information will also be recorded in your medical records at the hospital. Any identifiable information that is collected about you in connection with this study will remain confidential and will also comply with all relevant privacy legislation. In Australia this is the Privacy Act 1988 and relevant privacy laws. Your identifiable information will be disclosed only with your permission, unless otherwise required by law. Only the researchers named above that work in this hospital/health district and the regulatory authorities will have access to your personal details. The information collected will be entered into a secure database for analysis purposes, however prior to final analysis your personal details will be removed. The information collected will be stored securely for a minimum of 15 years in compliance with the National Health and Medical Research Council (NHMRC) guidelines.

17. Complaints and compensation

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital.

This study is non-commercial and performed by the hospital staff as a quality improvement initiative, hence in the event of loss or injury, we are unable to provide you any compensation.

18. Who is organising and funding the research?

The study is being conducted by investigators from the infectious diseases department and the infection prevention and control unit at Blacktown Hospital, and the microbiology laboratory at Westmead Hospital. The study has received research grant from the hospital to cover the expenses of swabs and the molecular testing. No member of the research team will receive a personal financial benefit from your involvement in this research project (other than their ordinary wages).

19. Who has reviewed the research project?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the HREC of Western Sydney Local Health District (WSLHD).

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

20. Further information and who to contact

The person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the principal study doctor on 02 9881 8000 or any of the following people.

Clinical contact person

Name	Dr. Ravindra Dotel
Position	Infectious Diseases Staff Specialist
Telephone	02 9881 8000
Email	ravindra.dotel@health.nsw.gov.au

For matters relating to research at the site at which you are participating, the details of the local site complaints person are:

Complaints contact person

Name	Office of the General Manager (GM), Blacktown-Mt Druitt Hospitals
Telephone	02 9881 8000 and ask to be connected to the GM's Office
Email	wsldh-bmdhexec@health.nsw.gov.au

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	Western Sydney Local Health District
Telephone	(02) 8890 9007
Email	Wslhd-researchoffice@health.nsw.gov.au

Consent Form - Adult providing own consent

Title *Molecular analysis guided infection control to determine the source of healthcare-associated Staphylococcus aureus bloodstream infections*

Short Title *MAGIC SABSI Study*

Principal Investigator *Dr. Ravindra Dotel*

Location *Blacktown Hospital, NSW*

Declaration by Participant

I have read the Participant Information Sheet or someone has read it to me in a language that I understand. I understand the purposes, procedures and risks of the research described in the project.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to **Blacktown Hospital** concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I acknowledge that the investigators and any regulatory authorities may have access to my medical records **specifically related** to this project to monitor the research in which I am agreeing to participate. However, I understand my identity will not be disclosed to anyone else or in publications or presentations.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I agree to have my data collected or the isolated "Staph" to be used in future studies aiming to improve treatments for "Staph" infections. YES ☐ NO ☐

I understand that I will be given a signed copy of this document to keep.

Name of Participant (please print) _____

Signature _____ Date _____

Name of Witness* to
Participant's Signature (please print) _____

Signature _____ Date _____

* Witness is not to be the investigator, a member of the study team or their delegate. In the event that an interpreter is used, the interpreter may not act as a witness to the consent process. Witness must be 18 years or older.

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print) _____

Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of, and information concerning, the research project.

Note: All parties signing the consent section must date their own signature.

Form for Withdrawal of Participation - *Adult providing own consent*

Title *Molecular analysis guided infection control to determine the source of healthcare-associated Staphylococcus aureus bloodstream infections*
Short Title *MAGIC SABSI Study*
Principal Investigator *Dr. Ravindra Dotel*
Location *Blacktown Hospital, NSW*

Declaration by Participant

I wish to withdraw from participation in the above research project and understand that such withdrawal will not affect my routine treatment, my relationship with those treating me or my relationship with Blacktown Hospital/WSLHD.

Name of Participant (please print) _____

Signature _____ Date _____

In the event that the participant's decision to withdraw is communicated verbally, the Study Doctor/Senior Researcher will need to provide a description of the circumstances below.

Declaration by Study Doctor/Senior Researcher[†]

I have given a verbal explanation of the implications of withdrawal from the research project and I believe that the participant has understood that explanation.

Name of Study Doctor/
Senior Researcher[†] (please print) _____

Signature _____ Date _____

[†] A senior member of the research team must provide the explanation of and information concerning withdrawal from the research project.

Note: All parties signing the consent section must date their own signature.

Study Ref Number:

HREC Number: **2020/ETH0155**

Surname

Given Name

Address

Date of Birth

Sex

Clinical Information:

Study Coordinators to fill:

Visit Day/week/time point: _____

RESEARCH

150/20 MAGIC SABSI Study - Blacktown

Patient Location/study details:

Research team to inform Collections Manager thru email or mobile prior to sample collections.

Email: Rajeshwari.Dotel@health.nsw.gov.au

Mobile: [REDACTION]

SRM Staff:

- Patient's with Hospital MRN provided, enter MRN in the Client Reference Number field when booking in
- For De-identified patients and no hospital MRN given, book in patient as new person under research and new research MRN will be generated

Financial Classification

Research

Phone

**Dr Ravindra Dotel
9881 8000**

SRM STAFF: - Any additional test requested which are not originally indicated on the request form and are hand written or not typed on the form, contact Zarah Timbol on 8890-5754 for clarification/confirmation.

Make a copy of Request Form and send it to Microbiology laboratory with the samples as per Trial Requirements

Test Requested:

Choose Specimen type:

Patient body site swabs:

- ☐ Nose
- ☐ Throat
- ☐ Hand
- ☐ Groin
- ☐ Wound

Environmental swabs:

- ☐ Bedside remote/ call button
- ☐ Bed side rails
- ☐ Ward computers on wheels desktops
- ☐ Keyboards
- ☐ Mouse

SRM staff to data enter each samples received for:

- ☐ Orderable test: **Trial MRSA**
- ☐ To data enter in the Comment section :
Microbiology staff to perform Primary culture, identification, confirmation of MRSA or MSSA by phoenix or PCR and storage of isolates

Study requirements:

Microbiology staff to perform Primary culture, identification, confirmation of MRSA or MSSA by phoenix or PCR and storage of isolate

Requesting Person: Dr Ravindra Dotel

Provider#: 283196EJ

Contact Number: 9881 8000

Signature: _____ Date: _____

Specimen Collected by:

Collection Date:

Collection Time:

Chapter 9:

1. Poster (ISSSI 2018)
2. CASSETTE RCT (the study based on the CASSETTE protocol presented in Chapter 9).

Protocol for Clindamycin Adjunctive Therapy for Severe Staphylococcus aureus Treatment Evaluation (CASSETTE STUDY)



Dotel R¹, Nelson J², Tong, S^{2,3}, Bowen A^{2,4}, O'Sullivan M¹, Davis J^{2,5}

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²Menzies School of Health Research, Charles Darwin University, Darwin, Australia

³Peter Doherty Institute for Infection and Immunity at the University of Melbourne, Melbourne, Australia

⁴The Telethon Kids' Institute, and University of Western Australia, Perth, Australia

⁵John Hunter Hospital, University of Newcastle, Newcastle, Australia



Australian New Zealand Clinical Trials Registry: ACTRN12617001416381

Introduction:

Exotoxins are an important virulence factor in *S. aureus*¹ (SA) (Box 1). They can lead to serious illness with a high mortality^{2,3}. Protein synthesis inhibitor (PSI) antibiotics⁴, of which clindamycin is the most frequently used, are thought to limit exotoxin production and improve outcomes in severe SA infections^{5,6}. Although in-vitro⁷, animal⁸ and observational human studies^{9,10} suggest a possible benefit of adjunctive clindamycin, a definite randomised controlled trial (RCT) to support this is lacking¹¹.

Aims of the study were to analyse the effects of adjunctive clindamycin in children and adults with severe methicillin-susceptible *S. aureus* (MSSA) infections. The pilot study will determine the feasibility and help refine the study design for a definitive RCT. Methicillin-resistant *S. aureus* (MRSA) infections will be excluded in the pilot study.

Study design*:

1. **Study settings:** 11 hospitals across Australia (both adult and paediatric centres)
2. **Participant eligibility criteria:** Box 3
3. **Interventions:** Box 4
4. **Criteria for discontinuing or modifying allocated interventions:** Dose adjustment in renal impairment and, if clinically necessary, change between flucloxacillin and cefazolin allowed. Use of clindamycin post Day 7 to Day 90 is not recommended.
5. **Strategies to improve protocol adherence:** Each site principal investigator (PI) will be trained in the study protocol, standard operating procedures, and their reporting requirements. Participants will be followed up to Day 90. The overall clinical management of patient is at the discretion of treating teams.

Outcomes assessment*:

1. **Primary outcomes:** Number of days alive and free of SIRS (Systemic Inflammatory Response Syndrome), i.e. meets <2 SIRS criteria simultaneously, within the first 14 days post-randomisation. SIRS will be defined as per the standard criteria in adults¹² and children¹³.
2. **Secondary outcomes:** All-cause mortality at 14, 42 and 90 days; microbiological relapse; microbiological treatment failure; number of surgical procedures needed to achieve source control; duration of intravenous (IV) treatment; development of *C. difficile* associated diarrhea or all-cause diarrhoea (>3 per day); and slope of CRP curve 1-14 days. Impacts of inducible clindamycin resistance, strains types, and presence of various exotoxins will also be analysed.
3. **Trial design and eligibility screening:** Box 5 (trial design). MSSA isolates collected within 48 hours of admission from blood culture or other invasive sites. Identification of SA as MSSA is acceptable if based on nucleic acid amplification, direct identification, or other phenotypic methods.
4. **Informed consent and randomisation:** Written informed consent will be obtained from participant or their next of kin. Randomisation stratified by age (child versus adult) in a 1:1 ratio, in permuted blocks of variable size.
5. **Endpoint assessment:** A panel blinded to treatment allocation will determine the primary endpoint.
6. **Discontinuation/Withdrawal:** Site PI can discontinue a participant. Reasons for discontinuation will be recorded. Participants can voluntarily withdraw.
7. **Sample size:** 60 patients, at least 20 of whom are aged <16 years (based on the number achievable in a time-frame of 12-18 months).
8. **Data management and statistical methods:** Data entry and storage in a purpose-built secure web-based database. Data reporting will follow CONSORT guidelines for reporting of RCTs. The primary analysis of both primary and secondary endpoints will be according to modified intention to treat principles.
9. **Safety aspects of the trial:** All adverse effects will be recorded. Serious adverse events will be notified appropriately.
10. **Ethics:** Site specific approval obtained for each participating hospital.

Summary:

The CASSETTE study is an investigator-initiated, open label, parallel group, superiority, multicentre, RCT. It will compare outcome differences in severe MSSA infection between a standard treatment (flucloxacillin or cefazolin) and a standard treatment plus an additional clindamycin given for 7 days. We will include a minimum of 60 participants (both adult and children) in the pilot study. We will assess the effect of adjunctive clindamycin on patient-centered outcomes in severe, toxin mediated, MSSA infections. The Pilot study will provide feasibility for a much larger RCT.

CASSETTE Study is currently recruiting (since July 2018)

*As per Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT 2013)

BOX 1: Known *S. aureus* exotoxins¹

A. Cytotoxins:

- Haemolysins (alpha, beta, gamma)
- Delta haemolysin and
- Leucocidins

B. Superantigens

- TSST-1 (Enterotoxin F)
- Enterotoxins (A-E, G)
- Enterotoxins-like (H, I, J-X)
- Exfoliative toxins (epidermolytic toxins A and B)

BOX 2: Protein Synthesis Inhibitor antibiotics⁴

A. 50s ribosomal subunit active:

Lincosamides (clindamycin, lincomycin), Linezolid, Macrolides, Streptogramins, Chloramphenicol, Fusidic Acid

B. 30s ribosomal subunit active:

Aminoglycosides, Tetracyclines, Tigecycline

BOX 3:

Inclusion criteria

1. Age – both children and adults will be eligible. Infants must have a corrected age of ≥28 days.
2. MSSA cultured from at least one clinically relevant specimen (including isolation in a polymicrobial culture when the other isolates are determined by the PI to be non-significant)
3. Able to be randomised within 72 hours of index culture
4. Likely to remain as inpatient for at least 7 days following randomisation (or is accessible for follow up by the site investigator, e.g. hospital in the home)
5. Index culture drawn no later than 48 hours after hospital admission
6. Severe disease – at least one of
 - 6.1 Septic shock (including Staphylococcal toxic shock syndrome)
 - 6.2 Necrotising lung/pleural space infection
 - 6.3 Complicated skin/soft tissue/osteoarticular infection which is multifocal and non-contiguous [e.g. pyomyositis, non-contiguous multifocal pyogenic skin infection (abscess/carbuncle)]

Exclusion criteria

1. Previous type 1 hypersensitivity reaction to β-lactams or lincosamides
2. Previous participation in the trial
3. Known pregnancy
4. Currently receiving a lincosamide or other potentially anti-toxin antibiotic which cannot be ceased or substituted (Box 2)
5. Participant's primary clinician unwilling to enrol patient
6. Moribund (expected to die in next 24 hours with or without treatment)
7. Treatment limitations which preclude the use of antibiotics
8. Significant immunosuppression (prednisolone >0.5mg/kg/day for ≥ 14 days in the last 30 days, other immunosuppressive medication, known HIV with CD4 count
9. Necrotising fasciitis
10. *C. difficile* associated diarrhoea or severe diarrhoea (>6 stools per day) of any cause

BOX 4: Interventions

Control group

IV flucloxacillin 50mg/kg/dose up to 2g q4-6 hourly OR IV cefazolin 50mg/kg/dose up to 2g q6-8 hourly (renal dose adjustment if necessary)⁶

Duration: 2-6 weeks IV in adults, 1-4 weeks IV in children; then oral if necessary

Combination therapy group

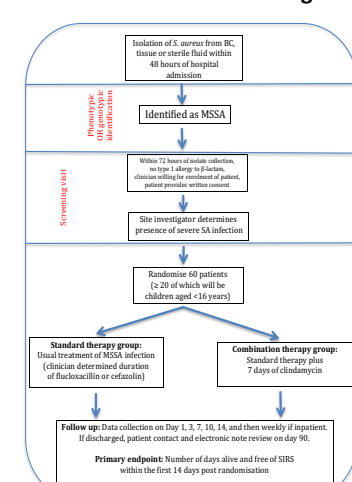
Standard therapy PLUS open-label IV clindamycin 10mg/kg/dose up to 600mg QID in both adults and children [PO clindamycin allowed – dose 450mg TDS for adults and 10mg/kg/dose TDS (max 450mg) for children]

Duration: 7 days of clindamycin; standard therapy as in Control group

References:

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12. Chest 1992; 101(6): 1644-55.
13. Pediatr Crit Care Med 2005; 6(1): 2-8.

BOX 5: Overview of trial design



Acknowledgements:

1. Other CASSETTE Study Investigators: Dr. Brendan McMullan, Dr. Owen Robinson, Dr. Laurens Manning, Dr. Damon Eisen, Dr. Joshua Francis, Dr. Sarah Lynar, Dr. Philip Britton
2. Funding Source: Improving Health Outcomes in the Tropical North: A Multidisciplinary Collaboration (HOT NORTH) Pilot Project: NHMRC ID GNT1131932
3. Study Sponsor: Menzies School of Health Research, Charles Darwin University, Darwin, Australia
4. The Australian Society for Infectious Diseases Clinical Research Network (ASID CRN)
5. Australian Postgraduate Awards (APA)/University of Sydney: Provided scholarships support to RD

Clindamycin adjunctive therapy for severe *Staphylococcus aureus* treatment evaluation (CASSETTE)—an open-labelled pilot randomized controlled trial

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Background: Combination antibiotic therapy with an antitoxin agent, such as clindamycin, is included in some guidelines for severe, toxin-mediated *Staphylococcus aureus* infections. The evidence to support this practice is currently limited to *in vitro*, animal and observational human case-series data, with no previous randomized controlled trials (RCTs).

Objectives: This pilot RCT aimed to determine the feasibility of conducting a clinical trial to examine if adjunctive clindamycin with standard therapy has greater efficacy than standard therapy alone for *S. aureus* infections.

Methods: We performed an investigator-initiated, open-label, multicentre, pilot RCT (ACTRN12617001416381p) in adults and children with severe *S. aureus* infections, randomized to standard antibiotic therapy with or without clindamycin for 7 days.

Results: Over 28 months, across nine sites, 127 individuals were screened and 34 randomized, including 11 children (32%). The primary outcome—number of days alive and free of systemic inflammatory response syndrome ≤ 14 days—was similar between groups: clindamycin (3 days [IQR 1–6]) versus standard therapy (4 days [IQR 0–8]). The 90 day mortality was 0% (0/17) in the clindamycin group versus 24% (4/17) in the standard therapy

group. Secondary outcomes—microbiological relapse, treatment failure or diarrhoea—were similar between groups.

Conclusions: As the first clinical trial assessing adjunctive clindamycin for *S. aureus* infections, this study indicates feasibility and that adults and children can be incorporated into one trial using harmonized endpoints, and there were no safety concerns. The CASSETTE trial will inform the definitive *S. aureus* Network Adaptive Platform (SNAP) trial, which includes an adjunctive clindamycin domain and participants with non-severe disease.

Introduction

Invasive *Staphylococcus aureus* infections are common and cause considerable mortality, particularly at the margins of the age continuum.¹ They are the most common infective reason for admission to an ICU in both adults² and children,³ with high mortality identified amongst these cohorts: 23%–33% in adults⁴ and 9% in children.⁵ Contributing to this severe phenotype is the ability of *S. aureus* to express multiple extracellular toxins that cause additional tissue damage.⁶

Despite this disease burden, fewer than 3000 adults⁷ and 300 children⁸ have been randomly assigned into clinical trials to assess the efficacy of treatments for *S. aureus* bacteraemia. Hence, opinions on best management vary widely,^{9,10} with an adjunctive protein synthesis inhibitor antibiotic suggested by some experts in severe, toxin-mediated *S. aureus* infections.^{11–13} The most frequently used protein synthesis inhibitor in this setting, clindamycin,¹⁰ decreases exotoxin production by binding to the 50S bacterial ribosomal subunit.⁶ The evidence to support adjunctive protein synthesis inhibitor use is currently limited to *in vitro*, animal and observational human case-series data, with no previous randomized controlled trials (RCTs).¹⁴

In this multicentre pilot RCT, we aimed to determine feasibility and to inform the design of a definitive RCT, the *S. aureus* network adaptive platform (SNAP) trial (<https://www.snaptrial.com.au/>). One of the aims of the SNAP trial will be to examine if the addition of clindamycin to standard antibiotic therapy (β -lactam, vancomycin or daptomycin) has greater clinical efficacy than standard therapy alone in adults and children with *S. aureus* infections. In addition, we aimed to provide proof of concept of including adults and children in the same RCT.

Methods

Study design and setting

We performed an investigator-initiated, open-label, multicentre, parallel-group, pilot RCT across four states in Australia, at six adult and three paediatric tertiary referral hospitals in adults and children with severe *S. aureus* infections from July 2018 to October 2020. This trial was endorsed by the Australasian Society of Infectious Diseases Clinical Research Network (ASID-CRN) and registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12617001416381p). The published protocol¹⁴ was prospectively approved at each participating site with ethical approval (HREC/17/HNE/411). Written legal guardian (<18 years) or participant/surrogate decision-maker (≥ 18 years) informed consent was required for all participants before enrolment by the site principal investigator (PI).

Participants

Eligibility criteria were: hospital inpatients ≥ 28 days old expected to remain an inpatient at the study site for ≥ 7 days post-randomization; *S. aureus* cultured from ≥ 1 clinically relevant site (defined as any

specimen where the site investigator determines the organism to be contributing to the patient's clinical syndrome); and index tissue (including blood) culture drawn within ≤ 48 h of hospital admission (to exclude nosocomial infections) and ≤ 72 h pre-randomization. Evidence of severe *S. aureus* disease was defined as at least one of the following: (i) septic shock (Appendix S1, available as Supplementary data at JAC-AMR Online);¹⁴ (ii) severe lung (necrotizing or multifocal pneumonia) or pleural space infection (Appendix S2); or (iii) multifocal disease (>1 non-contiguous site or >1 contiguous anatomical site involved).

To facilitate recruitment, after 12 months, the eligibility criteria for multifocal infection was broadened (initially it only included >1 non-contiguous site) to also include >1 contiguous anatomical site. The rationale for this change was supported in the literature, with an association of Panton–Valentine leucocidin (PVL)-positive *S. aureus* disease and complicated osteomyelitis (thrombosis and pyomyositis).^{15,16}

Exclusion criteria related to patients who: had significant immunosuppression (defined as prednisolone >0.5 mg/kg/day for ≥ 14 days in the last 30 days, other immunosuppressive medication, known HIV with CD4 cell count <200 cells/mm³ or congenital immunodeficiency); were expected to die within 24 h with or without treatment; had current severe diarrhoea (defined as >6 stools per day or clinician-determined severe diarrhoea in children) or onset of *Clostridioides difficile*-associated diarrhoea within 48 h prior to enrolment; had necrotizing fasciitis; were known to be pregnant; had a history of severe allergy to β -lactams, glycopeptides, lincosamides or daptomycin; had previously participated in the trial or were currently receiving a protein synthesis inhibitor antibiotic that could not be ceased or substituted. Other exclusions were the presence of polymicrobial culture containing other clinically significant isolates and where the primary clinician was unwilling to enrol the patient.

Randomization

Participants were randomized by a computerized central randomization schedule generated by a statistician in a 1:1 ratio to standard therapy (control group) or standard therapy plus clindamycin (adjunctive clindamycin group), stratified by age (<18 years versus ≥ 18 years), in permuted blocks of variable size. We pre-specified inclusion of one-third of children in our target sample.

Procedures

Standard therapy comprised IV flucloxacillin, 4–6 hourly or cefazolin, 6–8 hourly (both 50 mg/kg/dose for MSSA) or IV vancomycin [with dosing adjustments to maintain trough levels at 15–20 mg/L], daptomycin or ceftaroline (Appendix S3) for MRSA. Clindamycin was given IV 10 mg/kg/dose up to 600 mg four times daily or oral 450 mg three times daily in adults (10 mg/kg/dose in children) for 7 days. The choice and duration of standard antibiotic therapy and the decision to switch to oral clindamycin were clinician determined.

Outcomes and measurements

The primary outcome was number of days alive and free of systemic inflammatory response syndrome (SIRS) (defined as meeting <2 simultaneous SIRS criteria on a calendar day) ≤ 14 days post-randomization (Appendix S2). The secondary outcomes were: (i) all-cause mortality at

14, 42 and 90 days; (ii) time to first resolution of SIRS (number of days until the patient meets <2 simultaneous SIRS criteria); (iii) proportion with microbiological relapse by Day 90 (positive blood culture for *S. aureus* ≥ 72 h after a preceding negative culture); (iv) proportion with microbiological treatment failure by Day 90 (positive sterile site culture for *S. aureus* ≥ 14 days after randomization); (v) number of surgical procedures needed to achieve source control; (vi) duration of IV antibiotic treatment; (vii) *C. difficile*-associated diarrhoea (three or more loose stools per day with a positive laboratory test for *C. difficile* toxin); (viii) all-cause diarrhoea (three or more loose stools per day); and (ix) time to a $\geq 50\%$ decrease in C-reactive protein (CRP) in the first 14 days post-randomization (excluding participants missing a baseline CRP level).

Routine haematological parameters, repeat cultures, surgery for source control for *S. aureus*, patient progress and treatment were gathered from medical records, pathology results and treating teams onto an electronic case report form on Days 1, 3, 7, 10 and 14 and then weekly thereafter if an inpatient, and out to 90 days post-randomization (REDCap database).¹⁷ All-cause mortality data were collected through participant contact at 90 days or retrieved from medical records. Adverse events (AEs) were captured daily whilst an inpatient and up until 90 days post-randomization. All serious adverse events (SAEs) as defined in the protocol¹⁴ to any of the study drugs reported.

Laboratory methods

Identification of *S. aureus* was based on routine phenotypic and/or genotypic methods used in clinical laboratories of participating hospitals.

Statistical methods

The principal analysis of both primary and secondary endpoints was according to intention-to-treat principles. All participants with data available for the endpoint were analysed according to the treatment allocation, regardless of what treatment they received. Analyses of aggregated outcomes was initially performed by a statistician blinded to treatment groups, who was subsequently unblinded. Summary statistics were calculated for each outcome by trial arm and differences and 95% CIs were calculated using appropriate regression models. Given that this is a pilot study which is not powered for formal hypothesis testing, *P* values were not reported. All regression analyses were adjusted by the randomization covariate of age group (<18 years versus ≥ 18 years), and both unadjusted and adjusted estimates are reported.

For the primary outcome, days alive and free of SIRS, the difference between treatment arms was estimated using a zero-inflated negative binomial regression model, adjusted by days observed to account for participants with missing data. Binary secondary outcomes were assessed using logistic regression to estimate differences in proportions. All-cause mortality in both treatment arms was illustrated in a Kaplan-Meier survival curve. Competing risks regression was used to estimate HR for time-to-event outcomes. Difference in number of surgical procedures was estimated with negative binomial regression adjusted for days observed. All analyses were performed using Stata v16.0 (StataCorp LLC, College Station, TX, USA).

We planned to enrol 60 patients, at least 20 of whom were aged <18 years. As a pilot study, the sample size was based on the number achievable and the number needed to determine feasibility and to refine assumptions and study design. No assumptions were made about the

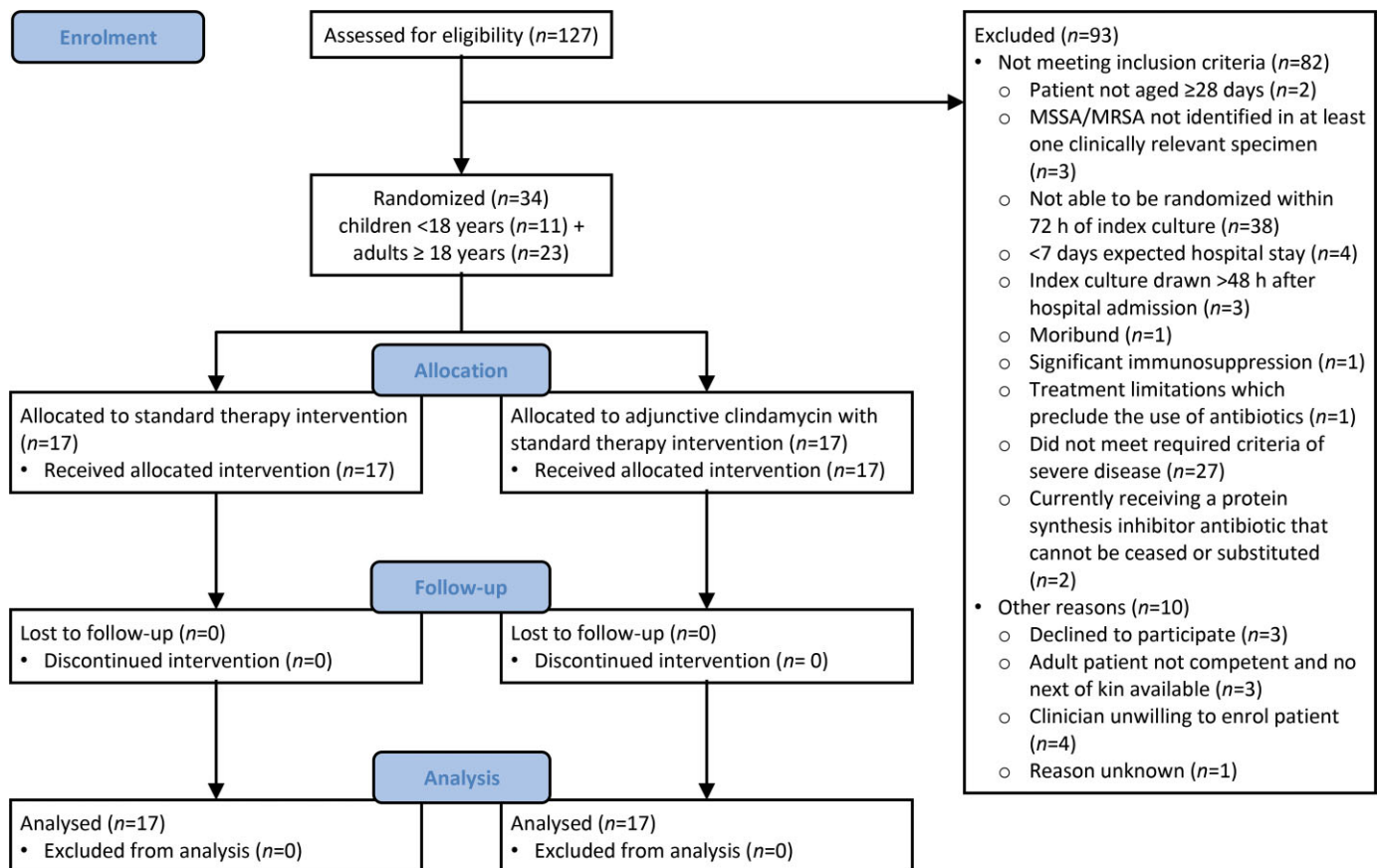


Figure 1. Study flow diagram.

Table 1. Baseline demographic, infection and treatment characteristics by study arm (overall and by age group [<18 years and ≥ 18 years])

Demographics	Overall $n = 34$				<18 years $n = 11$				≥ 18 years $n = 23$			
	standard therapy	adjunctive clindamycin therapy	total		standard therapy	adjunctive clindamycin therapy	total		standard therapy	adjunctive clindamycin therapy	total	
Age, years, median (IQR)	44 (11–63)	33 (12–60)	41 (11–63)		10 (10–11)	8 (0–12)	10 (0–11)		45 (41–68)	59 (33–75)	54 (37–70)	
Gender, female	4/17 (24)	7/17 (41)	11/34 (32)		3/5 (60)	2/6 (33)	5/11 (45)		1/12 (8)	5/11 (45)	6/23 (26)	
Weight, kg, median (IQR)	87 (48–90)	78 (44–87)	78 (44–90)		32 (28–36)	30 (7–44)	32 (7–44)		89 (84–98)	85 (77–102)	87 (78–102)	
Comorbid conditions												
Diabetes mellitus	3/17 (18)	2/17 (12)	5/34 (15)		0/5 (0)	0/6 (0)	0/11 (0)		3/12 (25)	2/11 (18)	5/23 (22)	
Chronic renal impairment (eGFR <50)	0/17 (0)	2/17 (12)	2/34 (6)		0/5 (0)	0/6 (0)	0/11 (0)		0/12 (0)	2/11 (18)	2/23 (9)	
Laboratory confirmed influenza ≤ 14 days prior to randomization	1/17 (6)	2/17 (12)	3/34 (9)		0/5 (0)	1/6 (17)	1/11 (9)		1/12 (8)	1/11 (9)	2/23 (9)	
Laboratory characteristics												
MRSA	5/17 (29)	3/17 (18)	8/34 (24)		2/5 (40)	2/6 (33)	4/11 (36)		3/12 (25)	1/11 (9)	4/23 (17)	
MSSA	12/17 (71)	14/17 (82)	26/34 (76)		3/5 (60)	4/6 (67)	7/11 (64)		9/12 (75)	10/11 (91)	19/23 (83)	
Infection site												
Multifocal	10/17 (59)	13/17 (76)	23/34 (68)		5/5 (100)	3/6 (50)	8/11 (73)		5/12 (42)	10/11 (91)	15/23 (65)	
Pleuropulmonary	7/17 (41)	6/17 (35)	13/34 (38)		3/5 (60)	3/6 (50)	6/11 (55)		4/12 (33)	3/11 (27)	7/23 (30)	
CNS	3/17 (18)	4/17 (24)	7/34 (21)		0/5 (0)	0/6 (0)	0/11 (0)		3/12 (25)	4/11 (36)	7/23 (30)	
Osteoarticular (native)	5/17 (29)	6/17 (35)	11/34 (32)		4/5 (80)	3/6 (50)	7/11 (64)		1/12 (8)	3/11 (27)	4/23 (17)	
Infective endocarditis	2/17 (12)	5/17 (29)	7/34 (21)		0/5 (0)	0/6 (0)	0/11 (0)		2/12 (17)	5/11 (45)	7/23 (30)	
Skin and soft tissue	6/17 (35)	3/17 (18)	9/34 (26)		1/5 (20)	1/6 (17)	2/11 (18)		5/12 (42)	2/11 (18)	7/23 (30)	
Bloodstream infection	8/17 (47)	12/17 (71)	20/34 (59)		3/5 (60)	3/6 (50)	6/11 (55)		5/12 (42)	9/11 (82)	14/23 (61)	
Infection severity												
ICU admission	12/17 (71)	10/17 (59)	22/34 (65)		2/5 (40)	2/6 (33)	4/11 (36)		10/12 (83)	8/11 (73)	18/23 (78)	
Septic shock	8/17 (47)	10/17 (59)	18/34 (53)		1/5 (20)	2/6 (33)	3/11 (27)		7/12 (58)	8/11 (73)	15/23 (65)	
SIRS on Day 1	16/17 (94)	16/17 (94)	32/34 (94)		4/5 (80)	5/6 (83)	9/11 (82)		12/12 (100)	11/11 (100)	23/23 (100)	
SOFA score ^a , median (IQR)	6 (3–12)	7 (5–11)	7 (5–11)		N/A	N/A	N/A		6 (3–12)	7 (5–11)	7 (5–11)	
Baseline CRP ^b mg/L, median (IQR)	245 (153–280)	206 (164–349)	235 (160–327)		146 (140–270)	249 (86–349)	170 (139–327)		261 (235–395)	206 (164–374)	243 (164–374)	
Surgical source control	6/17 (35)	9/17 (53)	15/34 (44)		3/5 (60)	2/6 (33)	5/11 (45)		3/12 (25)	7/11 (64)	10/23 (43)	
Antibiotic use ≤ 72 h prior to randomization												
Any antibiotics	16/17 (94)	16/17 (94)	32/34 (94)		4/5 (80)	5/6 (83)	9/11 (82)		12/12 (100)	11/11 (100)	23/23 (100)	
Any vancomycin	13/17 (76)	15/17 (88)	28/34 (82)		1/5 (20)	5/6 (83)	6/11 (55)		12/12 (100)	10/11 (91)	22/23 (96)	
Any beta lactam	16/17 (94)	16/17 (94)	32/34 (94)		4/5 (80)	5/6 (83)	9/11 (82)		12/12 (100)	11/11 (100)	23/23 (100)	
Any protein synthesis inhibitor antibiotic	8/17 (47)	8/17 (47)	16/34 (47)		1/5 (20)	1/6 (17)	2/11 (18)		7/12 (58)	7/11 (64)	14/23 (61)	

Predominant therapy Day 1–14	1/12 (8)	4/14 (29)	5/26 (19)	1/3 (33)	1/4 (25)	2/7 (29)	0/9 (0)	3/10 (30)	3/19 (16)
MSSA: cefazolin	11/12 (92)	10/14 (71)	21/26 (81)	2/3 (67)	3/4 (75)	5/7 (71)	9/9 (100)	7/10 (70)	16/19 (84)
MSSA: flucloxacillin	4/5 (80)	2/3 (67)	6/8 (75)	1/2 (50)	1/2 (50)	2/4 (50)	3/3 (100)	1/1 (100)	4/4 (100)
MRSA: vancomycin	1/5 (20)	1/3 (33)	2/8 (25)	1/2 (50)	1/2 (50)	2/4 (50)	0/3 (0)	0/1 (100)	0/4 (0)
MRSA: ceftaroline									
Non-study antibiotic use Day 1–90	8/17 (47)	N/A	N/A	2/5 (40)	N/A	N/A	6/12 (50)	N/A	N/A
Any non-study protein synthesis inhibitor in standard therapy arm									

Values are shown as n (%) unless otherwise specified.

ATSI; Aboriginal and Torres Strait Islander; eGFR; estimated glomerular filtration rate; LOS; length of stay; ID; infectious diseases; CRP; C-reactive protein.

^aSOFA scores are only calculated for adult participants. Two missing baseline platelet measurements, four missing baseline bilirubin measurements, one missing baseline creatinine and urinary output measurements, four missing baseline CRP measurements. Where values are missing, a score of zero was assumed.

^bFour participants missing baseline CRP measurements.

rate of occurrence of the primary outcome in the control group nor for anticipated treatment effects of the intervention.

Results

Over 28 months (July 2018–October 2020), across nine sites, 127 individuals were screened and 34 randomized from 40 eligible participants (85% consent rate). The recruitment rate was 1.2 participants/per month. Key reasons for exclusion included: inability to enrol participants within 72 h of the index culture (38/127, 30%) and not meeting the severe *S. aureus* disease definition (27/127, 21%). Consented participants were randomized to receive either standard therapy ($n=17$) or standard therapy plus adjunctive clindamycin ($n=17$) (Figure 1).

The trial was stopped prior to the anticipated sample size of 60 patients due to the slow recruitment rate and because the SNAP trial was planned to commence in 2021 and includes a domain for adjunctive treatment with clindamycin.

Baseline characteristics were similar between groups (Table 1). One third of the cohort were children <18 years (11/34, 32%) and female (11/34, 32%). All *S. aureus* infections (34/34, 100%) were community acquired and 24% (8/34) were caused by MRSA.

The majority of participants (32/34, 94%) met SIRS criteria on Day 1 of randomization (Table 1). The median SOFA score in adults was 7 (IQR 5–11) and underlying comorbidities were present in 35% (8/23) of adults and 9% (1/11) of children. Half (18/34, 53%) developed septic shock and 65% (22/34) required admission to the ICU, which was less frequent in children (4/11, 36%) compared with adults (18/23, 78%). Receipt of antibiotics prior to randomization was frequent overall (32/34, 94%), including with a β -lactam (32/34, 94%), vancomycin (28/34, 82%) or protein synthesis inhibitor antibiotic (16/34, 47%).

The primary endpoint, number of days alive and free of SIRS, was similar between the clindamycin group (median 3 days [IQR 1–6]) and standard therapy group (4 days [IQR 0–8]; adjusted difference [aD] -0.31 days [CI -3.20 , 2.57]) (Table 2). For the secondary outcome of all-cause mortality, there were no deaths (0/17, 0%) up to 90 days in the clindamycin group. In the standard therapy group, 14 day mortality was 18% (3/17) and 42 and 90 day mortality (all in adults) were 24% (4/17) (Figure 2). There were no differences in microbiological relapse (3/17, 18% versus 1/17, 6%; aD 12% [CI -10% , 33%]) or microbiological treatment failure (1/17, 6% in both; aD 0% [CI -16% , 16%]) in the clindamycin compared with standard therapy group.

All-cause diarrhoea occurred in 47% (8/17) of the clindamycin group compared with 35% (6/17) of the standard therapy group (aD 12% [CI -21% , 45%]), with one case (1/17, 6%) of *C. difficile*-associated diarrhoea occurring in the standard therapy group. The median days to first resolution of SIRS was equivalent between groups; 9 days (CI 2–10) in the clindamycin and 9 days (CI 5–12) in the standard therapy group; (adjusted hazard ratio [aHR] 1.57 [CI 0.75, 3.31]). The median days until CRP decreased by over 50% were similar between groups: 7 days (CI 3–14) in the clindamycin group and 5 days (CI 3–7) in the standard therapy group (aHR 0.59 [CI 0.30, 1.13]) (Table 2).

AEs were more common in the clindamycin group (10/17, 59%) compared with standard therapy (5/17, 29%) (Appendix S4). Study investigators determined most events to be unrelated to the study treatment and to be mild in severity.

Table 2. Primary, secondary and post-hoc outcomes in the standard therapy versus the adjunctive clindamycin therapy group, including summary statistics and estimates for the difference in outcome between groups

	Standard therapy, n = 17	Adjunctive clindamycin therapy, n = 17	Unadjusted		Adjusted	
			estimate	95% CI	estimate	95% CI
Estimated differences in count, including the primary outcome of days alive and free of SIRS						
Days alive and free of SIRS ^a , mean (SD)/ median (IQR)	4.59 (4.11)/4 (0–8)	4.76 (4.87)/3 (1–6)	0.05	–2.99, 3.09	–0.31	–3.20, 2.57
Number of surgical procedures, mean (SD)/ median (IQR)	0.65 (1.06)/0 (0–1)	1.18 (1.63)/1 (0–2)	0.35	–0.53, 1.23	0.35	–0.53, 1.23
Length of stay, days ^b , median (IQR)	15 (10–31)	29 (15–46)	—	—	—	—
Days of bacteraemia ^b , median (IQR)	2 (1–5)	3 (1–8)	—	—	—	—
Days febrile ^b , median (IQR)	2 (0–7)	3 (1–6)	—	—	—	—
Estimated differences in probability						
All-cause mortality ^c , n (%)						
Day 14	3/17 (18)	0/17 (0)	—	—	—	—
Day 42	4/17 (24)	0/17 (0)	—	—	—	—
Day 90	4/17 (24)	0/17 (0)	—	—	—	—
Microbiological relapse ^d , n (%)	1/17 (6)	3/17 (18)	12%	–10%, 33%	12%	–10%, 33%
Microbiological treatment failure ^e , n (%)	1/17 (6)	1/17 (6)	0%	–16%, 16%	0%	–16%, 16%
<i>C. difficile</i> -associated diarrhoea ^{c,f} , n (%)	1/17 (6)	0/17 (0)	—	—	—	—
All cause diarrhoea ^g , n (%)	6/17 (35)	8/17 (47)	12%	–21%, 45%	12%	–21%, 45%
Estimated hazard ratios, where standard therapy is considered the reference						
Days to first resolution of SIRS ^h , median survival (95% CI)	9 (5, 12)	9 (2, 10)	1.53	0.72, 3.27	1.57	0.75, 3.31
Days till CRP >50% decrease ⁱ , median survival (95% CI)	5 (3, 7)	7 (3, 14)	0.55	0.30, 0.99	0.59	0.30, 1.13

^aNumber of days patient is alive and meets <2 simultaneous SIRS criteria on a calendar day within ≤14 days post-randomization.

^bPost-hoc outcome: summary statistics presented only.

^cDifference in probability unable to be estimated due to zero events in one group.

^dPositive blood culture for MSSA or MRSA at least 72 h after a preceding negative culture within ≤14 days post-randomization.

^ePositive sterile site culture for MSSA or MRSA >14 days after randomization.

^fThree or more loose stools per day along with a positive laboratory test for *C. difficile* toxin.

^gThree or more loose stools per day.

^hNumber of days until the patient first meets <2 simultaneous SIRS criteria on a calendar day.

ⁱNumber of days till CRP decreases by >50% ≤14 days post randomization.

Discussion

This is the first clinical trial to assess adjunctive clindamycin for severe *S. aureus* infections. Key findings from the CASSETTE trial include that it is feasible to conduct an RCT examining adjunctive clindamycin therapy, adults and children can be incorporated into one trial using harmonized endpoints, and there were no serious safety concerns evident from this preliminary cohort.

This study took almost 2.5 years to recruit a relatively small number of participants despite a large cohort being screened (34/127, 27%). This was due, in part, to difficulty identifying patients that met the severe *S. aureus* infection definition within 72 h of the index culture (Figure 1), with trial recruitment performed during the working week by clinician study investigators. This information has provided future direction for the planned SNAP trial, which will expand inclusion criteria to all individuals with *S. aureus* bacteraemia, regardless of illness severity and

contain an adjunctive clindamycin domain. Importantly, clinician equipoise and patient acceptability were high, with 85% of eligible participants providing consent and no participant withdrawals or discontinued interventions noted (Figure 1). This trial experience confirms feasibility and has progressed the partnerships needed for a robust adult and paediatric infectious diseases trial network.

This pilot RCT has also provided insights into the utility of trial endpoints. The primary endpoint (number of days alive and free of SIRS), was pragmatically selected, given the availability of a validated scoring system that aligns in both adults¹⁸ and children.¹⁹ It was reasoned it would reflect decreased systemic inflammation due to decreased exotoxin production. Despite the rationale, the primary outcome, days alive and free of SIRS, were comparable, yet a difference in mortality was demonstrated (0% [0/17] 90 day mortality: clindamycin group versus 24% [4/17]: standard therapy group), although small numbers limit any definitive conclusions. Given these hypothesis-generating findings, and the much larger planned sample size for the SNAP trial, the more

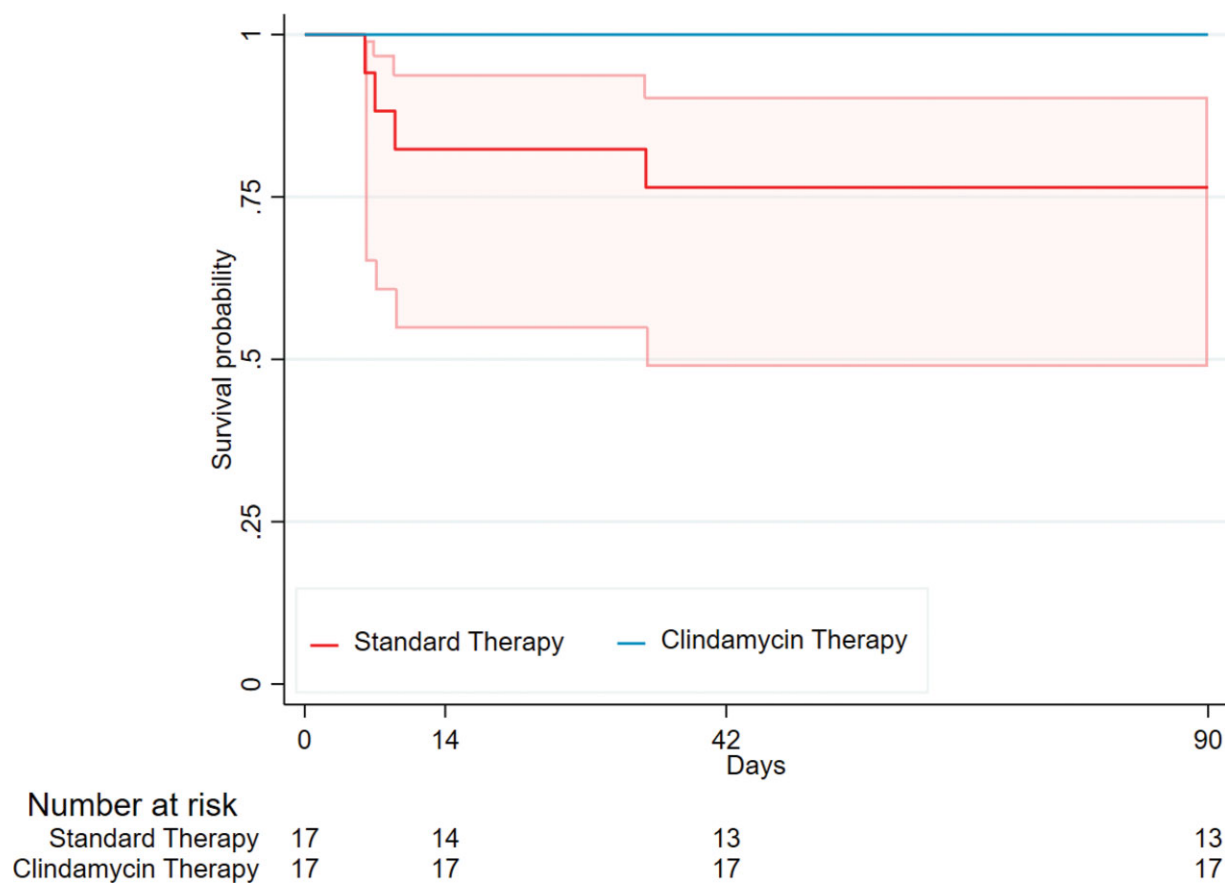


Figure 2. Kaplan–Meier survival curves and 95% CI for all-cause mortality in the standard therapy (n = 17) and clindamycin therapy (n = 17) treatment groups. We were unable to estimate 95% CIs for clindamycin therapy due to zero events in that group.

clinically relevant endpoint of all-cause mortality²⁰ has been selected as the primary outcome for this planned RCT.

In the CASSETTE trial, mortality in the standard treatment arm occurred only in adults and was consistent with mortality in other adult *S. aureus* bacteraemia trials.^{1,2} To have no deaths in the clindamycin arm despite balanced groups with severe disease suggests a possible benefit of clindamycin, but this could have occurred by chance given the small numbers. In the literature the human clinical studies previously examining adjunctive clindamycin include only four case series in the setting of necrotizing pneumonia (n = 92 adults/adolescents),²¹ skin and soft tissue infections (SSTIs) (n = 269 adults),²² severe influenza-MRSA pneumonia (n = 29 children)²³ and PVL-positive invasive *S. aureus* infections (n = 141 adults).²⁴ Two case series in the severe pneumonia setting demonstrated lower mortality with adjunctive protein synthesis inhibitor antibiotics compared with standard therapy alone,^{21,23} but all have major study design limitations. A definitive trial is needed to objectively determine if this observed mortality difference reflects a true benefit or a chance finding.

The burden of daily data collection for the primary endpoint was evident. This has since been refined to a single study time-point (Day 5) for the equivalent secondary outcome in SNAP.

In addition, the statistical model for SNAP acknowledges the infrequent occurrence of trial endpoints in children (particularly death, 0/11, 0%) and will incorporate Bayesian hierarchical models that specify borrowing of data from adult patients to children.^{25,26} This pilot RCT has importantly enabled significant refinement of trial design in preparation for the definitive SNAP trial.

Limitations of this study include a small sample size, limiting conclusions regarding efficacy and mortality between treatment groups. However, this was a pilot RCT designed primarily to assess feasibility. There were some missing data points, however primary and key secondary outcomes including death and relapse were complete. We were unable to assess the total duration of IV antibiotic therapy or timing of IV to oral switch due to incomplete data. Some of the standard therapy group received adjunctive clindamycin prior to enrolment, which may have impacted results. There was no placebo available in the standard therapy group.

Lessons from the CASSETTE trial will inform the SNAP trial, which includes an adjunctive clindamycin domain and will be powered to determine whether clindamycin reduces the likelihood of death. Incorporating both adults and children with *S. aureus* infections in an RCT is feasible, despite challenges in analysis and data interpretation.

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Transparency declarations

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Author contributions

J. S. Davis, S. Y. C. Tong and A. C. Bowen were involved in the conceptualization, methodology and supervision of this clinical trial. J. S. Davis, S. Y. C. Tong, A. C. Bowen, R. Dotel and A. J. Campbell were involved in the trial methodology. Statisticians N. Meagher and D. J. Price were involved in trial methodology, formal analysis and data visualization. Study coordinators J. Nelson and A. Whelan contributed to trial resources, data curation and project administration. A. J. Campbell, J. S. Davis, S. Y. C. Tong and A. C. Bowen wrote the original draft manuscript. All authors contributed to methodology, trial investigation and reviewing and editing of the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. A. C. Bowen was involved in funding acquisition for the project.

Supplementary data

Appendices S1 to S4 are available as [Supplementary data](#) at JAC-AMR Online.

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