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University of Southampton

Faculty of Medicine

School of Clinical and Experimental Sciences

**Investigating the efficacy of Mupirocin as a decolonisation agent in
preventing methicillin-resistant *Staphylococcus aureus* (MRSA)
healthcare related infections**

by

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Thesis for the degree of Doctorate of Medicine (DM)

April 2020

University of Southampton

Abstract

Faculty of Medicine

Clinical and Experimental Sciences

Thesis for the degree of Doctorate of Medicine

Investigating the efficacy of Mupirocin as a decolonisation agent in preventing methicillin-resistant *Staphylococcus aureus* (MRSA) healthcare related infections

by

Ali A. Salamat

Introduction

The burden of methicillin-resistant *Staphylococcus aureus* (MRSA) on health resources continues to be problematic. Health related infections (HAIs) attributed to MRSA are becoming increasingly difficult to treat, and at the heart of the problem is the sparsity of antimicrobial agents available due to antimicrobial/antibiotic resistance (AMR). Mupirocin is a key agent, and one of the very few anti-staphylococcal agents, currently utilised in the medical management of MRSA carriers. However, its use has been limited by the increasing degree of AMR exhibited by MRSA strains, and as a result its use has become increasingly restricted. The success of these agents is seen as crucial in the clearance of MRSA carriers prior to a surgical procedure for example or at the time of admission to hospital. Failure to successfully decolonise MRSA patients exposes them to HAIs, including surgical site infections (SSIs).

The aim of this project is three-fold. Firstly, I evaluate the efficacy of Mupirocin in preventing MRSA related surgical site infections using a systematic analysis. Secondly, I analyse 5-year data on MRSA prevalence in one English and one Welsh hospital sites retrospectively. Lastly, I investigate the antimicrobial profile of a novel engineered gel as a potential substitute to Mupirocin *in vitro*.

Hypothesis

Mupirocin is not effective in the decolonisation of MRSA carriers.

Methodology

A systematic review of the literature was undertaken to identify studies reporting the use of Mupirocin in decolonising surgical patients prior to their respective procedure. Utilising a clinical librarian service, a systematic search of the literature was conducted to include global trade names of Mupirocin. I analysed the quality and adherence of the studies to the review's inclusion criteria. In order to understand the prevalence of MRSA in the UK, I requested data over a five year period from Public Health Wales (PHW) and Public Health England (PHE), for the number of colonised MRSA patients in Swansea and Southampton, respectively. Almost 15,000 patients were eligible for this retrospective analysis. Clinical information such as demographics, antibiotic resistance patterns including Mupirocin were collected and analysed. Lastly, a laboratory-based *in vitro* study was undertaken to investigate the efficacy of Mupirocin and a novel engineered gel (Reactive Oxygen®) against established MRSA biofilms. Using a validated static biofilm and planktonic culture models, the efficacy of Mupirocin was compared against currently recommended nasal antibiotics, such as, Doxycycline and Clarithromycin.

Results

Due to the lack of the of a standardised framework for reporting MRSA-related HAIs, including SSIs, as well as the heterogeneity of the data, this project's hypothesis could only be partially confirmed. Using an *in vitro* model, Mupirocin was found to be ineffective against static nasal *Staphylococcus aureus* biofilms. The efficacy of Reactive Oxygen® against nasal *Staphylococcus aureus* biofilms was significant in comparison to Mupirocin and Clarithromycin, but not Doxycycline. The failure of Doxycycline and Clarithromycin as an adjuvant to Reactive Oxygen®, coupled with Mupirocin's limited *in vitro* efficacy, suggests a potential role for Reactive Oxygen® in becoming firstly a MRSA decolonisation agent and second, a targeted antibiotic-sparing therapy against nasal *Staphylococcus aureus* biofilms.

Conclusions

Current policy in reporting MRSA-related HAIs, including SSIs, is insufficient based on a systematic review and large population study of MRSA prevalence. A number of key reportable outcomes that would inform clinicians and policymakers are not currently recommended. However, in the race against AMR, a comprehensive MRSA reporting framework, in conjunction with digital technology,

is needed to inform future policy and preventative strategies, including the identification of potential novel therapies, such as Reactive Oxygen®.

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DECLARATION OF AUTHORSHIP

I, Ali A. Salamat declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

“Investigating the efficacy of Mupirocin as a decolonisation agent in preventing methicillin-resistant *Staphylococcus aureus* (MRSA) healthcare related infections”

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
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3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission

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PREFACE

Publications

Salamat AA, Radwan R, Harris D, Pender SLF. The efficacy of Mupirocin and Chlorhexidine Gluconate in preventing methicillin-resistant *Staphylococcus aureus* (MRSA) related surgical site infections; a systematic review. NHS National Institute of Health Research (NIHR) International prospective register of systematic reviews ([PROSPERO CRD 42020168082](https://doi.org/10.1016/j.jgar.2016.12.006)).

Dryden MS, Cooke J, Salib RJ, Holding RE, Biggs T, **Salamat AA**, Allan RN, Newby RS, Halstead F, Oppenheim B, Hall T. Reactive oxygen: a novel antimicrobial mechanism for targeting biofilm-associated infection. *Journal of Global Antimicrobial Resistance*. 2017 Feb 14. <http://dx.doi.org/10.1016/j.jgar.2016.12.006>

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Salamat AA, Ben-Ismaeil B, Pender SLF. A 5-year retrospective analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage and antimicrobial resistance patterns in two UK regional centres.

Salamat AA, Read RC, Salib RJ, Allan RN, Pender SLF. The efficacy of an engineered gel (Reactive Oxygen®) as a Mupirocin sparing therapy in treating methicillin-resistant and sensitive *Staphylococcus aureus* biofilms; an *in vitro* study.

AWARDS AND PRESENTATIONS

Grants and Fellowships

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Higher Education Innovation Fund (HEIF) Faculty of Medicine Enterprise Pump-Priming, 2015 [Co-applicant]

Awards & prizes

British Association of Paediatric Otorhinolaryngology (BAPO) Susannah Leighton Travelling Fellowship (€1000)

European Society for Paediatric Otolaryngology (ESPO), Lisbon, Portugal. June 2016

Junior ERS member Travelling Fellowship (€700)

European Rhinologic Society (ERS), Stockholm, Sweden. July 2016

1st prize for best oral presentation (£250)

British Rhinological Society (BRS), Leeds, UK. April 2016

1st prize for best oral presentation

British Association for Paediatric Otolaryngology (BAPO), Belfast, UK. September 2015

Oral Presentations

Surgihoney RO™ – A novel antibiotic sparing therapy for *Staphylococcus aureus* biofilm-mediated associated infections.

European Rhinologic Society (ERS), London, UK. April 2018

Development of an engineered honey (Surgihoney™) as a novel adjunctive biofilm-targeted therapy in *Staphylococcus aureus* related chronic rhinosinusitis.

European Rhinologic Society (ERS), Stockholm, Sweden. July 2016

The Science behind Surgihoney RO (Reactive Oxygen®): A new front in the fight against antimicrobial resistance.

Department of Health, Skipton House, London, UK. July 2016.

Investigating the role of DEA-CP as a nitric oxide adjuvant in treating *Streptococcus pneumoniae* biofilm mediated ENT infections.

European Society for Paediatric Otolaryngology (ESPO), Lisbon, Portugal. June 2016

Development of an engineered honey (Surgihoney™) as a novel adjunctive biofilm-targeted therapy in *Staphylococcus aureus* related chronic rhinosinusitis.

British Rhinological Society (BRS), Leeds, UK. April 2016

Investigating the role of Diazeniumdiolate nitric oxide-donor attached to cephaloram (DEA-CP) in treating *Streptococcus pneumoniae* biofilm mediated ENT infections.

British Association for Paediatric Otolaryngology (BAPO), Belfast, UK. September 2015

Eradication of biofilms in chronic rhinosinusitis.

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Poster presentations

Developing an engineered honey (SurgihoneyRO®) as a novel anti-MRSA treatment.

Royal College of Surgeons' of England, London, UK. July 2015

DEFINITIONS AND ABBREVIATIONS

| ABBREVIATION | DEFINITION |
|--------------|--|
| MRSA | Methicillin-resistant <i>Staphylococcus aureus</i> |
| MSSA | Methicillin-sensitive <i>Staphylococcus aureus</i> |
| HAI | Healthcare related infection |
| SSI | Surgical site infection |
| HA-MRSA | Healthcare/hospital-acquired MRSA |
| CA-MRSA | Community-acquired MRSA |
| PVL | Panton-Valentine Leukocidin |
| CRS | Chronic rhinosinusitis |
| EPOS | European Positional Paper on rhinosinusitis and nasal polyps |
| AMR | Antimicrobial and antibiotic resistance |
| WHO | World Health Organization |
| PHES | Public Health England Southampton |
| PHW | Public Health Wales |
| DoH | Department of Health (UK) |
| CDC | The Centers for Disease Control and Prevention |
| EUCAST | European Union Committee on Antimicrobial Susceptibility Testing |
| UK | United Kingdom |

CHAPTER 1: Introduction

The World Health Organization (WHO) has long warned about a post-antibiotic era and the urgent need for novel therapies, preventative strategies and diagnostic tests in combating antibiotic tolerance and subsequent resistance. (WHO 2016) Sexually transmitted diseases (*Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Treponema pallidum* (syphilis)), multi-drug resistant tuberculosis (MDR-TB) and *Staphylococcus aureus* are rapidly becoming resistant to our antibiotic armamentarium and pose a worldwide burden on resources. (WHO 2016) The over consumption, misuse and reliance on antibiotics has led us to uncharted territory about how we as scientists and clinicians will tackle common bacterial infections in generations to come.

1.1 Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) remains a problematic multi-drug resistant pathogen continuing to burden health care systems across the world. Shortly after beta lactam antibiotics were introduced into clinical practice in 1961, resistance of *Staphylococcus aureus* to methicillin was reported. (Jevons *et al.* 1961) Ever since, clinicians and scientists alike have endeavoured to develop novel therapies and strategies to contain and reduce MRSA related healthcare related infections (HAIs). The use of screening protocols, patient isolation and antimicrobial agents such as Mupirocin and Chlorhexidine Gluconate are all considered to be key in reducing the number of MRSA HAIs. (Schelenz *et al.*, 2005; Baratz *et al.*, 2015; Bode *et al.*, 2016) However, despite these interventions, MRSA strains are continuing to exhibit resistance to Mupirocin, not only low but at a high-level (McNeil *et al.*, 2011; Bathoorn *et al.*, 2012; McDanel *et al.*, 2013). As a result, the efficacy of Mupirocin in preventing MRSA HAIs has become compromised. Therefore, great emphasis has been placed by the WHO to develop novel agents and strategies to ease the burden of antimicrobial/antibiotic resistance (AMR) attributed to MRSA and the associated HAIs.

The infectious potential of MRSA is far reaching affecting not only hospitals, but in the community with an increasing trend of serious infections in patients with no traditional risk factors to explain colonisation. Key social focal points such as residential and nursing homes are becoming a large

conduit for MRSA re-cycling amongst residents, traditionally recognised as being high-risk for MRSA colonisation based on their health status and likely frequent visits and admissions to hospital. (Fraise *et al.*, 1997; Cox and Bowie, 1999; Barr *et al.*, 2007) Within the hospital, the burden of managing MRSA in all forms such as adopting a screening programme, promptly reporting positive MRSA swabs, commencing treatment and recording this data onto a national database is immensely time-consuming and expensive. It is estimated the cost of MRSA to the National Health Service (NHS) is in the range of £1 billion per annum. (Senior *et al.*, 2007) These costs are likely to have risen since 2007 considering the continued strain that MRSA HAIs are far reaching in cases clinical areas such as intensive care (Huang *et al.*, 2016) and haemodialysis (Gebreselassie, Lo Priore and Marschall, 2015). These facilities are reporting an increasing concern that the efficacy of current available decolonisation agents and strategies are becoming more regularly ineffective. (McNeil *et al.*, 2011; Bathoorn *et al.*, 2012; McDanel *et al.*, 2013)

1.1.1 MRSA related healthcare infections

MRSA is considered as one of the most important pathogens in developing HAIs. (Sader *et al.*, 2006; Anderson *et al.*, 2015) Surgical site infections (SSIs) are a class of HAIs and are defined by the National Healthcare Safety Network (NHSN) as those infections that occur up to 30 days post-surgery or up to 90 days after surgical implantation of a medical device (Mangram *et al.*, 1999) and these remain a significant clinical problem despite advances made in reducing the risk of SSIs. The cost of HAIs are immense. In a large meta-analysis analysing the number of HAIs in the United States of America, annual total costs were reported to be as high as \$9.8 billion (Zimlichman *et al.*, 2013), identifying central line-associated bloodstream infections to be the costliest at \$45 814 (95% CI, \$30 919-\$65 245) per patient. (Zimlichman *et al.*, 2013)

It is estimated that 40 million of the US population undergo surgery each year, 20% of whom acquire a nosocomial infection. (Anderson *et al.*, 2015) Infections commonly complicate the post-operative period and the frequency of SSIs can be up to 20%. (Anderson *et al.*, 2015) It is estimated that that these infections, by virtue of additional healthcare expenditure, cost the US economy an extra \$5-10 billion per annum. (Perl, Pfaller and Herwaldt, 2002)

Up to 30% of SSIs are attributed to *Staphylococcus aureus*, with almost half of these infections MRSA related. (Hidron *et al.*, 2008; Anderson *et al.*, 2015; Bode *et al.*, 2016) The implication of

MRSA colonisation is greater in patients receiving prosthetic implants in surgical specialities such as orthopaedic, vascular and spinal surgery. (Samad *et al.*, 2002)

Considering that central line-associated bloodstream infections were found to be the costliest, SSIs contribute the most to overall HAI costs. (Zimlichman *et al.*, 2013) Each SSI is estimated to cost \$20,785 (95% CI, \$18 902-\$22 667). (Zimlichman *et al.*, 2013) Studies considering the economics of MRSA SSIs postulate that the cost savings from preventing just a single case is in the region of \$25,000 to \$60,000 (Anderson *et al.*, 2007) and a reduction in the length of hospital stay by up to 50%. (Anderson *et al.*, 2007, 2015)

1.1.2 Healthcare/hospital and community acquired MRSA infections

The Centers for Disease Control and Prevention (CDC) helped distinguish and define CA-MRSA (community-acquired MRSA) from its HA-MRSA (healthcare/hospital-acquired MRSA) counterpart in 2000:- as any outpatient or within 48 hours of hospitalisation in the absence of HA-MRSA risk factors such as haemodialysis, surgery, percutaneous device or indwelling catheter, hospitalisation within the last year, or previous MRSA carriage. (Berríos-Torres *et al.*, 2017; David *et al.*, 2010) Whilst, HA-MRSA infections including bacteraemia, are disproportionately seen in patients with a medical device such as long-term catheter, stoma and Hickmann line (UK Department of Health, 2014), the degree of invasive infections attributed to CA-MRSA infections is troublesome. It is recognised that CA-MRSA infections have the tendency not only to cause cutaneous and soft tissue infections but significant and more invasive ones such necrotising pneumonia, osteomyelitis and infective endocarditis. (Tristan *et al.*, 2007)

The burden of MRSA in the UK is recognised to be high (Jumaa *et al.*, 2007; Ellington *et al.*, 2009). There is increasing prevalence of MRSA in the UK, such as children including those attending nurseries (Adedeji, Weller and Gray, 2007; Ellington *et al.*, 2009) and in nursing home residents. (Fraise *et al.*, 1997; Cox and Bowie, 1999; Barr *et al.*, 2007) However, there is sparse data informing the prevalence of CA-MRSA in the general United Kingdom population. (Abudu *et al.*, 2001) According to the Health Protection Agency (HPA), Colindale, London, UK, 100 cases of true/*de novo* CA-MRSA were recorded over a three year period. (Adedeji, Weller and Gray, 2007) Common risk factors for acquiring CA-MRSA include close contact between individuals such as households,

sharing personal items, social groups, athletes and gym users, military personnel, history of foreign travel and nursery attendance. (David et al., 2010; Ellington et al., 2009)

A study conducted in Birmingham, UK, in 2006, found 30/55 (62%) of children were colonised with MRSA from community sources, of which 10/55 (28%) attributed to HA-MRSA. (Adedeji, Weller and Gray, 2007) The prevalence of MRSA in Europe as a whole has seen a great deal of change following the influx of displaced people fleeing war in the Middle East and Africa from countries such as Syria, Afghanistan, Iran, Nigeria and Eritrea. (UNHCR 2016; Nellums et al., 2018) Whilst the risk of onward transmission to the host population is thought to be low, the migrant community are considered as vulnerable to acquiring antibiotic resistant organisms in host countries (Nellums *et al.*, 2018), including MRSA. The global movement of individuals is part of everyday life in the world today as we know it. This becomes applicable when considering the role of healthcare in the recycling of MRSA amongst patients, in two major forms. Firstly, by means of close contact needed in providing patient care exposes healthcare workers to becoming a carrier. Alternatively, healthcare workers may unknowingly act as a conduit for the transmission of MRSA to patients. Despite these hazards, current UK guidelines don't recommend screening of healthcare professionals. One exemplary case was reported in Kent, UK, where a healthcare professional who had returned to the UK following a vacation to visit his family back home in the Philippines. (Ali *et al.*, 2012) Subsequently, upon returning back to work in a neonatal intensive care unit, an outbreak of a South West Pacific clone of a PVL positive MRSA strain (ST30) which is uncommon in the UK (Ali *et al.*, 2012) was identified.

This wave of novel PVL positive MRSA strains being introduced into an already highly burdened country such as the UK, is concerning and appears to suggest a shift away from the traditional dogma that MRSA is as a result of HA-MRSA alone. Considering the spectrum of invasive infections attributable to CA-MRSA, there is one important hallmark and that is its carriage of the Panton-Valentine Leukocidin (PVL) gene.

1.1.3 Panton-Valentine Leukocidin

True CA-MRSA is characteristically susceptible to antimicrobials and resistant to beta-lactam antibiotics (Adedeji, Weller and Gray, 2007), distinguishing it from its HA-MRSA counterpart. At a molecular level, HA-MRSA strains carry a large staphylococcal chromosomal cassette *mec*

(SCC*mec*) belonging to types I, II and III and exhibits a high level of resistance to non- β -lactam antibiotics. (David *et al.*, 2010) HA-MRSA is thought to be the common form of transmission in the UK. (Jumaa *et al.*, 2007; Rollason *et al.*, 2008; Ellington *et al.*, 2009) Meanwhile, CA-MRSA carries a shorter chromosomal cassette, carrying type IV or V. (David and Daum, 2010) The clinical severity of CA-MRSA related infections are attributed to their carriage of the cytotoxin PVL gene. The virulence of PVL is explained by the synergism of two separate exoproteins, LukS-PV and LukF-PV. (Holmes *et al.*, 2005; Ellington *et al.*, 2009) Infections as a subsequence of a PVL positive CA-MRSA are known to be severe and invasive in the skin and soft tissues, and have been reported in cases of necrotising fasciitis, pneumonia and invasive osteomyelitis. (Holmes *et al.*, 2005; Tristan *et al.*, 2007)

Two epidemic MRSA (EMRSA) clones EMRSA-15 (ST22- MRSA-SCC*mec*IV) and EMRSA-16 (ST36-MRSA- SCC*mec*II) are known to be the predominant strains in the UK (Johnson, Pearson and Duckworth, 2005a; Ellington *et al.*, 2009). These clones differ from those from other high-prevalence countries with dominant MRSA clones such as the New York-Japan (USA), ST247 or ST239 (Portugal), USA300 (ST8) (USA) (Ali *et al.*, 2012; Ellington *et al.*, 2009; Kourbatova *et al.*, 2005; Patel *et al.*, 2009; Seybold *et al.*, 2006.; Tenover *et al.*, 2012) to name a few. In fact, USA300 clone (ST8), in particular, has become endemic amongst multiple North American healthcare facilities (Ali *et al.*, 2012; Kourbatova *et al.*, 2005; Patel *et al.*, 2009; Seybold *et al.*, 2006) and again is a PVL positive MRSA clone.

1.2 MRSA pathogenesis and decolonisation

1.2.1 Role of Mupirocin in MRSA decolonisation

Treatment protocols for MRSA colonised patients have largely remained unchanged with a status quo of the treatments currently available that shape the MRSA decolonisation regime. At present, MRSA carriers are prescribed a combination of a Chlorhexidine body wash and 2% Mupirocin calcium cream/ointment (Bactroban, GlaxoSmithKline) for nasal application for up to a combined total of 7 days. There are number of studies that report a benefit of intranasal Mupirocin in reducing the bioburden of *Staphylococcus aureus* including MRSA in preparation for major surgery, haemodialysis and critical care. (Watanakunakorn *et al.*, 1992; Samad *et al.*, 2002; van Rijen *et al.*, 2008; Baratz *et al.*, 2015; Gebreselassie, Lo Priore and Marschall, 2015; Huang *et al.*, 2016) van Rijen *et al.* conducted a systematic review and meta-analysis analysing the outcome of intranasal application of Mupirocin in preventing *Staphylococcus aureus* infections in nosocomial carriers. (van Rijen *et al.*, 2008) Pooled analysis of 3396 patients from nine randomised control trials, identified a statistically significant reduction in the rates of *Staphylococcus aureus* infection (RR 0.55, 95% CI 0.43-0.70) following the use of Mupirocin by *S. aureus* nasal carriers. This systematic review only included a single study examining the effects of Mupirocin use in MRSA nosocomial carriers. (Harbarth *et al.*, 1999) Harbarth *et al.* report that Mupirocin was marginally effective, in comparison to placebo, in the eradication of multisite MRSA. (Harbarth *et al.*, 1999) A more recent systematic review concludes that intranasal Mupirocin use have not led to a reduction in the number of MRSA carriers and are not effective at reducing *Staphylococcus aureus* related surgical site infections. (Liu *et al.*, 2017)

1.2.2 Emergence of Mupirocin resistance

Mupirocin calcium has been long been utilised as topical agent used in the decolonisation of *Staphylococcus aureus* in the anterior nares. (Perl, Pfaller and Herwaldt, 2002) An antibiotic produced by *Pseudomonas fluorescens* (Pseudomonic Acid), Mupirocin acts by inhibiting bacterial protein synthesis, (Uren, Psaltis and Wormald, 2008) and undergoes rapid degradation to become an inactive metanloite in human serum, supporting its use as a topical antibiotic. (Uren, Psaltis and Wormald, 2008) Its use in the nasal decolonisation of MRSA carriers has been well established worldwide as part of a treatment protocol and currently is part of the UK MRSA decolonisation

programme. (Nathwani *et al.*, 2008) Prophylactic decolonisation of MRSA carriers with Mupirocin has long been considered an important intervention of reducing the risk of *Staphylococcus aureus* related infections as a whole. (van Rijen *et al.*, 2008; Patel, Gorwitz and Jernigan, 2009; McDanel *et al.*, 2013; Huang *et al.*, 2016) However, the sole reliance on Mupirocin has led to the scenario of low-level (point mutation of the chromosomal *ileS* gene) and high-level (through plasmid exchange, conferred by the *mupA* gene which encodes an isoleucyl tRNA synthetase enzyme) Mupirocin resistance to MRSA. (Jones *et al.*, 2007) Systemic antibiotics such as Vancomycin and Gentamicin have become increasingly utilised for the prophylaxis of high risk MRSA patients such as those undergoing surgery. (Harbarth *et al.*, 1999)

1.2.3 Antimicrobial resistance and screening policy

MRSA has become a relentless pathogen capable of causing considerable morbidity and mortality. Global concerns regarding the virulence and AMR of MRSA have led to the establishment of surveillance and decolonisation programmes. These programmes have been key in establishing a strategy of reducing the bioburden of MRSA in HA-MRSA and CA-MRSA infections.

The degree of success achieved with the use of systemic antibiotics is becoming increasingly difficult to predict due to an increasing display of AMR by MRSA. The decolonisation process is only one part of a larger strategy to contain MRSA healthcare related infections. Additional infection control measures such as hand washing campaigns and patient isolation also play an important role in MRSA screening. Two approaches adopted by healthcare institutions has been to either swab all elective and emergency admissions, known as universal screening. Alternatively, targeted screening includes swabbing a subset of patients considered to have a high likelihood of MRSA carriage. (El-Bouri *et al.*, 2013; Department of Health 2014) Mandatory screening of all elective and emergency patients was enforced by the Department of Health between April 2009 and December 2010 in England only. Poor reporting compliance and the high number of patients required to screen in order to identify one new positive patient was not considered to be cost-effective. (UK Department of Health, 2014)

1.2.4 Novel therapies

There are reports identifying potential novel therapies capable at preventing MRSA colonisation and disrupting established biofilms. (Gould *et al.*, 2009; Dryden *et al.*, 2014, 2017; Cooke *et al.*, 2015;

Zipperer *et al.*, 2016) Lugdunin is a bactericidal agent, derived from *Staphylococcus lugdunensis*, which has been demonstrated to completely kill MRSA at 10 x MIC. (Zipperer *et al.*, 2016) The potential for using a probiotic bacterium that disrupts the nasal colonisation of *Staphylococcus aureus* is valuable in preventing MRSA related infections in vulnerable patient groups such as those undergoing surgery and haemodialysis. Whilst *Staphylococcus lugdunensis* is capable of causing opportunistic infections, the isolation of a mutant type (*S. lugdunensis* IVK28 wild type) lacking in virulence that has shown promise as a novel probiotic antimicrobial. (Zipperer *et al.*, 2016) Gould and colleagues report using Pomegranate rind extract (PRE) in combination with copper ions to reduce the biomass and viability of established MRSA and MSSA biofilms *in vitro*, as well as PVL positive MSSA strains. (Gould *et al.*, 2009) These treatments have not been introduced into clinical practice and the feasibility of their use is unknown.

1.3 *Staphylococcus aureus* mediated chronic rhinosinusitis

Chronic rhinosinusitis (CRS) is a common condition in the UK and European population, with an estimated prevalence of 11%. (Fokkens *et al.*, 2012) CRS patients are commonly burdened with a variety of intolerable symptoms such as nasal obstruction, purulent discharge, reduced sense of smell, headaches and facial pain. (Fokkens *et al.*, 2012) Naturally, these patients repeatedly report poor quality of life scores comparable with other chronic conditions such as ischaemic heart disease and chronic obstructive pulmonary disease. (Gliklich *et al.*, 1995) The management of CRS patients continues to be protracted due to their recurrent exacerbations when they present to general practice or specialist care. Whilst, the aetiology and pathophysiology of CRS remains unclear and likely multifactorial; MSSA bacterial biofilms have been recognised as a significant player in CRS recalcitrance, chronicity, poor surgical outcomes and antibiotic tolerance. (Foreman *et al.*, 2010) MRSA related CRS represents a small but an existing problem in the management of *Staphylococcus aureus* CRS biofilm mediated disease. (Jiang, Jang and Hsu, 1999; Solares *et al.*, 2006; Uren, Psaltis and Wormald, 2008) Treatment options in recent times have proposed the use of Mupirocin as an additive to the nasal saline wash used by CRS patients with proven *Staphylococcus aureus*. (Solares *et al.*, 2006; Uren, Psaltis and Wormald, 2008; Jervis-Bardy *et al.*, 2012)

Due to presence of biofilms as a key disease mediator in CRS, a number of patients will continue in being prescribed repeated courses of antibiotics, in the hope of establishing a level of symptom

control. This vicious cycle has led to the desperate clinical need for identifying novel antibiotic and antimicrobial substitutes. Doxycycline and Clarithromycin are recommended by European Position Paper on Rhinosinusitis and Nasal Polyps (Fokkens *et al.*, 2012) as part of the medical therapy ladder to CRS patients, avoiding the necessity and potential risks associated with endoscopic sinus surgery. The risks associated with endoscopic sinus surgery can be major and organ threatening such as retrobulbar haematoma, diplopia, epiphora, blindness and anterior skull base injuries leading to cerebrospinal fluid leak and meningitis. As such, optimising medical management in the form of reducing *Staphylococcus aureus* biofilms would be highly sought, in order to reduce and ultimately offset patients requiring surgery.

Therefore, based on these global concerns regarding the continued use of Mupirocin during a climate of AMR. I sought to further investigate the efficacy of Mupirocin in surgical practice as a decolonisation agent. Secondly, to understand the efficacy of Mupirocin under laboratories conditions against MRSA and MSSA biofilms. Lastly, to give a broad context of the prevalence of MRSA in the UK, focusing on the current methodology of reporting MRSA-related infection outcomes.

1.4 Hypothesis

Mupirocin is not effective in the decolonisation of MRSA carriers

1.5 Aims and objectives

Aims

To investigate the efficacy of Mupirocin in preventing MRSA infections

Objectives

1. To systematically review the literature evaluating the efficacy of Mupirocin and Chlorhexidine Gluconate in preventing methicillin-resistant *Staphylococcus aureus* (MRSA) related surgical site infections
2. To analyse the prevalence of MRSA carriage and antimicrobial resistance patterns at in two distinct UK regions
3. To evaluate the *in vitro* efficacy of Mupirocin alongside a novel agent (Reactive Oxygen®) against MRSA and MSSA biofilms

CHAPTER 2: The efficacy of Mupirocin and Chlorhexidine Gluconate in preventing methicillin-resistant *Staphylococcus aureus* (MRSA) related surgical site infections; a systematic review

2.1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) related infections remain a real and substantial threat to our hospitals and communities. Considerable budgets and infection control measures have been established in order to contain what is an antimicrobial epidemic. Amongst a number of other resistant pathogens, MRSA is repeatedly highlighted by the World Health Organization (WHO) as being an important bacterial species to treat whilst strongly advocating for the development of novel therapies in the face of an increasing pattern of antimicrobial resistance. Mupirocin remains a crucial anti-staphylococcal agent capable of decolonising MRSA carriers prior to surgical intervention and is commonly prescribed with Chlorhexidine Gluconate as part of the medical treatment of MRSA carriers, routinely known as decolonisation. However, a number of studies are frequently reporting more cases displaying Mupirocin resistance. The success of these agents is seen as crucial in the clearance of MRSA prior to a surgical procedure and failure to do so exposes the patient to surgical site infections (SSIs). MRSA-related SSIs are a devastating post-operative event for patients, surgeons and healthcare providers. Significant resources are needed to tackle MRSA SSIs in the form of systemic antibiotics, patient isolation, and at times the return to theatre. The financial burden of this disease is immense costing the US economy an estimated \$10 billion per year. (Zimlichman et al., 2013) Therefore, ensuring that current anti-MRSA therapeutic agents are effective is of paramount importance. There is a deficit in the literature detailing the efficacy of Mupirocin and Chlorhexidine Gluconate in preventing MRSA-related SSIs. Therefore, I sought to systematically review the literature in order to report the efficacy of Mupirocin and Chlorhexidine Gluconate in preventing MRSA-related SSIs.

2.2 Hypothesis

The decolonisation of MRSA carriers with Mupirocin and Chlorhexidine Gluconate is not effective in preparation for a surgical procedure.

2.3 Aim of this study

The main aim of this systematic review is to critically appraise the current literatures on the efficacy of Mupirocin and Chlorhexidine Gluconate in reducing the number of MRSA associated SSIs.

2.4 Methods

2.4.1 Criteria for considering studies

2.4.1.1 Types of studies

Randomised controlled trials (RCTs), quasi-experimental studies and observational studies irrespective of language or publication status.

2.4.1.2 Types of participants

Studies of MRSA carriers (identified by microbiological culture and/or polymerase chain reaction (PCR)) from any anatomical site including anterior nares, axilla, genitalia and perineum undergoing a surgical procedure were included. Patients irrespective of their gender, age and ethnicity were included in the study.

2.4.1.3 Types of interventions

Trials in which participants were randomly allocated Mupirocin and Chlorhexidine Gluconate treatment were included. Studies pre-determining the use of control groups with either placebo or no treatment were also included. Studies using Mupirocin or Chlorhexidine Gluconate alone were excluded, as were studies using systemic antibiotics alone as a comparator.

2.4.2 Types of outcome measures

2.4.2.1 Primary outcomes

Based on the definition of The Centres for Disease Control and Prevention (CDC), surgical site infections (SSIs) can be classified into superficial incisional, deep incisional or organ/space

(<https://www.cdc.gov/hai/ssi/ssi.html>). The rate of MRSA related SSIs reported within 30 days of a surgical intervention were included.

2.4.2.2 Secondary outcomes

Where reported, the following outcomes were recorded:

1. The pre and post-operative use of systemic antibiotics
2. Return to theatre
3. Mortality
4. Adverse events
5. Infections attributed to non-MRSA species.

2.4.3 Search methods for identification of studies

For details of the search methods used in this chapter, please refer to Appendix (A).

2.4.3.1 Electronic searches

For this systematic review, I invited the expertise of a trained clinical librarian (Ms Jennie Roe, Morriston Hospital, Swansea Bay University Health Board, Swansea, Wales) to assist me in conducting a comprehensive review of the literature.

In order to include the widest possible and clinically relevant reports including Mupirocin and Chlorhexidine Gluconate, a search of the National Institute of Clinical Excellence (NICE) Micromedex database was considered an important initial step in the systematic review to identify all the relevant alternative nomenclature of both Mupirocin and Chlorhexidine Gluconate and their respective trade names adopted around the world. By doing so, and in combination with medical subject headings (MeSH) terms, a systematic search of the literature was conducted using the following databases:

- Cochrane Wounds Group Specialised Register (searched 8 February 2020);
- The Cochrane Central Register of Controlled Trials (CENTRAL) - *The Cochrane Library* 2020 Issue;
- Ovid MEDLINE (R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions (R) 1946 to February 07, 2020
- Ovid EMBASE (1974 to 2020 February Week 05)

- Pubmed

No language or publication restrictions were applied for this systematic review. The following search strategy was used for CENTRAL and modified, where appropriate, for other databases: The search strategies for Ovid MEDLINE and Ovid EMBASE can be found in Appendix A. The MEDLINE search was guided by the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in MEDLINE. I also searched the following clinical trial registries: -

- (www.ClinicalTrials.gov) (searched 8th February 2020)
- World Health Organization (WHO) International Clinical Trials Registry Platform (<http://apps.who.int/trialsearch/AdvSearch.aspx>) (searched 8th February 2020)

2.4.3.2 Searching other resources

Citation lists from the studies identified by the above methodology were also searched.

2.4.4 Data collection and analysis

2.4.4.1 Selection of studies

The titles and abstracts of studies retrieved by the systematic review were assessed by me (AAS) for their eligibility for inclusion into the review. Full-text versions of all potentially relevant studies were identified. In order to ensure that all the relevant papers were included in this systematic review, the bibliography of all relevant studies were hand checked and traced. Papers deemed compliant with the review's inclusion criteria were subsequently added (Figure 2.1) In the event of data considered to be missing from reports, attempts to contact study authors were made to obtain the missing information.

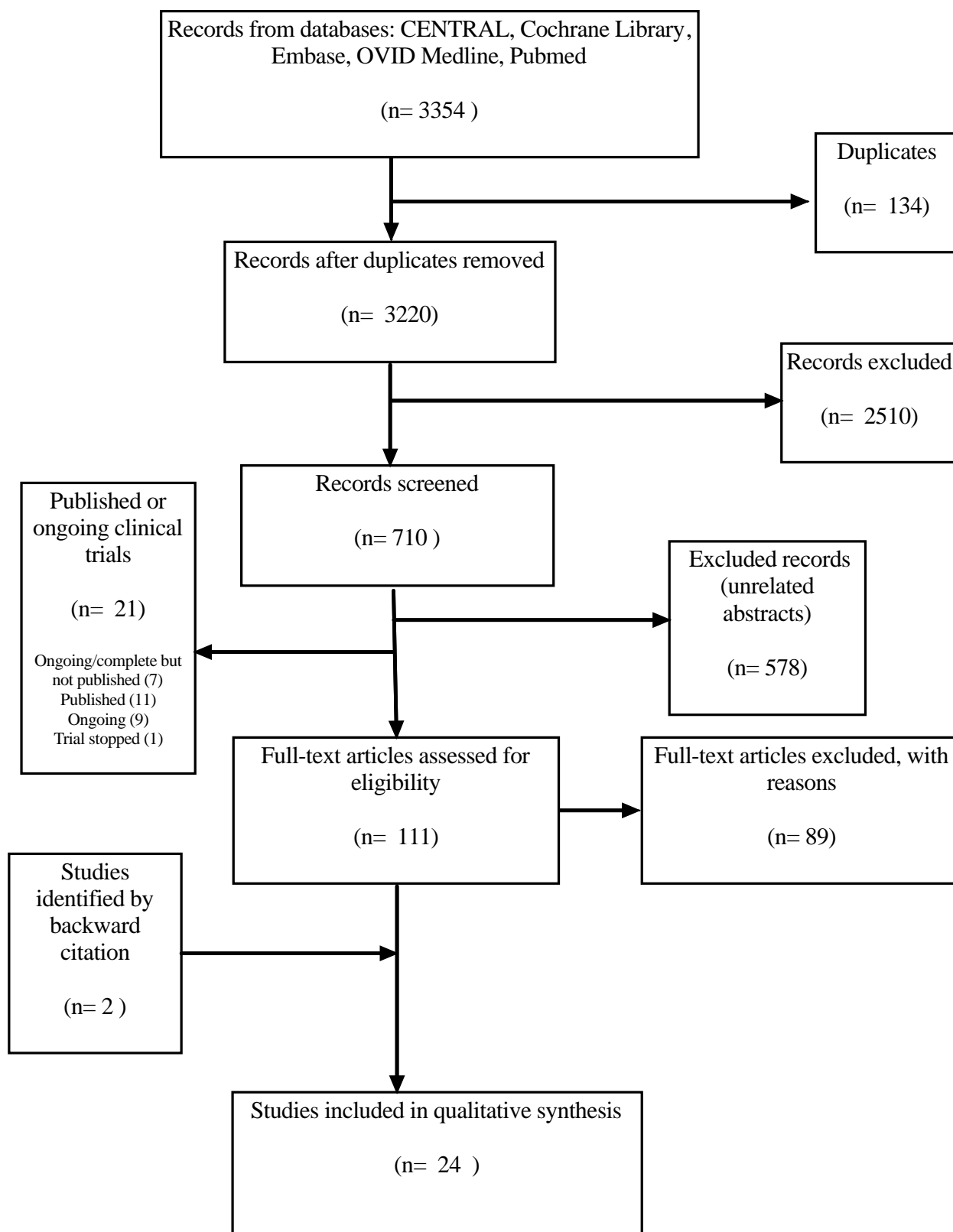


Figure 2. 1 Flow diagram of the systematic review

2.4.4.2 Data extraction and management

Types of information and data extracted included the following:

1. Study authors
2. Year of publication
3. Country where the study was performed
4. Study design (RCT or cohort)
5. Laboratory methods used for identifying MRSA species
6. Length, dose and timing of Mupirocin and Chlorhexidine Gluconate treatment prior to surgical intervention
7. Surgical speciality of operative procedure.
8. Definition of surgical site infection and criteria
9. Numbers of MRSA carriers in Mupirocin/Chlorhexidine Gluconate and placebo treated patient groups.
10. Number of MRSA related surgical site infections among Mupirocin/Chlorhexidine Gluconate and control treated patients
11. Adverse events

2.4.4.3 Assessment of risk of bias in included studies

In the event that the majority of the studies included in the review are cohort studies, instead of randomised control trials, then an assessment of bias, including the quality of the study, will be performed using the Newcastle Ottawa Score.

2.4.4.4 Data synthesis

This study aimed to analyse the primary and secondary outcomes of the studies identified from the systematic review. Studies were analysed for primary and secondary outcomes and then included as part of a quantitative meta-analysis using the Cochrane Review Manager (RevMan) software (version 5.3).

MRSA proven SSIs, time to infection, adverse events and further surgical treatment will be expressed as relative risk (RR) with 95% confidence intervals (CI). Using the random-effects model, data from all the studies will be pooled using a Forest plot. Thereafter, the level of study heterogeneity will be analysed using the chi-squared test (χ^2) test and the I^2 statistic, using the Cochrane RevMan software

(version 5.3). Should the values of I^2 be over 50%, this will likely represent a high degree of study heterogeneity.

2.4.4.5 Subgroup analysis and investigation of heterogeneity

Subgroup analyses will be planned to assess for differences between treatment versus placebo/no treatment groups. Variables such as discipline of surgical procedure (e.g. cardiothoracic, orthopaedic, etc.), doses and lengths of treatment, use of systemic antibiotics and the return to theatre for further surgical intervention, will be submitted for analysis.

2.5 Results

The characteristics of the excluded studies with written rationale for exclusion can be found in Appendix B.

2.5.1 Results of the search

The literature search identified 3354 articles using the inclusion selection process shown in Figure 2.1. After the screening process of this systematic review, 24 studies in accordance to the inclusion criteria were identified. These included two published randomised control trials, 13 retrospective and 9 prospective studies. The studies were conducted over a broad range of surgical centres around the world including the United Kingdom (Sott *et al.*, 2001; Schelenz *et al.*, 2005; Murphy *et al.*, 2011; Akhtar, Kadir and Chandran, 2014; Tandon *et al.*, 2017), India (Sasi *et al.*, 2015; Agarwala *et al.*, 2016), Portugal (Sousa *et al.*, 2016), Japan (Takahashi *et al.*, 2014; Nakamura *et al.*, 2017), Switzerland (Harbarth *et al.*, 2008) and the USA (Baratz *et al.*, 2015; Hadley *et al.*, 2010; Kim *et al.*, 2010; Phillips *et al.*, 2014; Price *et al.*, 2008; Rao *et al.*, 2008; Richer *et al.*, 2009; Schweizer *et al.*, 2015; Shuman *et al.*, 2012; Thompson *et al.*, 2013; Torres *et al.*, 2016; Walsh *et al.*, 2011). The characteristics of these studies can be found in Table 3.1. Only one multi-national study was included in this systematic review. (Lee *et al.*, 2013)

2.5.2 Patient populations

A total of 20 studies were performed in one speciality alone, and these include Orthopaedics (n=14)

(Sott *et al.*, 2001; Price *et al.*, 2008; Rao *et al.*, 2008; Hadley *et al.*, 2010; Kim *et al.*, 2010; Murphy *et al.*, 2011; Akhtar, Kadir and Chandran, 2014; Phillips *et al.*, 2014; Baratz *et al.*, 2015; Agarwala *et al.*, 2016; Sousa *et al.*, 2016; Torres *et al.*, 2016; Nakamura *et al.*, 2017; Tandon *et al.*, 2017), Cardiac (n=2) (Schelenz *et al.*, 2005; Walsh *et al.*, 2011), Otorhinolaryngology (n=2) (Richer *et al.*, 2009; Shuman *et al.*, 2012), General/abdominal Surgery (n=2) (Takahashi *et al.*, 2014; Sasi *et al.*, 2015).

The four remaining studies reported the patient pool from multiple specialties, including the aforementioned within their studies, as well as patients from neurosurgery and vascular. (Harbarth *et al.*, 2008.; Lee *et al.*, 2013; Schweizer *et al.*, 2015; Thompson *et al.*, 2013)

Lee *et al.* reports a study involving 33 surgical wards from 10 hospitals in nine countries in Europe (Scotland, France, Italy, Spain (two hospitals), Germany, Greece, Serbia, Switzerland) and Israel. In total, 8 orthopaedic, 8 general and abdominal, 6 vascular, 5 cardiothoracic, 4 urology, 2 neurosurgery and one plastic surgical wards were included in the only multi-national study included in this systematic review. (Lee *et al.*, 2013) The remaining studies were performed at their respective host institution. The total number of patients included in this systematic review is 74,037.

2.5.3 Interventions

There was a wide degree of variance in the duration and frequency in the prescription of Mupirocin and Chlorhexidine Gluconate treatment in the preparation for surgery amongst the studies. The regimens and timing of Mupirocin and Chlorhexidine Gluconate treatment (intervention) given prior to surgery for each of the included studies are detailed in Table 3.2.

2.5.4 Mupirocin

Out of the 24 included studies, 11 studies (Kim *et al.*, 2010; Murphy *et al.*, 2011; Phillips *et al.*, 2014; Richer *et al.*, 2009; Sasi *et al.*, 2015; Schelenz *et al.*, 2005; Shuman *et al.*, 2012; Sott *et al.*, 2001; Sousa *et al.*, 2016; Takahashi *et al.*, 2014; Tandon *et al.*, 2017) specified the use of Mupirocin with a treatment concentration of 2%. The remaining studies did not report the concentration of Mupirocin used.

21 studies reported the use of a 5-day course of intranasal Mupirocin application. Amongst this group, 7 studies (Baratz *et al.*, 2015; Phillips *et al.*, 2014; Richer *et al.*, 2009; Sousa *et al.*, 2016; Thompson and Houston, 2013; Torres *et al.*, 2016; Walsh *et al.*, 2011) specified a twice daily frequency of Mupirocin application. Five studies (Schelenz *et al.*, 2005; Murphy *et al.*, 2011; Akhtar, Kadir and

Chandran, 2014; Takahashi *et al.*, 2014; Tandon *et al.*, 2017) reported a thrice daily regimen of Mupirocin application for 5 days. Nine studies (Hadley *et al.*, 2010; Harbarth *et al.*, 2008.; Kim *et al.*, 2010; Lee *et al.*, 2013; Rao *et al.*, 2008; Sasi *et al.*, 2015; Schweizer *et al.*, 2015; Shuman *et al.*, 2012; Sott *et al.*, 2001) did not report the frequency of use amongst the intervention group.

Two studies (Agarwala *et al.*, 2016; Nakamura *et al.*, 2017) reported the use of a shorter duration of Mupirocin treatment of 3 days course of Mupirocin treatment instead of 5. Neither study reported the concentration of treatment used. One study (Price *et al.*, 2008) reported that participants received in total six doses of Mupirocin but the number of days was unclear. The frequency and concentration of Mupirocin treatment in this study was not specified.

2.5.5 Chlorhexidine Gluconate

A greater daily of variability in the reporting of Chlorhexidine Gluconate with little in the way of commonality between the descriptions of use, to report this in a comparative way. The frequency of Chlorhexidine Gluconate concentration, formulations (body wash versus mouthwash versus wipes) and timing of treatment were considered too heterogeneous to report. However, it remains important to respect the data and report it accordingly and will be discussed further.

2.5.6 Systemic antibiotics

In the same vain, the heterogeneity of systemic antibiotics reporting amongst the studies makes it a challenge to present the data in any meaningful way without bias or misreporting. The timing, duration and rationale behind the choice of antibiotic treatment (when available) can be referred to in Table 3.2.

2.5.7 Definition of SSI

Thirteen studies (Baratz *et al.*, 2015; Hadley *et al.*, 2010; Kim *et al.*, 2010; Lee *et al.*, 2013; Nakamura *et al.*, 2017; Phillips *et al.*, 2014; Price *et al.*, 2008; Sasi *et al.*, 2015; Schweizer *et al.*, 2015; Shuman *et al.*, 2012; Thompson and Houston, 2013; Torres *et al.*, 2016; Walsh *et al.*, 2011) reported the use of The Centers for Disease Control and Prevention (CDC) guidelines (Berríos-Torres *et al.*, 2017) as their pre-defined protocol for recording surgical site infections. Three studies (Murphy *et al.*, 2011; Akhtar, Kadir and Chandran, 2014; Tandon *et al.*, 2017), all from the UK, reported the use of the World Health Organization (WHO) guidelines in defining of SSIs. Three studies (Agarwala *et al.*, 2016; Harbarth *et al.*, 2008.; Sousa *et al.*, 2016) reported using a clinician assessment to

diagnose a SSI. Two studies (Schelenz *et al.*, 2005; Takahashi *et al.*, 2014) referred to their national guidelines. The remaining three studies (Rao *et al.*, 2008; Richer *et al.*, 2009; Sott *et al.*, 2001) did not report their preferred methodology to diagnose MRSA-related SSIs.

2.5.8 Microbiological Culture and MRSA identification

All the studies apart from four (Thompson and Houston, 2013; Torres *et al.*, 2016; Nakamura *et al.*, 2017; Tandon *et al.*, 2017) reported the location of the anatomical site sampled. 11 studies (Baratz *et al.*, 2015; Hadley *et al.*, 2010; Kim *et al.*, 2010; Phillips *et al.*, 2014; Price *et al.*, 2008; Rao *et al.*, 2008; Richer *et al.*, 2009; Schweizer *et al.*, 2015; Sousa *et al.*, 2016, 2016; Takahashi *et al.*, 2014; Walsh *et al.*, 2011) reported sampling the anterior nares only. The remaining 9 studies reported the sampling of the anterior nares and another anatomical site. The combinations of these can be found in Table 2.2.

Twelve studies (Sott *et al.*, 2001; Schelenz *et al.*, 2005; Price *et al.*, 2008; Rao *et al.*, 2008; Hadley *et al.*, 2010; Shuman *et al.*, 2012; Phillips *et al.*, 2014; Sasi *et al.*, 2015; Agarwala *et al.*, 2016; Torres *et al.*, 2016; Tandon *et al.*, 2017) reported the characterisation of MRSA using direct microbiological culture, one of which was a randomised control trial (RCT) that reported the use the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines including the Cefoxitin screen test. (Sousa *et al.*, 2016) Seven studies (Baratz *et al.*, 2015; Harbarth *et al.*, 2008; Kim *et al.*, 2010; Lee *et al.*, 2013; Richer *et al.*, 2009; Schweizer *et al.*, 2015; Takahashi *et al.*, 2014) confirmed the use of polymerase chain reaction (PCR) to identify MRSA, with four studies (Kim *et al.*, 2010; Lee *et al.*, 2013; Takahashi *et al.*, 2014; Schweizer *et al.*, 2015) using this in conjunction with direct culture. Five studies (Akhtar *et al.*, 2014; Murphy *et al.*, 2011; Nakamura *et al.*, 2017; Thompson *et al.*, 2013; Walsh *et al.*, 2011) failed to report their methodology in this domain.

2.5.9 Outcomes

All the studies included in the systematic review reported the number of confirmed cases of MRSA proven SSIs. These can be found in Table 3.2. All but four studies (Price *et al.*, 2008; Murphy *et al.*, 2011; Akhtar, Kadir and Chandran, 2014; Agarwala *et al.*, 2016) reported the risk of MRSA SSIs in a pre-defined control group.

2.5.10 Risk of bias in included studies

22 studies out of the 24 included in this systematic review were non-randomised control trials. Therefore, an assessment of quality using the Newcastle-Ottawa Scale for Cohort Studies was performed. Ten studies (Baratz et al., 2015; Harbarth et al., 2008.; Kim et al., 2010; Lee et al., 2013; Phillips et al., 2014; Rao et al., 2008; Sasi et al., 2015; Schelenz et al., 2005; Schweizer et al., 2015; Takahashi et al., 2014) were stratified as being “good quality” based on their scores of selection, compatibility and outcome. Three (Hadley et al., 2010; Richer et al., 2009; Tandon et al., 2017) studies were considered to be “fair quality”. The remaining studies were classified as being “poor quality” according to the Newcastle-Ottawa Scale. The score for each study within each of the domains of assessment are presented in Table 2.1.

The risk of bias assessment for two published randomised controls (Shuman *et al.*, 2012; Sousa *et al.*, 2016) was performed using the Cochrane Risk of Bias tool. The results of this assessment can be found in Tables 2.3 and 2.4 respectively.

2.5.11 Effect of intervention

Due to the heterogeneity of the quality amongst the studies, wide confidence intervals and high I^2 score, a single combined prevalence estimate of sub-group outcome was not performed in order to avoid confusion and incorrect conclusions.

Newcastle-Ottawa Scale

| No. | Study | Year | Country | Speciality | Journal | Study Design | S | C | O | Quality of evidence |
|-----|------------------------|------|----------------|---------------------|--|-----------------------|---|---|---|---------------------|
| 1 | Agarwala <i>et al.</i> | 2016 | India | Orthopaedics | <i>Journal of Clinical Orthopaedics and Trauma</i> | Retrospective | 2 | 0 | 1 | Poor |
| 2 | Akhtar <i>et al.</i> | 2014 | UK | Orthopaedics | <i>Journal of Orthopaedics</i> | Retrospective | 2 | 0 | 1 | Poor |
| 3 | Baratz <i>et al.</i> | 2015 | USA | Orthopaedics | <i>Clinical Orthopaedics and Related Research</i> | Retrospective | 3 | 1 | 2 | Good |
| 4 | Hadley <i>et al.</i> | 2010 | USA | Orthopaedics | <i>Arthritis</i> | Retrospective | 2 | 1 | 1 | Fair |
| 5 | Harbarth <i>et al.</i> | 2008 | Switzerland | Multi-speciality | <i>Journal of the American Medical Association</i> | Prospective crossover | 4 | 2 | 2 | Good |
| 6 | Kim <i>et al.</i> | 2010 | USA | Orthopaedics | <i>The Journal of Bone and Joint Surgery</i> | Prospective | 3 | 2 | 2 | Good |
| 7 | Lee <i>et al.</i> | 2013 | Multi-national | Multi-speciality | <i>British Medical Journal</i> | Prospective | 3 | 1 | 2 | Good |
| 8 | Murphy <i>et al.</i> | 2011 | UK | Orthopaedics | <i>The Journal of Bone and Joint Surgery</i> | Retrospective | 2 | 0 | 1 | Poor |
| 9 | Nakamura <i>et al.</i> | 2017 | Japan | Orthopaedics | <i>Journal of Orthopaedic Science</i> | Retrospective | 1 | 0 | 1 | Poor |
| 10 | Phillips <i>et al.</i> | 2014 | USA | Orthopaedics | <i>Infection Control and Hospital Epidemiology</i> | Prospective | 3 | 1 | 2 | Good |
| 11 | Price <i>et al.</i> | 2008 | USA | Orthopaedics | <i>Clinical Orthopaedics and Related Research</i> | Retrospective | 2 | 0 | 1 | Poor |
| 12 | Rao <i>et al.</i> | 2008 | USA | Orthopaedics | <i>Clinical Orthopaedics and Related Research</i> | Prospective | 4 | 1 | 3 | Good |
| 13 | Richer <i>et al.</i> | 2009 | USA | Otorhinolaryngology | <i>Otolaryngology – Head and Neck Surgery</i> | Retrospective | 2 | 1 | 2 | Fair |

| | | | | | | | | | | |
|----|-------------------------|------|----------|---------------------|---|---------------|-------------------------------|---|---|------|
| 14 | Sasi <i>et al.</i> | 2015 | India | General Surgery | <i>The International Journal of Clinical Practice</i> | Prospective | 3 | 2 | 3 | Good |
| 15 | Schelenz <i>et al.</i> | 2005 | UK | Cardiac | <i>Journal of Hospital Infection</i> | Retrospective | 4 | 2 | 3 | Good |
| 16 | Schweizer <i>et al.</i> | 2015 | USA | Multi-speciality | <i>Journal of the American Medical Association</i> | Prospective | 3 | 2 | 3 | Good |
| 17 | Shuman <i>et al.</i> | 2012 | USA | Otorhinolaryngology | <i>The Laryngoscope</i> | RCT | Not applicable. See Table 3.3 | | | |
| 18 | Sott <i>et al.</i> | 2001 | UK | Orthopaedics | <i>Hip International</i> | Retrospective | 2 | 1 | 1 | Poor |
| 19 | Sousa <i>et al.</i> | 2016 | Portugal | Orthopaedics | <i>The Journal of Arthroplasty</i> | RCT | Not applicable. See Table 3.4 | | | |
| 20 | Takahashi <i>et al.</i> | 2014 | Japan | General Surgery | <i>Journal of Hospital Infection</i> | Prospective | 3 | 2 | 2 | Good |
| 21 | Tandon <i>et al.</i> | 2017 | UK | Orthopaedics | <i>The Journal of Arthroplasty</i> | Retrospective | 2 | 1 | 2 | Fair |
| 22 | Thompson <i>et al.</i> | 2013 | USA | Multi-speciality | <i>American Journal of Infection Control</i> | Prospective | 1 | 0 | 0 | Poor |
| 23 | Torres <i>et al.</i> | 2006 | USA | Orthopaedics | <i>The Journal of Arthroplasty</i> | Retrospective | 1 | 0 | 1 | Poor |
| 24 | Walsh <i>et al.</i> | 2011 | USA | Cardiac | <i>The Archives of Internal Medicine</i> | Retrospective | 1 | 2 | 1 | Poor |

Table 2. 1 Demographics and quality scoring of included studies for systematic review.

A quality scoring system for cohort and non-randomised control trials was performed using the Newcastle-Ottawa Scale. Maximum stars for each study criteria are 4 for selection (s), 2 for comparability (c), and 3 for outcome (o), with a possible total score of 9. Studies scoring <5 were considered low, 5 to 6 medium, and 7 to 9 high quality. Two randomised control trials (RCT) (Shuman *et al.* 2012, Sousa *et al.* 2016) were assessed using the Cochrane Risk of Bias Tool (Tables 2.3 and 2.4).

| Study | MRSA Identification | | Intervention | | Primary outcome | | |
|------------------------|----------------------------------|----------------------------------|--|--|--------------------------------|---------------------------|----------------|
| | Anatomical site(s) swabbed | Method(s) of MRSA identification | MUP & CHG protocol (days and frequency) | Systemic antibiotic used and timing of administration from time of incision (t = hours) | MRSA SSIs (Intervention group) | MRSA SSIs (Control) group | SSI Definition |
| Agarwala <i>et al.</i> | Nasal and axillae | Morphology | MUP - 3/7 <i>tds</i> CHG - night before surgery and knife to skin | Cefazolin 1g <i>tds</i> (t = 0, 6, 18) | 0/7 (0%) | Not reported | Clinician |
| Akhtar <i>et al.</i> | Nasal, axillae and groin | Not specified | MUP - 5/7 <i>tds</i> 4% CHG 5/7 (surgery on day 3 during Rx) | Gentamicin and Teicoplanin (doses unreported) at t = 0 | 2/88 (2.27%) | Not reported | WHO |
| Baratz <i>et al.</i> | Nasal only | PCR | MUP - 5/7 <i>bd</i> 4% CHG 5/7 (pre-op) | Cefazolin <i>tds</i> (t = 0, 8, 16) <i>or</i> Vancomycin (β -lactam allergy) | 4/158 (2.5%) | 17/2763 (0.6%) | CDC |
| Hadley <i>et al.</i> | Nasal only | Culture | 2% MUP - 5/7 CHG - night before surgery only | IV Vancomycin 1g <i>bd</i> (MRSA +ve) Cefazolin or Clindamycin (if MRSA/MSSA status unknown) | 3/58 (5.17%) | 1/414 (0.24%) | CDC |
| Harbarth <i>et al.</i> | Nasal, perineum, medical devices | qPCR | MUP - 5/7 4% CHG 5/7 (pre-op) | Not reported 115/266 (identified pre-op were truly given antibiotics) | 70/515 (13.6%) | 60/10910 (0.55%) | Clinician |
| Kim <i>et al.</i> | Nasal only | PCR & Culture | 2% MUP - 5/7 2% CHG - 5/7 <i>od</i> | IV Vancomycin 1g <i>bd</i> (MRSA +ve) IV Vancomycin AND Cefazolin (if MRSA carriage eliminated) or MSSA +ve or unknown) | 3/309 (0.97%) | 1/5122 (0.02%) | CDC |
| Lee <i>et al.</i> | Nasal and perineum | PCR & Culture | MUP - 5/7 4% CHG 5/7 (pre-op) | Variable protocol | 15/99 (15%) | 14/54 (27.8%) | CDC |

| | | | | | | | |
|------------------------|--------------------------|---------------|---|---|----------------|----------------|--------------|
| Murphy <i>et al.</i> | Nasal, throat and groin | Not specified | 2% MUP - 5/7 <i>tds</i> Or Fusidic acid + polymyxin (Mupirocin resistant) 2% CHG (body and hair) pre-op 5/7 | 65/68 (95.6%) Cefuroxime; 3/68 (4.4%) given Vancomycin | 4/90 (4.4%) | Not reported | WHO |
| Nakamura <i>et al.</i> | Not specified | Not specified | MUP - 3/7 <i>tds</i> CHG wipes 3/7 (body washes restricted). | IV Vancomycin (regimen unspecified) | 2/140 (1.43%) | 1/3112 (0.03%) | CDC |
| Phillips <i>et al.</i> | Nasal only | Culture | 2% MUP - 5/7 <i>bd</i> 2% CHG wipes - night before and morning of surgery | Vancomycin 1g (MRSA +ve) (t = 0-2) Cefazolin (1g); Clindamycin 600mg (if β -lactam allergy) (t = 0-1) | 24/763 (3.15%) | 21/776 (2.71%) | CDC |
| Price <i>et al.</i> | Nasal only | Culture | ≥ 6 doses of MUP CHG wipes to prep. No bodywash | Cefazolin or Clindamycin (if β -lactam allergy) (t = -1 and up to t =24) Doses and frequency not reported | 1/9 (11.1%) | Not reported | CDC |
| Rao <i>et al.</i> | Nasal only | Culture | MUP - 5/7 CHG 5/7 (pre-op) | Cefazolin 2g (t = -1) AND Cefazolin 1g <i>tds</i> (t = 6, 12, 18) OR Vancomycin 1g (t = -1) AND Vancomycin 1g <i>bd</i> (t = 12, 24) ** | 0/17 (0%) | 8/741 (1.08%) | Not reported |
| Richer <i>et al.</i> | Nasal only | PCR | 2% MUP - 5/7 <i>bd</i> CHG washes on day 1, 3 and 5 of MUP. | Not reported | 0/24 (0%) | 2/241 (0.83%) | Not reported |
| Sasi <i>et al.</i> | Nasal, axillae and groin | Culture | 2% MUP - 5/7 2% CHG <i>bd</i> 5/7 | Cefazolin 1g ((t ≤ 0) for prosthesis) Ceftriaxone & Metronidazole (clean-contaminated) | 10/21 (47.6%) | 9/24 (37.5%) | CDC |

| | | | | | | | |
|-------------------------|-----------------------------|----------------|--|--|-------------------|--------------------|--------------|
| Schelenz <i>et al.</i> | Nasal, axillae and perineum | Culture | 2% MUP - 5/7 <i>tds</i> (Chlorhexidine/neomycin if Mupirocin resistant) 4% CHG hair and bodywash 5/7 | Cefuroxime 1.5g (t=0) Change within study to Teicoplanin 800mg and 5mg/kg Gentamicin | 20/956 (2.09%) | 44/1075 (4.09%) | National |
| Schweizer <i>et al.</i> | Nasal only | qPCR & Culture | MUP - 5/7 (≥ 10 doses) CHG 5/7 (pre-op) | Cefazolin or Cefuroxime (MRSA/MSSA -ve) If MRSA +ve Vancomycin added | 14/29 (48.2%) | 45/101 (44.6%) | CDC |
| Shuman <i>et al.</i> | Nasal, oropharynx and neck | Culture | 2% MUP - 5/7 2% CHG od - 5/7 | Ampicillin/sulbactam or Clindamycin (clean-contaminated) or Cefazolin (clean) (t -1 and <24) Vancomycin (MRSA +ve) | 1/4 (25%) | 1/10 (10%) | CDC |
| Sott <i>et al.</i> | Nasal, axillae and perineum | Culture | 2% MUP - 5/7 4% CHG - 5/7 | 2nd gen Cephalosporin | 1/123 (%) | 5/113 (%) | Not reported |
| Sousa <i>et al.</i> | Nasal only | Culture | 2% MUP - 5/7 <i>bd</i> CHG - 5/7 | Cefazolin 2g (t = -1) AND Cefazolin 1g <i>tds</i> (t = 6, 12, 18) If MRSA +ve addition of Vancomycin 1g (t = -1) AND Vancomycin 1g <i>bd</i> (t = 12, 24) | 0/89 (0%) | 6/1028 (0.58%) | Clinician |
| Takahashi <i>et al.</i> | Nasal only | PCR & culture | 2% MUP - 5/7 <i>tds</i> 2% CHG (body and hair) pre-op 5/7 | Vancomycin 1g (MRSA +ve) (t = Cefazolin (1g) (t = -1) | 2/49 (4.1%) | 31/613 (5.1%) | National |

| | | | | | | | |
|------------------------|---------------|---------------|--|---|---------------------|--------------------|------|
| Tandon <i>et al.</i> | Not specified | Culture | 2% MUP - 5/7 <i>tds</i> 4% CHG body 5/7 <i>od</i> , hair (day 1 and 3 of MUP Rx) | Multiple (Teicoplanin, Flucloxacillin/gentamicin, Augmentin and cefuroxime) | 5/79 (6.3%) | 83/6530 (1.27%) | WHO |
| Thompson <i>et al.</i> | Not specified | Not specified | MUP - 5/7 <i>bd</i> CHG wipes 5/7 (no wipes above chin). | Not reported | 33/19886 (0.17%) | 39/9976 (0.39%) | CDC† |
| Torres <i>et al.</i> | Not specified | Culture | MUP - 5/7 <i>bd</i> CHG 5/7 body wash pre-op and wipe at surgery | Cefazolin (weight-based) Clindamycin 600mg (β-lactam allergy) | 6/849 (0.71%) | 8/1004 (0.80%) | CDC |
| Walsh <i>et al.</i> | Nasal only | Not specified | MUP - 5/7 <i>bd</i> CHG wipes at surgery | Cefazolin 48 hours (i.e. 6 doses) Vancomycin for MRSA +ve | 2/2496 (0.08%) | 32/2766 (1.15%) | CDC |

Table 2. 2 Description of the doses, frequency and timing of interventions, methodology of identifying MRSA species and MRSA SSI rates.

CDC = The Centers of Disease Control and Prevention, WHO = World Health Organization, MUP = Mupirocin, CHG = Chlorhexidine Gluconate, *tds* = thrice daily, *bd* = twice daily, *od* = once daily, PCR = Polymerase chain reaction, pre-op = pre-operatively, +ve = positive, † = HIPAC (agency under the auspices of CDC), Rx = treatment course.

| | | |
|-------------------------------------|--|--|
| Shuman et al. 2012 | | |
| Methods | Prospective, randomised control trial | |
| Participants | Patients undergoing head and neck surgery | |
| Interventions | <p>2% Intranasal Mupirocin 5 days (frequency not reported); 2% Chlorhexidine Gluconate 5 days (frequency not reported).</p> <p>Reports the use of perioperative Vancomycin (dose not reported) in the prophylaxis of MRSA carriers at the time of surgery.</p> <p>MRSA -ve and/or MSSA +ve/-ve carriers received Ampicillin/sulbactam or Clindamycin (clean-contaminated procedures) or Cefazolin (clean) at three time points (within 1 hour before incision, redosed intraoperatively and a further dose no more than 23 hours post-operatively). Doses not reported. Practice in accordance to institution and in accordance to CDC</p> | |
| Outcomes | <p>The efficacy of Mupirocin and Chlorhexidine on rates of SSI</p> <p>Incidence of <i>S. aureus</i> (MRSA and MSSA) SSIs</p> <p>Prevalence of MRSA and MSSA in study group</p> | |
| Notes | | |
| Bias | Authors' judgement | Support for judgement |
| <i>Selection bias</i> | | |
| random sequence generation | Unclear | |
| allocation concealment | Unclear | |
| <i>Reporting bias</i> | Low | Clearly defined protocol for defining SSIs (in accordance with CDC). |
| <i>Other sources of bias</i> | High | |

| | | |
|-------------------------|---------|---|
| | | Doses of antibiotics and frequency of Mupirocin and Chlorhexidine Gluconate not reported |
| <i>Performance bias</i> | High | Unblinded. Small sample size. |
| <i>Detection bias</i> | Unclear | |
| <i>Attrition bias</i> | Low | Electronic prospectively collected perioperative database. Violations of protocol recorded. |

Table 2. 3 Cochrane Risk of Bias Tool analysis (Shuman *et al.* 2012)

| | | |
|---------------------------------|---|--|
| Sousa <i>et al.</i> 2016 | | |
| Methods | Prospective, randomised control trial | |
| Participants | Patients undergoing elective total hip or knee arthroplasty | |
| Interventions | <p>2% Intranasal Mupirocin 5 days (twice a day); Chlorhexidine Gluconate 5 days (concentration and frequency not reported).</p> <p>Reports the use of perioperative Vancomycin 1g given 1 hour prior to procedure, 12 and 24 hours post-operatively to identified MRSA carriers.</p> <p>All patients, including MRSA carriers, received Cefazolin 2g 1 hour prior to procedure and three further doses at 6, 12 and 18 hours post-operatively (Cefazolin 1g).</p> | |
| Outcomes | <p>Prevalence of MRSA and MSSA in study group</p> <p>The efficacy of Mupirocin and Chlorhexidine on rates of MRSA and MSSA SSIs</p> | |
| Notes | Detailed microbiological techniques from patient swab technique by dedicated nurse, handling of bacteria and characterisation using EUCAST guidelines using Cefoxitin screen test | |
| Bias | Authors' judgement | Support for judgement |
| <i>Selection bias</i> | | |
| random sequence generation | Low | Good detail of randomisation process and concealment. |
| allocation concealment | Low | Electronic ID for <i>S. aureus</i> carriers assigned by microbiology laboratory. Assigned by sequence in advance by first author |
| | | |

| | | |
|------------------------------|---------|--|
| <i>Reporting bias</i> | High | Use of a clinician-based assessment of SSI |
| <i>Other sources of bias</i> | Low | |
| <i>Performance bias</i> | Unclear | |
| <i>Detection bias</i> | Unclear | |
| <i>Attrition bias</i> | Low | |

Table 2. 4 Cochrane Risk of Bias Tool analysis (Sousa *et al.* 2016)

2.6 Discussion

The implications of a surgical site infection (SSI) on patients and surgeons can be devastating. The consequence of a SSI and in particular MRSA-related SSIs has the potential to not only compromise the results of the initial surgery but render the patient to long-term healthcare problems. SSI as a subsequence of a nosocomial infection, such as MRSA, are common and are thought to be one of the leading contributors to infection after pneumonia and urinary sepsis (Perl, Pfaller and Herwaldt, 2002). The economic burden of these new infections is thought to require an additional funding in the region of \$5 to 10 billion in the US economy as a result of additional healthcare costs (Perl, Pfaller and Herwaldt, 2002). The emergence of community-acquired MRSA in the US has led to 94,000 invasive infections and 18,650 deaths in 2005 alone (Pofahl *et al.*, 2009).

The anterior nares are a recognised ecological reservoir for nasal *Staphylococcus aureus* carriage, including MRSA (Wertheim *et al.*, 2005; van Rijen *et al.*, 2008). The prevalence of nasal *Staphylococcus aureus* carriage has been reported as high as 6.3% (Pofahl *et al.*, 2009). Considering that Mupirocin is not effective in the clearance of nasal *Staphylococcus aureus* in around 15% of cases (Pofahl *et al.*, 2009), this raises alarm at the fact MRSA SSIs are clearly associated with pre-operative MRSA carriage and is reported to be as common in 44% of carriers (Pofahl *et al.*, 2009). In addition, a number of patients are likely to stop Mupirocin treatment as a result of common local side-effects such as headache, rhinorrhoea, congestion, sore throat and localised symptoms attributed to treatment (Rieser *et al.*, 2018).

The heavy reliance on the use of Mupirocin and Chlorhexidine Gluconate in the decolonisation of MRSA carriers pre-operatively has been threatened owing to concerns of an increasing number of studies reporting Mupirocin resistance and the lack of availability and supply of these two important agents. Such is their importance; the prescription of Mupirocin for MRSA carriers has become increasingly scrutinised and managed by healthcare providers. As nasal *Staphylococcus aureus* carriers are two to nine times more likely to develop a SSI in comparison to non-carriers (van Rijen *et al.*, 2008; Sasi *et al.*, 2015), there is palpable concern at the lack of no viable alternative anti-staphylococcal agents, in particular to Mupirocin, that are currently available, reinforcing the importance of reporting the current state of affairs on the efficacy of Mupirocin and Chlorhexidine Gluconate in preventing MRSA-related SSIs.

To the best of my knowledge, this is the most comprehensive and up-to-date systematic review of original research articles presenting data solely on patients decolonised with Mupirocin and

Chlorhexidine Gluconate amongst surgical patients. This systematic review found that the quality of the current evidence to be heterogenous and varied and precluded the opportunity to perform a meta-analysis. Description of study quality and risk of bias of the studies included in this systematic review are attached in Tables 3.1-3.4. All the intended primary outcomes outlined in this review were achieved. Some secondary outcomes intended for report were affected by the quality of the studies and prevented a unique opportunity to perform a sub-group analysis on the risk of MRSA-related SSIs. In failing to do so, the systematic review was unable to comment on the efficacy of Mupirocin and Chlorhexidine Gluconate, an important intended outcome and the hypothesis of which this systematic review was based on.

Similarly, differences in risk between specialities, countries, treatment regimens including the use of systemic antibiotics has been desperately missed. The heterogeneity of not only the studies but the description of the information presented within the studies would benefit from further discussion. However, this does give the opportunity to pause and re-consider the approach and consider standardising the reported outcomes desired for MRSA SSIs. As a result of not being able to perform a meta-analysis based on the heterogeneity of the data, the next chapter will refer to using a retrospective approach to further this discussion.

CHAPTER 3: A 5-year retrospective analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) and reportable outcomes in two UK regional centres

3.1 Introduction

Staphylococcus aureus continues to dominate as a leading bacterial species in healthcare-acquired infections (HAIs). This burden of *Staphylococcus aureus* on HAIs is vast, and contributes to 30% of surgical site infections (SSIs) (Kline *et al.*, 2018). Methicillin-resistant *Staphylococcus aureus* (MRSA) related SSIs in particular are becoming increasingly problematic and difficult to treat (Bocchini, 2006; Pofahl *et al.*, 2009; Green *et al.*, 2010; Tandon *et al.*, 2017). As a whole, the burden of MRSA related infections in the UK is often underestimated but considered to be one of the highest in the developed world (Jumaa *et al.*, 2007; Ellington *et al.*, 2009). Traditionally, hospital/healthcare acquired MRSA (HA-MRSA) has been commonly reported as being the more common form of acquisition (Rollason *et al.*, 2008). The UK sustains a high level of HA-MRSA (Ellington *et al.*, 2009) and this includes patients undergoing haemodialysis, surgery and hospitalisation in the past 12 months (Berríos-Torres *et al.*, 2017; David *et al.*, 2010, Department of Health 2014). Two epidemic MRSA (EMRSA) strains dominate the phenotype in the UK: EMRSA-15 (ST22-MRSA-SCCmecIV) and EMRSA-16 (ST36-MRSA-SCCmecII) (Ellington *et al.*, 2009).

However, the emergence of community acquired MRSA (CA-MRSA) cases are becoming increasingly reported around the world (Como-Sabetti *et al.*, 2009; Cunnington *et al.*, 2009; Ellington *et al.*, 2009). CA-MRSA related infections have been reported to be on the severe form of the infective spectrum, leading to cases of osteomyelitis, necrotising pneumonia and even infective endocarditis (Tristan *et al.*, 2007). The USA in particular has continued to report a rising number of CA-MRSA cases in recent years, with one study reported a 22% increase in the number cases in just 5 years (Como-Sabetti *et al.*, 2009).

Whilst not entirely synonymous with CA-MRSA, the presence of the Pantone-Valentine Leukocidin (PVL) toxin is estimated to be present in 1-2% of all *Staphylococcus aureus* strains (Health Protection Agency, 2008). The number of PVL +ve (positive) MRSA strains isolated in 2006 represented a 0.4-fold increase, from 117 to 158, in comparison to 2005 from a total of 1087 MRSA isolates received by the Health Protection Agency (HPA) *Staphylococcus* Reference Unit from nearly 40 geographically dispersed centres across England and Wales (Ellington *et al.*, 2009). Case series of

severe PVL positive SA infections in children has already been reported in the UK (Cunnington *et al.*, 2009). New guidance regarding the management of PVL SA infections is currently awaited.

The fear is that, CA-MRSA will continue to add to the burden of MRSA-related SSIs, unchecked and with little standardised data available to inform future practice and contingency planning.

At present, the UK Department of Health (DoH) publishes monthly rates of MRSA bacteraemias, as mandatory, from individual hospital Trusts on the UK Government website (<https://www.gov.uk/government/statistics/mrsa-bacteraemia-monthly-data-by-location-of-onset>).

However, there is an increasing desire for the inclusion of the rates of PVL, CA-MRSA and HA-MRSA in order to inform the process. Therefore, a UK based population study analysing the prevalence of MRSA and current practice is needed.

3.2 Hypothesis

CA-MRSA prevalence is underestimated amongst the UK population

3.3 Aims and Objectives

The primary outcome of the study is to present the current landscape of CA-MRSA and HA-MRSA in two representable regional centres in the United Kingdom.

Secondary outcomes include reporting the frequency of PVL carriage, staphylococcal cassette chromosomal typing, antibiotic susceptibility of MRSA isolates as well as anatomical swab site(s) of positive MRSA culture, age, methods of MRSA identification and clinical location of positive MRSA samples, when available.

3.4 Methods

3.4.1 Population characteristics

The Swansea and Neath Port Talbot area are served by three hospitals. Morriston Hospital is Wales' second largest hospital, a major trauma centre and a tertiary referral centre for burns, plastic and reconstructive, cardiothoracic, paediatric, otorhinolaryngology, oral and maxillofacial surgery in south west Wales. In addition to providing a tertiary level service for renal and haemodialysis patients, Morriston Hospital houses a 30-bed intensive care unit (ICU) supporting Swansea's only Emergency

Department and acute general medicine wards. (<https://sbuhb.nhs.wales/about-us/swansea-bay-uhb>, accessed 24th August 2019).

Singleton Hospital, Swansea's second largest hospital with 330 beds, acts as a further tertiary referral centre for the region providing an obstetrics and gynaecology neonatal intensive care unit (NICU) to West Wales. In addition, Singleton Hospital is the hub for acute medical admissions including haematology/oncology as well as day case surgery. Neath Port Talbot Hospital has 200 beds providing inpatient care, day case surgery and neurorehabilitation in the region.

Princess of Wales Hospital in Bridgend is a district general hospital with 200 beds serving a population of approximately 160,000. It provides acute health care services such as an emergency department, obstetrics and gynaecology, medical, surgical and paediatric services.

However, all MRSA swab samples from Princess of Wales hospital, as well as all the other three Swansea group hospitals, were analysed and handled in one central microbiological laboratory at Singleton Hospital during the study period up to 2016 (AAS personal communication with Consultant Microbiologist Dr Bassam Ben-Ismaeil (Swansea); <https://sbuhb.nhs.wales/about-us/swansea-bay-uhb/>, accessed 24th August 2019).

University Hospital Southampton (UHS), is a tertiary referral centre providing for nearly 4 million people in central southern England and the Channel Islands, including the city of Southampton. The hospital provides specialist care for paediatrics including intensive care (PICU), neurosurgery and cardiothoracic services as well as renal and dialysis, obstetrics and gynaecology and NICU. Up to seven regional hospitals including Winchester, Basingstoke, Portsmouth, Salisbury, Poole, Dorset and Isle of Wight all refer to UHS at any given time. (<https://www.uhs.nhs.uk/AboutTheTrust/AboutTheTrust.aspx> , accessed 24th August 2019).

3.4.2 Data acquisition

Consecutive MRSA positive patients from these two UK regional centres were included in this retrospective analysis over a five-year period from April 2011 to November 2016. Anonymised patient data was provided by the Public Health of England Southampton Microbiological Laboratory (PHES) and the Public Health of Wales (PHW). Parameters such as patient demographics including age, gender, clinical location at the time of specimen taken, anatomical origin of the specimen, antibiotic susceptibility patterns and Panton Valentine Leukocidin (PVL) status (when available) were collected and analysed using Microsoft Excel. Ethical approval was deemed unnecessary by the local health boards as the data received from both public health bodies was anonymised at source with no identifiable patient information.

3.4.3 Bacterial handling and testing

Citing the specialist training required to handle and characterise MRSA isolates, microbiology technicians employed by each hospital carried out testing during the five-year study period between 2011 and 2016. My role in this study was to approach the respective public health bodies (PHES and PHW) that record and store the demographics of every recorded MRSA case within their jurisdiction. A standardised methodology of handling and identifying MRSA isolates was confirmed following personal communication with a Consultant Microbiologists at each regional centre. (AAS personal communication with Consultant Microbiologists Dr Bassam Ben-Ismaeil (Swansea) and Dr Graeme Jones (Southampton)).

3.4.3.1 Swansea group of hospitals

The MRSA screening process in the Swansea group of hospitals is based on swabs taken from three anatomical sites – the anterior nares, groin and throat. Additional swabs from medical devices, sputum, urine and wounds were taken if there was a clinically suspicion. Once collected, these samples were transported in charcoal transport medium (Amies, Thermo Scientific, UK), bacterial isolates were plated onto chromogenic MRSA agar plates (P&O Laboratories). Presumptive colonies were sub-cultured onto Columbia blood agar plates (Oxoid Ltd, UK) and identified with a latex agglutination kit (Pro-Lab, Canada). De novo MRSA isolates were further characterised using the Becton-Dickenson Phoenix ID system and mass spectrometry, (MALDI-TOF) (El-Bouri and El-Bouri, 2013, p.)

3.4.3.2 Southampton

MRSA screening using a minimum of two anatomical sites, the anterior nares and groin, was the standard at UHS during the study period. Additional swabs from sputum, medical device exit sites, urine and wounds were also undertaken in the event of clinical suspicion. Bacterial isolates were plated onto *Staphylococcus aureus* chromogenic agar plates (P&O Laboratories). Presumptive colonies were sub-cultured onto Columbia blood agar plates (Oxoid Ltd, UK) and identified with a latex agglutination kit (Pro-Lab, Canada) (AAS personal communication with Consultant Microbiologist Dr Graeme Jones (Southampton)).

3.4.4 Antimicrobial susceptibility

Antimicrobial susceptibility of MRSA is carried out to identify the antibiotic susceptibilities of isolates using a disk diffusion method or titrating the antibiotics until a minimum inhibitory concentrations (MIC) has been achieved. Whilst PCR remains the gold standard for the detection of

MRSA, its expense and time-consumption preclude its use in clinical practice. Therefore, MRSA resistance to Cefoxitin using a disk diffusion test, was used by both regional centres as a confirmatory test to detect MRSA. Antimicrobial susceptibility protocols at both sites were in accordance with the British Society of Antimicrobial Chemotherapy (BSAC) until the introduction of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The Swansea group of hospitals adopted these guidelines in 2015, whilst University Hospital Southampton joined in 2018 (AAS personal communication with Dr Graeme Jones, Consultant Microbiologist, Southampton).

3.4.5 Statistical analysis

Statistical analysis of the mean number of MRSA cases between the clinical venues (in-patient, general practice and out-patients) were evaluated a two-way ANOVA referring to Tukey's multiple comparison test. Data reporting a p value of ≤ 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism statistical software (release 8.0c; GraphPad Prism, Inc., California, USA)

3.5 Results

MRSA is most prevalent amongst males and inpatients in the Swansea group of hospitals

Amongst a study population of 12,138 proven MRSA swabs, the majority of these cases arose from male patients (55.5%). The mode of acquisition was made based on the referral sources from where MRSA swabs were taken. The majority (6607/12138) of these samples came from hospital/healthcare MRSA related sources such as established inpatients >48 hours and high-risk areas such as intensive care (ICU) and haemodialysis. The remaining samples came from community associated sources such as general practice, outpatients, emergency department and others listed in Table 3.1.

The clinical location of where the MRSA samples were taken from was widened in 2015 to include ante-natal and private patients. Due to the retrospective nature of the data provided by Public Health of Wales, further analysis of the inpatient MRSA swabs received over this period was not possible.

A lot of change is witnessed over the years of 2014 and 2015. This is may be explained by a number of local and important factors such as the widening of the number of clinical locations recorded to have sent the MRSA sample. Second, the Trust changed combination of anatomical swabs based on a published departmental retrospective study. Third, there was a change to the information and analytical system used in the Trust, adopting a Laboratory Information Management System (LIMS) in 2015. Lastly, the regional laboratory changed from using British Society of Antimicrobial Chemotherapy (BSAC) and adopting the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines on reporting antibiotic susceptibilities and breakpoints. These are represented in Figure 3.5

However, a number of important MRSA reportable domains were not readily available and could not be established from the Trust and/or the Public Health of Wales. These include MRSA-related bacteraemia, SSIs and the number of samples tested for PVL carriage during the study period from 2011 to 2016. The total number of MRSA samples and the number of patients sampled was not provided. However, the anatomical swab site(s) routinely sampled were provided. (Source of data: Public Health of Wales)

| | Year | | | | | | Total |
|-------------------------------------|------|------|------|------|------|------|-----------------|
| | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | |
| In-Patient | 1138 | 1274 | 1176 | 1290 | 797 | 666 | 6341 |
| General Practice | 619 | 694 | 658 | 589 | 441 | 328 | 3329 |
| Out-Patient | 254 | 265 | 288 | 269 | 190 | 150 | 1416 |
| Emergency Department | 41 | 46 | 30 | 54 | 59 | 85 | 315 |
| Intensive Care Unit | | | | | 77 | 96 | 173 |
| Renal & Dialysis | | | | | 37 | 43 | 80 |
| Outpatient Rapid Turn-around | | | | | 12 | 19 | 31 |
| Private Patient | 2 | 3 | 1 | 10 | 4 | 2 | 22 |
| Day Case | 3 | 1 | 2 | 1 | 4 | 3 | 14 |
| Ante-natal | | | | | 6 | 7 | 13 |
| Environmental | | | | | 2 | 5 | 7 |
| Community | | | | | 2 | 5 | 7 |
| Occupational Health | | | | | 2 | | 2 |
| Other | | | | | 106 | 19 | 125 |
| Unknown | 77 | 48 | 68 | 69 | 1 | | 263 |
| Total | 2134 | 2331 | 2223 | 2282 | 1740 | 1428 | 12138 |
| | | | | | | | |
| CA-MRSA | 917 | 1006 | 978 | 913 | 712 | 595 | 5121 (42.4%) |
| HA-MRSA | 1138 | 1274 | 1176 | 1290 | 917 | 812 | 6607 (54.4%) |
| Unknown | 79 | 51 | 69 | 79 | 111 | 21 | 410 |
| Total | 2134 | 2331 | 2223 | 2282 | 1740 | 1428 | 12138 |
| | | | | | | | |
| Male | 1204 | 1218 | 1181 | 1305 | 956 | 858 | 6722 (55.4%) |
| Female | 912 | 1093 | 1018 | 959 | 774 | 563 | 5319 (43.8%) |

Table 3. 1 MRSA prevalence, mode of acquisition and gender according to clinical venue from 2011 to 2016 (Swansea).

MRSA is most prevalent and statistically significant amongst hospital inpatients in the Swansea group of hospitals

Statistical significance is observed between the three commonest locations of MRSA carriage (inpatients, general practice and outpatients). Using a Tukey's multiple comparison test, a significant difference in the number of MRSA patient carriage is observed between inpatients versus general practice population (mean difference 502 cases, 95% CI 240.3 - 763.7, adjusted $p < 0.0005$) and inpatient versus outpatient (mean difference 820 cases, 95% CI 559.1 - 1083, adjusted $p < 0.0001$). Due to MRSA reporting in ICU and renal and dialysis only starting in 2015, further subgroup analysis was not possible. (* $p = 0.016$; *** $p = 0.0005$).

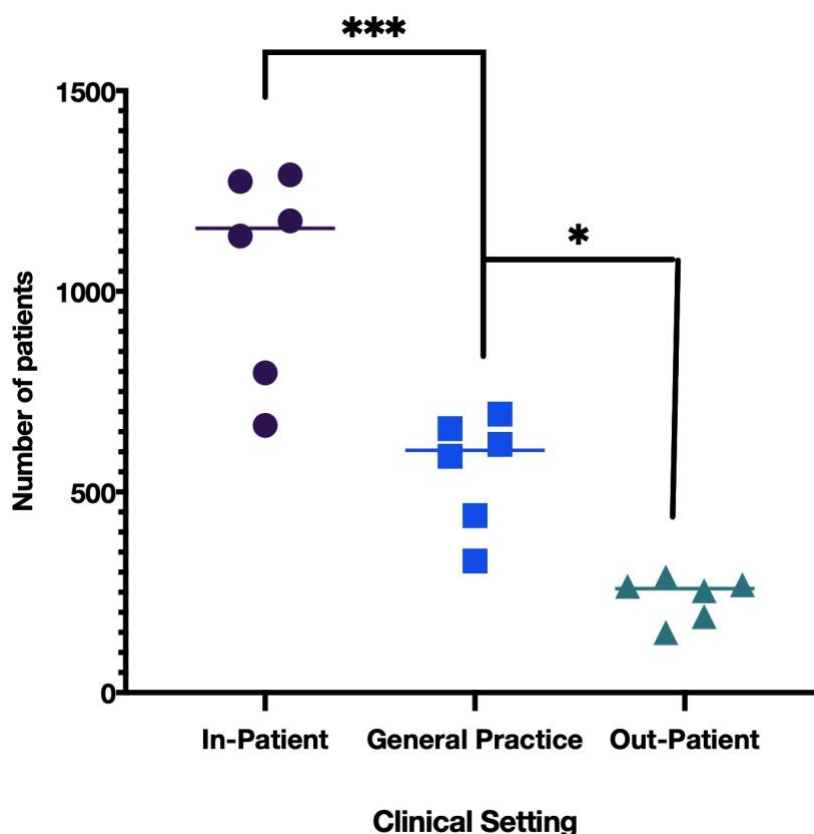


Figure 3. 1 Prevalence of MRSA amongst study population (Swansea)

CA-MRSA is more prevalent in the Swansea group of hospitals up to the age of 40. HA-MRSA is most prevalent in the patients aged 71-90 years old and statistically significant in comparison to the younger population.

Considering the decline of the total number of MRSA cases between 2011 to 2016, an isolated rise in HA-MRSA was observed in 2015, accounting for 56.5% of cases, a 3.6% rise in comparison to 2014. CA-MRSA accounted for 42.2% of cases. During the study period, a peak of 44% CA-MRSA cases were recorded in 2014. Over the course of the following two years, the lowest rates of CA-MRSA carriage were recorded, 40% and 40.9% respectively. HA-MRSA carriage over the five-year study period, in total, accounted for 52.2% of all cases.

Using the mean difference between the sub-groups of age, there is no statistical significance in the rise of the CA-MRSA cases between the age of 0-5 up to 41-50 years old mean difference -49.17, 95% CI (-105.3 to 6.952), $p=0.14$. In comparison, this rise in CA-MRSA prevalence becomes significant when comparing the 41-50 age group to 61-90 age group; 41-50 to 61-70 years old mean difference -72.67, 95% CI (-128.8 to -16.55), $p=0.021$; 41-50 to 71-80 years old mean difference -135.7, 95% CI (-191.8 to -79.55), $p<0.0001$; 41-50 to 81-90 years old mean difference -126.3, 95% CI (-182.5 to -70.21), $p<0.0001$.

Meanwhile, HA-MRSA prevalence becomes statistically significant in the population aged 51-60 years old, in comparison to the ages of 0-40 years old; 0-5 to 51-60 years old mean difference -102.5, 95% CI (-158.6 to -46.38), $p<0.0001$; 6-10 to 51-60 years old mean difference -113.5, 95% CI (-169.6 to -57.38), $p<0.0001$; 11-20 to 51-60 years old mean difference -109.3, 95% CI (-165.5 to -53.21), $p<0.0001$; 21-30 to 51-60 years old mean difference -92.50, 95% CI (-148.6 to -36.38), $p<0.0001$; 31-40 to 51-60 years old mean difference -87.83, 95% CI (-144.0 to -31.71), $p<0.0001$.

Within the population with HA-MRSA, those aged 71-90 years have the highest prevalence. When comparing this to the 51-60 years old group, this difference is statistically significant mean difference -177.7, 95% CI (-233.8 to -121.5), $p<0.0001$.

In those aged >91 years old, the prevalence of both CA-MRSA and HA-MRSA is significantly lower than those aged 81-90 years old (CA-MRSA 81-90 versus >91 years old mean difference 131.2, 95% CI (75.05 to 187.3), $p<0.0001$; HA-MRSA mean difference 216.5, 95% CI (160.4 to 272.6), $p<0.0001$).

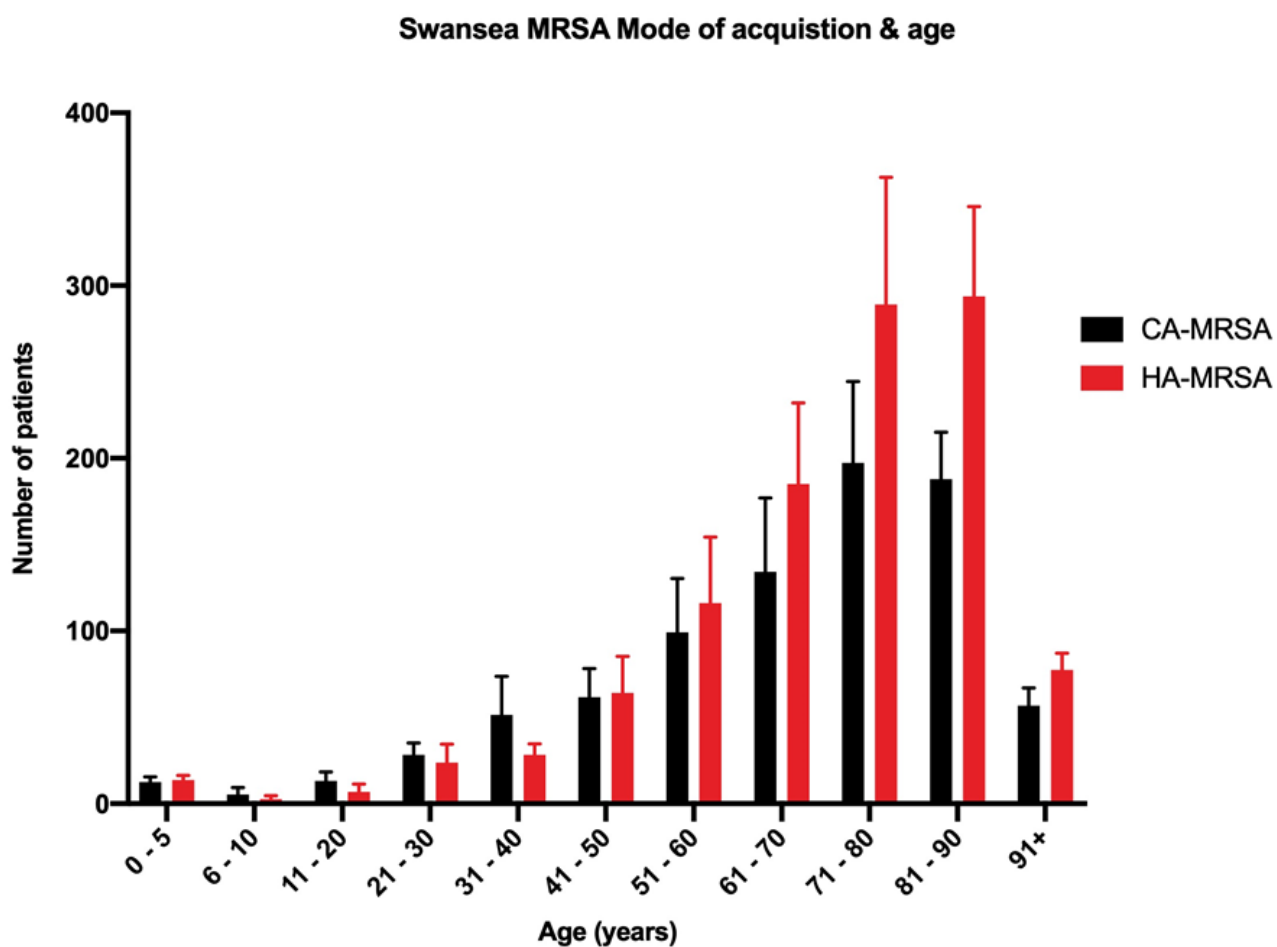


Figure 3. 2 HA-MRSA and CA-MRSA prevalence by age (Swansea)

More than two-thirds of all MRSA positive swabs in the Swansea group of hospitals came from screening and wounds.

A targeted screening process (patients with a high-risk or clinical suspicion of MRSA carriage) was adopted by the Swansea group of hospitals before and during the study period. As stated earlier, the Trust changed combination of anatomical swabs based on a published departmental retrospective study (El-Bouri *et al.* 2013). Since 2015, the standardised set of swabs sites sampled for targeted MRSA screening has included the anterior nares, groin and throat (El-Bouri *et al.* 2013). In total, 8506 (70%) MRSA swabs were identified from targeted screening swabs and wounds.

Positive MRSA swabs samples taken from joints, synovial fluid, limbs and amputations, classified as bone, contributed to a large portion to the total number of positive MRSA samples 2011 (n=422) and 2012 (n=448). The number of MRSA positive blood cultures and urine samples remained static over the study period (blood culture n=29 (median), interquartile range (IQR) 23 - 34.25); urine (n=42.5 (median), IQR 37-57). Other common anatomical sites, such as the head and neck, genitalia and the abdomen, witnessed a decline in the number of reported cases during the study period.

Swansea MRSA (anatomical site)

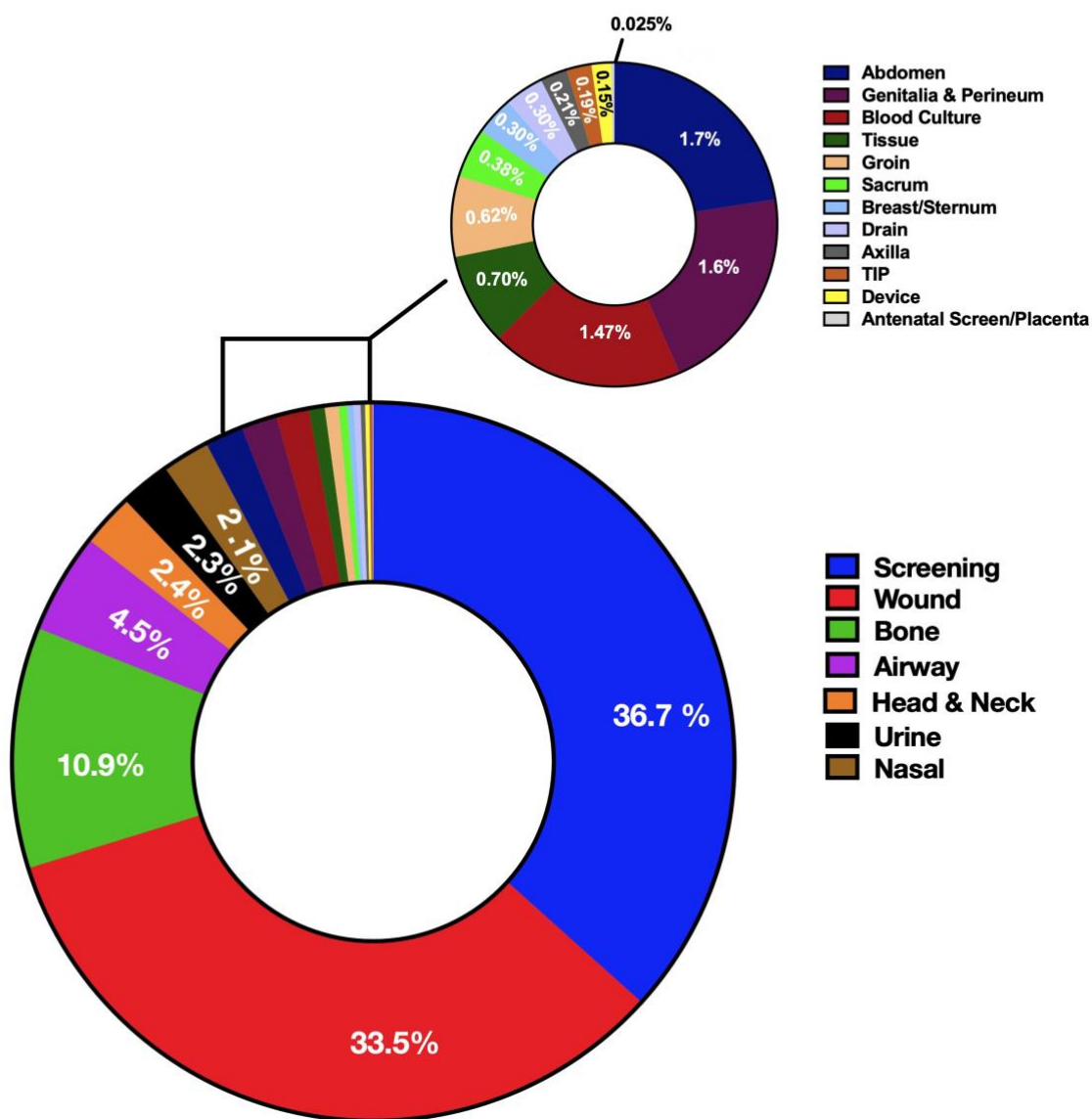


Figure 3. 3 Anatomical origin of positive MRSA samples (Swansea).

Prevalence of MRSA amongst the elderly population fell in 2015 with reporting bias likely to be a contributing factor.

As described in Figure 3.2, MRSA is most prevalent in the elderly population within the Swansea group of hospitals. However, a great variance in the number of cases reported during the study period can be identified. An incremental rise in the prevalence of MRSA is seen with age peaking between the ages of 71 to 90, accounting for 5980 (49.3%) of all cases from 2011 to 2016 in Swansea. During this period, a 42% reduction in the number of cases in the 71-80 age group is observed from 505 cases in 2011 to 289 in 2016. Meanwhile, a 16% reduction in prevalence over the course of the six-year study period is witnessed in the 81-90 age group. A sharp decline in prevalence is seen in the 91+ age group (6.8%). Due to the change of a number of standard operating procedures implemented to MRSA testing and reporting in 2015, this may represent an element of bias in the reporting. Similarly, due to the retrospective data provided by Public Health of Wales, further interrogation and validity of the data was not possible.

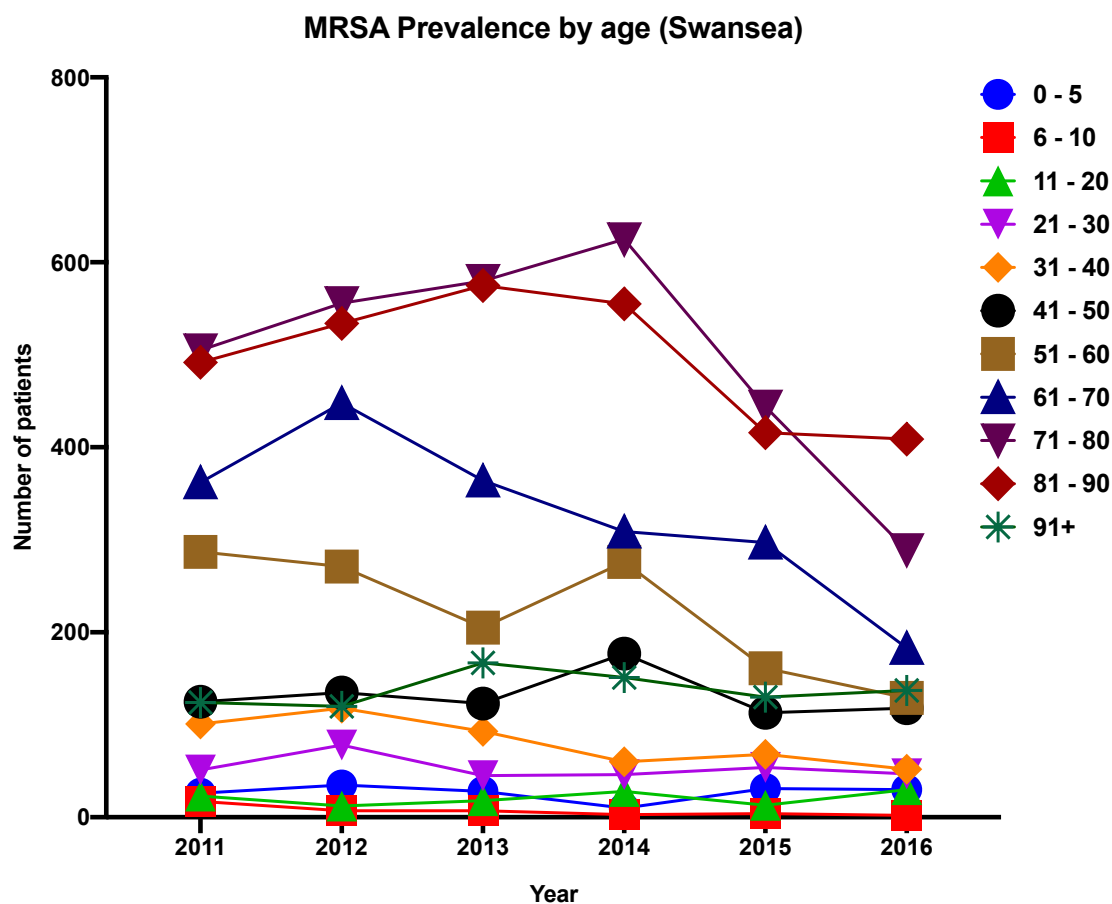


Figure 3. 4 Age distribution of MRSA prevalence (Swansea)

Commonly utilised antibiotics for the prophylaxis of surgical MRSA carriers such as Vancomycin and Gentamicin remain

The data acquired from PHW contained the antibiotic sensitivities of the MRSA isolates received during the study period. These included the list of current antibiotics considered to be sensitive in the treatment of MRSA patients requiring systemic antibiotics. The sensitivity of MRSA strains to Vancomycin, Gentamicin and Mupirocin remained consistent over the five-years with little variation.

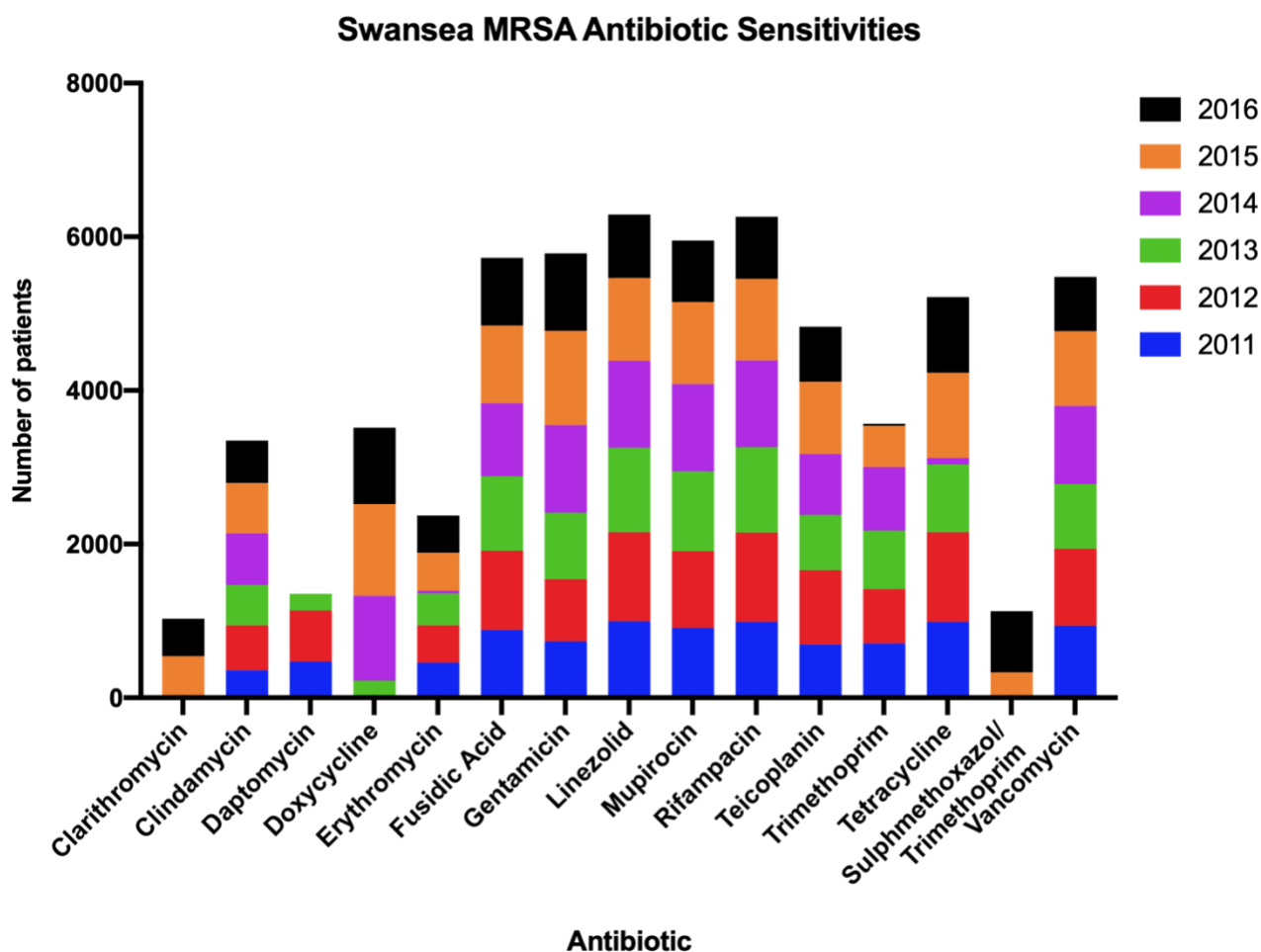


Figure 3. 5 Antibiotic resistance and susceptibility patterns (Swansea)

CA-MRSA is the dominant form of MRSA acquisition in Southampton.

Since April 2009, a universal policy of MRSA screening has been adopted and implemented at University Hospital Southampton of all elective patients. This was extended to all emergency patients in December 2010. Upon request, a number of desired MRSA domains and outcomes were available from the Public Health of England Southampton (PHES) Microbiological Laboratory. These include the number of CA-MRSA and HA-MRSA cases (defined at source by PHES) and the number of samples tested for PVL carriage (Table 3.2). The epidemic strain of MRSA isolates (EMRSA) was also recorded and results found in Table 3.3. Whilst the rate of MRSA bacteraemia was routinely recorded by the PHES (Figure 3.7), other desired MRSA-related outcomes such as SSIs and the clinical location of where samples came from was not available.

A total of nearly 1.2 million MRSA screens from 443,233 patients were taken over the study period, with 2032 positive MRSA carriers. A total MRSA prevalence of 0.46% is calculated from the dataset provided by the Public Health of England Southampton (PHES) Microbiological Laboratory from April 2011 to November 2016 (Table 3.2). The majority (51.6%) of samples came from male patients. The primary outcome of this study of confirming the prevalence of CA-MRSA (68%) and HA-MRSA (26%) was readily available and defined at source by the PHES according to the clinical presentation and within 48 hours of hospital admission in the absence of HA-MRSA risk factors.

PVL testing was performed. 56.8% of all samples were tested for PVL carriage, with 4.9% of these positive for detection of the PVL exotoxin. Only 878 out of the 2032 MRSA cases were not tested for PVL carriage.

| | Year | | | | | | Total |
|--------------------------|--------|--------|--------|--------|--------|--------|-----------------|
| | 2011 | 2012 | 2013 | 2014 | 2015 | 2016 | |
| 0 - 5 | 28 | 39 | 32 | 24 | 17 | 17 | 157 |
| 6 - 10 | 4 | 11 | 3 | 5 | 3 | 4 | 30 |
| 11 - 20 | 7 | 10 | 12 | 13 | 20 | 8 | 70 |
| 21 - 30 | 20 | 16 | 20 | 19 | 26 | 14 | 115 |
| 31 - 40 | 25 | 26 | 34 | 21 | 26 | 25 | 157 |
| 41 - 50 | 22 | 34 | 33 | 18 | 27 | 28 | 162 |
| 51 - 60 | 23 | 39 | 50 | 31 | 31 | 22 | 196 |
| 61 - 70 | 39 | 52 | 35 | 46 | 46 | 35 | 253 |
| 71 - 80 | 58 | 66 | 69 | 67 | 57 | 44 | 361 |
| 81 - 90 | 67 | 87 | 67 | 58 | 75 | 39 | 393 |
| 91+ | 24 | 41 | 21 | 20 | 20 | 8 | 134 |
| Unknown | 2 | 1 | 0 | 1 | 0 | 0 | 4 |
| | 319 | 422 | 376 | 323 | 348 | 244 | 2032 |
| | | | | | | | |
| Male | 161 | 212 | 212 | 178 | 189 | 132 | 1084 (53.5%) |
| Female | 158 | 207 | 163 | 145 | 158 | 112 | 943 (46.5%) |
| | | | | | | | |
| Patients screened | 56678 | 76681 | 77930 | 78907 | 79736 | 73301 | 443233 |
| Number of screens | 151035 | 202324 | 207330 | 210426 | 220730 | 202178 | 1194023 |
| | | | | | | | |
| CA-MRSA | | | | | | | 1382 (68%) |
| HA-MRSA | | | | | | | 529 (26%) |
| Not recorded | | | | | | | 121 (6%) |
| | | | | | | | |
| PVL +ve | | | | | | | 99 (4.9%) |
| PVL -ve | | | | | | | 1055 (51.9%) |
| Not tested | | | | | | | 878 (43.2%) |

Table 3. 2 MRSA prevalence from 2011 to 2016 (Southampton)

The antibiotic susceptibility of MRSA samples in Southampton were tested against a limited panel of antibiotics (Ciprofloxacin, Mupirocin, Neomycin and Fusidic Acid).

The epidemic strain of MRSA strains (EMRSA) was tested in 43.9% of the Southampton population. Of all the samples tested, 834 (93.4%) were phenotyped as EMRSA-15. The remaining cases were typed as EMRSA-16. In total, 1139 cases (56%) remained untested.

Antibiotic susceptibility, a desired MRSA-reported outcome, was performed using a limited panel of antibiotics. Details of which are tabulated below (Table 3.3)

| MRSA phenotype & Antibiotic susceptibility | n= |
|--|-------------|
| EMRSA 15 | 834 |
| MRSA 15* | 606 |
| MRSA 15 Ciprofloxacin Sensitive | 189 |
| MRSA 15 Ciprofloxacin Sensitive Neomycin Resistant | 1 |
| MRSA 15 Ciprofloxacin Resistant | 1 |
| MRSA 15 Ciprofloxacin Sensitive Fusidic Acid Sensitive | 1 |
| MRSA 15 Mupirocin Resistant | 21 |
| MRSA 15 Neomycin Resistant | 15 |
| EMRSA 16 | 59 |
| MRSA 16* | 50 |
| MRSA 16 Mupirocin Resistant | 1 |
| 16 Ciprofloxacin Sensitive | 4 |
| 16 Gentamicin Resistant | 1 |
| 16 Mupirocin Resistant | 1 |
| 16 Neomycin Resistant | 2 |
| MRSA (Unclassified) | 1139 |
| MRSA (Unclassified)* | 630 |
| MRSA Ciprofloxacin Sensitive | 287 |
| MRSA Ciprofloxacin Resistant | 1 |
| MRSA Gentamicin Resistant | 1 |
| MRSA Mupirocin Resistant | 14 |
| MRSA Ciprofloxacin Sensitive and Mupirocin Resistant | 3 |
| Not recorded | 203 |

Table 3. 3 Epidemic strain and phenotype of MRSA samples.

CA-MRSA is prevalent in Southampton in neonates and young children up to the age 5 and the elderly. The prevalence of HA-MRSA and CA-MRSA in the elderly aged 71-90 years old is statistically significant in comparison to the remaining population, including those aged >91 years old.

CA-MRSA carriage over the five-year study period, in total, accounted for 68% of all cases. In contrast, only 529 cases (24%) were attributed to HA-MRSA sources. The decision on whether MRSA isolates were community acquired (CA-MRSA) and hospital acquired (HA-MRSA) was made at source by the PHES Microbiological Laboratory. Therefore, information relating to which clinical location (general practice, outpatients, haemodialysis, ICU etc.) the positive MRSA samples were received from was not readily available and could not be differentiated from the dataset provided by the PHES.

Subgroup analysis using a Tukey's multiple comparison test, there is a statistically significance in the number of CA-MRSA cases in children aged 0-5 years compared to those aged 6-10 years old (mean difference 12.83, 95% CI (3.678 to 21.99), $p=0.0005$). Up to the age of 60, there is no significant change in CA-MRSA prevalence in comparison to those aged 0-5 (mean difference -6.000, 95% CI (-15.16 to 3.155), $p=0.54$). No statistical significance in the prevalence of CA-MRSA between those aged 21-30 up to 51-60 years old is observed (mean difference -7.667, 95% CI (-16.82 to 1.489), $p=0.19$). A similar picture is observed when comparing those aged 31-40 up to 61-70 years old (mean difference -5.500, 95% CI (-14.66 to 3.655), $p=0.66$). However, the prevalence of CA-MRSA becomes significant when comparing those aged 31-40 years old to patients 71-80 years old (mean difference -11.33, 95% CI (-20.49 to -2.178), $p=0.0041$) and 81-90 years old (mean difference -24.00, 95% CI (-33.16 to -14.84), $p<0.0001$) respectively. This trend of increasing CA-MRSA prevalence with age remains up to the those aged 81-90 years old and in comparison, to those aged 71-80 years old is significant (mean difference -12.67, 95% CI (-21.82 to -3.511), $p=0.0007$).

In comparison, HA-MRSA prevalence between neonates and children up to the age of 5 years is not statistically significant up to the age of 70 (mean difference -4.500, 95% CI (-13.66 to 4.655), $p=0.87$). HA-MRSA in Southampton, during the study period, is most prevalent in those aged 71-80 years old. The prevalence of HA-MRSA in this subgroup is not significant to those aged 81-90 (mean difference 6.333, 95% CI (-2.822 to 15.49), $p=0.46$), however becomes significant in comparison to those aged 91 years and older (mean difference 17.33, 95% CI (8.178 to 26.49), $p<0.0001$) (Figure 4.6).

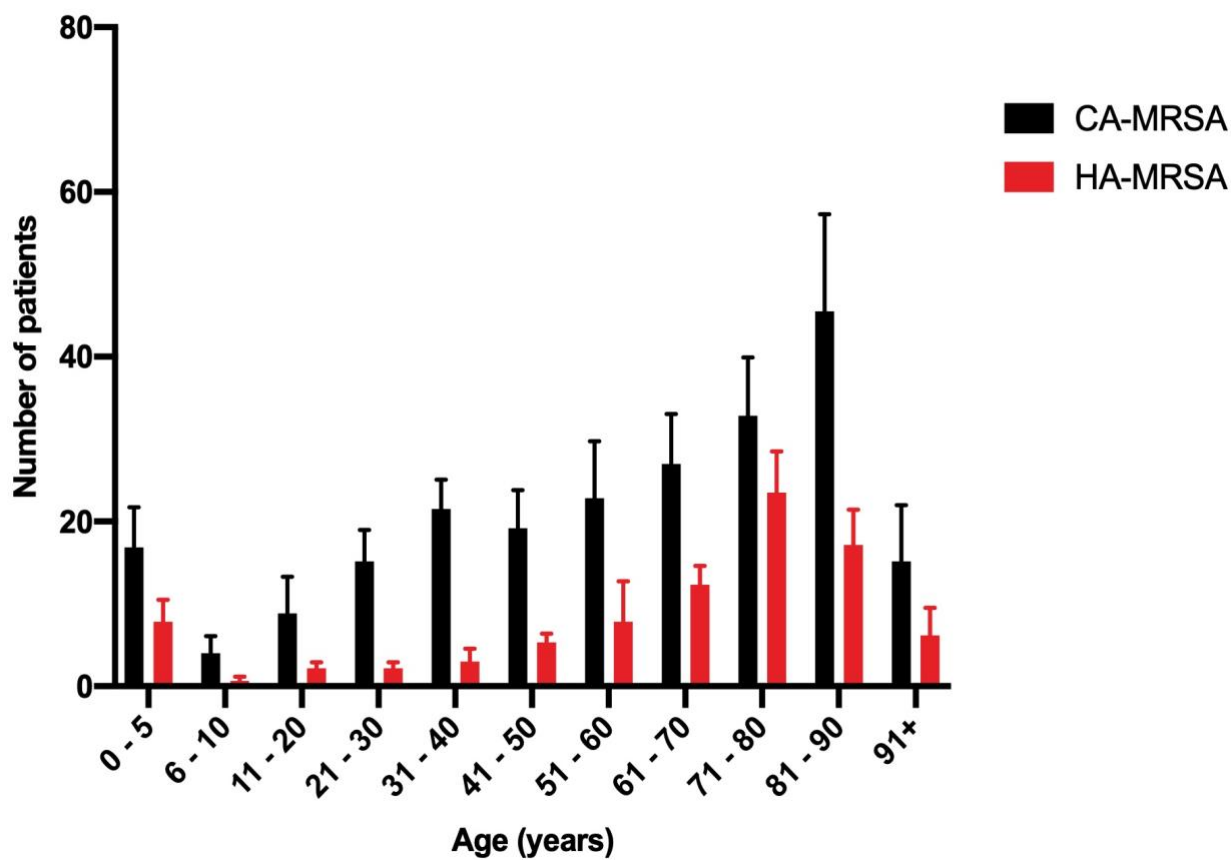
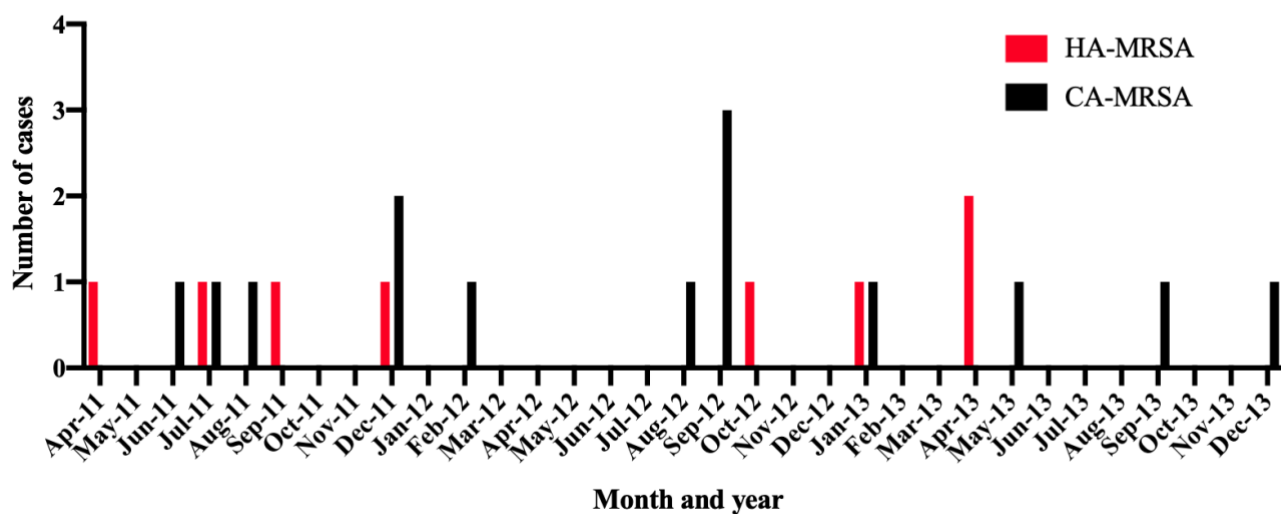
Southampton MRSA Mode of acquisition by age

Figure 3. 6 HA-MRSA and CA-MRSA prevalence by age (Southampton)

MRSA related bacteraemia in Southampton are more prevalent in patients with HA-MRSA

During the study period, 60% (24/40) of all total bacteraemia were attributed to CA-MRSA. Meanwhile, 16 HA-MRSA related bacteraemia were related during this time. Considering the proportions of CA-MRSA cases versus HA-MRSA in Southampton, the rate of bacteraemia is higher in those with HA-MRSA (3%) than CA-MRSA (1.73%). Data pertaining to MRSA related surgical site infections was not available.

Southampton MRSA Bacteraemias 2011 - 2013



Southampton MRSA Bacteraemias 2014 - 2016

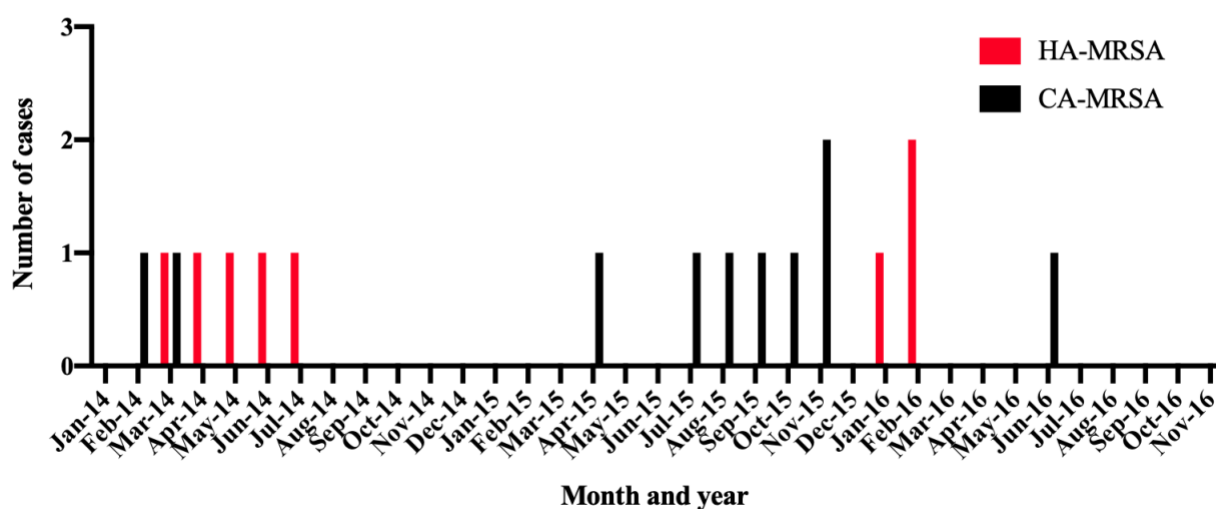


Figure 3. 7 The prevalence of MRSA related bacteraemia from 2011 to 2016 (Southampton).

Swabs from the anterior nares and groin yield the highest number of MRSA positive screens in Southampton area.

Local departmental policy favours a universal method of screening (all emergency and elective patients admitted to hospital) using a combination of two-site swabs (anterior nares and groin) at the time of screening at the University Hospital Southampton. In combination, 76% of all MRSA positive screens were from the anterior nares and groin. During the study period, 15% of positive MRSA swabs, classified as a wound, were taken from samples labelled as skin, abscess, pus and gastrostomy (Figure 3.8)

Southampton MRSA (anatomical site)

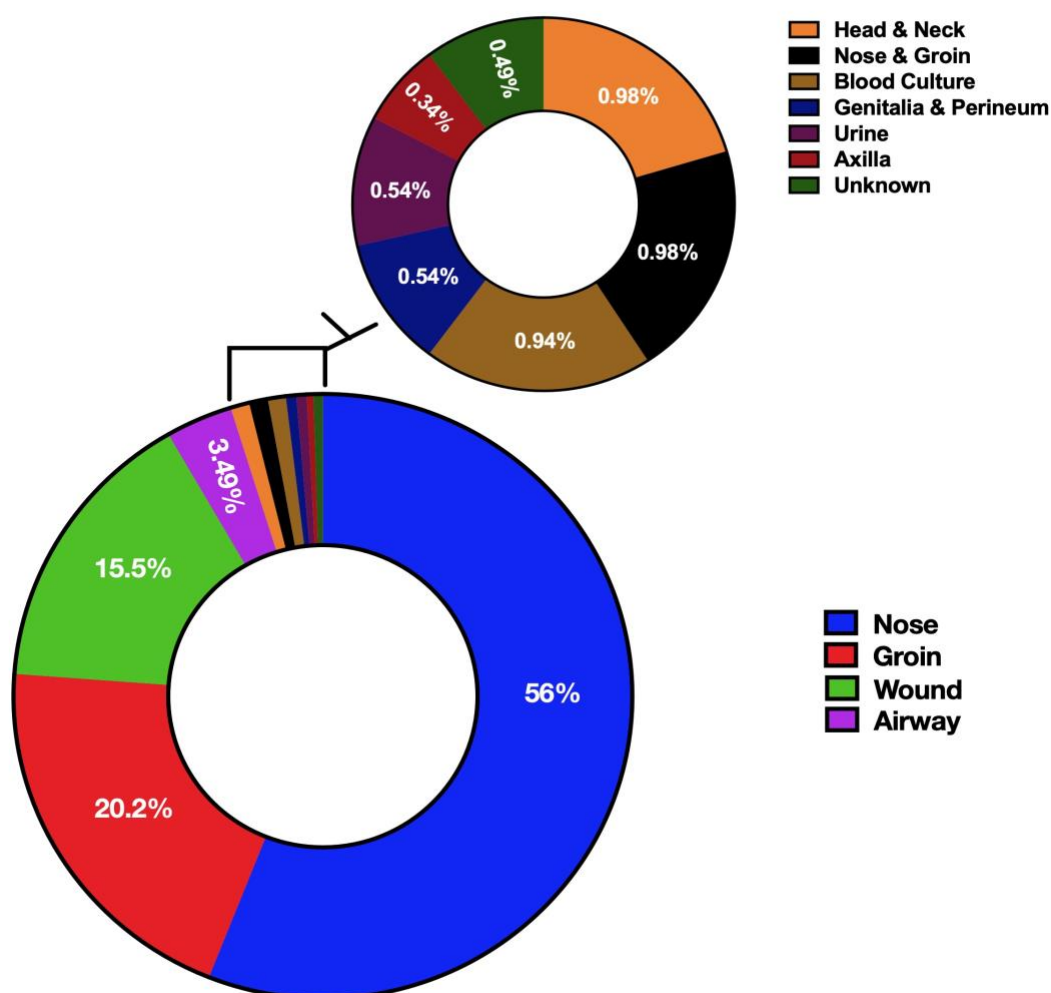


Figure 3. 8 Anatomical origin of positive MRSA samples (Southampton).

MRSA is largely prevalent in the elderly of Southampton, however, the number of CA-MRSA cases in those aged 0-5 and 31-70 also contribute to the burden of MRSA in the region.

UHS is a tertiary referral centre to a number of district general hospitals in the region, especially for paediatric surgical and intensive care services. This axis of referrals may be hypothesised as a contributor in the spike in numbers of children aged 0-5 testing positive for MRSA in this age group. The burden of disease from those aged 71-90 years old is high. In 2013, 2014 and 2016, prevalence was highest amongst those aged 71-80 in comparison to those aged 81-90 years old. The roles are reversed in 2011, 2012 and 2015. The age group of those aged 91 years and older represent an interesting finding of significantly lower prevalence than in the previous two decades. The immune status, fitness/mobility and clinical history of this sub-group and reasons behind the low prevalence of MRSA is not well understood and represents a potential research question.

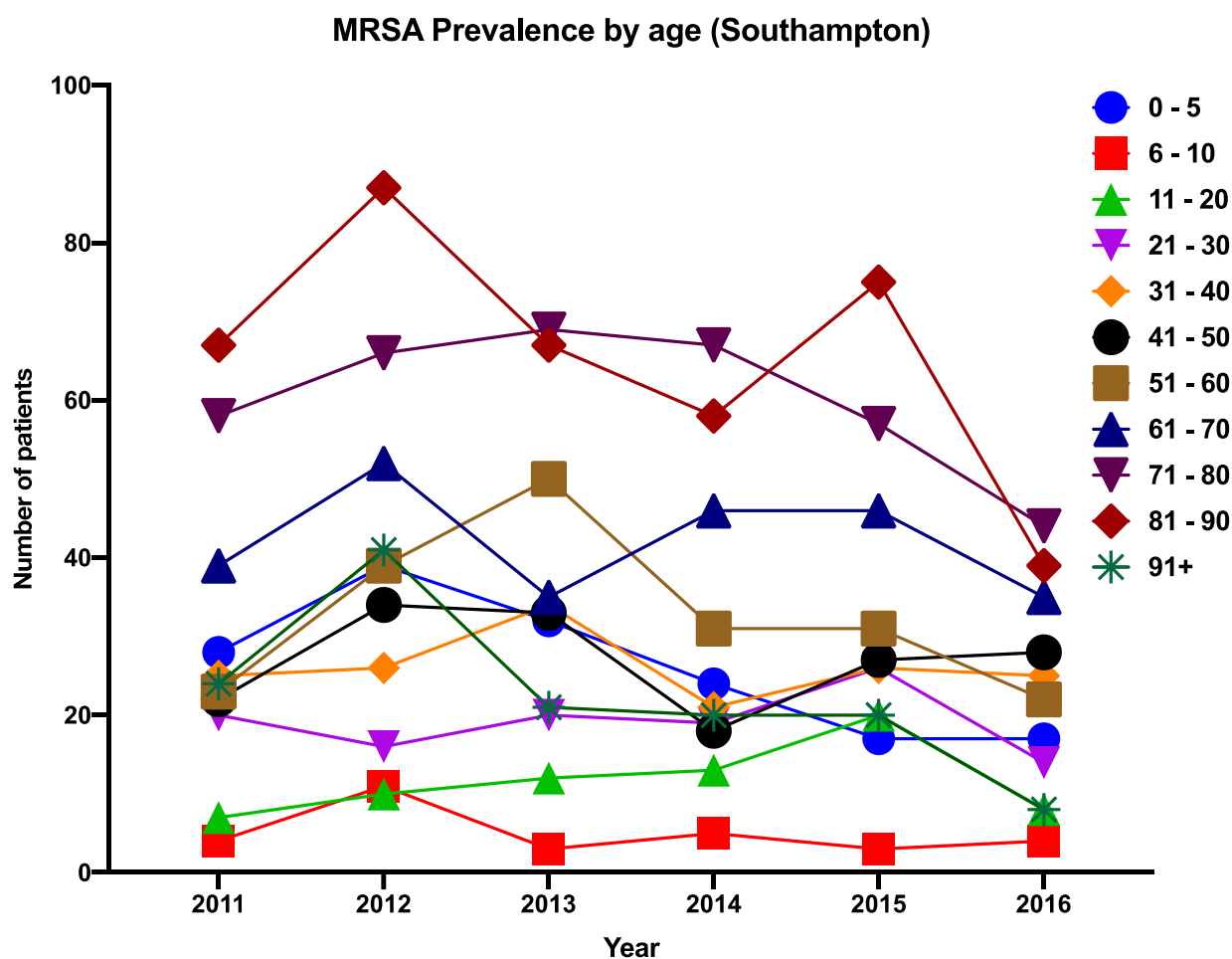


Figure 3. 9 Age distribution of MRSA prevalence (Southampton).

3.6 Discussion

The call to understand the prevalence of HA-MRSA and CA-MRSA in the context of MRSA HAIs including SSIs has been repeatedly mentioned in the literature (Bode *et al.*, 2016; Berríos-Torres *et al.*, 2017; Tandon *et al.*, 2017; Kline *et al.*, 2018; Tsang *et al.*, 2018). There is a common concern that SSIs attributed to *Staphylococcus aureus*, including MRSA, are occurring frequently, becoming clinically complex and, over time, economically strenuous.

Built on these concerns, this study proposed to consider the lack of MRSA-related reportable outcomes. Based on a retrospective cohort study, covering two UK regions, one in England and the other in Wales, the hope is that with presenting the commonalities and differences in practice, will encourage debate about future planning and strategies needed improve the level of success associated with the treatment of MRSA-related infections.

To my knowledge, this type of comparative study, over a five-year period, has not been performed in the UK. Considering that CA-MRSA related infections are becoming increasingly reported worldwide, this study aims to add into that conversation based on the experiences of two UK regions. This study was only possible with the co-operation from the respective Public Health bodies, Public Health of Wales (PHW) and Public Health of England Southampton (PHES) Microbiological Laboratory. Based on their information, a clear difference of data collection and reporting of MRSA-related outcomes was evident.

The primary outcome, to report the prevalence of CA-MRSA and HA-MRSA, were achieved from both regional centres. As described in the results section of this chapter, CA-MRSA is the more prevalent form of MRSA acquisition in the Southampton region, based on the data provided. However, due to the format in which the data was provided, this precluded further interrogation of the population characteristics. Therefore, the requirement to refer to the Office of National Statistics (ONS) data from the 2011 Census was felt to be needed. According the ONS, the number of residents born outside the UK (from EU member states and other countries) was estimated to be around 17% based at the time of the 2011 census. Whilst, this fact may be relevant in understanding the preponderance of CA-MRSA in the Southampton region, it only represents one point of view and explanation for this finding.

Meanwhile, in the Swansea group of hospitals, HA-MRSA (55.4%) was the more prevalent form of MRSA acquisition. In a similar fashion, using the ONS data from the 2011 Census, the population in this area is different from Southampton. HA-MRSA is the predominant form amongst the Swansea and Bridgend population, with 6722 (56.3%) reported cases. This finding is in keeping with previous

reports until now implicating HA-MRSA as the dominant strain in the UK (Jumaa *et al.*, 2007; Saeed *et al.*, 2014). Closer inspection of the 2011 census alludes to two likely reasons for this. Firstly, this Welsh region has a high proportion of residents reporting long-term health difficulties and or disabilities. Nearly a quarter of the population in Swansea (23.4%) and Bridgend (24.7%) report a limitation to their activities based on their health. Secondly, a larger number of residents in this area are housed in communal accommodation with or without nursing care. Residents of nursing and care home are a high risk group for HA-MRSA carriage (Grundmann, 2002; Millership, Harris and Batchelor, 2006; Barr *et al.*, 2007). The reported prevalence of MRSA in nursing homes has ranged from 0.8 to 22% (Barr *et al.*, 2007; Cox *et al.*, 1999; Fraise *et al.*, 1997). A significant proportion of these patients have an invasive device, long-term wound or previous exposure to healthcare associated facilities. Thus, it is has long been established that nursing homes are a reservoir for not only *de novo* MRSA colonisation but a high-risk arena for further recycling and re-infection (Barr *et al.*, 2007; Cox *et al.*, 1999; Fraise *et al.*, 1997; Grundmann, 2002; Millership *et al.*, 2006).

Within this Welsh population, samples collected from CA-MRSA related sources such as general practice, outpatients and emergency departments, are considerable nevertheless accounting for 42% of all MRSA cases. Whilst these MRSA specimens were obtained from CDC defined community associated sources, there is a school of thought that argues that these are merely an escape or overspill of MRSA from hospital and healthcare associated sources (Bygott *et al.*, 2008; Rollason *et al.*, 2008; David and Daum, 2010)

The pheno and genotype of MRSA carriers in a UK community was been performed in 2008 (Bygott *et al.*, 2008; Rollason *et al.*, 2008). In one study (Rollason *et al.*, 2008), a total of 199 MRSA samples were obtained from patients presenting to their general practice with a skin or soft tissue infection. There was a common theme in 173 (87%) of isolates, all harbouring the SCC*mec* IV type, similar in genetic lineage to their region's hospital related strains, EMRSA-15 and 16 (Rollason *et al.*, 2008). The authors also report that 17% of the samples came from patients living at a long-term facility and 74% of samples came from patients aged 65 or above, with the vast majority (62%) of the study group reporting either inpatient or outpatient contact up to a year preceding the study (Rollason *et al.*, 2008). As expected, none of the MRSA samples in the study carried the PVL gene, reinforcing the idea of healthcare associated MRSA escaping into the community (Rollason *et al.*, 2008).

True CA-MRSA is characteristically susceptible to antimicrobials and resistant to beta-lactam antibiotics (Adedeji, Weller and Gray, 2007), distinguishing it from its HA-MRSA counterpart. At a

molecular level, HA-MRSA strains carry a large staphylococcal chromosomal cassette *mec* (SCC*mec*) belonging to types I, II and III and exhibits a high level of resistance to non- β -lactam antibiotics (David et al., 2010) and is thought to be the common form of transmission in the UK (Jumaa et al., 2007; Rollason et al., 2008; Ellington et al., 2009). In comparison, CA-MRSA carries a shorter chromosomal cassette, carrying type IV or V (David et al., 2010). The clinical severity of CA-MRSA related infections is attributed to their carriage of the cytotoxin PVL gene. The virulence of PVL is explained by the synergism of two separate exoproteins, *LukS-PV* and *LukF-PV* (Holmes et al., 2005; Ellington et al., 2009). Infections as a subsequence of a PVL positive CA-MRSA are known to be severe and invasive in the skin and soft tissues, and have been reported in cases of necrotising fasciitis, pneumonia and invasive osteomyelitis (Holmes et al., 2005; Tristan et al., 2007). As the rates of PVL positive CA-MRSA cases in the UK are thought to be low (Adedeji, Weller and Gray, 2007), the referral to a notable systematic review (Nellums et al., 2018) offering an evidence-base to the risk of CA-MRSA and PVL carriage amongst migrants to Europe is critical. Nellums et al calculated a pooled prevalence of MRSA in migrants was 7.8% (Nellums et al., 2018). Countries such as Sweden, Germany, Italy, Spain, Switzerland and The Netherlands (Angeletti et al., 2016; Broseta et al., 2006; Casado-Verrier et al., 2012; Cercenado et al., 2008; Dudareva et al., 2011; Frick et al. 2010; Gustaffson et al., 2006; Hagleitner et al., 2012; Heudorf et al., 2016; Kruger et al., 2016; Manzur et al., 2007; Piso et al., 2017; Rauensburgen et al., 2015; Reinheimer et al., 2016; Steger et al., 2016; Stenheim et al., 2010; Tenebaum et al., 2016) all reported a high prevalence of community-associated MRSA amongst refugees and asylum seekers (Nellums et al., 2018). As a result, southern European countries, such as Greece (39.2%), Italy (34.1%) and Spain (25.3%) have found a surge, in not only the total population prevalence of MRSA, but a higher proportion of PVL positive CA-MRSA isolates (Angeletti et al., 2016; Nellums et al., 2018; Piso et al., 2017).

Back to the UK, PVL testing is not routinely performed unless of clinical suspicion or contact with a PVL +ve case (HPA 2012). In Southampton PVL testing was performed in 56.8% (1054/2032) of all positive MRSA patients. PVL carriage was proven in 4.9% of all samples received. Due to the lack of heterogeneity in MRSA testing patterns reported MRSA outcomes, in the UK as a whole, prevents further analysis and comparison. Despite this, a PVL +ve rate of 5% is significant and although there are no comparators to understand the significance of this rate, it does shed light on a potentially increasing problem. Also, this figure may represent an underestimation of the true prevalence of PVL, as 878/2032 (43.2%) of samples were not tested for PVL, with no reasons provided. One further

strength of the dataset from Southampton was the inclusion and reporting of the MRSA phenotype, by performing Staphylococcal cassette chromosomal typing. This was performed on 893 out of the 2032 (43.9%) positive MRSA patients. As a result, it is evident that EMRSA-15 is the main MRSA phenotype in the region and helps shape an idea of the population and their carriage of these epidemic strains, currently not routinely tested.

In comparison, PVL testing and Staphylococcal cassette chromosomal typing, in the Swansea group of hospitals, was not routinely performed. Failure to capture the rate of PVL positive MRSA samples from this region raises the level of uncertainty at the true numbers of CA-MRSA cases in the Swansea group of hospitals. Whilst, CA-MRSA cases represented the less dominant form of acquisition in this region, the data provided risks bias based on the selection and representation of the patient cohort.

Another risk of selection bias based on the Swansea cohort is that the mode of acquisition (CA-MRSA versus HA-MRSA) was derived on the clinical location of where the sample was taken, only. In comparison, this decision was at source by the PHES, based on the clinical information provided at the time of the sample receipt. However, on a positive note, the dataset provided from the PHW was comprehensive in its reporting of antibiotic susceptibilities. A standard operating procedure of reporting MRSA susceptibilities was clearly in place in the Swansea group of hospitals, using the British Society of Antimicrobial Chemotherapy (BSAC) guidance before a change of practice in 2015 adopting the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. This was not replicated in the Southampton dataset, where a limited panel of antibiotics were reported.

Another limitation of this study was the lack of the end reportable outcomes for MRSA, in particular, MRSA-related bacteraemia and SSIs. Data for MRSA bacteraemia was the only received outcome and that came from the PHES. The number of SSIs was unobtainable from either regional centre for the study period. In failing to do so, the risk of comparability and outcome bias come in to play and again prevents a meaningful comparison between the two regional centres.

The discussion of who to screen and when to screen has been hotly debated for years. Up until 2009, targeted screening was widely adopted as the preferred method of screening (Department of Health 2014). During this time, there was a reported decline in the number of MRSA-related SSIs, where MRSA was the causative organism (27% in 2004-06 versus 4% in 2011/12) (Department of Health 2014). By April 2009, The UK Department of Health (DoH) introduced the mandatory screening of all elective patients for MRSA carriage in England, and by December 2010 this was rolled out to

included emergency patients (Department of Health 2014). According to the DoH, these decisions were made on the cost-effectiveness of different screening and decolonisation strategies in preventing MRSA bacteraemia, wound infections and deaths (Department of Health 2014). During that period, data captured by the Public Health of Wales (PHW) reported a marked reduction in MRSA cases (Department of Health 2014). The study period covered in this retrospective analysis, between April 2011 and November 2016, make the study results, despite the heterogeneity of the data, perhaps relevant when considering the implications and decisions made by the Department of Health.

As outlined in this study, key reportable outcomes such as PVL carriage, staphylococcal cassette chromosomal typing and antibiotic susceptibility patterns are not routinely reported. Citing the fact of the ever-evolving face of MRSA prevalence and the prominence of CA-MRSA in the picture should set the agenda for all Public Health bodies in the UK to commit to standardising their practices and in particular the MRSA-related reportable outcomes outlined in this study.

CHAPTER 4: The efficacy of an engineered gel (Reactive Oxygen®) as a Mupirocin sparing therapy in methicillin-resistant and sensitive *Staphylococcus aureus* biofilms; an *in vitro* study

4.1 Introduction

Staphylococcus aureus remains a challenging bacterial species in clinical practice. Nosocomial infections attributed to MRSA have been repeatedly and unequivocally shown as a risk factor for MRSA-related HAIs including SSIs (Baratz et al., 2015; Rao et al., 2008; Rieser et al., 2018; Sasi et al., 2015; Tandon et al., 2017; Tsang et al., 2018). Meanwhile, the presence of methicillin-sensitive *Staphylococcus aureus* (MSSA) in the nasal cavity has been strongly associated with chronic infection. Chronic rhinosinusitis (CRS) is the second most common chronic condition in the UK affecting between 10-15% of the population (Fokkens et al., 2012; Hopkins et al., 2006; Philpott et al., 2013).

CRS remains an underappreciated but equally debilitating disease for sufferers. One of the main challenges in the treatment of nasal MRSA and MSSA infections is the limited pool of effective antimicrobial and antibiotic agents available. One common agent, Mupirocin, has been the main treatment for the nasal decolonisation of MRSA carriers, but has also been muted as a further agent of use in recalcitrant CRS (Jervis-Bardy *et al.*, 2012). Based on the guidance of the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) from 2012, the use of Doxycycline and Clarithromycin is recommended as part of the medical management of CRS patients, in the hope of preventing or delaying surgery (Fokkens *et al.*, 2012).

The biology of nasal *Staphylococcus aureus* has been a hot topic of research, especially in MSSA related diseases, such as CRS. The formation, presence and importance of MSSA biofilms in the chronicity and failure of treatment has been repeatedly highlighted (Desrosiers et al., 2007; Foreman et al., 2011; Foreman et al., 2010; Jervis-Bardy et al., 2012; Ramadan et al., 2005).

Cases of CRS attributed to MRSA have also been reported and are thought to affect 4.75 – 9.3% of the CRS population (Jiang, Jang and Hsu, 1999; Manarey, Anand and Huang, 2004) and represent an increasingly difficult subset of patients to treat. The over prescription of antibiotics is believed to be a key factor in the development of MRSA-related CRS (Manarey, Anand and Huang, 2004).

In light of an increasing display of antimicrobial and antibiotic resistance (AMR), the clinical need to identify firstly the efficacy of current treatments such as Mupirocin, Doxycycline, Clarithromycin, but more importantly, potential novel agents, has been a long-standing call from the World Health Organization (WHO 2016). One potential agent, Reactive Oxygen®, has been reported to be

effective in the treatment of polymicrobial wounds, in selected cases. However, little scientific basis for the use of Reactive Oxygen® in nasal *Staphylococcus aureus* conditions has precluded its use for nosocomial MRSA infections as well as MRSA and MSSA-related CRS. In this chapter, I am going to investigate these.

4.2 Hypothesis

Mupirocin is not effective at reducing the viability of nasal *Staphylococcus aureus* biofilm

4.3 Aims and objectives

This study aimed to examine the efficacy of Mupirocin and Reactive Oxygen® on nasal MRSA and MSSA isolates.

The objectives of this study were to: -

1. Report the efficacy of currently used nasal *Staphylococcus aureus* treatments, Mupirocin, Doxycycline and Clarithromycin based on their planktonic and biofilm activity
2. Investigate the anti-biofilm efficacy of Reactive Oxygen® on CRS-related MRSA and MSSA isolates.

4.4 Methods

4.4.1 Ethical approval

This project received ethical approval by The Isle of Wight, Portsmouth and South East Hampshire Research Ethics Committee (NHS REC 09/H0501/74) prior to me joining the research group. As part of a study running in parallel to this study, patients with CRS (with and without polyps) were recruited having been diagnosed according to the criteria previously outlined in the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2012) undergoing endoscopic sinus surgery.

4.4.2 Bacterial strains and acquisition

Eighteen *Staphylococcus aureus* isolates, nine MRSA and nine MSSA, were used in this study. Eight of the nine MRSA isolates were acquired from the Public Health England of Southampton (PHES) Microbiology reference laboratory. The remaining MRSA isolate was collected at the time of endoscopic sinus surgery at the University Hospital Southampton NHS Foundation Trust under the care of one senior rhinologist (A/Prof R Salib) and later characterised. Sino nasal tissue was sampled by the senior rhinologist from either i) the region of the middle meatus and ethmoid bulla and/or iii) nasal polyps. This tissue was aseptically handled and placed in 20ml of 20% Hank's balanced salt solution (HBSS) in a white-top universal container and put on wet ice at the time of surgery. Samples were then immediately transferred to the National Institute for Health Research (NIHR) Wellcome Trust Clinical Research Facility (WTCRF) laboratories at the University Hospital Southampton for characterisation.

In total 5 *Staphylococcus aureus* samples were collected at the time of endoscopic sinus surgery and characterised by the PHES. One out of the five CRS samples was determined to be MRSA (CRS-MRSA1). The remaining four CRS samples were categorised as MSSA. Three of these four MSSA isolates were cultured from nasal polyps (CRS-P3, 4 and 5). The remaining CRS MSSA isolate was from a biopsy from the middle meatus (CRS-M2). These five samples were re-checked and confirmed by PHES at the time of acquiring the remaining 13 *Staphylococcus aureus* samples; 8 MRSA and 5 MSSA. Pulse field gel electrophoresis (PGFE) polymerase chain reaction (PCR) testing by the PHES Microbiology Southampton Laboratory identified all MRSA isolates used in the study were Pantone-Valentine Leukocidin (PVL) negative. Genomic characterisation of all nine MRSA isolates displaying a staphylococcal cassette chromosome mec (SCCmec) type IV, synonymous with the epidemic MRSA variant, EMRSA-15 (AAS personal communication with Dr Steve Green, Nusreen Ahmad-Saeed and Dr Peter Marsh at PHES).

4.4.3 Blood agar plate inoculation

Ten μl inoculation of MRSA or MSSA (n=3) were streaked using onto chocolate horse blood agar plates (Fisher Scientific, UK) and incubated overnight at 37°C 5% CO₂ for 14 hours. During this pilot run, an improved yield was observed (AAS & Dr Ray Allan (RNA) observation) when Colombia blood agar (CBA) plates (Fisher Scientific, UK) instead of chocolate horse blood agar was used as a medium. Subsequently, inoculation of respective MRSA and MSSA isolates were performed using CBA (Fisher Scientific, UK) plates alone. The incubation period overnight remained unchanged.

4.4.4 Preparation of Brain Heart Infusion (BHI) broth

36g of Brain Heart Infusion (BHI) (Sigmoid-Aldrich, UK) was dissolved in 1000ml of distilled water, autoclaved and kept sterile in an incubator at 37°C 5% CO₂ as a master stock. BHI was handled aseptically and discarded if not used within 3-5 days.

4.4.5 Preparation of bacteria suspension in BHI

Three individual colonies of *Staphylococcus aureus* (MRSA or MSSA respectively) were chosen and picked from the CBA (Fisher Scientific, UK) plates using bacterial inoculation loops and suspended in 5mls of 100% BHI (53286; Sigmoid-Aldrich, UK).and incubated at 37°C 5% CO₂ for 3 hours. This broth was considered as the starting point for planktonic and biofilms assays which are described further in this chapter.

4.4.6 Planktonic assay

Planktonic assays were performed in triplicate in clear flat-bottomed 96-well plates (Fisher Scientific, UK). 20 μl of inoculum (MRSA or MSSA) were added to 180 μl 100% BHI to make up to a maximum volume of 200 μl and designed for control (i.e. non-treated) wells. In the event that a treatment was to be added to a well, 20 μl of the inoculum was added to 160 μl of 100% BHI instead of 180 μl . Once the 96-well plate (Fisher Scientific, UK) was prepared and with the inoculum and 100% BHI, 20 μl of a treatment (described later in this chapter) would be added as the last step before the assay would begin. The moment the treatment was added to a well, the experiment was considered to have started and considered as t (time) = 0. The 96-well plates were transferred for incubation for 24 hours at 37°C 5% CO₂. The optical density (OD₆₀₀) of wells was quantified using a microplate reader (Biochrom Ltd., UK) at two points of time, t = 0 (start of the experiment) and t = 24 (end of the experiment).

4.4.7 Treatment preparation

4.4.7.1 Preparation of Reactive Oxygen® stock solution

A 10-gram sachet of Reactive Oxygen®, donated by Matoke Holdings Ltd. was pre-warmed at 37°C for 5 minutes to improve viscosity and practicality in expressing it from the sachet packaging (AAS & RNA observation). This was aseptically diluted in 10mls of Brain Heart Infusion (BHI) broth (Sigmoid-Aldrich, UK) and vortexed, until homogenous. This master solution of Reactive Oxygen® of 1000g/L (100% solution) was then diluted further with BHI, when required, to attain the desired concentration of Reactive Oxygen®.

4.4.7.2 Preparation of Acacia honey stock solution

10ml Acacia honey was used as vehicle control for Reactive Oxygen®, donated by Matoke Holdings Ltd. Ten mls of vehicle, aseptically weighed, were diluted with 10mls BHI (Sigma-Aldrich, UK), vortexed for 60 seconds thus creating a 1000g/L final concentration. In a similar fashion to the methodology adopted when handling Reactive Oxygen®, serial dilution was performed with 100% pre-warmed fresh BHI (Sigma-Aldrich, UK).

4.4.7.3 Preparation of Mupirocin stock solution

20mg of 2% Mupirocin (Sigma-Aldrich, UK) dissolved and mixed in 1ml of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, UK) was vortexed for 60 seconds. Due to the appearance of sedimentation, a further vortex of 60 seconds was required. 0.25ml of this master solution was diluted with 9.75mls of 100% BHI (Sigma-Aldrich, UK). This *in vitro* concentration (0.05%) of Mupirocin has been reported (Uren *et al.* 2008).

4.4.7.4 Preparation of Doxycycline stock solution

2mg of Doxycycline hyclate (Sigma-Aldrich, UK) was diluted in 1ml of sterile distilled water (dH₂O) creating a 0.2% master solution. This master stock was vortexed for 60 seconds before serial dilution with dH₂O to the desired concentrations required for each individual assay.

4.4.7.5 Preparation of Clarithromycin stock solution

2mg of Clarithromycin (Sigma-Aldrich, UK) in 1ml of 50mg/ml acetone forming 0.2% master solution. Following a 60 second vortex, serial dilution with acetone to the desired concentration was performed.

4.4.8 Growing *in vitro* *Staphylococcus aureus* biofilms

Six individual colonies of *Staphylococcus aureus* (MRSA or MSSA respectively) were chosen and picked from the CBA (Fisher Scientific, UK) plates using bacterial inoculation loops and suspended in 20mls of sterile pre-warmed 20% BHI (Sigmoid-Aldrich, UK) and incubated at 37°C 5% CO₂ for 4 hours. 2mls of this broth were then added to a sterile 6-well plate (Costar, UK) and supplemented with 20% fresh and pre-warmed BHI (Sigmoid-Aldrich, UK). A total of 4mls (2mls of inoculum and 20% BHI) were then repeated in each well. Care was taken when handling and transferring the 6-well plate (Costar, UK) for incubation at 37°C, 5% CO₂ for 24 hours. The first stage of biofilm feeding occurred at 24-hour timepoint, 2mls of media was replaced by 2mls of 20% fresh pre-warmed BHI and returned to the incubator at 37°C, 5% CO₂ for another 24 hours. A further round of biofilm feeding occurred at 48-hour timepoint, once the biofilm became established and prior to introducing a treatment(s) to a well.

4.4.9 Treatment of 48-hour old *in vitro* *Staphylococcus aureus* biofilms

At 48 hours, the 6-well plate (Costar, UK) was handled with care in order not to disturb the biofilm formation at the bottom of the plate when removed from the incubator and transferred by hand to the laminar flow cabinet. For wells planned not to receive treatment, 2mls of the well's media was replaced by 2mls of 20% fresh pre-warmed BHI.

Whilst for treatment wells, each well was replenished with 2mls of freshly prepared aliquots of the respective treatment (1000g/L Reactive Oxygen®; 0.05% Mupirocin; 0.01mg/ml Doxycycline; Clarithromycin 0.01mg/ml) and incubated at 37°C 5% CO₂ for a further 24 hours. Experiments assessing an adjuvant response, 1ml of 0.01mg/ml of Doxycycline or Clarithromycin was added first before adding 1000g/L of Reactive Oxygen and similarly incubated at 37°C 5% CO₂ for 24 hours.

4.4.10 Biofilm washing

Following 24 hours of treatment, all wells (non-treated and treated) were washed twice with pre-warmed 20% BHI. Each individual well was designated a sterile cell scraper (Fisher Scientific, UK)

to mechanically disrupt biofilm adherent to the bottom of the well. 1mL of pre-warmed 20% BHI ensuring that all the media containing the biofilm was re-suspended and homogenised before transferring this to a 1ml aliquot for serial dilution.

4.4.11 Dilution series

The homogenate remaining from the wash was then used to perform 10-fold serial dilutions to 1.0×10^7 in a 96-well plate (Fisher Scientific, UK).

4.4.12 Spot plating and CFU enumeration

Five 20 μ l spots of each dilution were plated onto one half of a CBA (Fisher Scientific, UK) plate and incubated overnight at 37°C 5% CO₂. Following the removal of the CBA (Fisher Scientific, UK) plate from the incubator, manual counting of the plates was undertaken recording the number of colonies within each spot for each respective dilution. The mean number of colonies at every respective dilution was then enumerated (CFU). This was then normalised to CFU per cm² and expressed in a log scale when reporting biofilm viability. Each experiment was conducted in triplicate and performed on separate days to ensure reliable biological and technical repeats.

4.4.13 Calculating the remaining viable population within the supernatant

In order to determine the effect of treatment on overall biofilm biomass and the biofilm-associated viable cell population, an evaluation of the supernatant was performed. The supernatant is the remaining media that is usually discarded before washing the biofilm before serial dilution, spot plating and CFU enumeration. Crucially, the biofilm was strictly not to be disturbed or scraped. The supernatant was collected and transferred to a 1ml aliquot. To quantify the viability of the supernatant a dilution series followed by spot plating and CFU enumeration, described in 2.3.11 and 2.3.12 was undertaken. The supernatant population of wells treated with Reactive Oxygen® and Acacia honey were assessed. A rise in the CFU/cm² would suggest a dispersal effect of the treatment on the biofilm. Conversely, a reduction in the CFU/cm² and thus the viable population would confer a cidal effect of the treatment on the biofilm. The factors of time and financial cost precluded the analysis of the supernatant following treatment with Mupirocin, Clarithromycin and Doxycycline.

4.4.14 Confocal laser scanning microscopy (CLSM)

CLSM was used to study planktonic and static biofilm cultures (Allan *et al.*, 2016). Planktonic cultures of MRSA (CRS-MRSA1) and MSSA (CRS-P3) were grown in BHI and used to inoculate a sterile 35/10mm CELL view cell culture dish (627870; Greiner Bio One, United Kingdom) supplemented with fresh pre-warmed 20% BHI. Biofilms were grown using static incubation

conditions for 48 hours at 37⁰C/5% CO₂ and the medium replaced daily with fresh pre-warmed 20% BHI. The biofilms were then treated with Reactive Oxygen® Mupirocin, Doxycycline or Clarithromycin or a combination of Reactive Oxygen® **and** Doxycycline or Clarithromycin for another 24 hours. Treatments were then removed, and the remaining biofilm rinsed twice with HBSS. Biofilms were stained using a LIVE/DEAD BacLight bacterial availability kit (L7012; Fisher Scientific, UK) and immediately analysed under an inverted Leica SP8 confocal laser-scanning microscope using a 63-oil immersion lens (Leica Microsystems, Milton Keynes, UK). Three representative images were then taken to assess biofilm response to treatment This was then subsequently analysed using the Leica LCS software.

4.4.15 Statistical analyses

Statistical analysis of planktonic and biofilm bacterial viability were evaluated using Mann-Whitney U test and ANOVA. Comparative data assessing the CFU response of MRSA and MSSA biofilms to each treatment, in comparison to non-treated wells, was evaluated using the one-way and two-way ANOVA referring to Tukey's multiple comparison test. Data reporting a *p* value of ≤ 0.05 was considered statistically significant.

The Mann-Whitney U test was used to measure the difference in the effect of one treatment (e.g. Reactive Oxygen®) to another (e.g. Mupirocin). All statistical analyses were performed using GraphPad Prism statistical software (release 8.0c; GraphPad Prism, Inc., California, USA).

4.5 Results

Mupirocin is highly effective and statistically significant at reducing the planktonic viability of nasal *Staphylococcus aureus*, exhibiting a greater effect on MSSA than MRSA.

The addition of 0.05% Mupirocin to planktonic wells of MRSA and MSSA wells leads to a large and significant reduction in the viable population of MRSA (mean difference 0.6018, 95% CI (0.5580 to 0.6455), $p < 0.0001$) and MSSA (mean difference 0.8679, 95% CI (0.8419 to 0.8939), $p < 0.0001$) (Figure 4.1). All eighteen nasal *Staphylococcus aureus* (MRSA 9; MSSA 9) were tested to ensure reliable biological replicability. Absorbance (OD600) readings are presented as the mean \pm standard deviation. Tukey's multiple comparisons tests between treatment and no treatment for MRSA and MSSA (**** = $p < 0.0001$). These findings applied to clinical practice, would explain the success of Mupirocin in eliciting a short-term response of patients with nasal *Staphylococcus aureus* – mediated infections.

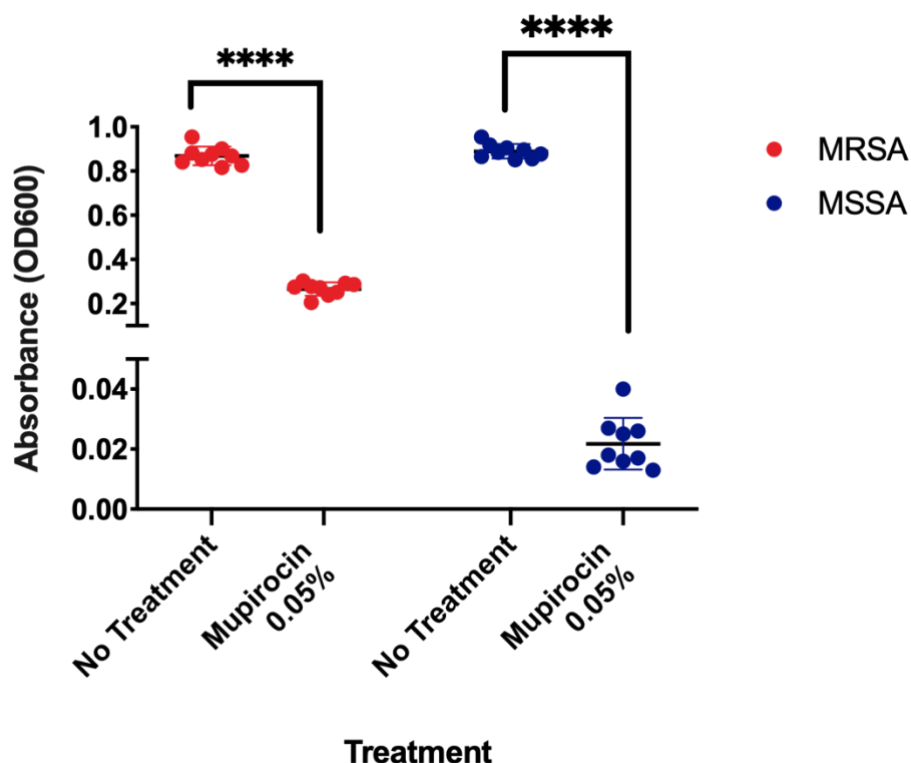


Figure 4. 1 The planktonic response of MRSA and MSSA isolates to Mupirocin in vitro.

***In vitro*, low concentrations of Doxycycline reduce the growth rate of planktonic nasal *Staphylococcus aureus* populations.**

Using a range of concentrations, the response of planktonic MRSA and MSSA to CRS recommended antibiotics, Doxycycline and Clarithromycin was conducted. Absorbance (OD600) readings are presented as the mean +/- standard deviation.

As illustrated in Figure 4.2, Doxycycline is the more effective antibiotic of the two EPOS antibiotics. The response of MSSA to Doxycycline is the most significant at concentrations of 0.025 (mean difference 0.4813, 95% CI (0.3566 to 0.6061), $p=0.0018$) and 0.01mg/ml respectively (mean difference 0.6583, 95% CI (0.4428 to 0.8739), $p=0.0062$). In comparison, the response of the viable planktonic MRSA population is also significant. Doxycycline was effective at reducing the viable MRSA population, to statistical significance, only at concentrations of 0.0025mg/ml (mean difference 0.4046, 95% CI (0.07264 to 0.7365), $p=0.034$), 0.05mg/ml (mean difference 0.5093, 95% CI (0.05239 to 0.9661), $p=0.0405$), 0.01mg/ml (mean difference 0.5648, 95% CI (0.2054 to 0.9241), $p=0.0140$) and 0.1mg/ml (mean difference 0.5745, 95% CI (0.1904 to 0.9586), $p=0.0161$) respectively.

Meanwhile, Clarithromycin was not effective at reducing the viable planktonic MSSA population *in vitro* but not MRSA. At a concentration of 0.005mg/ml (mean difference 0.2863, 95% CI (0.1003 to 0.4724), $p=0.0209$), 0.01mg/ml (mean difference 0.2867, 95% CI (0.03945 to 0.5339), $p=0.0375$) and 0.025 mg/ml (mean difference 0.2717, 95% CI (0.1731 to 0.3702), $p=0.0084$) respectively.

Up to a concentration of 1mg/ml, Clarithromycin failed to reduce the viable planktonic MRSA population (mean difference 0.1880, 95% CI (-0.02529 to 0.4013), $p=0.0637$). Statistical analysis of planktonic bacterial viability was evaluated using Tukey's multiple comparisons tests.

These results represent an important finding in explaining the clinical response of CRS patients to these routinely prescribed antibiotics.

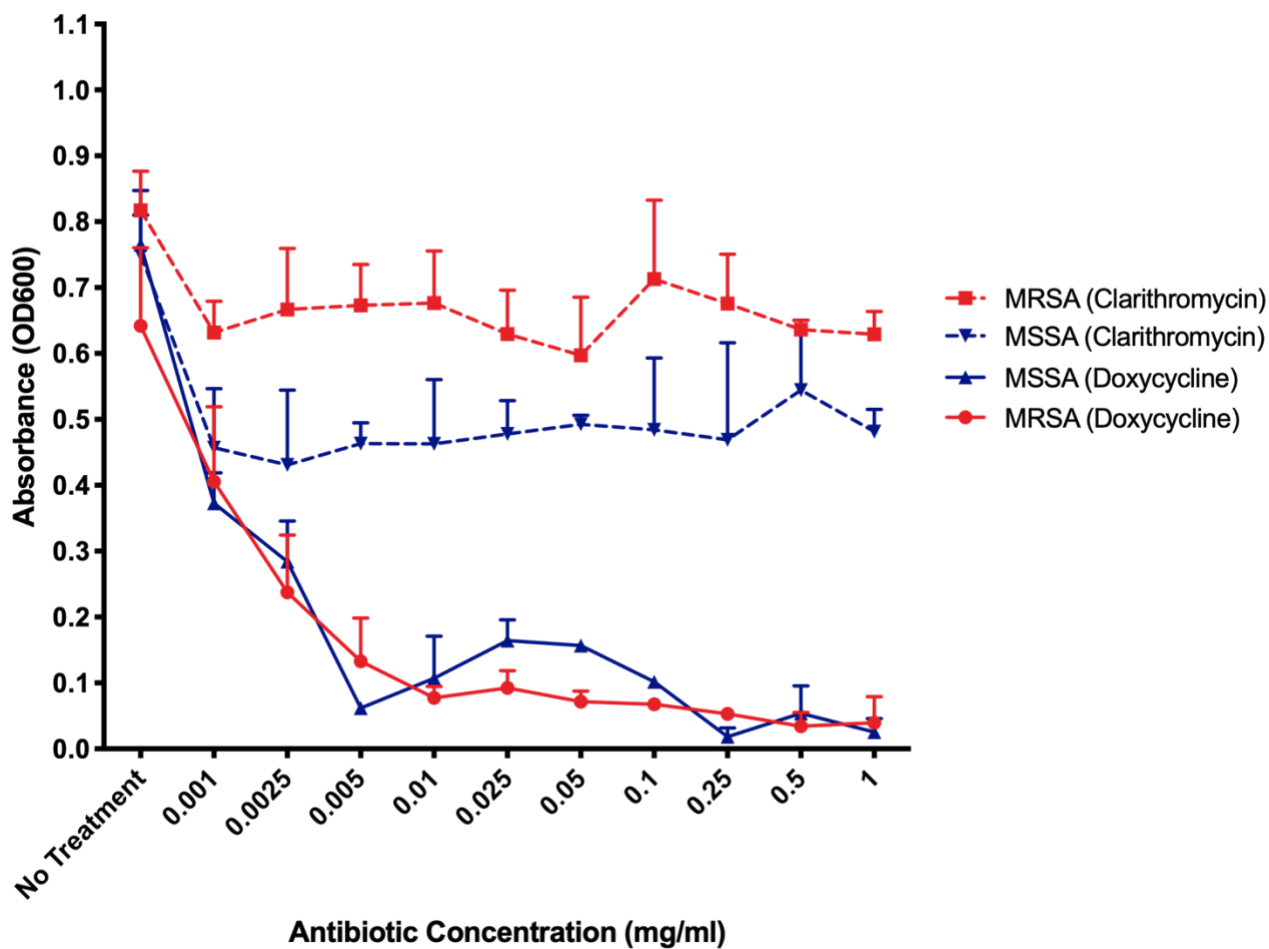


Figure 4. 2 The planktonic response of MRSA and MSSA to Doxycycline and Clarithromycin *in vitro*.

The treatment of nasal *Staphylococcus aureus* biofilms *in vitro* with Mupirocin fails to reduce the viability of the biofilm population.

A 24-hour treatment with Mupirocin failed to reduce the viability of established nasal *Staphylococcus aureus* biofilms. Established *in vitro* MRSA biofilms treated with Mupirocin appear to be unaffected in comparison to untreated wells (mean difference -2436648 (95% CI -5926181 to 1052885, $p=0.2$). This response is mirrored when Mupirocin is added to established *in vitro* MSSA biofilm wells (mean difference -155946, 95% CI (-1477578 to 1165687), $p=0.95$). Colony forming units/cm² (CFU) readings are presented as a log scale using the mean \pm standard deviation with 95% confidence interval. Comparative data was evaluated using the one-way ANOVA referring to Tukey's multiple comparison test. (ns = not significant)

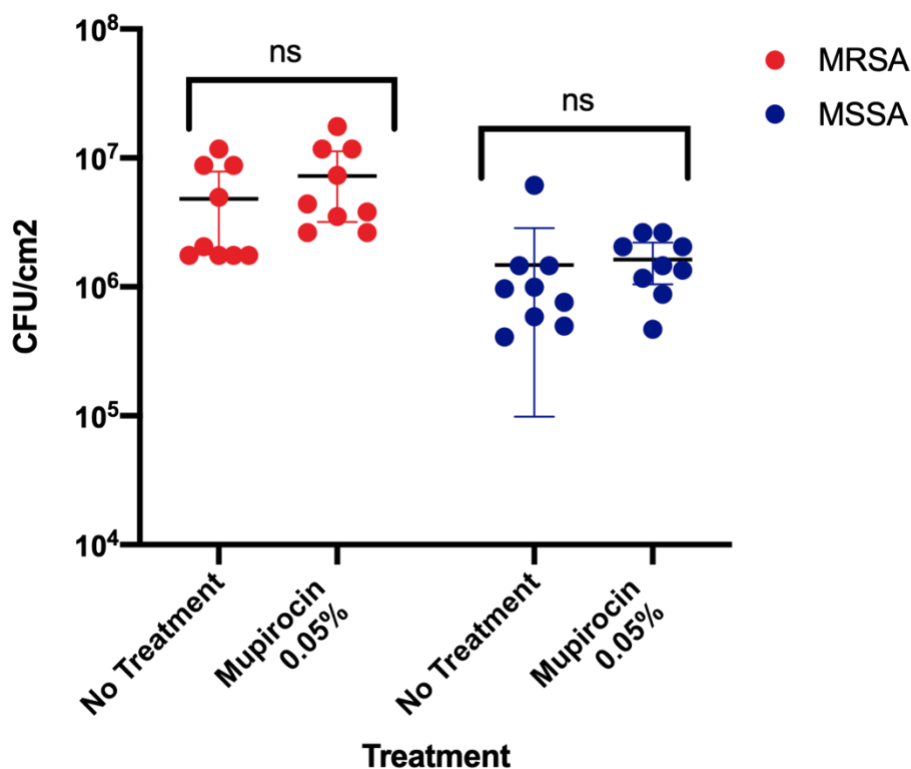


Figure 4. 3 The efficacy of Mupirocin on established *in vitro* MRSA and MSSA biofilms.

Doxycycline is highly effective and statistically significant at reducing the viability of established nasal *Staphylococcus aureus*, exhibiting a greater effect on MSSA biofilms

Upon exposure to all concentrations of Doxycycline, the viability of established *in vitro* MRSA biofilms is significantly reduced. CFU readings are presented as a log scale using the mean \pm standard deviation with 95% confidence interval.

In comparison to non-treated wells, a statistically significant reduction in the viability is witnessed using a concentration of 0.001mg/ml (mean difference 8304095 (95% CI 387358 to 16220833, $p=0.0398$), 0.01mg/ml (mean difference 10847954 (95% CI 2931217 to 18764692, $p=0.0094$), 0.1mg/ml (mean difference 12652730 (95% CI 4735993 to 20569468, $p=0.0037$) and 1mg/ml (mean difference 12669495 (95% CI 4752757 to 20586232, $p=0.0036$) respectively.

Established MSSA biofilms were highly susceptible to treatment with Doxycycline at similar concentrations and statistically higher so than their MRSA counterpart; 0.001mg/ml (mean difference 18508774 (95% CI 16405263 to 20612286, $p<0.0001$), 0.01mg/ml (mean difference 18187137 (95% CI 16083626 to 20290649, $p<0.0001$), 0.1mg/ml (mean difference 19468326 (95% CI 17364815 to 21571838, $p<0.0001$) and 1mg/ml (mean difference 19492205 (95% CI 17388694 to 21595717, $p<0.0001$). . (* = $p<0.05$; ** = $p<0.01$; **** = $p<0.0001$).

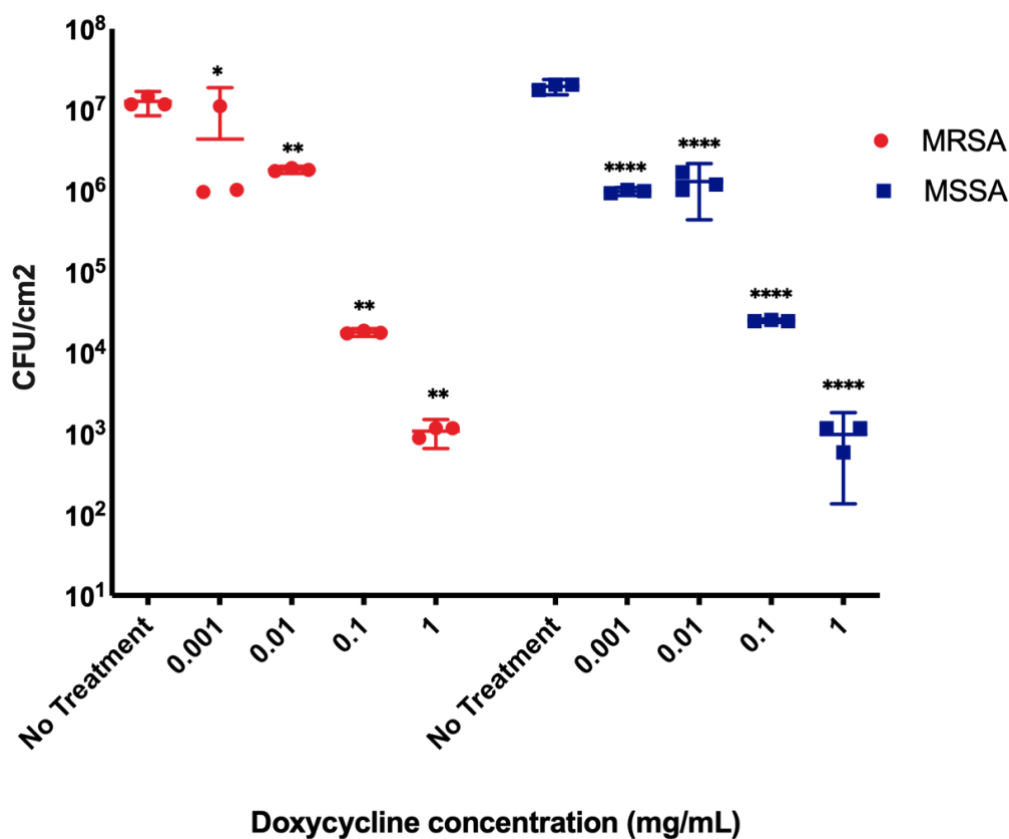


Figure 4. 4 The response of nasal *Staphylococcus aureus* biofilms to Doxycycline.

Established nasal *Staphylococcus aureus* biofilms fail to respond to treatment with Clarithromycin

The treatment of nasal *Staphylococcus aureus* biofilms with Clarithromycin failed to reduce the viable biofilm population, despite the use of a super physiological dose (1mg/ml). Using this concentration, no significant reduction of viability is seen in the *in vitro* MRSA biofilm model (mean difference -2923947 (95% CI -28722100 to 22874206, $p= 0.9940$). A similar picture is seen in the MSSA biofilm model, using the same concentration of Clarithromycin (mean difference -11695893 (95% CI -45669611 to 22277824, $p= 0.7576$). CFU readings are presented as a log scale using the mean +/- standard deviation with 95% confidence interval.

These results are highly relevant in understanding the response of CRS patients to EPOS recommended antibiotics. The use of Clarithromycin, in particular, warrants further discussion amongst the rhinology community. Citing the side-effects associated with the use of Clarithromycin and its lack of anti-biofilm response on CRS-related nasal *Staphylococcus aureus* isolates, as demonstrated in this *in vitro* study. (ns = not significant)

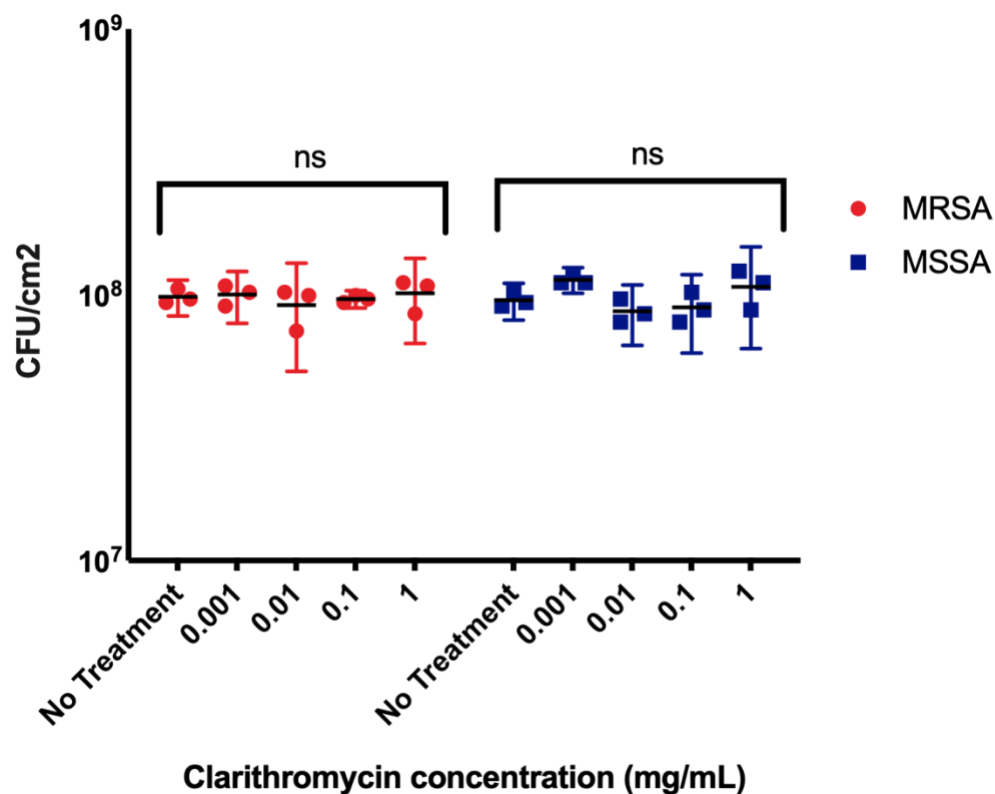


Figure 4. 5 The response of nasal *Staphylococcus aureus* biofilms to Clarithromycin

The minimum inhibitory concentration (MIC) of nasal MRSA isolates to Reactive Oxygen® is statistically higher to that of nasal MSSA isolates.

Due to the novelty of Reactive Oxygen® on treating the planktonic and biofilm populations of nasal *Staphylococcus aureus*, an assay establishing the MIC (the minimum concentration of treatment required to inhibit growth) of these isolates following treatment with Reactive Oxygen® was performed. By titrating the dose of Reactive Oxygen® in 50g/L increments, the MIC of each of the eighteen *Staphylococcus aureus* included in this study were tested and are presented in Figure 4.6. A higher concentration of Reactive Oxygen® was required in order to achieve a MIC in MRSA samples in comparison to MSSA, and this was statistically significant (Two-way ANOVA test, mean difference 60, 95% CI (25.41 to 94.59), $p=0.0039$).

Of interest, CRS-related nasal *Staphylococcus aureus* (red) appear to show no difference in their MIC. Meanwhile, the MIC on non-CRS (black) isolates are more varied in their response to Reactive Oxygen®. From herein, based on these results, only CRS-related MRSA and MSSA samples were used to test the efficacy of Reactive Oxygen®. The rationale and importance of using these CRS samples is explained in the discussion chapter. (** = $p<0.05$)

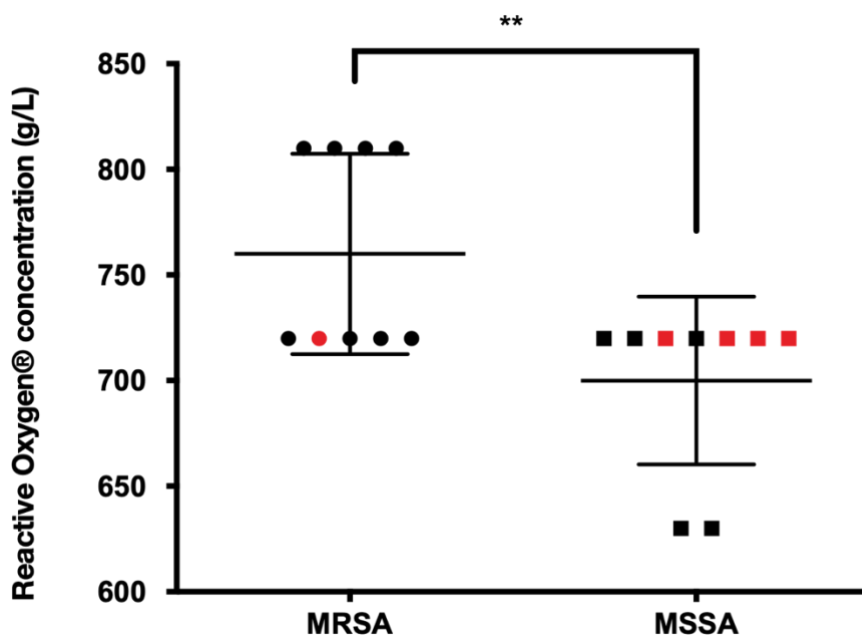


Figure 4. 6 The minimum inhibitory concentration of nasal MRSA and MSSA isolates.

At a concentration of 1000g/L, Reactive Oxygen® is highly effective at reducing the viability of planktonic nasal *Staphylococcus aureus*

A dose-dependent reduction in the growth rate of planktonic MSSA and MRSA populations is observed, up to a concentration of 1000g/L of Reactive Oxygen®. This concentration was deemed to be the minimum bactericidal concentration (MBC) (i.e. the lowest concentration of treatment capable of preventing physical growth of the inoculum). At this concentration, Reactive Oxygen® was significantly active against the planktonic populations of MRSA (one-way ANOVA, mean difference 0.7910, 95% CI 0.6043 to 0.9777), $p < 0.0001$) and MSSA (mean difference 0.8023, 95% CI 0.6156 to 0.9891), $p < 0.0001$). The planktonic MRSA population was more sensitive to Reactive Oxygen® in comparison to MSSA with a lower concentration (200g/L) required to bear a statistical significance (two-way ANOVA, mean difference 0.2420, 95% CI (0.05528 to 0.4287), $p = 0.0022$). Only by using higher concentration of Reactive Oxygen® (300g/L), that a significant response from the viable planktonic MSSA population is seen (two-way ANOVA, mean difference 0.2670, 95% CI 0.08028 to 0.4537), $p = 0.0004$), Figure 4.7.

Acacia honey, the base product (vehicle) from which Reactive Oxygen® has been engineered from, was used as the control, has no effect at reducing the viability of the planktonic population of MRSA (two-way ANOVA, mean difference -0.1777, 95% CI (-0.3644 to 0.009058), $p = 0.0768$) and MSSA (two-way ANOVA, mean difference -0.09400, 95% CI (0.2807 to 0.09272), $p = 0.8494$). Absorbance (OD600) readings are presented as the mean +/- standard deviation.

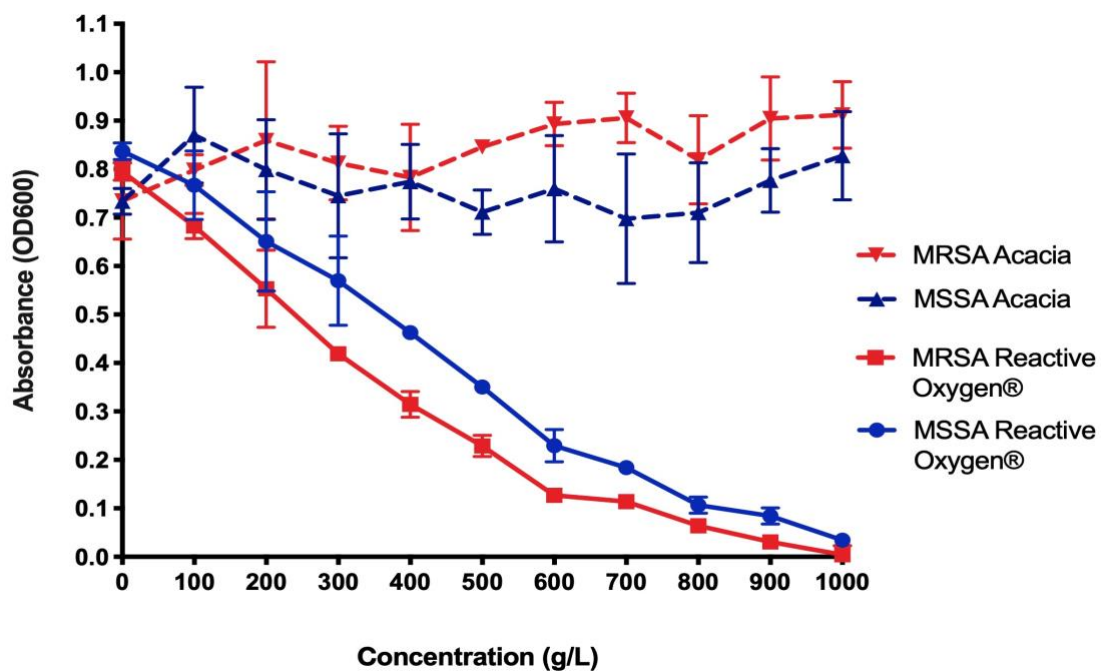
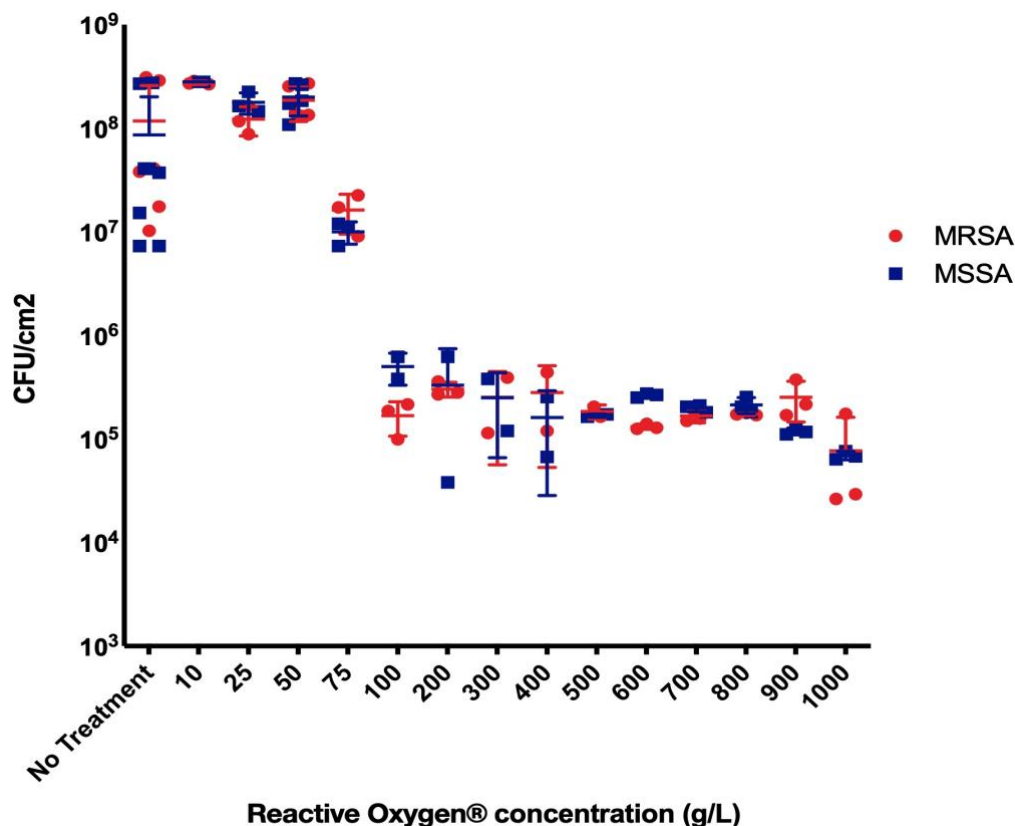


Figure 4. 7 The planktonic response of MRSA and MSSA to Reactive Oxygen® *in vitro*

Treatment of nasal *Staphylococcus aureus* biofilms *in vitro* with Reactive Oxygen® leads to a significant reduction in the viable population.



The addition of Reactive Oxygen® to established *in vitro* wells of static MRSA and MSSA biofilms leads to a significant reduction in viability. At lower concentrations (10 to 50g/L), there is a marked statistical reduction in the viability of MRSA and MSSA biofilms. Treatment with 50g/L of Reactive Oxygen® (Mann-Whitney U test, mean difference -91154954, 95% CI (-139037101 to -43272806), $p=0.0001$), lead to a statistical reduction in viability. However, it is at concentrations of ≥ 100 g/L, where the largest reduction in viability is demonstrated (Two-way ANOVA test, mean difference 101785335, 95% CI (53903187 to 149667482), $p<0.0001$), in comparison to non-treated wells. CFU readings are presented as a log scale using the mean \pm standard deviation with 95% confidence interval. Despite increasing the concentration of Reactive Oxygen® to 1000g/L, there was no further reduction in the viability of the biofilm, across all concentrations, and as such no statistical significance in comparison to 100g/L. This may suggest a persistent and resistant nasal *Staphylococcus aureus* population not penetrated by Reactive Oxygen®.

Figure 4. 8 The anti-biofilm profile of Reactive Oxygen® on established *in vitro* MRSA and MSSA biofilms.

Comparison of the treatment of nasal *Staphylococcus aureus* biofilms *in vitro* with Mupirocin and Reactive Oxygen®

Figure 4.9 shows Mupirocin has no anti-biofilm effect on established MRSA (Tukey's multiple comparison test, mean difference -2436648 (95% CI -5926181 to 1052885, $p=0.2$) and MSSA (mean difference -155946, 95% CI (-1477578 to 1165687), $p=0.95$) biofilms, in comparison to non-treated wells. The viability of nasal *Staphylococcus aureus* biofilms treated with Reactive Oxygen® 1000g/L was reduced by 3 log fold (Tukey's multiple comparison test, MRSA mean difference 7052957, 95% CI (3563425 to 10542490), $***p<0.0002$; MSSA (Tukey's multiple comparison test, mean difference 1602235, 95% CI (280603 to 2923868), $*p<0.01$) when compared to biofilm wells treated with Mupirocin. CFU readings are presented as a log scale using the mean \pm standard deviation with 95% confidence interval. (* = $p<0.05$; *** = $p<0.001$).

These findings represent a novel and important finding in the treatment of nasal *Staphylococcus aureus* biofilms and will be explored further in the discussion section of this chapter.

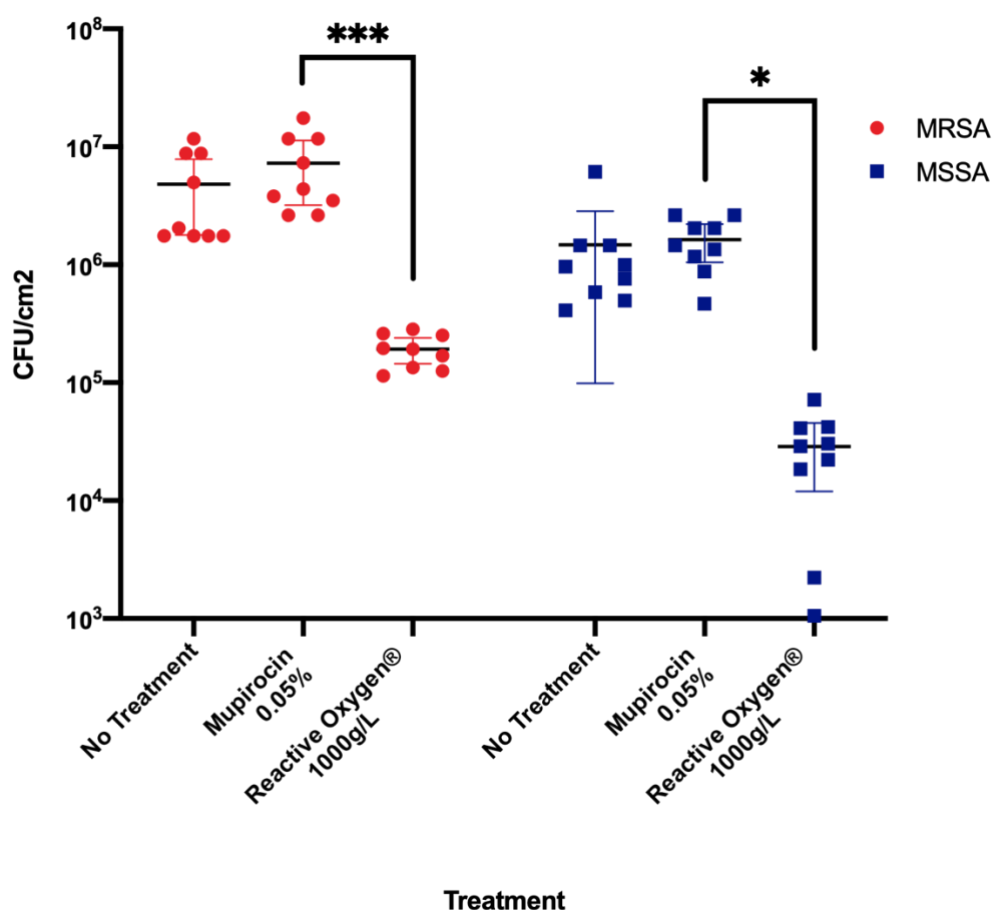


Figure 4. 9 Comparison of the efficacy of Mupirocin versus Reactive Oxygen® on established *in vitro* MRSA and MSSA biofilms.

Confocal laser scanning microscopy of nasal *Staphylococcus aureus* biofilms confirm a hypertrophy and increased viability of the biofilm after treatment with Mupirocin.

CLSM images (Figure 4.10 A-F), are presented to visually confirm the CFU/cm² response of treated biofilm wells. Images A-C display the MRSA biofilm response to treatment with 0.05% Mupirocin (B) and 1000g/L Reactive Oxygen® (C). In comparison to MRSA biofilms that remained untreated (A), MRSA biofilms treated with Mupirocin 0.05% demonstrate little to no dead (red) cells that can be identified with CLSM. In fact, this appears to show a more viable (Live (green)) MRSA population, that appears hypertrophied and uniform in comparison to untreated (A) wells. Despite this appearance suggesting a rise in MRSA biofilm viability, there was no statistical significance in CFU/cm² between non-treated and Mupirocin treated wells (Tukey's multiple comparison test, mean difference -2436648, 95% CI (-5926181 to 1052885), $p=0.2$).

Images Figure 4.7 D-E illustrate the response of MSSA biofilms to treatment with 0.05% Mupirocin (E) and 1000g/L Reactive Oxygen® (F). In comparison to MSSA biofilms that remained untreated (D), MSSA biofilms treated with Mupirocin 0.05%, again, demonstrate little to no dead (red) cells that can be identified with CLSM. Similar to MRSA biofilms treated with Mupirocin, MSSA biofilms also appear fuller in comparison to untreated (D) wells. Despite this appearance suggesting a rise in MRSA biofilm viability, there was no statistical significance in CFU/cm² between non-treated and Mupirocin treated wells (Tukey's multiple comparison test, mean difference -155946, 95% (CI -1477578 to 1165687), $p=0.9$).

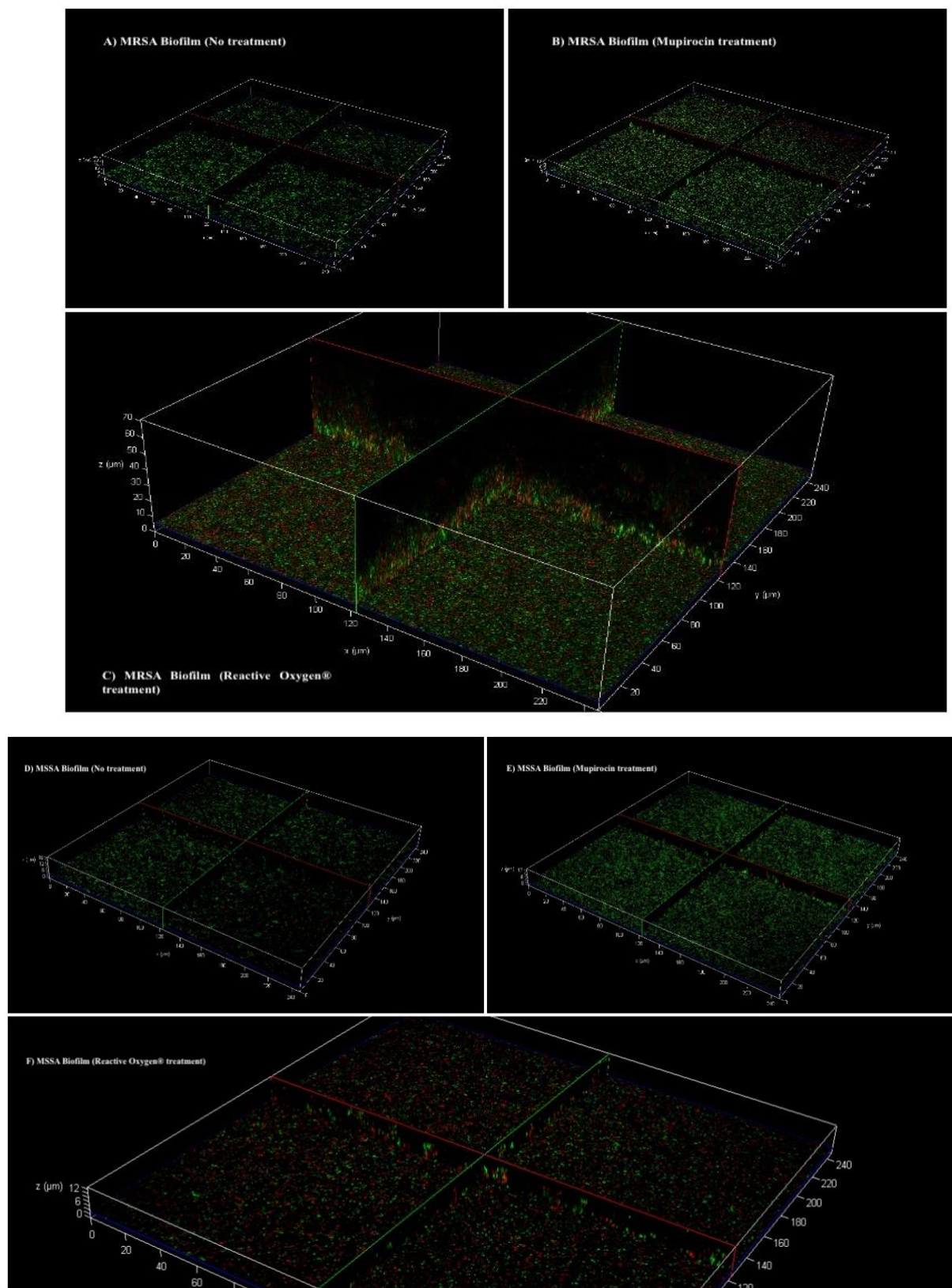


Figure 4. 10 Representative confocal laser scanning microscopy of biofilm structure following Mupirocin and Reactive Oxygen® treatment

Reactive Oxygen® alone is sufficient in its *in vitro* activity as a potential targeted antibiotic-sparing therapy against nasal MRSA biofilms, but not MSSA.

In order to avoid eliciting an exaggerated response to super physiological doses of an antibiotic *in vitro*, the smallest but most effective dose of Doxycycline and Clarithromycin, was used (0.01mg/ml). This concentration of antibiotic treatment was used when combining Doxycycline and Clarithromycin, respectively, as an adjuvant therapy to Reactive Oxygen®.

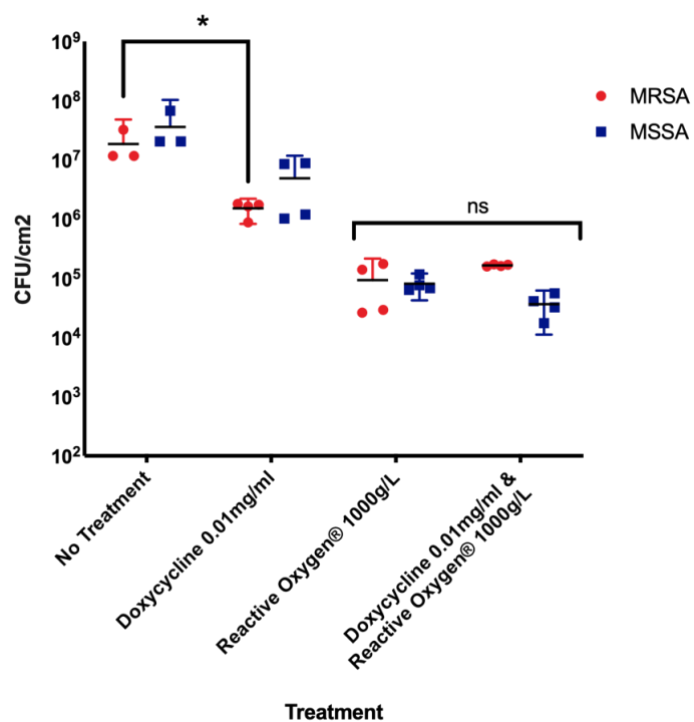
The viability of MRSA biofilms is significantly reduced by a dose of 0.01mg/ml of Doxycycline alone (A), (Two-way ANOVA, mean difference 17076025, 95% CI (159586 to 33992463), $p=0.0482$) in comparison to untreated wells. Established MSSA biofilms treated with a similar concentration of Doxycycline fail to reduce the viable population to any degree of significance (Two-way ANOVA, mean difference 29912283, 95% CI (-7658179 to 67482745), $p=0.1153$) in comparison to non-treated biofilm wells.

In comparison to MRSA biofilm wells treated with Doxycycline alone, the combination of Doxycycline with Reactive Oxygen® failed to ameliorate the anti-biofilm response (Two-way ANOVA, mean difference 1278753, 95% CI -15637686 to 18195191), $p=0.9931$. The response of this adjuvant treatment, in comparison to MRSA wells treated with Reactive Oxygen® alone, was also not significant (Two-way ANOVA, mean difference -98441, 95% CI (-17014879 to 16817998), $p=>0.9999$). Reactive Oxygen® alone was also effective at reducing the viable MRSA biofilm population (Two-way ANOVA, mean difference 18453218, 95% CI 1536780 to 35369656), $p=0.0350$), compared to non-treated wells.

Using a concentration of 0.01mg/ml Clarithromycin (B), however, fails to reduce the viability of established MRSA biofilm (mean difference 26315800, 95% CI (-54661279 to 107292879), $p=0.6892$) and MSSA biofilm population (Two-way ANOVA, mean difference 38011693, 95% CI -24042332 to 100065719), $p=0.2475$).

In comparison to MRSA biofilm wells treated with Clarithromycin alone, the combination of Clarithromycin with Reactive Oxygen® alone failed to ameliorate the anti-biofilm response (Two-way ANOVA, mean difference 36019885, 95% CI -26034141 to 98073910), $p=0.2819$). The response of this adjuvant treatment, in comparison to MRSA wells treated with Reactive Oxygen®, was also not significant (Two-way ANOVA, mean difference 15984, 95% CI -62038041 to 62070010), $p=>0.9999$).

A)



B)

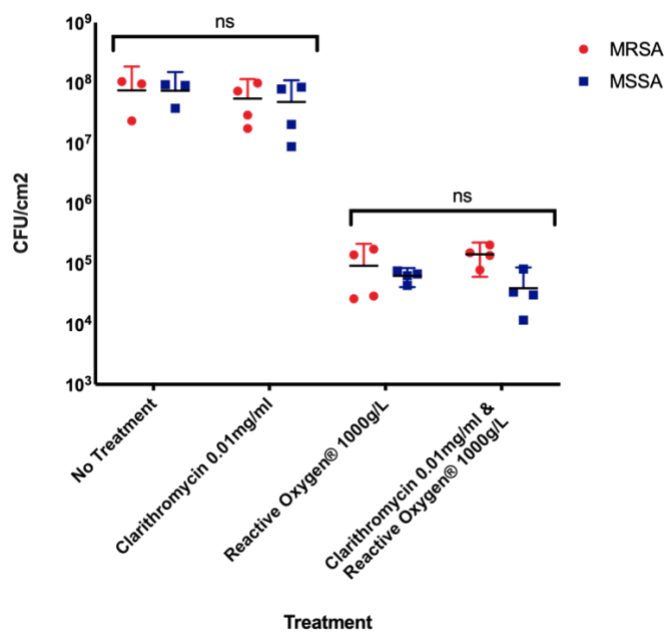
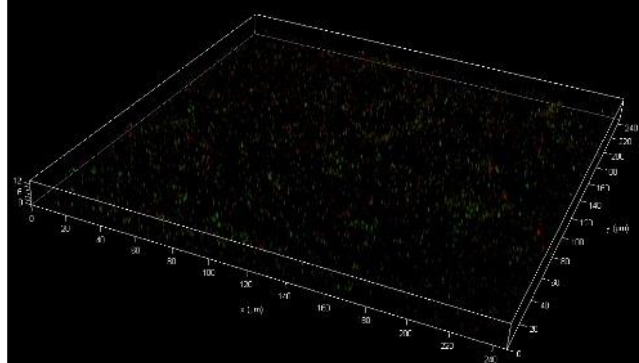
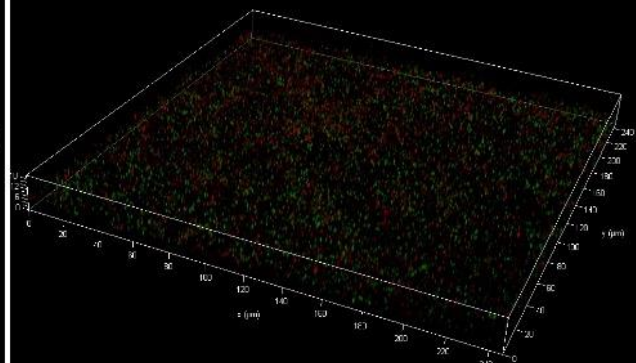
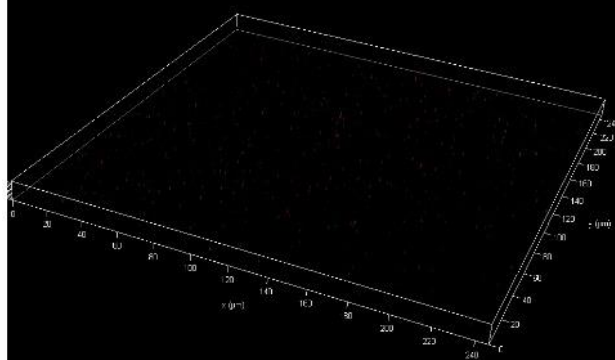
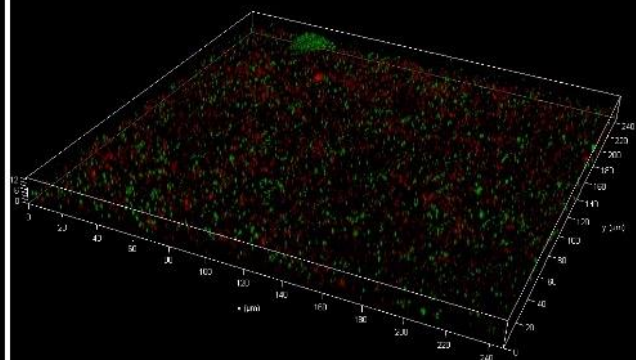


Figure 4. 11 The response of *in vitro* MRSA and MSSA biofilms to adjuvant therapy with Doxycycline (A) or Clarithromycin (B) versus Reactive Oxygen® alone.

Confocal laser scanning microscopy of nasal *Staphylococcus aureus* biofilms with treated with Doxycycline and Reactive Oxygen® confirm a hypertrophy but reduced viability of the biofilm

CLSM images (Figure 4.12 G-N), are presented to visually confirm the CFU/cm² response of treated biofilm wells. CLSM images show a short biofilm with sparse dead (red) bacteria visible of MRSA (G) and MSSA (I) biofilms treated with 0.01mg/ml of Doxycycline alone, respectively. In comparison, wells treated with Reactive Oxygen® and Doxycycline combination, biofilms appear to show a taller biofilm structure with a broad spread of dead cells (red) appearing to encase the biofilm in the peripheries, superficial to what appears to be a live underlying MRSA (H) and MSSA (J) biofilm population.

Meanwhile, CLSM images (K-N) illustrate the response of MRSA biofilm response to Clarithromycin alone (K) and as an adjuvant to Reactive Oxygen® (L). Meanwhile, images (M) and (N) depict the response of MSSA biofilms to treatment of Clarithromycin alone and as an adjuvant to Reactive Oxygen®, respectively. In comparison, to wells (H) and (J), CLSM images (L) and (N), those treated with Clarithromycin and Reactive Oxygen® combination has a more dead (red) population following treatment is illustrated. Additionally, the biofilm height in these groups appears taller than wells treated with Clarithromycin alone.

G) MRSA Biofilm (Doxycycline alone)**H) MRSA Biofilm (Doxycycline & Reactive Oxygen®)****I) MSSA Biofilm (Doxycycline alone)****J) MSSA Biofilm (Doxycycline & Reactive Oxygen®)**

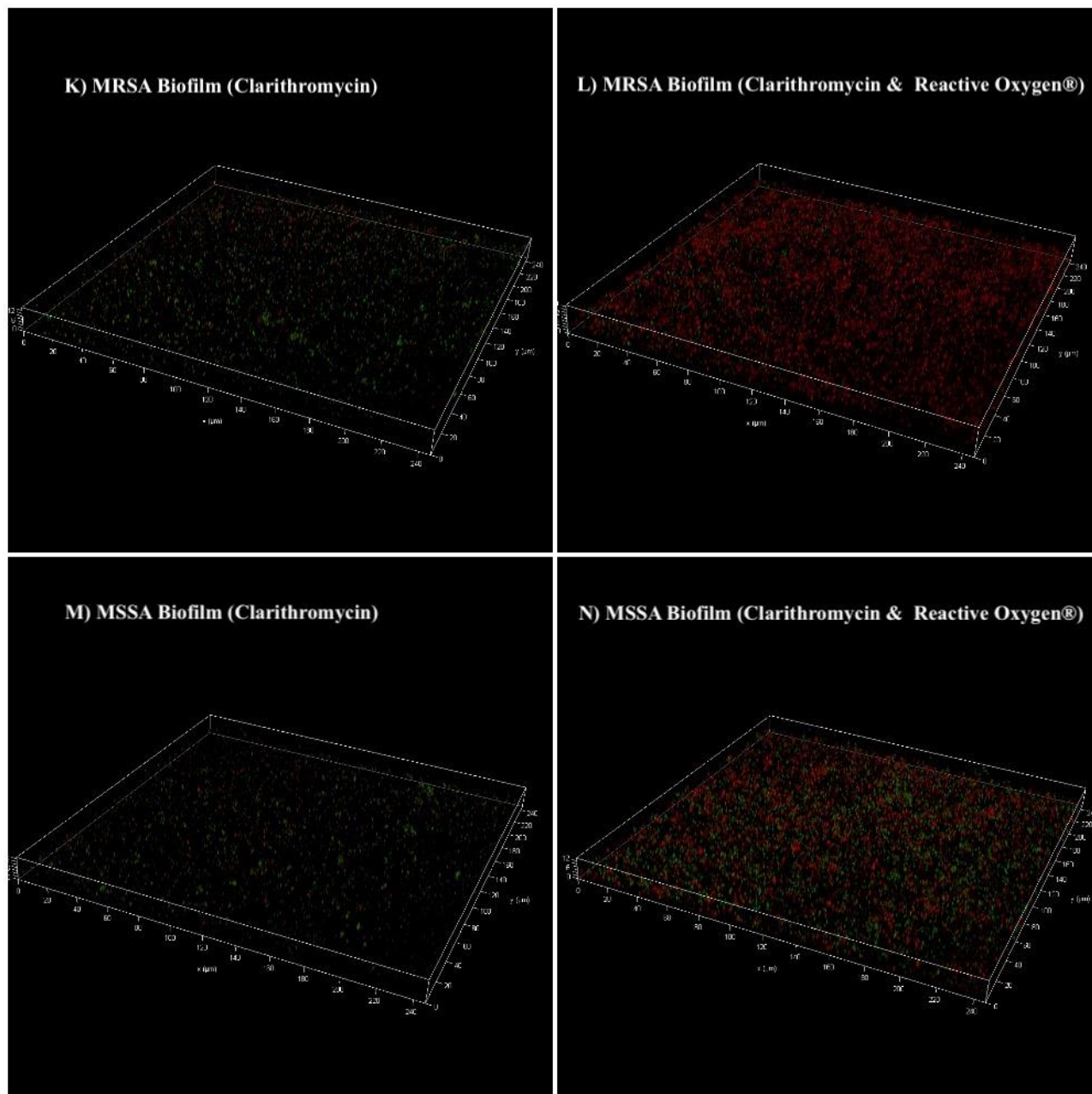


Figure 4. 12 Representative confocal laser scanning microscopy of biofilm structure following adjuvant therapy with Reactive Oxygen®.

The proprietary technology used to engineer Reactive Oxygen® is responsible for the anti-biofilm effect demonstrated and not dependent on the vehicle, Acacia honey.

To fully understand the mechanisms and potential for the response of nasal *Staphylococcus aureus* biofilms to Reactive Oxygen®, analysis of the base honey (Acacia honey) used to engineer this gel was performed. The static biofilm model was used within these series of experiments, a direct comparison of Acacia honey versus to Reactive Oxygen® is presented in Figure 4.13. CFU readings are presented as a log scale using the mean +/- standard deviation with 95% confidence interval.

In response to 1000g/L of Acacia honey, no reduction in the viable population of established *in vitro* MRSA (Tukey's multiple comparison test and two-way ANOVA, mean difference -21832359, 95% CI (-134365273 to 90700555), $p=0.58$) is demonstrated. However, a significant increase in the viable MSSA (Tukey's multiple comparison test and two-way ANOVA, mean difference -42884989, 95% CI (-67302634 to -18467344), $p=0.004$) biofilms population is seen. (Tukey's multiple comparison test and two-way ANOVA ** = $p<0.01$; *** = $p<0.0005$)

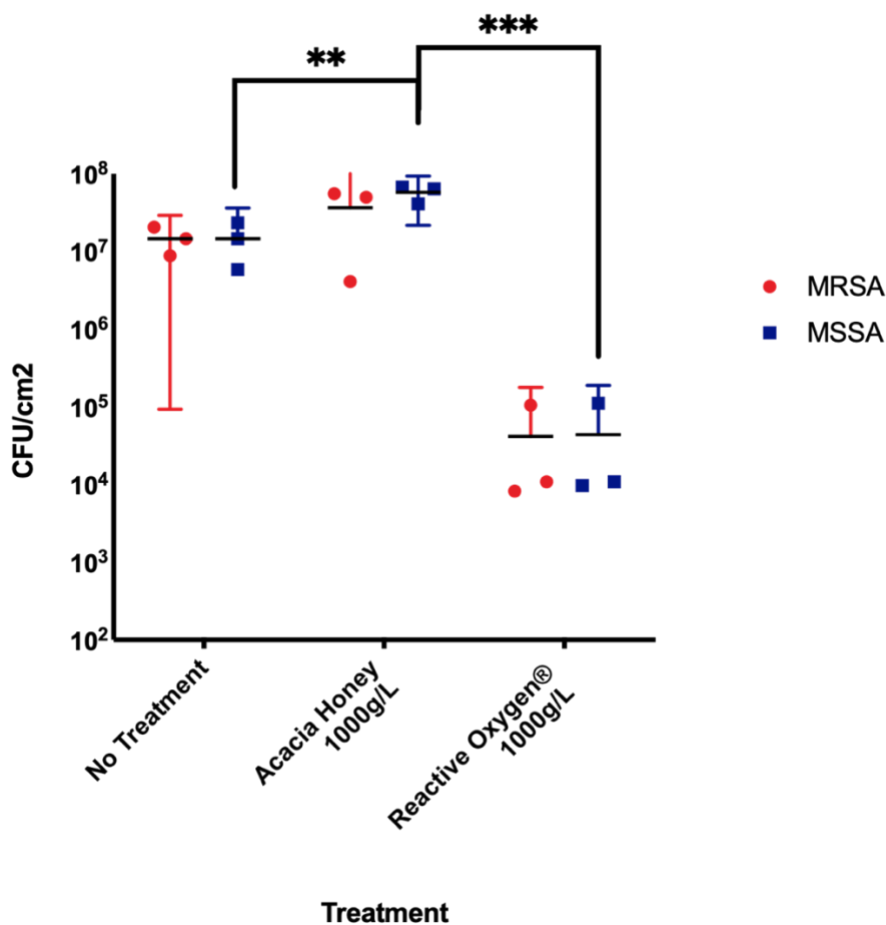


Figure 4. 13 Comparison of the efficacy of Acacia honey versus Reactive Oxygen® on established *in vitro* MRSA and MSSA biofilms.

A rich provision of carbohydrate vehicle source leads to a significant rise in nasal *Staphylococcus aureus* biofilm height after treatment with Reactive Oxygen®

Measurements of biofilm height (μm) were performed at a single CLSM sitting for the assessment of biofilm structure following Reactive Oxygen® treatment. At each sitting a triplicate of biofilm heights were measured. A significant rise in biofilm height was seen after the exposure of nasal *Staphylococcus aureus* biofilms to treatment with Reactive Oxygen®. MSSA biofilms, in particular, experienced a large and statistically significant rise in the biofilm height (mean difference -19.25, 95% CI (-23.69 to -14.81), $p < 0.0001$). MRSA, by virtue of being a potentially weaker biofilm former, witnessed a smaller but nonetheless statistically significant rise in biofilm height after Reactive Oxygen® treatment (mean difference -10.00, 95% CI (-15.18 to -4.816), $p = 0.004$). Biofilm heights are represented as the mean \pm standard deviation with 95% confidence interval, Figure 4.14. (Mann Whitney U test ** $p < 0.01$; **** = $p < 0.0001$).

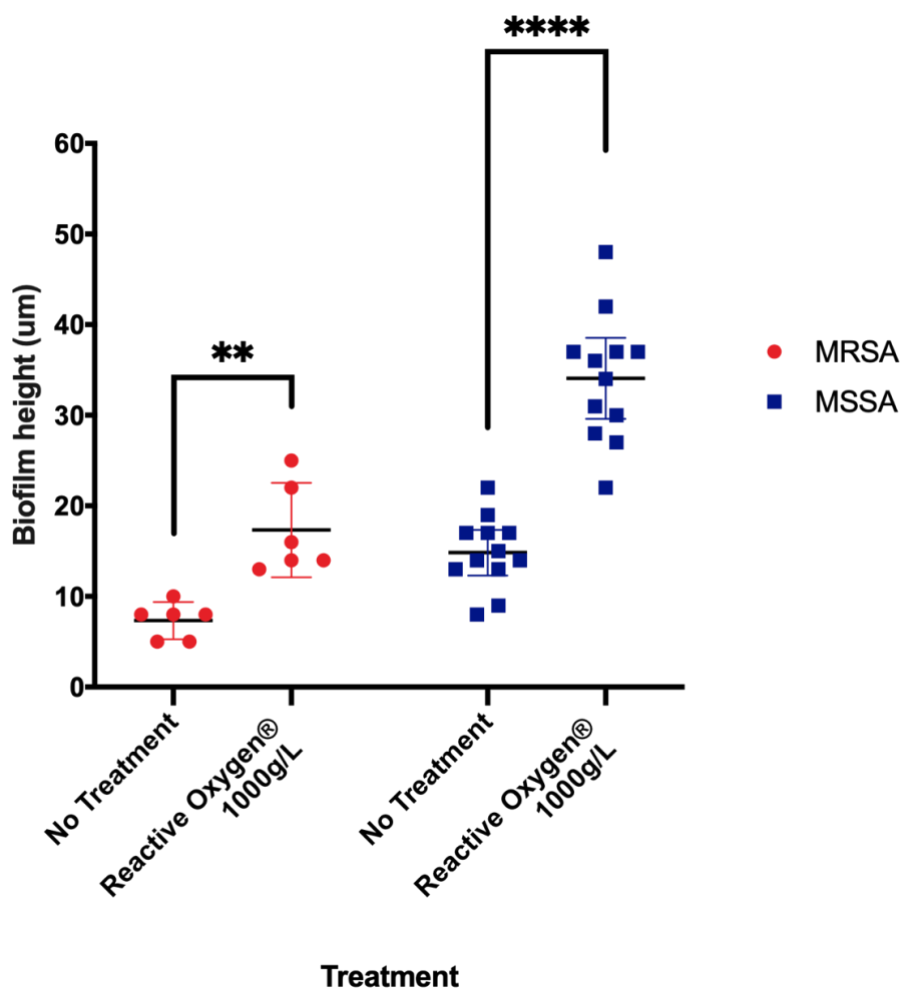


Figure 4. 14 MRSA and MSSA biofilm height following treatment with Reactive Oxygen®.

The supernatant of MRSA and MSSA biofilms treated with Reactive Oxygen® suggests a direct cidal effect.

The supernatant of nasal *Staphylococcus aureus* Reactive Oxygen® treated wells was collected and incubated for 24 hours. The supernatant of MSSA biofilm wells treated with Reactive Oxygen®, demonstrated a 5 log-fold reduction in CFU/cm² in comparison to wells treated with Acacia honey (one-way ANOVA, mean difference 57461010, 95% CI 33043365 to 81878656), $p=0.0009$). In comparison to non-treated wells, a significant rise in the viability of MSSA biofilm wells treated with Acacia honey (one-way ANOVA, mean difference -42884989, 95% CI -67302634 to -18467344), $p=0.004$). Meanwhile, whilst the viability of the MRSA biofilm supernatant treated with Reactive Oxygen is reduced 3 log-fold, this was statistically not significant (Mann-Whitney U test, mean difference 14578460, 95% CI -27043254 to 56200175), $p=0.5621$). The supernatant collected from wells of MRSA biofilms treated with Acacia demonstrated no significant rise in comparison to non-treated wells (Ordinary one-way ANOVA, mean difference -21832359, 95% CI (-63454073 to 19789355), $p=0.3124$). CFU readings are presented as a log scale using the mean +/- standard deviation with 95% confidence interval, Figure 4.15. This reduction in bacterial viability of the biofilm in the supernatant would appear to suggest that Reactive Oxygen® leads to direct killing of the biofilm, rather than to its dispersal.

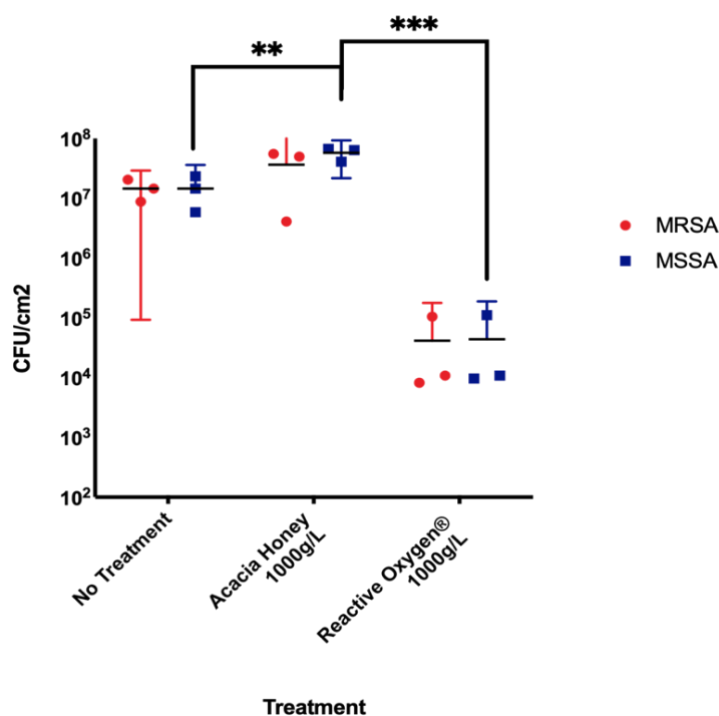


Figure 4. 15 Assessment of the supernatant of nasal *Staphylococcus aureus* biofilms with Reactive Oxygen®.

A summary of the treatment effects on nasal *Staphylococcus aureus*

Considering the results of this study, a summary in the form of a table is presented to report the response of the respective treatments against nasal *Staphylococcus aureus* biofilm populations. (-) no response; (+) mild response (++) moderate response; (+++) strong response.

| Treatment | Planktonic | Biofilm |
|------------------|---------------|---------|
| Doxycycline | +++ | ++/+++ |
| Clarithromycin | + (MSSA only) | - |
| Mupirocin | ++/+++ | - |
| Reactive Oxygen® | +++ | ++/+++ |

Table 4. 1 *In vitro* efficacy of treatments on nasal *Staphylococcus aureus* biofilms.

4.6 Discussion

This study, according to my knowledge, is one of the few studies to conduct a comprehensive analysis of the *in vitro* efficacy of Mupirocin and EPOS recommended antibiotics, Doxycycline and Clarithromycin, on nasal MRSA and MSSA biofilms. Further to this, few studies quote the use of CRS-related MRSA and MSSA isolates, therefore, the scope of this study, I hope, will be wide enough to stimulate discussion on the findings presented. One of the most significant findings of this study was the failure of Mupirocin and Clarithromycin in reducing the viability of CRS-related MRSA and MSSA biofilms. These novel findings represent a potential gateway for the further understanding of these treatments on nasal *Staphylococcus aureus*. It is worth reporting that Mupirocin was found to be highly effective in reducing the viable planktonic MRSA and MSSA populations. However, its lack of anti-biofilm effect is the most important point and within clinical practice may present a question to policymakers and researchers in the field of MRSA-related research. By relating these findings to clinical practice, these *in vitro* findings would explain the short-term response and “success” in decolonising MRSA carriers. Once MRSA carriers have completed a course of Mupirocin treatment, repeat swabs are usually reported not to re-grow MRSA. However, direct culture, and in fact any current diagnostic testing, cannot confirm the presence of biofilm formation. Without this knowledge, patients may return in the future with further exacerbations. Therefore, the knowledge that Mupirocin fails to reduce the viability of both MRSA and MSSA biofilms, *in vitro*, is a highlight of this study.

Considering that the use of Mupirocin in the treatment of CRS-related MSSA and MRSA infections has been previously reported (Solares *et al.*, 2006; Uren, Psaltis and Wormald, 2008; Jervis-Bardy *et al.*, 2012). The results of this study serve to inform against the liberal use of Mupirocin in the context of CRS biofilm related infections. Returning to the discussion of the efficacy of current EPOS recommended antibiotics, Doxycycline and Clarithromycin, our studies outlines a number of key messages. Doxycycline is a highly effective antibiotic on both CRS-related MRSA and MSSA, in planktonic and biofilm form. The discussion of the evidence behind the decisions of using these antibiotics is well documented in the EPOS guidelines and are not within the mandate of this study. However, the strong performance of Doxycycline is important when informing rhinologists and policymakers involved in the process of reviewing the available evidence on the success of these antibiotics.

Meanwhile, the limited planktonic activity of Clarithromycin, coupled with its poor anti-biofilm profile on CRS-related nasal *Staphylococcus aureus*, question its efficacy in clinical practice especially with the knowledge that the side-effects of Clarithromycin can be serious, inducing cardiac arrhythmias in a selection of patients (NICE British National Formulary).

In response to the calls from the WHO to identify potential novel agents, Reactive Oxygen® presents itself as a potential substitute to these aforementioned agents. As demonstrated, its planktonic and anti-biofilm efficacy on both MRSA and MSSA CRS-related isolates are encouraging considering the exhaustion and AMR of current used nasal antimicrobial/antibiotic treatments (Mupirocin, Doxycycline and Clarithromycin). The use of this engineered gel, noted to release hydrogen peroxide and reactive oxygen species as by-products (Dryden *et al.*, 2014, 2017; Cooke *et al.*, 2015; Halstead *et al.*, 2016), represents an exciting opportunity in the treatment of CRS-related nasal *Staphylococcus aureus* infections.

Reactive Oxygen® through its proprietary engineering, has been noted to release hydrogen peroxide and reactive oxygen species for a longer period of time than conventional honeys (Dryden *et al.*, 2014, 2017; Cooke *et al.*, 2015; Halstead *et al.*, 2016). Reactive Oxygen®, synthesised from a honey-based vehicle (Acacia honey) represents a new domain of therapeutics in the field of nasal *Staphylococcus aureus* treatment. To my knowledge, this is the first study to analyse the planktonic and anti-biofilm profile of Reactive Oxygen® against MRSA and MSSA isolates. The use of honey-based products has been trialled on infected burns, chronic wounds and caesarean wounds (Dryden *et al.*, 2014).

The mechanism of action behind Reactive Oxygen® can be explained by the presence of reactive oxygen species (ROS). Through a bio-engineering process (exact mechanisms unreported), the producers of Reactive Oxygen® report their ability of mimicking the natural properties of conventional honeys in a concentrated manner (Dryden *et al.*, 2014).

Based on the outcomes of this study, Reactive Oxygen® was superior in its planktonic ability to Mupirocin and Clarithromycin. Extending this to the anti-biofilm model, it was highly effective and superior to Mupirocin and Clarithromycin but not Doxycycline. The addition of the two studied EPOS antibiotics as an adjuvant to Reactive Oxygen®, failed to ameliorate the anti-biofilm response. These results represent two key findings. Firstly, the superior response of Reactive Oxygen® on CRS-related MRSA and MSSA *in vitro* biofilms, in comparison to Clarithromycin, qualify it as a direct

potential substitute to Clarithromycin. Secondly, considering the equal efficacy of Doxycycline and Reactive Oxygen® in the treatment of *in vitro* nasal *Staphylococcus aureus* biofilms, as well as the failure to demonstrate an adjuvant response from these two agents, lends to the suggestion of using Reactive Oxygen® as an antibiotic sparing therapy in the treatment of CRS patients. It is worth reporting the strong efficacy of Doxycycline on MRSA and MSSA biofilms. Due to the nature of CRS, the repeated use of Doxycycline and the effect that has on long-term outcomes on AMR in CRS is unknown. Therefore, providing a potential solution or substitute for Doxycycline is highly important in limiting the exposure of CRS-related *Staphylococcus aureus* to Doxycycline and the associated repercussions of AMR.

However, in order to understand the likelihood of the success of not only current, but novel antimicrobials in the treatment of nasal *Staphylococcus aureus*, attention should be focused on understanding the underlying mechanisms behind nasal colonisation. These include the ability of *Staphylococcus aureus* to firstly colonise the vestibulum nasi (anterior nares), secondly its capability to form a biofilm in this anatomical region and lastly whether there is a relationship between the presence of *Staphylococcus aureus* in the anterior nares and more upstream in the sinonasal mucosa.

Staphylococcus aureus, as well as other bacterial species, have been visualised as large biofilm-like bacterial aggregates have been found on mucosal samples of CRS patients. (Sanderson, Leid and Hunsaker, 2006) Unlike the strong correlation between the presence of *Staphylococcus aureus* in the anterior nares and the likelihood of developing a surgical site infection (SSI) (Perl, Pfaller and Herwaldt, 2002; van Rijen *et al.*, 2008), the bacterial relationship between the anterior nares and sinonasal disease, namely CRS, is not so clear cut and remains an area of continuing research. As the nasal microbiota is regarded as being diverse, complex and not yet fully understood, the presence of other bacterial organisms in the nose has been well established and are considered to be detrimental to the attachment and nasal colonisation by *Staphylococcus aureus*. (Krismer and Peschel, 2011; Sakr *et al.*, 2018)

Species such as *Streptococcus pneumoniae* and *Staphylococcus lugdunensis* have been found to have a bactericidal effect on *Staphylococcus aureus*, the former by producing H₂O₂ (Regev-Yochay *et al.*, 2006) and the latter through the means of a non-ribosomal synthesised bioactive compound, lugdunin (Zipperer *et al.*, 2016) One study investigating this subject found from 178 adult participants, 90% of participants, were found to have intranasal presence of *Staphylococcus epidermidis*. (Liu *et al.*, 2015;

Sakr *et al.*, 2018) The importance of this organism has been highlighted for a number of reasons. The presence of *Staphylococcus epidermidis* in the anterior nares is considered as crucial in the steps of disrupting the initial attachment of *Staphylococcus aureus*, preventing the aggregation of a biofilm matrix and lastly its capability to eliminate *Staphylococcus aureus* from the nose of colonised human subjects in a matter of days. (Krismer and Peschel, 2011; Liu *et al.*, 2015) Conversely, *Staphylococcus aureus* colonisation is associated negatively with the presence of other bacterial species in the nose such as *Staphylococcus epidermidis*. (Frank *et al.*, 2010; Sakr *et al.*, 2018) The explanation behind *Staphylococcus epidermidis*' ability to facilitate this disruption to the adherence of *Staphylococcus aureus* to the nasal mucosa is considered to be multifactorial.

Firstly, the release of Esp protease by *Staphylococcus epidermidis* strains provides the capacity to degrade the proteinaceous biofilm matrix of *Staphylococcus aureus*. (Krismer and Peschel, 2011) It is also hypothesised that Esp has the potential to degrade keratinised squamous epithelium and therefore blocking a pathway related to *Staphylococcus aureus* nasal colonisation. (Krismer and Peschel, 2011)

Secondly, the role of the Staphylococcal *agr* system has been demonstrated as another mechanism which interferes with the biofilm formation of *Staphylococcus aureus*. The *agr* system has been shown to be important in the formation of *Staphylococcus aureus* biofilms and the quorum sensing that biofilm communities rely on for continued survival. The fact that this mechanism is downregulated to the extent that it is switched off has been demonstrated by a number of studies reporting the status of colonisation and non-colonisation in human and cotton rat noses. (Burian *et al.*, 2010; Burian, Wolz and Goerke, 2010; Krismer and Peschel, 2011) In fact, a histological analysis examining the nasal tissue from human corpses and cotton rats found no evidence of biofilm formation in the anterior nares of the study subjects. (Burian *et al.*, 2010; Burian, Wolz and Goerke, 2010)

Furthermore, host conditions such as the presence and absence of certain proteins appear to be important not only in the initial colonisation but the continuation of this colonisation in the long-term. One such protein is *wall teichoic acid* (WTA). One study examining this protein found that WTA deficient *Staphylococcus aureus* mutants could not adhere to nasal cells and thus were unable to colonise the host (cotton rat model), in comparison to wild type control strains. (Weidenmaier *et al.*, 2004)

Lastly, the intracellular residence of *Staphylococcus aureus* (ICSA) has become a topic of increasing interest in understanding not only the colonisation of this bacterium in the nose, but also the efficacy of treatments. The internalisation of *Staphylococcus aureus* into a cell is a protective mechanism with the aims of evading from the host defence mechanisms but also to support the establishment of infection, even in the presence of antibiotics and antimicrobials. (Sakr *et al.*, 2018) As a result, the presence of ICSA is advantageous in the continued bacterial survival, re-colonisation, infection recurrence and even failure of decolonisation. (Sakr *et al.*, 2018) Therefore, it comes as no surprise that Mupirocin has been reported to have a weak effect against ICSA. (Sakr *et al.*, 2018) However, based on the design of this series of experiments, a comment about Mupirocin's ability to reduce the intracellular resident population of *Staphylococcus aureus* can not be made. Nevertheless, this would be an important avenue of further research for a number of reasons. Firstly, we need to understand the effect of Mupirocin on the host's mechanisms associated with nasal colonisation. Secondly, further laboratory and histological analysis is needed to not only understand Mupirocin's ability to target ICSA but the other panel of treatments used in this series of experiments, especially Reactive Oxygen®. By doing so, could help extract answers in explaining the relationship of nasal colonisation (of the anterior nares) and the development of biofilms in the sinonasal cavity. This remains an important avenue in rhinological research to explain the pathogenicity, recalcitrance of CRS and crucially the therapeutic targets of current and future therapies.

One notable study considering the persistence of ICSA in the sinonasal cavity has alluded to a number of key findings thus far. (Ou *et al.*, 2017) Whilst, the long-term consequence of ICSA in CRS remains under review, there are reports of increased ICSA prevalence in CRS patients with surface biofilm (Tan *et al.*, 2012) and in those with recalcitrant disease necessitating the need for revision sinus surgery. (Tan *et al.*, 2013; Ou *et al.*, 2016, 2017) The basis behind the current notion that ICSA acts as a reservoir for causing recurrent infections in CRS, (Ou *et al.*, 2017) has been based on the *in vivo* persistence of a single *Staphylococcus aureus* strain dominating the sinonasal microbiome (Clement *et al.*, 2005) and over a significant period of time (Drilling *et al.*, 2014). One of the further challenges in tackling ICSA mediated disease, is that the antibiotics (e.g. β -lactams) currently used to treat CRS MSSA infections, have low intracellular activity and are considered ineffective in eliminating ICSA. (Barcia-Macay *et al.*, 2006) Therefore, the *in vitro* findings of this chapter need further exploration in the context of their activity not only on ICSA but the response of the nasal microbiome in general.

Within this series of experiments we witnessed a rise in biofilm height following Reactive Oxygen® treatment. The rich provision of carbohydrates from a honey vehicle is a considerable factor behind

this rise. However, taking into account the CLSM images following treatment, viability of the biofilms after treatment and the direct cidal effect, demonstrated in the supernatant assay, suggest that this rise in biofilm contains no live but only dead cells. However, further studies to specifically answer this question, as well as the ability of Reactive Oxygen® to reduce the reservoir of ICSEA are needed. The practical nature of Reactive Oxygen® as a gel lends itself as topical therapy in the treatment of nasal MRSA and MSSA-mediated infections. Especially when Mupirocin is becoming increasingly unreliable, this represents an exciting and important finding in the treatment of MRSA-related HAIs and SSIs. The scope and magnitude of introducing Reactive Oxygen® as a potential new alternative in the decolonisation of MRSA carriers and CRS patients warrants further research and discussion. I discuss the implications of this in the next chapter.

Concluding remarks

I'm extremely grateful for the support of the funding bodies that have recognised the importance of this work, namely the Royal College of Surgeons' of England, British Medical Association and the Rhinology societies. Finally, I hope the bigger concern of MRSA and AMR become an integral part of the discussions that can influence change and ultimately safeguard patients and improve their care.

CHAPTER 5: Discussion

The main aim of this thesis was to investigate the efficacy of Mupirocin, which remains one of the very few available agents in the decolonisation of MRSA carriers. I have used three different approaches; firstly, a systematic review of the current literatures investigating the present efficacy of Mupirocin alongside Chlorhexidine Gluconate in preventing SSIs. Secondly, a retrospective analysis including almost 15,000 patients in two regions of the UK, highlighting the clinical challenges in reporting MRSA-related outcomes. Lastly, I undertook an *in vitro* study of directly testing Mupirocin against MRSA and MSSA biofilms. Collectively, these series of studies have highlighted the difficulties that AMR poses to clinical practice with the exclusive focus on MRSA. These studies, I hope, will be well accepted and acknowledged for the rigor in MRSA-related research and to be a catalyst for national change in policy and recognition of the level of funding and resources required to tackle MRSA-related infections

5.1 Summary of findings

Studies reporting the healthcare costs and mortality of as a result of SSIs paint a bleak picture. Healthcare costs associated with SSIs in the USA is estimated to be in be in the region of \$5-10 billion per year (Kline *et al.*, 2018). Post-operative mortality rates as a result of SSI increase two-fold (Kline *et al.*, 2018). Within 60 years from when it was first isolated, MRSA has become a leading cause of HAIs and SSIs in particular (Pofahl *et al.*, 2009; Tandon *et al.*, 2017). Over recent years, research commenting on MRSA infections have focused on a number of key factors considered to be important in understanding the prominence of MRSA in a relatively short period of time. Studies linking the nasal carriage of SA with the rate of MRSA-related SSIs have been well supported over the years (Pofahl *et al.*, 2009; Baratz *et al.*, 2015; Sasi *et al.*, 2015). MRSA colonisation is associated with subsequent MRSA-related SSI in as many as 44%, whilst the rate of MRSA SSIs in patients not colonised to be 2% (Pofahl *et al.*, 2009). It is therefore unsurprising that nasal *Staphylococcus aureus* carriers are two to nine times more likely to develop a SSI in comparison to non-carriers (Perl, Pfaller and Herwaldt, 2002; Sasi *et al.*, 2015). The general consensus based on the available evidence, is that the development of MRSA SSIs is usually preceded by MRSA colonisation (Sasi *et al.*, 2015).

Considering that *Staphylococcus aureus* contributes 30% of the total SSI burden (Kline *et al.*, 2018) raises the alarm about an increasing exhibition of Mupirocin resistance, one of the few topical anti-

staphylococcal treatments, especially in the treatment of MRSA carriers, has accelerated the need for novel agents at a time of widespread AMR .

With the clock ticking to find a solution, I systematically reported the key messages from a multi-faceted project considering a number of key themes involving the impact of MRSA in the UK and applicable to the wider audience. Firstly, a significant portion of the conversation is afforded to the discussion of MRSA SSIs and HAIs as a whole. Second, to discuss the complexity of MRSA research, which at present is highly affected by reporting bias owing to heterogeneity of the data and lack of standardised reported outcomes in MRSA related research.

5.2 MRSA in the United Kingdom (UK) and SSI reporting

In the UK, the cost of MRSA on the taxpayer is immense. One study in 2007, estimated the cumulative costs associated with the suppression, surveillance and treatment of MRSA to be in the region of £1 billion per annum (Senior et al., 2007). Taking into account the time-lapse since that report, until now, the expectation is that the cost of dealing with MRSA-related healthcare infections as a whole will exponentially rise. This is supported in fact by the UK Government and the Public Health bodies reporting that the costs attached with tackling antimicrobial and antibiotic resistance as a whole is estimated to be £66 trillion by 2050 (UK Government 2017).

Currently in the UK, MRSA bacteraemia are mandatory for national reporting (Department of Health 2014). KH03, is a fiscal measure of bed availability and occupancy per 100,000 of the population (Department of Health 2014, UK Government 2017). Its use has been widely adopted across the UK and by Trusts in England and Wales. An annual report of monthly MRSA bacteraemia are published on the UK Government website (Department of Health 2014, (Johnson et al., 2005), UK Government 2017). These reports are easily accessible, accurate and routinely updated. However, further scrutiny suggests that all rates of MRSA-related SSIs are not routinely published in the UK. To the best of my knowledge, there is no UK or international register recording MRSA-related SSI data using standardised reporting outcomes.

Based on an up-to-date systematic review into the topic of MRSA SSIs, the majority of studies reporting MRSA SSIs arise from the USA. The UK is consistent in its reporting of MRSA SSIs, as demonstrated by three included studies (Murphy *et al.*, 2011; Akhtar, Kadir and Chandran, 2014; Tandon *et al.*, 2017) standardising their use of the WHO guidelines to define a SSI. One further UK

based study (Schelenz *et al.*, 2005) included in that systematic review, reported the use of combined National Guidelines from the UK and USA (Mangram *et al.*, 1999).

In fact, 13 out of the 24 studies systematically included to examine the rates of MRSA SSIs (using Mupirocin and Chlorhexidine Gluconate as primary decolonisation agents) used the CDC definition of SSIs (Berríos-Torres *et al.*, 2017).

This mismatch may be explained by the fact that more than half (13) of the studies included in this systematic review were reported by authors from the USA. However, further interrogation identified that 10 out of the 12 American studies reported using the CDC guidelines as a reference for MRSA SSIs (Baratz *et al.*, 2015; Hadley *et al.*, 2010; Kim *et al.*, 2010; Lee *et al.*, 2013; Phillips *et al.*, 2014; Price *et al.*, 2008; Schweizer *et al.*, 2015; Shuman *et al.*, 2012; Thompson *et al.*, 2013; Torres *et al.*, 2016; Walsh *et al.*, 2011). The remaining two American studies (Rao *et al.*, 2008; Richer *et al.*, 2009) did not report their preferred method of reporting MRSA SSIs. Of note, the remaining 3 studies to use the CDC definition of MRSA SSIs were from India (Sasi *et al.*, 2015), Japan (Nakamura *et al.*, 2017) and one multi-site study (Lee *et al.*, 2013).

5.3 The threat of CA-MRSA in MRSA SSIs

Studies reporting the prevalence of HA-MRSA and CA-MRSA is becoming an increasingly desired in the context of MRSA SSIs. As mentioned earlier, SSIs attributable to *Staphylococcus aureus* are a large portion of the problem. An increasing number of these cases identifying PVL positive *Staphylococcus aureus* (PVL +ve SA) SSIs are increasing (Cunnington *et al.*, 2009) and represent a new challenge to surgeons dealing with SSIs. CA-MRSA infections are associated with more invasive infections and are repeatedly associated with poorer outcomes (Tristan *et al.*, 2007). A school of thought argues that carriage of the PVL (*LukS-PV* and *LukF-PV*) genes are synonymous with CA-MRSA carriage. However, in the UK, testing for PVL carriage is not routinely performed as a minimum and is used in clinically suspicious cases (HPA 2012). This may be in part explained by that, the UK has traditionally reported the higher prevalence of HA-MRSA in the UK (Rollason *et al.*, 2008).

In contrast, the rates of CA-MRSA in the UK are not routinely reported and likely to be unknown. Therefore, a population study examining a number of key reportable MRSA outcomes was felt to be highly important. The primary outcome of the study was to present the current landscape of CA-MRSA and HA-MRSA in the UK. Further MRSA relatable outcomes, some currently reported such as anatomical swab site(s) of positive MRSA culture, age, methods of MRSA identification were also reported. MRSA relatable outcomes, which not currently reported in the UK, such as PVL carriage

and antibiotic susceptibility of MRSA isolates were also included and reported in this study. This study was conducted from two regions in the UK. The composition of these populations including the hospitals can be found in Chapter 2.

Despite including a large cohort of patients, retrospectively, from two regional centres in England and Wales; the data recorded and stored by Public Health bodies of these respective countries precluded the ability to make meaningful and accurate comparisons between patient groups to add the body of evidence in the topic.

This heterogeneity in the data reported by studies, I believe, is a significant obstacle in the current process of MRSA infection reporting. Therefore, this landscape needs an evidence-based framework to highlight the relevant research outcomes that should be reported as a minimum. The inclusion of novel technologies to help lighten the heavy load of MRSA research should be welcomed, and as such, the debate about the use of digital technology including the use of augmented intelligence (AI) should be encouraged. This topic of AI in healthcare as whole is being passionately debated and the inclusion of MRSA research in this field of technology is discussed briefly later in this chapter.

5.4 Bacterial biofilms

Whilst, the aetiology and pathophysiology of CRS remains unclear and likely multi-factorial; MSSA bacterial biofilms have been recognised as a significant player in CRS recalcitrance, chronicity, poor surgical outcomes and antibiotic tolerance (Desrosiers *et al.*, 2007; Foreman *et al.*, 2011; Foreman *et al.*, 2010; Jervis-Bardy *et al.*, 2012; Ramadan *et al.*, 2005).

Bacterial biofilms are characterised by their innate capacity to form a matrix of organised cellular bacteria adherent to a mucosal surface (Høiby *et al.*, 2010). Following the attachment of free-floating (planktonic) bacteria, a phase of bacterial proliferation occurs and a self-formed extracellular matrix consisting of polysaccharides, nucleic acid and proteins (Høiby *et al.*, 2010; Wilkins *et al.*, 2014). This provides a protective capsule for the biofilm in a challenging environment (Wilkins *et al.*, 2014). This encapsulating matrix creates a physical diffusion barrier, creating a number of favourable conditions for survival including: 1) a gradient of nutrients and oxygen, 2) a medium for bacterial proliferation 3) a reduction in metabolic rate and 4) an ability to transfer genetics horizontally evading the host's immune response (Høiby *et al.*, 2010; Wilkins *et al.*, 2014).

As a result, these mechanisms contribute to the tolerance and subsequent resistance that biofilms exhibit when faced by antibiotics and antimicrobials, emphasising the urgent development of novel therapeutic options.

5.5 *Staphylococcus aureus* biofilms and AMR

Methicillin sensitive *Staphylococcus aureus* (MSSA) remains an important nasal bacterial pathogen implicated in the recalcitrance of CRS. The prevalence of CRS in the UK and population is estimated to be 11% (Fokkens *et al.*, 2012). CRS patients are commonly burdened with a variety of intolerable symptoms such as nasal obstruction, purulent discharge, reduced sense of smell, headaches and facial pain (Fokkens *et al.*, 2012). Naturally, these patients repeatedly report poor quality of life scores comparable with other chronic conditions such as ischaemic heart disease and chronic obstructive pulmonary disease (Gliklich *et al.*, 1995). As described by the European position paper on rhinosinusitis and nasal polyps (Fokkens *et al.*, 2012), CRS has been broadly classified into two distinct patient phenotypes, those with nasal polyposis (CRSwNP) and those without polyposis (CRSsNP) (Fokkens *et al.*, 2012). Whilst the aetiology of CRS and nasal polyps have yet to be fully established clinically, CRSwNP patients tend to have poorer outcomes in comparison to their CRSsNP counterparts. The presence of MSSA biofilms is thought to be a major driver in the aetiology and pathophysiology of CRS and has been a fast evolving rhinology research theme since this was first reported over a decade ago (Ramadan, Sanclement and Thomas, 2005; Foreman and Wormald, 2010; Al-Mutairi and Kilty, 2011; Ou *et al.*, 2014).

Maximal medical therapy of CRS patients is usually the first line of management. The basis of medical therapy is in the form of nasal saline irrigation and topical nasal steroids, in addition to EPOS recommended antibiotics, namely Doxycycline and Clarithromycin (Fokkens *et al.*, 2012). The eventuality of endoscopic sinus surgery usually follows a failure of maximal medical therapy. The intended benefit of performing surgery is to improve nasal airflow, widen the sinus pathways for ventilation and drainage, but crucially, in order to create a nasal cavity ready to be delivered with topical therapies, usually lifelong, to control nasal symptoms and abate recurrence of disease. According to the UK national sino-nasal audit, it is thought that 20% of CRS patients undergo revision surgery within 5 years (Hopkins *et al.*, 2006). As MSSA biofilms are strongly associated with recalcitrance of disease, the identification of a MSSA-specific anti-biofilm agent, in the form of Reactive Oxygen® represents a potential disease specific agent.

5.6 Antibiotic Breakpoints

The reporting of antibiotic susceptibility patterns for MRSA has witnessed a revolution of sorts ever since the introduction of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) under the auspices of the European Centre for Disease Control (ECDC). In fact, the

ECDC will only accept data for surveillance processes generated by laboratories adopting the EUCAST breakpoints and methodology from 2020. It is encouraging to know that since 2019 all the European countries are using these guidelines. Previous disc diffusion tests from Sweden, France and the UK have been abandoned and laboratories advised and subsequently have switched over to adopting EUCAST recommended breakpoints and methods. There appears to be an appetite for collaboration, evident by the ethos and message displayed by the ECDC, encouraging those interested in research into antimicrobial agents and their breakpoints contact the EUCAST and affiliated committees.

However, reliance on one entity to push the agenda of MRSA research forward is unrealistic. In fact, the committee is clear in its mandate that it cannot deal with policies relating to antimicrobial antibiotic resistance, surveillance, containment, resistance or infection control. Therefore, this transmits a reliance on national bodies such as national public health bodies, infection control prevention teams and fiscal bodies to take the lead in these domains and like the example of EUCAST, standardise the method of reporting antibiotic breakpoints.

5.7 Mupirocin and Chlorhexidine Gluconate

Attention should now focus on the use of Mupirocin and Chlorhexidine Gluconate as primary decolonisation agents in the clearance of MRSA. Their combined prescription is commonly used by healthcare providers to decolonise carriers. Increasing scrutiny about the efficacy of these agents is ongoing. One part of this process includes the systematic review of these agents investigating their efficacy to effectively decolonise patients prior to a surgical procedure by critically appraising the current literatures. Within the systematic review, 24 studies adhering to the inclusion criteria were analysed, including two randomise control trials. A broad number of countries were included such as the UK, India, Portugal, Switzerland and the majority of studies from the USA. One multinational prospective cohort study reported the rate of MRSA related SSIs from a further eight countries including Scotland, France, Italy, Spain, Germany, Greece, Serbia and Israel (Lee *et al.*, 2013). . Despite a total of nearly 75,000 (74,037 in total) included in the most up-to-date systemic review conducted in this topic, the heterogeneity of the data, owing to wide confidence and a high I^2 score, precluded the ability to perform a meta-analysis. This represents a desperately missed opportunity to report the highest level of evidence on the risk of MRSA SSIs in patients given the importance of the question and in response to the hypothesis posed that these agents are not effective. The systematic review highlighted the heterogeneity amongst the reporting of key outcomes such the number of days, frequency and concentrations of not only Mupirocin and Chlorhexidine Gluconate, but increasingly

precious antibiotics such as Vancomycin, Linezolid and Teicoplanin. Only 11 out of the 24 studies included, managed to report the concentration of Mupirocin used in their practice. Even fewer studies (n=5) reported Mupirocin use thrice a day for 5 days before surgery. 7 studies opted for a twice daily regimen. These small, but key, nuances could not be investigated further for two main reasons. Firstly, the application and availability of Chlorhexidine Gluconate preparations was diverse and could not be standardised. Second, the risk of bias amongst a number of studies was varied, with a mixture of good, fair and poor scoring studies according to the Newcastle-Ottawa Scale. Therefore, this bears a great deal of significance on the quality of evidence available to all stakeholders, and these include patients, surgeons, healthcare providers and fiscal bodies.

5.8 Reactive Oxygen®

Considering that *Staphylococcus aureus* contributes 30% to the burden of SSIs (Kline *et al.*, 2018) and with concerns regarding the true efficacy of Mupirocin resistance, the development of novel agents, displaying promise, in this current climate of AMR resistance is highly desired. The efficacy of Reactive Oxygen®, as demonstrated in Chapter 5, on the viability of MRSA and MSSA biofilm was superior to Mupirocin and Clarithromycin. However, no significance was found its anti-biofilm profile to Doxycycline. Considering the fact that only a few anti-staphylococcal agents are currently effective in clinical practice, the call for the development of novel agents with the potential to aid the fight against AMR from the WHO is becoming increasingly desired. Reactive Oxygen® seems to that profile. Its efficacy against not only planktonic, but established MRSA and MSSA biofilms, even *in vitro*, signals the potential and the importance in the fight against *Staphylococcus aureus* health-care related infections and on a wider scale, AMR. The idea of using Reactive Oxygen® as firstly a novel agent and substitute to Mupirocin in the decolonisation of MRSA is highly attractive. The cost savings and prevention of HAIs including SSIs would be immense. Based on the UK Government's prediction that the cost of AMR by 2050 is likely to cost £66 trillion, worldwide, suggests that not only the identification of potential novel agents is needed, but their development within a framework capable of continually assessing the response of MRSA and MSSA, in this case, and in a cautious manner. Due to the scarcity of data available about the resistance of *Staphylococcus aureus* to reactive oxygen species, further testing is required to understand underline mechanisms of the role of Reactive Oxygen®. Therefore, and despite the optimism of these results, further scientific rigor and testing is needed in order to prevent the exhaustion of a novel agent prior to being introduced in the field. Thus, it is vital to learn the lessons of Mupirocin-related AMR, considering that it was only introduced to clinical practice in the 1980's. Additionally, the reliance of a sole treatment in the treatment and

containment of a global burden such as MRSA and MSSA, is unrealistic and dangerous citing the lack of viable antimicrobials and antibiotics as a whole. As explained previously the lack of a standardised set of reportable outcomes for nasal *Staphylococcus aureus* mediated infections is concerning and in the fight against AMR requires a fresh approach in how we firstly handle large datasets and secondly ensure the adoption of a system or technology capable of disseminating the data in a coherent and organised fashion.

5.9 Concluding remarks

Augmented intelligence (AI) is quickly evolving and encompasses the potential to help in the fight of AMR, and that will undoubtedly include MRSA. By recognising that there is an “escape” of MRSA from hospital sources to the community, there is the challenge of adopting new ways of managing MRSA. AI has the potential to act as a framework and the basis of creating a personalised patient health passport detailing all the necessary parameters needed to understand and accurate MRSA-related health infections. By tapping into this technology, vast libraries of MRSA data relating to each patient, region, country at any given time, could be made available for regular analysis. By standardising the reporting and a minimum to the necessary tests required on MRSA samples on a large scale could also help unify our efforts in containing MRSA. However, development of AI is beyond the scope of this study. However, in having this tool, a passport of sorts detailing the infectious disease history, admissions, surgeries and all treatments in one secure portal would be highly attractive to clinicians monitoring MRSA prevalence, and antibiotic resistance patterns on a more regular and accurate basis. Additionally, policymakers would become better informed on the scale of funding and resources required to effectively tackle and contain MRSA related healthcare infections.

The reporting of the variance of the use of SSI definitions was unintended but helps to inform about the tendencies of practice adopted by different countries in tackling a global problem. The failure of the systematic review to conduct a meta-analysis as a result of the heterogeneity of the data, including the choice of national or global health organisation to refer to in order to define a MRSA SSI. A call to standardise the reported outcomes for MRSA SSIs needs to be made to the experts in the field to collectively rise to this global challenge.

CHAPTER 6: Limitations

These series of studies were designed to exhibit and demonstrate the broad impact MRSA has on our health systems and resources. By doing so, we have a contemporary and up to date analysis on the odds of a MRSA SSI in MRSA carriers. Secondly, the current landscape of MRSA prevalence as well as the challenges affecting two tertiary referral hospitals in the United Kingdom; Lastly, the response of MRSA biofilms to potential novel therapies including Mupirocin. There are a few important factors to consider when interpreting the results of these studies and the limitations that they pose.

It is clear that PVL positive MRSA cases pose a significant risk due to their virulence and capability to cause serious infection in the community. The samples used in our *in vitro* studies were all PVL negative and confirmed as such by the Public Health England Southampton (PHES) Microbiological Laboratory. The significance of our results using PVL negative MRSA strains in relation to PVL positive strains is unknown. However, further experiment repeats comparing the efficacy of Mupirocin, Reactive Oxygen® and Doxycycline on PVL negative versus PVL positive MRSA is desired. There would be a lot of answers to questions such as, are PVL positive MRSA strains better biofilm formers to their PVL negative counterpart? *In vitro*, are PVL positive strains more resistant to Mupirocin?

Using a similar concentration of Mupirocin (0.05%) as reported by Uren et al., we sought to examine the efficacy of Mupirocin on the viability of CRS related MSSA in the planktonic and biofilm forms. Mupirocin is very effective at reducing the viable planktonic MSSA population ($p < 0.0001$), however, when faced with an established MSSA biofilm, no reduction in bacterial viability is witnessed ($p = 0.95$). Therefore, in the clinical setting, the recalcitrance of CRS following Mupirocin use can be expected, based on these *in vitro* findings, as Mupirocin displays no anti-biofilm effect and would therefore not reduce nor interrupt the bacterial reservoir responsible for driving repeated infective exacerbations. It is worth considering the concentration of Mupirocin used in this *in vitro* analysis. The recommended dose of Mupirocin, in clinical practice, is 2%. However, there are no reports, according to our knowledge, reporting the use of Mupirocin at this clinical concentration. Uren et al. explain that the effective concentration of Mupirocin used (0.05%) is substantially greater than the mean inhibitory and bactericidal concentration of Mupirocin against *Staphylococcus aureus*. It is worth considering the concentration of Mupirocin used in this *in vitro* analysis. The recommended dose of Mupirocin, in clinical practice, is 2%. However, there are no reports, according to my knowledge, reporting the use of Mupirocin at this clinical concentration. Uren et al. explain that the

effective concentration of Mupirocin used (0.05%) is substantially greater than the mean inhibitory and bactericidal concentration of Mupirocin against *Staphylococcus aureus*.

Mupirocin and Chlorhexidine Gluconate are essential antimicrobials and have been important in preventing numerous MRSA related infections. Despite its inefficacy against MRSA biofilms *in vitro*, based on our model, Mupirocin still retains an impressive planktonic ability. However, the efficacy of Chlorhexidine Gluconate on MRSA biofilms, as well as in planktonic, was not explored. Great importance was placed on relating the efficacy of treatments to Mupirocin, and rightly so, considering its role in the decolonisation of MRSA carriers and its precarious position at being rendered ineffective due to increasing patterns of high-level resistance. Yet, without knowing the efficacy of Chlorhexidine Gluconate *in vitro* on established MRSA biofilms in comparison to our data from the other treatments, leaves a further future research opportunity. For example, can Chlorhexidine Gluconate act as an adjuvant to Reactive Oxygen® or Doxycycline? Does Chlorhexidine Gluconate disperse or actively kill MRSA biofilm populations?

The data provided from Public Health of Wales and Public Health England Southampton (PHES) Microbiological Laboratory was immense and detailed. Maintaining the records of MRSA patients is a challenge in itself, and for that reason the inception of a mandatory reporting register is vital. The level of detail that can be gained from the Department of Health MRSA monthly register is sufficient but lacks some important data that should be made available at all times. Ideally, the staphylococcal cassette type, antibiograms and PVL status of all MRSA strains could be examined at any given moment in time.

All in all, an agreement in vision followed-up with practical solutions, treatment and strategies to contain MRSA at a time of AMR should become a global priority. Novel therapies and innovations in the standardisation of the reported outcomes related to MRSA infection are desperately needed from clinicians, surgeons, scientists, researchers, microbiologists, epidemiologists and ministerial authorities including governments.

Appendices

APPENDIX A

Systematic review search strategy

Database: Embase <1974 to 2020 Week 05> and Ovid MEDLINE (R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions (R) 1946 to February 07, 2020

- 1 exp pseudomonic acid/ (6852)
- 2 mupirocin.ab,ti,tn. (2483)
- 3 arocin.ab,ti,tn. (0)
- 4 bacoderm.ab,ti,tn. (0)
- 5 bacskin.ab,ti,tn. (0)
- 6 bactestop.ab,ti,tn. (0)
- 7 bactex.ab,ti,tn. (1)
- 8 bactifree.ab,ti,tn. (0)
- 9 bactocin.ab,ti,tn. (1)
- 10 bactoderm.ab,ti,tn. (1)
- 11 bactokil.ab,ti,tn. (0)
- 12 bactreat*.ab,ti,tn. (4)
- 13 bacriderm.ab,ti,tn. (0)
- 14 bactroban*.ab,ti,tn. (633)
- 15 bactrocin*.ab,ti,tn. (1)
- 16 bactroneo.ab,ti,tn. (1)
- 17 balaban.ab,ti,tn. (130)
- 18 banbact.ab,ti,tn. (0)
- 19 banogon.ab,ti,tn. (0)
- 20 bantix.ab,ti,tn. (0)
- 21 bonderm.ab,ti,tn. (0)
- 22 brodisym.ab,ti,tn. (0)
- 23 celefer.ab,ti,tn. (0)
- 24 centany.ab,ti,tn. (7)
- 25 dermoban.ab,ti,tn. (0)
- 26 dermucor*.ab,ti,tn. (0)

- 27 derpix.ab,ti,tn. (0)
- 28 dextrocin.ab,ti,tn. (0)
- 29 dimsa.ab,ti,tn. (4)
- 30 eismycin.ab,ti,tn. (5)
- 31 flutibact.ab,ti,tn. (0)
- 32 foskina*.ab,ti,tn. (0)
- 33 hevronaz.ab,ti,tn. (0)
- 34 infectopyoderm.ab,ti,tn. (5)
- 35 mertus.ab,ti,tn. (0)
- 36 micoban.ab,ti,tn. (0)
- 37 Mpower.ab,ti,tn. (186)
- 38 mu-oint.ab,ti,tn. (0)
- 39 mufect.ab,ti,tn. (0)
- 40 mupax*.ab,ti,tn. (0)
- 79 antebor.ab,ti,tn. (9)
- 80 "antiseptic plus".ab,ti,tn. (0)
- 81 assepmed.ab,ti,tn. (0)
- 82 benodent.ab,ti,tn. (0)
- 83 bexident.ab,ti,tn. (7)
- 84 "bi Jie".ab,ti,tn. (0)
- 85 bucasmol.ab,ti,tn. (0)
- 86 burnheal.ab,ti,tn. (0)
- 87 burnoff.ab,ti,tn. (24)
- 88 "cetal aerosol".ab,ti,tn. (0)
- 89 chlorohex.ab,ti,tn. (13)
- 90 citrosteril.ab,ti,tn. (0)
- 91 clioron.ab,ti,tn. (0)
- 92 clomirex.ab,ti,tn. (2)
- 93 curasept.ab,ti,tn. (13)
- 94 cyteal.ab,ti,tn. (34)
- 95 dentaton.ab,ti,tn. (1)
- 96 dentosan.ab,ti,tn. (9)
- 97 "derma care".ab,ti,tn. (3)

- 98 dermoswab.ab,ti,tn. (0)
- 99 "DP hand rub".ab,ti,tn. (0)
- 100 duxidina.ab,ti,tn. (0)
- 101 effaclar.ab,ti,tn. (7)
- 102 elogerme.ab,ti,tn. (0)
- 103 eludental.ab,ti,tn. (0)
- 104 ferisept.ab,ti,tn. (0)
- 105 "flex-care".ab,ti,tn. (5)
- 106 "fresh-n-free".ab,ti,tn. (0)
- 107 hexide.ab,ti,tn. (1)
- 108 hexiprep*.ab,ti,tn. (0)
- 109 hexisol.ab,ti,tn. (0)
- 110 ivan.ab,ti,tn. (904)
- 111 "jiu tai".ab,ti,tn. (0)
- 112 "kou tai".ab,ti,tn. (0)
- 113 lactigriet.ab,ti,tn. (0)
- 114 levaknel.ab,ti,tn. (0)
- 115 melam.ab,ti,tn. (5)
- 116 merthiolate.ab,ti,tn. (306)
- 117 mertisept.ab,ti,tn. (0)
- 118 mexidin.ab,ti,tn. (0)
- 119 mouden.ab,ti,tn. (1)
- 120 "neba-sept".ab,ti,tn. (0)
- 121 neogyn.ab,ti,tn. (5)
- 122 neostrata.ab,ti,tn. (25)
- 123 octrene.ab,ti,tn. (0)
- 158 148 or 149 or 151 or 152 or 157 (53590)
- 159 exp surgical wound/ (6837)
- 160 exp surgical infection/ (43806)
- 161 (surg* adj3 infect*).ab,ti. (33291)
- 162 (surg* adj3 wound*).ab,ti. (13017)
- 163 (surg* adj3 site*).ab,ti. (28974)
- 164 (surg* adj3 incision*).ab,ti. (11155)

- 165 (surg* adj3 dehisce*).ab,ti. (890)
 166 exp wound dehiscence/ (17036)
 167 exp wound infection/ (44195)
 168 exp surgery/ (4638824)
 169 166 or 167 (58449)
 170 168 and 169 (47464)
 171 159 or 160 or 161 or 162 or 163 or 164 or 165 or 170 (135369)
 172 70 or 143 (28275)
 173 158 and 171 and 172 (413)

Database: Cochrane Wounds Group Specialised Register (searched 8 February 2020) and The Cochrane Central Register of Controlled Trials (CENTRAL) - *The Cochrane Library* 2020 Issue

MeSH terms exploding all trees:

- 1 Mupirocin (217) (6 reviews and 211 trials);
- 2 MRSA (204) (10 reviews and 194 trials);
- 3 Chlorhexidine Gluconate (2155) (23 reviews and 2132 trials);
- 4 Surgery (348) (5 reviews and 343 trials)

.....
 Database: Pubmed (searched 8 February 2020)

MeSH terms exploding all trees:

- 1 Mupirocin (1205) ["mupirocin"[MeSH Terms] OR "mupirocin"[All Fields]];
- 2 MRSA (13813) ["methicillin-resistant staphylococcus aureus"[MeSH Terms] OR ("methicillin-resistant"[All Fields] AND "staphylococcus"[All Fields] AND "aureus"[All Fields]) OR "methicillin-resistant staphylococcus aureus"[All Fields] OR "mrsa"[All Fields]];

3 Chlorhexidine Gluconate (8152) ["chlorhexidine gluconate, lidocaine drug combination"[Supplementary Concept] OR "Chlorhexidine"[Mesh];

4 Surgery (3884384) ["General Surgery"[Mesh] OR "Surgical Procedures, Operative"[Mesh] OR "surgery" [Subheading] OR "Conversion to Open Surgery"[Mesh] OR "Microvascular Decompression Surgery"[Mesh] OR "Orthognathic Surgery"[Mesh] OR "Sex Reassignment Surgery"[Mesh] OR "Natural Orifice Endoscopic Surgery"[Mesh] OR "Vitreoretinal Surgery"[Mesh] OR "Failed Back Surgery Syndrome"[Mesh] OR "Bariatric Surgery"[Mesh] OR "Corneal Surgery, Laser"[Mesh] OR "Surgery, Computer-Assisted"[Mesh] OR "Second-Look Surgery"[Mesh] OR "Thoracic Surgery, Video-Assisted"[Mesh] OR "Video-Assisted Surgery"[Mesh] OR "Filtering Surgery"[Mesh] OR "Mohs Surgery"[Mesh] OR "Thoracic Surgery"[Mesh] OR "Surgery, Veterinary"[Mesh] OR "Surgery, Plastic"[Mesh] OR "Surgery, Oral"[Mesh] OR "Surgery Department, Hospital"[Mesh] OR "Stapes Surgery"[Mesh] OR "Colorectal Surgery"[Mesh] OR "Enhanced Recovery After Surgery"[Mesh] OR "Transanal Endoscopic Surgery"[Mesh] OR "Body Contouring"[Mesh] OR "Orthognathic Surgical Procedures"[Mesh] OR "Ambulatory Surgical Procedures"[Mesh] OR "Piezosurgery"[Mesh] OR "Mastectomy, Segmental"[Mesh] OR "Tissue Adhesions"[Mesh] OR "Ulnar Collateral Ligament Reconstruction"[Mesh] OR "Dermatologic Surgical Procedures"[Mesh] OR "Bloodless Medical and Surgical Procedures"[Mesh] OR "Medical Errors"[Mesh] OR "Angioscopy"[Mesh] OR "Urologic Surgical Procedures, Male"[Mesh] OR "Fetoscopy"[Mesh] OR "Reconstructive Surgical Procedures"[Mesh] OR "Orthopedic Procedures"[Mesh] OR "Nasal Surgical Procedures"[Mesh] OR "Hand-Assisted Laparoscopy"[Mesh] OR "Endoscopy, Digestive System"[Mesh] OR "Obstetric Surgical Procedures"[Mesh] OR "Gynecologic Surgical Procedures"[Mesh] OR "Reoperation"[Mesh] OR "Laparoscopy"[Mesh] OR "Contraindications, Procedure"[Mesh] OR "Robotic Surgical Procedures"[Mesh] OR "Mandibular Reconstruction"[Mesh] OR "Operative Time"[Mesh] OR "Endoscopy, Gastrointestinal"[Mesh] OR "Cytoreduction Surgical Procedures"[Mesh] OR "Ultrasonic Surgical Procedures"[Mesh] OR "Refractive Surgical Procedures"[Mesh] OR "Laser Therapy"[Mesh] OR "Cervicoplasty"[Mesh] OR "Unnecessary Procedures"[Mesh] OR "Minimally Invasive Surgical Procedures"[Mesh] OR "Ureteroscopy"[Mesh] OR "Hysteroscopy"[Mesh] OR "Eye Enucleation"[Mesh] OR "Thoracoscopy"[Mesh] OR "Minor Surgical Procedures"[Mesh] OR "Sigmoidoscopy"[Mesh] OR "Proctoscopy"[Mesh] OR "Palliative Care"[Mesh] OR "Mediastinoscopy"[Mesh] OR "Laryngoscopy"[Mesh] OR "Gastrosocopy"[Mesh] OR "Esophagoscopy"[Mesh] OR "Duodenoscopy"[Mesh] OR "Cystoscopy"[Mesh] OR

"Culdoscopy"[Mesh] OR "Colposcopy"[Mesh] OR "Colonoscopy"[Mesh] OR
"Bronchoscopy"[Mesh] OR "Arthroscopy"[Mesh] OR "Coronary Artery Bypass"[Mesh] OR "Maze
Procedure"[Mesh] OR "Sutureless Surgical Procedures"[Mesh] OR "Heller Myotomy"[Mesh]

5 #1 AND #2 AND #3 AND #4 ; (7 studies identified)

APPENDIX B

Characteristics of excluded studies

| Study | Year | Country | Reason for exclusion |
|--------------------------|------|-------------|--|
| Abi-Haidar <i>et al.</i> | 2011 | USA | Did not mention use of Mupirocin. The use of Vancomycin and Chlorhexidine wipes. |
| Adogwa <i>et al.</i> | 2018 | USA | Did not mention use of Mupirocin. Use of Cefazolin as primary MRSA prophylaxis agent in spinal surgery |
| Aggarwal <i>et al.</i> | 2010 | UK | Abstract only. |
| Anderson <i>et al.</i> | 2015 | USA | <i>In vitro</i> study |
| Bebko <i>et al.</i> | 2015 | USA | Used Chlorhexidine cloths/oral rinse and nasal iodine. |
| Bessesen <i>et al.</i> | 2013 | USA | Screening bundle study. Not specific to surgical patients |
| Boccardo <i>et al.</i> | 2018 | Argentina | Abstract only. |
| Bode <i>et al.</i> | 2010 | Netherlands | No MRSA cases. Uses Mupirocin and Chlorhexidine |
| Bode <i>et al.</i> | 2016 | Netherlands | No MRSA cases. Long-term follow up of patients in 2010 RCT (Bode 2010) |
| Bulanda <i>et al.</i> | 1989 | Poland | Doesn't specify MRSA or SSI. |
| Byrne <i>et al.</i> | 1994 | UK | Doesn't specify MRSA or SSI. |
| Camus <i>et al.</i> | 2011 | France | Medical ICU setting. |
| Carrier <i>et al.</i> | 2002 | Canada | Whilst uses Mupirocin +/- Vancomycin for MRSA cardiac patients, no clear mention of Chlorhexidine (mentions alcohol gel) |
| Carroll <i>et al.</i> | 2011 | USA | Abstract only. |
| Chen <i>et al.</i> | 2013 | USA | Abstract only. Used Chlorhexidine impregnated cloths. No clear indication to suggest Mupirocin use. |
| Chiang <i>et al.</i> | 2017 | USA | Abstract only. Senior author contacted |
| Cho <i>et al.</i> | 2016 | South Korea | Whilst includes the use of Mupirocin and Chlorhexidine in MRSA carriers - performed in a surgical ICU. |

| | | | |
|-------------------------------------|------|-------------|--|
| Cordova <i>et al.</i> | 2010 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, SSI data not sufficient. |
| Coskun <i>et al.</i> | 2004 | Turkey | Letter |
| Coskun <i>et al.</i> | 2005 | Turkey | Letter |
| Deng <i>et al.</i> | 2019 | USA | No use of Mupirocin in pre-op. |
| Denning <i>et al.</i> | 1988 | USA | Case report. |
| Dupeyron <i>et al.</i> | 2002 | France | Chronic liver disease. Not surgical patients. |
| Fawley <i>et al.</i> | 2006 | UK | Whilst uses Mupirocin +/- Cephadrine or Cefuroxime for MRSA surgical patients. Instead of Chlorhexidine, authors use Triclosan (discontinued in UK 2004) |
| Foster <i>et al.</i> | 2018 | USA | Abstract only. MSSA paediatric infections |
| García <i>et al.</i> | 2003 | Colombia | Spanish text. Translated. Uses Mupirocin only. No Chlorhexidine use reported. |
| Gernaat-van der Sluis <i>et al.</i> | 1998 | Netherlands | Whilst uses Mupirocin +/- cefazoline, no mention of Chlorhexidine. <i>Staphylococcus aureus</i> instead of MRSA |
| Gulack <i>et al.</i> | 2018 | USA | Study analyses SSI risk factors and incidence post CABG. No intervention. |
| Gupta <i>et al.</i> | 2011 | USA | Vancomycin and Chlorhexidine use only. No Mupirocin use |
| Hamson <i>et al.</i> | 2010 | UK | Letter |
| Harbarth <i>et al.</i> | 1999 | Switzerland | Includes the use of Mupirocin and Chlorhexidine in MRSA carriers; not specific to surgery or SSIs |
| Harbarth <i>et al.</i> | 2000 | Switzerland | Analysis of 1999 study and associated risk factors |
| Hardy <i>et al.</i> | 2010 | UK | Screening protocol MRSA prevalence on surgical wards (large number of patients). Uses Mupirocin and Triclosan. |
| Harold <i>et al.</i> | 2017 | USA | Whilst uses Mupirocin and Chlorhexidine wipes. Focus on risk factors and effect of blanket decolonisation SSIs and not MRSA specific. No data re swab methodology |
| Huang <i>et al.</i> | 2019 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, ICU setting assessing response of MRSA or VRE (Vancomycin resistant enterococci). SSI not reported. |
| Huang <i>et al.</i> | 2019 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, follows-up the risk of MRSA infection following treatment versus education alone. SSI not reported. |
| Immerman <i>et al.</i> | 2011 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, follows-up the persistence of MRSA carriage despite treatment. MRSA related SSIs not reported. |
| Jabbour <i>et al.</i> | 2010 | Lebanon | French Text. Translated. Use of Mupirocin prior to cardiac surgery. Patients bath with Betadine instead of Chlorhexidine |
| Jervis-Bardy <i>et al.</i> | 2012 | Australia | The use of Mupirocin as an additive in the nasal wash in chronic rhinosinusitis. No clear use of Chlorhexidine or SSI. |

| | | | |
|--------------------------|------|-------------|---|
| Kahler <i>et al.</i> | 2019 | USA | Abstract only. Correspondence with senior author, publication later this year |
| Kline <i>et al.</i> | 2018 | USA | Whilst uses Mupirocin and Chlorhexidine incl. mouthwash on <i>Staphylococcus aureus</i> eradication (primary outcome). Effect of this bundle yet to be trialled with SSI. |
| Kluytmans <i>et al.</i> | 1998 | Netherlands | Review paper |
| Kluytmans <i>et al.</i> | 1998 | Netherlands | Use of Mupirocin as comparator between intervention and historical control group under Cardiothoracic Surgery. Chlorhexidine and pre-op Cefuroxime. <i>Staphylococcus aureus</i> rather than MRSA |
| Konvalinka <i>et al.</i> | 2006 | Canada | <i>Staphylococcus aureus</i> rather than MRSA. Authors report Mupirocin and Chlorhexidine washes +/- Cefazolin use. |
| Larkin <i>et al.</i> | 2008 | USA | Review paper |
| Lindeque <i>et al.</i> | 2008 | USA | No access to full text. Prevalence rather than interventional study. |
| Lipke <i>et al.</i> | 2010 | USA | Review paper |
| Lising <i>et al.</i> | 2014 | USA | Abstract only. Authors contacted for full text. |
| Maxwell <i>et al.</i> | 2017 | USA | Abstract only. Whilst includes the use of Mupirocin and Chlorhexidine in MRSA carriers - performed in an ICU for trauma patients. No SSIs |
| Mest <i>et al.</i> | 1994 | USA | Surgical ICU setting. Did not use Chlorhexidine |
| Moroski <i>et al.</i> | 2015 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, follows-up the effect of MRSA on carriage despite treatment. MRSA related SSIs not reported. |
| Morisaki <i>et al.</i> | 2011 | Japan | Whilst uses Mupirocin for MRSA post sternotomy prevention, no clear mention of Chlorhexidine. |
| Murphy <i>et al.</i> | 2001 | UK | Retrospective audit. Use of Mupirocin and Triclosan +/- systemic antibiotics (rifampicin, vancomycin) |
| Neelakanta <i>et al.</i> | 2019 | USA | Abstract. Uses Iodine instead of Mupirocin, therefore excluded. |
| Nilsson <i>et al.</i> | 2015 | Sweden | Phase I trial of LTX-109 (hydrogel) in decolonisation of <i>Staphylococcus aureus</i> nasal carriers. |
| Nocchi <i>et al.</i> | 2009 | Italy | Abstract only. Case report of <i>Staphylococcus aureus</i> endocarditis |
| Oh <i>et al.</i> | 2011 | Singapore | Poster presentation. Surgical ICU setting. |
| Paocharoen <i>et al.</i> | 2009 | Thailand | No use of Mupirocin. Use of 4% Chlorhexidine as sole treatment |
| Park <i>et al.</i> | 2016 | South Korea | Reports SSIs but not MRSA specific. No use of Mupirocin. Comparison of Povidone - Iodine versus Chlorhexidine. |
| Patel <i>et al.</i> | 2001 | USA | Letter. |

| | | | |
|--------------------------|------|-----------|---|
| Perl <i>et al.</i> | 2002 | USA | <i>Staphylococcus aureus</i> SSI rather than MRSA specifically. Use of Mupirocin. Cardiac patients only used Chlorhexidine. Multi-speciality. Incision cleansed with an iodophor product |
| Pofahl <i>et al.</i> | 2009 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, SSI data insufficient to extract number of MRSA SSI cases. |
| Pofahl <i>et al.</i> | 2011 | USA | Abstract only. Authors contacted for full text. |
| Reynolds <i>et al.</i> | 2013 | USA | Abstract only. No response from author. |
| Rieser <i>et al.</i> | 2018 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, follows-up the cost-savings using povidone/iodine for MRSA decolonisation in comparison to Mupirocin and Chlorhexidine. SSI data not sufficient. |
| Rivera <i>et al.</i> | 2016 | USA | Abstract only. Chlorhexidine wipes and on the day of surgery iodine nasal decolonisation. Mupirocin not used. |
| Robicsek <i>et al.</i> | 2008 | USA | Surveillance study. ICU setting |
| Roggenkamp <i>et al.</i> | 2004 | Germany | Microbiological assay study |
| Rohr <i>et al.</i> | 2003 | Germany | Whilst includes the use of Mupirocin, it combines it with Octeonidine Dihydrochloride instead of Chlorhexidine |
| Saraswat <i>et al.</i> | 2017 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, follows-up the risk of admission to cardiac ICU post CABG. SSI data insufficient to extract number of MRSA SSI cases. Only |
| Shrem <i>et al.</i> | 2016 | Israel | Whilst uses Mupirocin and Chlorhexidine. <i>Staphylococcus aureus</i> SSIs and not MRSA specific |
| Simor <i>et al.</i> | 2007 | Canada | Uses Mupirocin, Chlorhexidine, Rifampicin and Doxycycline in treatment of MRSA. However, no surgery undertaken, and no SSI reported |
| Sporer <i>et al.</i> | 2016 | USA | Whilst includes the use of Mupirocin and Chlorhexidine, SSI data not sufficient. |
| Stegmeier <i>et al.</i> | 2019 | USA | Abstract only. Authors contacted for full text. |
| Suzuki <i>et al.</i> | 2003 | Japan | Whilst includes the use of Mupirocin and Chlorhexidine, follows-up the effect of <i>Staphylococcus aureus</i> SSIs. MRSA related SSIs per se not reported. |
| Tai <i>et al.</i> | 2013 | Australia | Whilst uses Mupirocin and Chlorhexidine <i>Staphylococcus aureus</i> SSIs and not MRSA specific (refers to group study Cordova 2010 and not included in this analysis. |
| Takatsuki <i>et al.</i> | 2010 | Japan | Whilst uses Mupirocin +/- Vancomycin for MRSA carriers in liver transplant patients, no mention of Chlorhexidine body wash use. |
| Talon <i>et al.</i> | 1995 | France | Whilst studies MRSA prevalence after Mupirocin and Chlorhexidine treatment. Doesn't measure SSIs. Setting - Surgical ICU. |
| Tanaka <i>et al.</i> | 2000 | Japan | Japanese text. Translated. Prevalence of MRSA in oesophageal cancer patients. Treated with Mupirocin but not Chlorhexidine. SSIs over 12 months. |
| Thakkar <i>et al.</i> | 2014 | USA | Uses Mupirocin in preoperative decolonisation +/- Vancomycin or cefazolin. Chlorhexidine not used. Data on nasal decolonization prior to surgery using topical antibiotics was not available during the study period. |

| | | | |
|------------------------|------|-----------|---|
| Tsang <i>et al.</i> | 2018 | UK | Uses both Mupirocin and chlorhexidine gluconate + cefuroxime in preoperative decolonisation. Focuses on compliance. Not SSIs |
| Uren <i>et al.</i> | 2008 | Australia | Rhinology specific outcomes following treatment with Mupirocin as an additive to the nasal wash. <i>Staphylococcus aureus</i> instead of MRSA specifically. No Chlorhexidine use. |
| Walsh <i>et al.</i> | 2016 | USA | Doses/duration of Mupirocin and Chlorhexidine not specified |
| Wendt <i>et al.</i> | 2007 | Germany | MRSA prevalence study after intervention with Mupirocin and Chlorhexidine. Hospital and nursing home residents. No SSIs reported. |
| White <i>et al.</i> | 2018 | USA | Abstract only. Whilst includes the use of Mupirocin and Chlorhexidine in cardiac MRSA carriers. No reduction in SSIs. Author uncontactable |
| Wilcox <i>et al.</i> | 2003 | UK | Uses Mupirocin and Tricolosan washes (discontinued in UK in 2004) |
| Williams <i>et al.</i> | 2015 | UK | Abstract only. Whilst includes the use of Mupirocin and Chlorhexidine in paediatric neurosurgical MRSA carriers. No reduction in SSIs. Author uncontactable |
| Wong <i>et al.</i> | 1989 | Singapore | MRSA primary dermatological infections treated with Mupirocin or Tetracycline |
| Zamani <i>et al.</i> | 2017 | USA | Abstract only. Study of a MRSA bundle including risk of groin staples as risk of SSI. Chlorhexidine preparation recorded. Mupirocin not mentioned |

APPENDIX C

Reagents used in Chapter 4

10g Reactive Oxygen® (donated by Matoke Holdings Ltd.)

10g Acacia honey (vehicle) (donated by Matoke Holdings Ltd.)

Mupirocin (M7694;) 20mg/ml, 2%; Sigmoid-Aldrich, UK)

Clarithromycin (A3487; 2mg/ml, 0.2%, Sigmoid-Aldrich, UK)

Doxycycline hyclate (D9891; 2mg/ml, 0.2% C9742; Sigma-Aldrich, UK)

Brain Heart Infusion broth (53286; Sigmoid-Aldrich, UK)

LIVE/DEAD staining kit

DAPI counterstain

96-well plates (Fisher Scientific, UK)

Cell scrapers

Pipetting reservoirs

Centrifuge tubes

Syringe filters

Inoculating loops

Microcentrifuge tubes

6-well plates (Costar)

Hank's balanced salt solution

List of references

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