Natural Honey to Eradicate Nasal Methicillin resistant Staphylococcus aureus (MRSA) A Randomised Control Trial

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Natural Honey to Eradicate Nasal Methicillin resistant *Staphylococcus aureus* (MRSA): A Randomised Control Trial

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Declaration

I declare that this thesis, which I submit to the RCSI for examination in consideration of the award of a higher degree of Doctor of Philosophy, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research program this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed_____

Student number: **13150065**

Date: 14th October 2016

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Summary

Methicillin-resistant *Staphylococcus aureus (*MRSA) is an endemic pathogen of public health concern in Ireland, as in many other health systems. For the nasal clearance of MRSA, the site that is often colonised in humans, the antibiotic mupirocin remains one of the most successful topical antibiotics to date. However, increasing bacterial resistance to mupirocin and limited effective alternate antibiotic options necessitate the need for unconventional approaches to eradicate nasal MRSA. Colonisation is a precursor for infection, and infections due to MRSA are associated with a greater risk of treatment failure, increased patient mortality and higher costs.

Natural honey has been used by many traditional systems of medicine as a healing agent. In modern medicine, it is used as a wound healing agent. A recent Cochrane review reports that honey appears to heal partial thickness burns more quickly than conventional treatment, and infected post-operative wounds more quickly than antiseptics and gauze.

Interest in an alternative agent for nasal decolonisation of MRSA led the researcher to the pilot study that employed medical grade honey (MGH). The results of the pilot study were encouraging which lead to the conduct of a clinical study 'Natural Honey to Eradicate Nasal MRSA (NHNMRSA) a Randomised Control Trial (RCT)'. Patients were recruited from Beaumont Hospital to the single centre open label RCT, which investigated the comparative efficacy of nasal decolonisation of MRSA using MGH and mupirocin 2% nasal application.

Patient characteristics, including age, gender, comorbidity, dependency of care, presence of invasive and indwelling devices, skin integrity, colonisation with multi-resistant drug resistant organisms, MRSA status on study enrolment, past decolonisation attempts and mupirocin use, as well as infection prevention and control practices during the study period were assessed, to determine the impact if any, on the outcome of intervention on nasal MRSA. The data were then analysed to establish the correlation, if any, between the outcomes of the

intervention, and between the intervention and control groups. The relationship between nasal as well as non-nasal MRSA colonisation was assessed, in addition to other factors that are previously reported as factors associated with failed decolonisation.

Five specific objectives formed the foundation of the RCT, the key findings of which are summarised. The first objective a literature review on mupirocin resistance (MR), presented a comprehensive picture on the prevalence of MR, which ranges from 1% - 81%, associated chlorhexidine resistance, which ranges from 0.6% - 91%, as well as multi drug resistance among MRSA isolates. The emergence of high-level MR amongst coagulase negative Staphylococci (CoNS) isolates indicates an expanding reservoir of plasmids encoding MR, which can be transferred to other CoNS strains as well as to *S. aureus* including MRSA. HLMR and resistance to other antibiotics amongst CoNS curtails the oral antibiotic options for prolonged treatment of prosthetic infections with CoNS. Resistance to mupirocin and chlorhexidine limits the options for patients who may benefit from MRSA suppression or decolonisation therapy. Alternative agents such as octenidine dihydrochloride, polyhexanide, ethanol (70%), sodium hypochlorite, lysostaphin, omiganon pentahydrochloride, natural honey, tea tree oil, silver and bacteriophages have been investigated with varying success for MRSA decolonisation. However, therapeutic trials of alternative agents that show some promise must be further evaluated in clinical trials before they can be recommended for use in clinical practice.

In the RCT, robust comparability of the study participants in the intervention and control groups was confirmed on univariate analysis. The univariate analysis also confirmed that none of the patient variables analysed was of statistical significance on the patient outcome, i.e. eradication of nasal MRSA. On an intention to treat (ITT) analysis, 18 (36%) in the intervention group and 25 (50%) in the control group were decolonised of nasal MRSA. A χ^2 test was performed to assess the difference in the rate of decolonisation of MRSA between the intervention and control group. There was no statistically significant difference between the two groups (χ^2=1.999, p=0.157). On a per-protocol (PP) analysis,

in the intervention group 18 (43%) participants and in the control group 25 (57%) participants were decolonised of nasal MRSA, however, a χ^2- test showed no significant difference (χ^2=1.675, p=0.196). Based on the ITT and PP analysis, as there was no statistical significance difference in the outcome of nasal MRSA decolonisation between the intervention and control groups, the null hypothesis was not rejected. On multivariate logistic regression, concomitant non-nasal MRSA colonisation was significantly associated, (χ^2=7.241, p=0.008) with persistent nasal MRSA. In addition, altered skin integrity and the application of more than two courses of mupirocin 2% nasal ointment prior to RCT enrolment were also associated with persistent nasal carriage of MRSA. However the less than anticipated number of patients enrolled in the study impacted on the power to detect significant differences between the intervention and control groups.

The third objective was to determine and compare bacterial susceptibility to mupirocin and changes over time from first identification to completion of the RCT. Of the historic, baseline and final isolates, mupirocin susceptibility (MS) was 91%, 88% and 77% respectively. The prevalence of MR progressively increased from the time initially identified to the end of the study, 8% on first time identification, 12% at study enrolment, and 23% at the end of study. New acquisition of MR amongst RCT participants was 10% and of all the cases who newly acquired MR, 75% were HLMR. The acquisition of MR necessitates the monitoring of MS amongst MRSA isolates, risk assessment, the judicious use of mupirocin as well as the use of alternative agents for MRSA decolonisation.

Laboratory investigations of MRSA isolates and assays to determine antimicrobial efficacy of MGH together formed the fourth study objective. The MRSA isolates were characterised using *spa* typing and compared where available, at three time points; historic, baseline and on study completion. Of the baseline and persistent MRSA isolates (143), 26 different *spa* types were identified. The common *spa* types were; t032, 59 (41%), t515, 19 (13%), t127, 17 (11.8%) and t4599, 10 (7%). Based on the *spa* types, a sequence-type (ST) could be inferred for 110 (77%) isolates. The common STs were ST22, 91

(63.6%), followed by ST1, 17 (11.8%), and one isolate each of the ST5 and ST8 type. In summary, the *spa* type of the carriage isolates did not appear to influence the outcome of the nasal decolonisation. Persistent colonisation with the same *spa* type was evident even over relatively long time-spans. However, replacement of the colonising *spa* type was also identified.

The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of MGH (test honey) was determined using laboratory assays. The MIC determined using the agar well diffusion method demonstrated antibacterial activity of test honey at 5% and higher concentrations for the clinical and reference MRSA isolates. Using the broth micro-dilution method to differentiate bactericidal or bacteriostatic action, it was established that the MIC and MBC of the test honey were both 12.5% to the MRSA isolates tested. The findings are in concordance with reports by other investigators who have reported MIC at concentrations of 4% and higher to antibacterial honey.

The participant's perception on MRSA carriage and their experience following the use of MGH and mupirocin 2% was evaluated which formed the basis of the fifth objective. An adapted brief illness perception questionnaire (BIPQ) that composed of nine elements was utilised to collate patient perceptions of MRSA. In summary the participants perceived MRSA colonisation as a chronic condition and that it did not have serious consequences on their daily lives. They were in general emotionally detached from the condition and few had MRSA related symptoms. Although most participants felt they had limited or no control over carriage, decolonisation was considered beneficial, indicating the importance attached to treatment/control of MRSA. The survey result shows a sub-optimal understanding of MRSA among 40% of patients that necessitates measures to target improving knowledge about MRSA. Such an intervention should enable patients to understand MRSA acquisition and transmission as well as adherence to treatment/decolonisation, potentially leading to better outcomes.

A Likert scale type rating was used in the product experience questionnaire (PEQ) to collate participants' experiences of MGH cream and mupirocin nasal

application. In the investigative group, most patients (95%) concurred that Medihoney™ Derma Cream was easy to apply, not sticky (90%), did not lead to a runny nose (85%) and they did not experience an unpleasant sensation (95%). Based on the participants' response it could be inferred that Medihoney™ Derma Cream may be applied to the human nasal passages with minimal undesirable effects. In the control group, most respondents (87%) concurred that mupirocin 2% nasal ointment was easy to apply, but it was sticky (20%), and a runny nose was experienced by 33% of respondents. Almost all (95%) respondents agreed that they did not experience any unpleasant sensation following its application. Overall the patients' preferred choice was a natural alternative to an antibiotic, if available, for MRSA decolonisation.

The NHNMRSA RCT, I believe, is the first study that has used MGH for nasal MRSA decolonisation. The RCT results offer potential but larger and multisite studies must be conducted to confirm the results to facilitate the development of MGH as an alternate agent for nasal decolonisation of MRSA.

Chapter 1 Introduction and background

Natural Honey to Eradicate Nasal MRSA A Randomised Controlled Trial

1.1 Introduction

Methicillin resistant *Staphylococcus aureus (*MRSA) was first identified in England in 1961 and has since emerged as an important nosocomial pathogen. MRSA is an endemic pathogen of public health concern in Ireland, as in many other European countries and elsewhere. The antibiotic mupirocin is one of the most successful topical antibiotics for the clearance of nasal MRSA. However, increasing bacterial resistance to mupirocin and a dearth of effective antibiotic options necessitates the need for alternative approaches to eradicate human nasal MRSA.

1.2 *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*) was discovered in 1880 by the Scottish surgeon Sir Alexander Ogston, who coined the name *Staphylococcus aureus*, '*Staphylococcus*' from the Greek expression *staphylé*, which means 'bunch of grapes' and '*aureus'* from the Latin golden, due to the yellow-orange appearance of the colonies. (1) *S. aureus* is a member of the *Staphylococcus* genus, which are Gram-positive cocci. *Staphylococci* typically appear in clusters, but can be seen in pairs, tetrads or short chains. *Staphylococci* are non-motile, non-spore forming, facultative anaerobes. There are at least 40 different species in the *Staphylococcus* genus of which more than half are found in humans. *S. aureus* is the most virulent member of the genus *Staphylococci* and can be differentiated from other *Staphylococci* by coagulase production, which converts fibrinogen to fibrin. *S. aureus* is both a coloniser and a pathogen of humans. It is a ubiquitous organism which colonises a variety of different body sites in humans such as the anterior nares, throat, groin and axilla.

The most frequent carriage site is the anterior nares with 20-30% of the human population being persistent carriers of *S. aureus* and a further 60% carrying *S. aureus* intermittently. (2-4) Nasal carriage is probably due to a combination of

host and organism mediated factors. The ability of particular strains of *S. aureus* to adhere to the desquamated cells of the nasal epithelial surface may be due to the presence of certain proteins on the organism. *S. aureus* behaves as a commensal in the majority of the people, i.e. colonisation. However, colonisation with *S. aureus* significantly increases the risk of infection in the host if their defences become compromised. (2)

S. aureus is a virulent organism capable of causing pathologic effects in the host (human and animals) resulting in a range of infections from superficial to fatal. Virulence is defined by Dorland's Medical Dictionary for Health Consumers as the degree of pathogenicity of a microorganism, as indicated by the severity of disease produced and the ability to invade the tissues of the host, or as the ability of any infectious agent to produce pathogenic effects. Clinical diseases caused by *S. aureus* can range from more superficial skin and soft tissue infections to more invasive diseases such as bone and joint infections, bloodstream infections (BSI), pneumonia, infective endocarditis and the toxin mediated toxic shock syndrome. In most cases *S. aureus* infection is endogenous in origin, with the anterior nares being the most important reservoir of *S. aureus* in humans.

The incidence of *S. aureus* infection has increased over the last 25 years. (5) *S. aureus* was the most common cause of BSI, skin and soft tissue infection and pneumonia in the United States, Canada, Europe, Latin America and Western Pacific in the 1990's. (6) *S. aureus* is also a cause of medical-device related infection, and can infect a variety of prosthetic devices such as central vascular catheters (CVCs), prosthetic joints and implantable cardiac devices. (7)

1.3 Methicillin-resistant *Staphylococcus aureus*

A number of factors have contributed to the success of *S. aureus* as a pathogen. One of the factors that has enhanced the virulence potential of this pathogen has been the evolution of antibiotic resistance. The first strains of MRSA were identified in England in 1961, two years after the introduction of methicillin, the first anti-staphylococcal penicillin. (8) This organism was also found to be resistant to most other β-lactam antibiotics, such as flucloxacillin, cefuroxime and co-amoxiclav. Other methicillin resistant strains were soon identified in various parts of the world. (8) MRSA emerged as a nosocomial pathogen in the 1980's and its prevalence increased dramatically worldwide. MRSA has been endemic in Ireland since the 1970's and is a major public health concern, resulting in increased morbidity and mortality, and is responsible for increased healthcare costs. (9)

Infections due to MRSA, in comparison with methicillin-susceptible *S. aureus* (MSSA), are associated with greater risk of treatment failure, increased patient mortality and higher costs. (10-11) In Scotland, the overall prevalence of MRSA colonisation in patients being admitted to hospital was 7.5% during 2008. (12) This translates to around one in 13 patients presenting to hospital were colonised with MRSA, and in some specialties this was as high as one in five patient admissions.

The methicillin resistance gene (*mecA*) is carried on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). SCC*mec* consists of the *mecA* gene and cassette chromosome recombinase (*ccr*) gene complexes, which integrates into the *S. aureus* chromosome. Eleven different SCC*mec* types (SCC*mec*1-XI) have been identified in MRSA to date, with different combinations of *mec* and *ccr* gene complexes. The *mecA* gene codes for an alternative penicillin binding protein (PBP) 2a, which has reduced affinity for β-lactam antibiotics and in turn facilitates cell wall synthesis when native PBPs have been inactivated by β-lactam antibiotics. (13) The majority of healthcare-

associated MRSA (HA-MRSA) strains belong to one of the five genetic lineages or clonal complexes (CCs), CC5, CC8, CC22, CC30 and CC45. (8) ST22-MRSA-IV (MRSA sequence type 22, *mec* type IV), the predominant HA-MRSA strain circulating in Ireland and the UK, at present belongs to CC22. (14)

Community-acquired MRSA (CA-MRSA), which is increasing in prevalence worldwide, is often associated with more severe infections than HA-MRSA. (15) CA-MRSA is not the result of HA-MRSA strains spreading into the community but the more recent acquisition of SCC*mec* elements with distinct MSSA lineages e.g. ST1-MRSA-IV (CC1), ST80-MRSA-IV) (CC80) and ST5-MRSA-IV (CC5). (16)

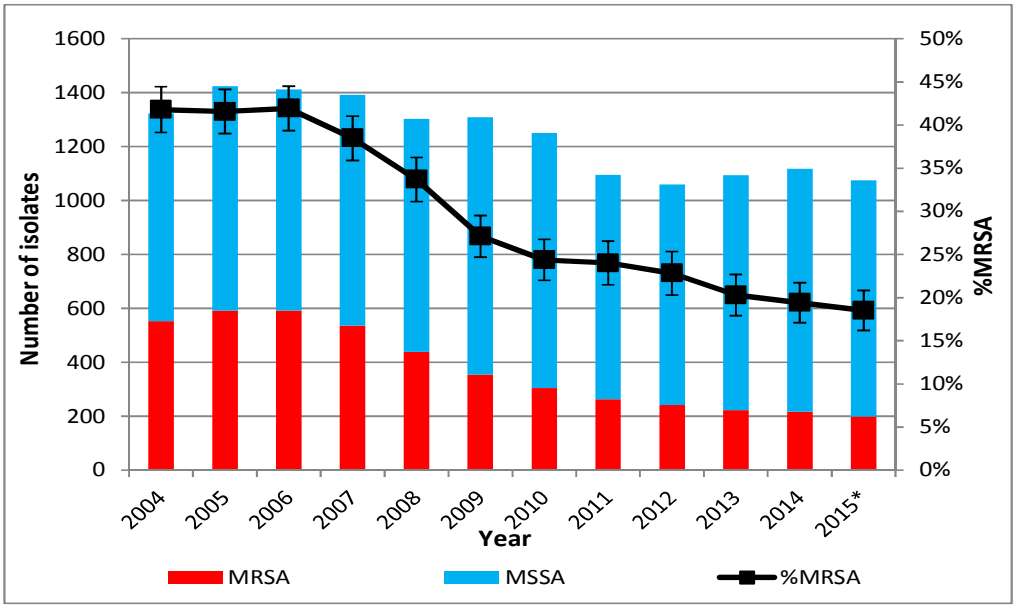
The epidemiology of MRSA is continuously evolving as exemplified by the emergence of CA-MRSA and its spread within healthcare settings. The emergence of livestock-associated MRSA among farmers in Europe underpins the changing epidemiology of MRSA. (17) With increasing resistance, not only to glycopeptides but to older antimicrobials such as fusidic acid and rifampicin, the management of MRSA is also challenging. (18-20)

S. aureus bloodstream infection surveillance

In Ireland, the Health Protection Surveillance Centre (HPSC) collects data on invasive isolates of *S. aureus* as part of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Data are collected on the first isolate from blood per patient per quarter (i.e. no duplicates or second strains are included) in accordance with the EARS-Net case definition. In Ireland between 1,100 and 1,400 *S. aureus* BSIs are reported annually (EARS-Net) data Q4, 2015, Figure 1.1) with the majority of BSIs caused by MSSA. The proportions of *S. aureus* that are MRSA have decreased from 42% in 2006 to 18.5% in Q4 2015. In 2014, the overall number of *S. aureus* BSIs increased compared to 2013, however, it coincided with a 0.9% reduction in MRSA BSI. The numbers

reported to EARS-Net represent only a proportion of the total number of people infected and/or colonised with MRSA in Ireland.

Figure 1.1 Trends in *S. aureus* bloodstream infections showing %MRSA in Ireland



*2015 data provisional to the end of Q4; and missing data from 3 laboratories for 2 quarters each

The EARS-Net surveillance provides data on the burden of MSSA and MRSA BSIs which enables the ongoing monitoring of BSIs as well as the impact of various infection prevention and control (IPC) efforts. However, in the absence of a national surveillance program monitoring the incidence of MRSA from non BSI sources, BSIs represents the 'tip of the iceberg' i.e. the burden of both susceptible and resistant *S. aureus,* infection and colonisation.

Prevention and control of MRSA

Colonisation with MRSA increases the risk of adverse health outcomes and it is estimated that 10% to 30% of carriers subsequently developing infection. (21) The nose as well as extra-nasal sites such as the throat and perineum, skin ulcers and skin lesions e.g. wounds and eczema, are commonly colonised. (22-24) Eradication of MRSA carriage reduces the risk of infections due to MRSA.

Nasal and non-nasal MRSA eradication is attempted with varying success. A multipronged strategy is advocated for the control and prevention of transmission of MRSA. This includes screening of high risk patients, early detection, isolation, decolonisation, education of health care workers, cleaning and disinfection of the environment and most importantly, practices such as hand hygiene among all healthcare workers, which is one of the components of standard precautions in infection prevention and control. (25)

Standard precautions

Standard precautions (SP) are a set of infection control practices used to prevent transmission of diseases that can be acquired by contact with blood, body fluids, non-intact skin (including rashes), and mucous membranes. Standard precautions are designed to reduce the risk of transmission of microorganisms from both recognized and unrecognized sources of infection in hospitals. (26) The elements of SP are; hand hygiene, use of personal protective equipment such as; gloves, apron and facial protection, prevention of needle stick injuries, safe injection practices, safe practices for lumbar puncture procedure, respiratory hygiene and cough etiquette, environmental hygiene, safe management of linen, safe disposal of waste and decontamination of patent equipment. While the practice of SP are essential in the care of all patients regardless of their infective status, additional precautions are required to prevent and control transmission of pathogens from patients colonised or infected with infectious organisms.

Transmission-based precautions

Transmission-based precautions are additional infection control precautions in health care, and the latest routine infection prevention and control practices are applied for patients who are known or suspected to be infected or colonised with infectious agents, including certain epidemiologically important pathogens. These include contact, droplet and airborne precautions. (26)

Contact precautions (CP) are intended to prevent transmission of infectious agents, including epidemiologically important microorganisms, which are spread by direct or indirect contact with the patient or the patient's environment. A single-patient room is preferred or, when not available, cohorting of patients with similar pathogens, who require CP. Healthcare personnel caring for patients on CP's must wear a gown and gloves for all interactions that may involve contact with the patient or potentially contaminated areas in the patient's environment.

Droplet precautions are intended to prevent transmission of pathogens spread through close respiratory or mucous membrane contact with respiratory secretions. In addition to contact precautions, isolation or cohorting is essential and a spatial separation of more than three feet is especially important for patients in multi-bed rooms, with infections transmitted by the droplet route. In addition to gloves and apron healthcare personnel must wear a mask for close contact with patients who are infectious.

Airborne precautions prevent transmission of infectious agents that remain infectious over long distances when suspended in the air, and the preferred placement for patients who require airborne precautions is in an airborne infection isolation room (AIIR). Healthcare workers must use the personal protective equipment used for CP in addition to a high filtration mask such as N95 or higher level respirators while caring for patients on airborne precautions.

Patient decolonisation of MRSA

Eradication of MRSA carriage from the nose and from other sites forms an integral part of the strategies to prevent and control of MRSA in many countries. (27-29) This includes nasal decolonisation, decolonisation from intact and non-intact skin, as well as from other MRSA colonising sites.

The warm moist environment in the human nasal passages provides a conducive environment for the survival and growth of *S. aureus* including MRSA. Mupirocin calcium ointment was clinically introduced in the late 1980s

and has proved to be one of the most successful topical antibiotics for the clearance of nasal *S. aureus*, including MRSA. (30,24,31-33)

Mupirocin nasal ointment

The antibiotic mupirocin (*pseudomonic acid A*) is produced by the bacteria *Pseudomonas fluorescens*. Mupirocin is a competitive inhibitor of bacterial isoleucyl transfer-RNA synthetase, and is active against most Gram positive and some Gram negative bacilli. Mupirocin-mediated inhibition of isoleucyl-tRNA synthetase impedes protein and RNA synthesis, ultimately leading to bacterial death. There is very little systemic absorption following the topical application of mupirocin. After systemic administration, mupirocin has a short half-life (15 mins) and is rapidly converted into inactive monic acid, which is excreted principally through the kidney. The therapeutic indication for mupirocin is the elimination of the nasal carriage of staphylococci, including MRSA. The method of application is nasal ointment, usually 2%, applied to the anterior nares two to three times a day. Nasal carriage is then normally cleared within five to seven days of commencing treatment. (30,33)

Mupirocin 2% ointment is used for nasal decolonisation alone or as part of a comprehensive MRSA decolonisation strategy along with skin antiseptics such as chlorhexidine. The impact of the application of mupirocin to the nose has been investigated by various researchers with varying success, in terms of immediate as well as medium to long term sustained nasal MRSA decolonisation. (24,34-36)

Neomycin sulphate (Naseptin Nasal 0.5% v/v Cream) is an alternate option for eradicating staphylococcal carriage in the nose. For nasal decolonisation, it is applied four times a day for ten days. In clinical practice, a single course of neomycin is generally used for nasal decolonisation of mupirocin resistant *S. aureus* carriage.

Non-nasal MRSA decolonisation

MRSA colonises nasal as well as non-nasal body sites such as the throat, groin/perineum, axilla and non-intact skin, namely ulcers and wounds. Antiseptics such as 4% chlorhexidine, 7.5% povidone iodine or 2% triclosan are reported to be equally efficacious for decolonisation of non-nasal sites. (25)

In most MRSA IPC programs, chlorhexidine is a major component and is often used in various forms as part of oral care, skin antisepsis prior to intravascular device placement, before surgical procedures, during patient bathing, and as a component of some antimicrobial-impregnated catheters and dressings. Chlorhexidine is a biguanide cationic bactericidal agent which is rapidly taken up by *S. aureus.* (37-38) At low concentrations, it disrupts the integrity of the cell wall and membranes, resulting in leakage of the intracellular contents, and at high concentrations chlorhexidine causes coagulation of the intracellular contents.

Attempts at non-nasal MRSA decolonisation involves the use of antiseptic preparations such as chlorhexidine soap / triclosan and includes daily body washes and shampoo for at least two days during a consecutive five day period. More than one course of antiseptic body washes are reported to be beneficial for some patients. However, decolonisation is challenging where multi-site colonisation is present.

1.4 Mupirocin resistance

In modern medicine the discovery and use of antibiotics to treat bacterial infections has been largely successful since their introduction. However, the use of antibiotics has also lead to the development of bacterial resistance which limits the effective use of several antibiotics.

For nasal MRSA eradication a significant limitation to the use of mupirocin is resistance, which ranges from 1% to 81%. (39-46) Mupirocin resistance (MR) is clinically important for IPC personnel who are engaged in MRSA control efforts

and also in the management of individual patients such as before major surgery to minimise post-operative MRSA infection.

Laboratory detection of mupirocin resistance

Phenotypically, resistance to mupirocin is determined according to minimum inhibition concentration (MIC) breakpoints with susceptible being ≤4 mg/L, an MIC of 8-256 mg/L indicating low level resistance and MIC >512 mg/L indicating high level resistance. (46-47) Mupirocin MICs of 8-64 mg/L are usually due to non-synonymous changes in the native isoleucyl-tRNA synthetase gene.

Categories of mupirocin resistance

There are two types of MR reported; low level (LLMR) and high level mupirocin resistance (HLMR). A MIC of 8-256 mg/L reflects LLMR and MIC of ≥512 mg/L reflect HLMR. HLMR is mediated by the acquisition of a conjugative plasmid containing *mupA* (*ileS2*), which encodes an alternate isoleucyl tRNA synthetase. (47-48)

In addition to the *mupA* gene, another mechanism of HLMR, mediated by a novel locus, *mupB,* has been reported. (49) The *mupB* gene (3,102 base pairs) shares 65.5% sequence identity with *mupA* but only 45.5% with *ileS*. The resultant MupB protein shares 72.3% similarity and 41.8% similarity with MupA and *ileS*, respectively. These findings support the presence of non-*mupA*-mediated HLMR as reported by others. (49,46,50) Molecular studies of MR in *S. aureus* populations indicate that nearly all *S. aureus* isolates with HLMR have the *mupA* gene. (51) However, low or non-expression of the *ileS2* gene has been described amongst LLMR isolates. (52)

1.5 Alternative agents to mupirocin

While mupirocin remains the most successful topical antibiotic for the clearance of nasal *S. aureus*, including MRSA, increasing resistance to mupirocin along with concurrent resistance to antiseptics used for non-nasal eradication in addition to multidrug resistance, necessitates the need for alternatives. There are very few antibacterial agents with new mechanisms of action under development to meet the challenge of multidrug resistance. The EU identifies a widening gap between the burden of infections due to multi-drug anti-bacterial resistance and the development of new systemic agents, as a key priority. (53) This also applies to topical agents used in decolonisation.

Tea tree oil (*Melaleuca alternifolia*) exhibits antimicrobial and anti-inflammatory properties, and has been investigated as a potential agent for MRSA decolonisation. In a clinical trial, a tea tree oil (10%) based regimen was compared with standard treatment consisting of mupirocin, chlorhexidine or silver sulfadiazine. (54) Of the patients who received standard treatment, overall 56/114 (49%) were cleared of MRSA carriage. Of the patients who received the tea tree oil regimen, 46/110 (41%) were cleared. The results show that mupirocin was significantly more effective at clearing nasal carriage (n=58/74), than tea tree oil cream (n=36/76), (78% *vs* 47%; $P = 0.0001$). However, tea tree oil treatment was more effective than chlorhexidine or silver sulfadiazine in clearing superficial skin sites and skin lesions of MRSA. Variable success on the use of tea tree oil for MRSA decolonisation is also reported. (55)

Sodium hypochlorite or bleach is another agent that has been used extensively as a topical antimicrobial for the treatment of wounds and burns since it was originally described in 1915 by Dakin. The Infectious Diseases Society of America guidelines recommend nasal mupirocin and dilute bleach baths for 15 min twice weekly for three months, as eradication of colonisation for patients with refractory MRSA skin and soft tissue infections (SSTI). (56) A randomised controlled trial (RCT) comparing various decolonisation regimens using mupirocin, chlorhexidine and bleach on patients with community based SSTI and multisite *S. aureus* colonisation revealed that the highest rate of successful

S. aureus eradication (71%) in patients occurred with a combination of nasal mupirocin and daily bleach baths. (57)

Honey has been used for centuries as a topical treatment for a wide range of wounds. Honey is once again of interest to healthcare practitioners involved with wound management. Medical grade honey based dressings are used with reported wound healing stimulating properties in human burns, wounds and ulcers. In burns there is also reported evidence for its antibacterial capacity. The antibacterial action of honey leads to a lower incidence of infection and the elimination of MRSA. Researchers claim that honey has deodorizing, debridement, anti-inflammatory and wound pain reducing properties, although the evidence for these properties is rather limited. (58)

1.6 Honey and its medical importance

This section will consider the historic as well as evidence-based scientific literature on the use of honey for various medicinal purposes. It will provide the rationale for the use of honey in the pilot study, and later in the randomised controlled trial. The composition of honey and its antibacterial properties will be presented. The use of honey for healing ulcers and wounds by various investigators will be considered along with the Cochrane review conclusion (2015) on the use of honey for the management of wounds, ulcers and burns.

What is honey and its composition?

Honey is a viscous, supersaturated sugar solution derived from nectar gathered and modified by the honeybee, *Apis mellifera*. Honey is an acidic, hygroscopic and hyperosmolar solution. (59) It is composed of water, sucrose, glucose, fructose, amino acids, wax, pollen, pigments, minerals and enzymes. (60) Honey contains low levels of sugars, other than the four main sugars (fructose, glucose, sucrose and maltose), organic acids, proteins and minerals in addition to water molecules which comprise less than 20% of the weight of honey, which renders it a supersaturated sugar solution. (61)

Honey has a pH of 3.2-5.5, which is mainly due to the presence of gluconic acid. (62-63) Various enzymes are present in honey, namely diastase also known as α amylase, glucose oxidase and invertase. The enzyme invertase converts sucrose in the honey into simpler glucose and fructose, and glucose oxidase, oxidizes glucose in honey producing gluconic acid and hydrogen peroxide. (60) Various honeys have been studied for their chemical composition, almost all of which has demonstrated low pH, hydrogen peroxide production, and hyperosmolarity. However, Manuka honey is distinctive with high concentrations of the enzyme methylglyoxal (MGO). (64)

The beneficial role of honey for medicinal purposes is ascribed to its high osmolarity, acidity, content of hydrogen peroxide and non-peroxide

components, i.e., the presence of phytochemical components like MGO. (64-65) Most types of honey generate hydrogen peroxide when diluted, due to the activation of the enzyme glucose oxidase that oxidises glucose to gluconic acid, which in turn yields hydrogen peroxide. (66) Several components are known to contribute to the nonperoxide activity, such as methyl syringate and MGO. (64) Honey originating from *Leptospermum* trees contains a number of aromatic acids of which syringic acid and phenyllactic acid are the most abundant. (64)

A study examining the antimicrobial properties of honey *in vitro* concluded that hydrogen peroxide, MGO and an antimicrobial peptide, bee defensin-1, are distinct components contributing to the bactericidal activity of honey. (67) Of the different types of honeys studied, darker honeys are richer in phenolic compounds like flavonoids and tannins, and posses the strongest antioxidant and radical scavenging activities. (68) The antimicrobial characteristics of honey will be explored in detail later on in the chapter.

Source of honey and recognised characteristics

The flora origin, bee species, geographical source and post-harvesting conditions impacts on the properties of honey. (69) The variations in the antimicrobial activity of some natural honeys are attributed to spatial and temporal variation in the sources of nectar. Honey derived from *Leptospermum* trees (Manuka) is perhaps the most studied monofloral honey, which has been shown to have significant antibacterial properties independent of hydrogen peroxide and osmolarity as confirmed during *in vitro* studies. (70-71) Manuka honey contains a number of aromatic acids and high concentrations of the enzyme MGO. Honey from the Ulmo tree (*Eucryphia cordifolia*), is another honey that has been investigated for antimicrobial activity.

Honey use in human history

The medicinal importance of honey has been documented in the world's oldest medical literatures. The healing properties of honey is recorded in the

Ayurveda, a system of medicine in ancient Hindustan, the modern day Indian sub-continent, Hippocrates, Aristotle, ancient Greece, and the Egyptian medical literatures. (72-73) A Sumerian tablet writing, dating back to 2100-2000 BC, is one of the first known written references to honey, which mentions honey's use as a drug and an ointment. One of the world's oldest surgical texts, the Edwin Smith papyrus, dates back to 1600–2200 BC, describes treating a *head wound with an oil-and-honey–soaked linen bandage*. (74) Aristotle (384-322 BC) refers to pale honey as being *good as a salve for sore eyes and wounds*. (75) The belief that honey is a nutrient, a drug and an ointment thus has been passed on to generations. In contemporary times the use of honey for medicinal purposes has developed into an alternate branch of medicine called apitherapy.

In the modern era honey's anti- microbial qualities were first documented in 1892 by B.A. Van Ketel, a Dutch scientist, and research in the United States and Europe has noted its worth in treating infected wounds. (76) The use of honey for medicinal purposes fell out of favour in modern medicine, perhaps with the advent of antibiotics. However, the emergence of bacteria that are resistant to antibiotics such as MRSA and the limited armour on hand in the fight against antimicrobial resistance has seen a resurgence of interest in natural agents that have demonstrated antibacterial properties such as honey. (69)

Honey for skin care

Honey is considered particularly suitable for skin care, and its regular use is thought to keep the skin juvenile and retard wrinkle formation. Honey is hygroscopic, antibacterial and fungicidal, nurtures skin and contributes to regulating the mildly acid pH of the upper protective skin layer. (77) The mechanisms of action of honey on skin cells are deeply conditioned by the botanical sources and include antioxidant activity, the induction of cytokines and matrix metalloproteinase expression, as well as epithelial-mesenchymal transition in wounded epidermis. (77) The hydrating effect of honey is mainly

linked to the high content of fructose and glucose, forming hydrogen bridges with water and maintaining the moisture of the skin's horny layer. (78) The hydrating ability of honey also derives from the presence of amino acids and of organic acids which can supplement the natural moisturizing factors of the horny layer of the skin.

The dermatological uses of honey are generally based on empirical knowledge. However there is a growing interest in demonstrating the scientific evidence supporting empirical knowledge for its use of in dermatological applications. Due to large variations in the qualities of honeys from different natural sources, much interest is paid to the development of medical-grade honey, especially for the treatment for skin infections and wound healing. (67)

In cosmetic formulations, honey is used for its emollient, humectants, soothing, and hair conditioning effects, and because it regulates pH and prevents infections. Honey-based cosmetic products include lip ointments, cleansing milks, hydrating creams, after sun, tonic lotions, shampoos, and conditioners.

The physical and chemical properties of honey and the fact that it can be painlessly removed along with the use of a non-adherent dressing is particularly suitable for its use in the care of wounds and burns. Honey is also used in the treatment of pityriasis, tinea, seborrhea, dandruff, diaper dermatitis, psoriasis, hemorrhoids, and anal fissure. (77)

Honey and wounds

The healing properties of honey are believed to be a combination of antibacterial, antioxidant and immuno-modulatory properties. (79,76,58)

Honey when applied to wounds maintains a moist wound condition, and its high viscosity helps to provide a protective barrier to prevent infection, by inhibiting bacterial growth, in addition to preventing adhesion of dressings to wounds. (80-81) Honey is thought to decrease oedema, allowing better circulation and delivery of oxygen and essential nutrients for wound repair. (63) The high sugar content of honey draws fluid by osmosis from deep in the wound bed, which

enhances debridement and aids in wound healing. This high osmotic pressure is also thought to be responsible for extracting water from bacteria thus contributing to its anti-bacterial activity. (70) The osmotic pressure from honey also aids in removal of necrotic and devitalized tissue by drawing out lymphatic fluid from the wound base. (80)

The sugary wet environment may also improve local nutrition and epithelialisation of the wound, in addition to an acidic environment that provides an optimal medium for fibroblast activity, which aids in wound healing. (59) During the inflammatory and proliferative wound healing phase, honey is able to either stimulate or inhibit the release of certain factors (cytokines, Matrix metalloproteinase 9 (MMP-9), reactive oxygen species (ROS) from immune and cutaneous cells, depending on wound condition. (82) When wound inflammation is uncontrolled, honey prevents prolonged wound inflammation and reduces the elevated levels of pro-inflammatory cytokines, ROS, and MMP-9. It is also speculated that honey can act as an immuno-modulator with both pro-inflammatory and anti-inflammatory properties, inducing or stimulating the release of tumour necrosis factor-α (TNF-α), interlukin (IL), IL-1β, and IL-6 from monocytic cell line Mono Mac 6 (MM6) cells and peripheral blood monocytes. The production of pro-inflammatory cytokines, decreases biofilm formation, inhibits bacterial cell cycle production, decreases pain perception, reduces malodour, and decreases exudates, all factors that are relevant to wound repair and healing. (82)

One of the other attributes of honey that aids wound healing is its ability to produce hydrogen peroxide which is both antibacterial but at concentrations that are nontoxic to granular cells. (59) The low levels of hydrogen peroxide in honey are thought to promote growth of new cells, such as fibroblasts that are important in early wound healing. However, even in the presence of catalase, which has the potential to inactivate hydrogen peroxide, some researchers claim honey has antimicrobial properties which are beneficial for the healing of wounds. (83)

Honey and diabetic foot ulcers

The management of diabetic ulcers and their healing is challenging. Wound care experts have investigated the potential for various types of dressings that are used in routine wound care as well as other natural cures, including honey, to improve clinical outcomes.

A RCT investigated the effect of Manuka honey-impregnated dressings compared with conventional dressings in the healing of neuropathic diabetic foot ulcers (NDFU) and followed up 59 patients on a weekly basis for up to 16 weeks. The investigators reported that, the proportion of ulcers that healed did not differ significantly between Manuka honey 97% (n=31) and for conventional dressings 90% (n=28). However, Manuka honey was associated with a significant reduction in the time to healing and with rapid decolonisation of ulcers (78% *vs* 35%). (84)

The efficacy of topically applied Royal jelly (RJ), of honey origin, on the healing of 64 diabetic foot ulcers (DFU) was evaluated by Siavash and colleagues in a double blinded placebo controlled clinical trial. They found that 5% topical RJ was not superior to placebo for the treatment of DFU's. (85) In another clinical study that employed honey dressings for DFU's for up to three months, it was observed that 43.3% (n=13/ 30) of the DFU were completely healed, with a decrease in ulcer size and healthy granulation was observed. The bacterial load of all ulcers from the 30 patients was significantly reduced after the first week of honey dressing. (86)

Comparing the outcome from the three clinical studies alluded to above in the management of DFU's with honey based products, it appears that honey is non-inferior to routine care and conventional dressings. In addition to the healing properties of honey, antimicrobial properties may offer an advantage by which bacterial colonisation and subsequent infection of the DFU's may be prevented or reduced by the use of honey based dressings. However, inconsistencies in the outcome of DFU's are evident from the studies examined, which warrants further systematic investigation.

Honey and venous leg ulcers

Similar to that of diabetic ulcers, the management and healing of venous ulcers is taxing. A RCT evaluated the outcome of calcium alginate dressings impregnated with Manuka honey (n=187), in comparison to a range of conventional dressings (n=181) in a community setting. There was no difference in complete healing, time to healing, or reduction in ulcer area between the two groups. Moreover, patients in the honey treated group reported >/= 1 adverse event and ulcer pain than in the usual care group. (87) In another similar RCT comparing desloughing efficacy in venous leg ulcers (n=108) treated with a Manuka honey product (Woundcare 18+) (n=54) compared with standard hydrogel therapy using IntraSite Gel (n=54), the WoundCare 18+ group had improved healing at or within 12 weeks more effective desloughing within four weeks and a lower incidence of infection than the control group. (88)

In 2013, the German Society for Wound Healing and Wound Treatment published a revised guideline on chronic wound management. The recommendations included hydrogel, hyperbaric oxygenation, and integrated care, and but advised against the use of medicinal honey and growth factors for the treatment of chronic wounds in patients with peripheral vascular disease, chronic venous insufficiency, and diabetes. (89)

A recent Cochrane review (2015), evaluated the effect of honey compared with alternative wound dressings and topical treatments on the healing of acute and chronic wounds, including leg ulcers and burns. (90) Twenty six trials were included in the review of which, three trials evaluated the outcome of honey in minor acute wounds, 11 trials evaluated honey in burns, 10 trials on different chronic wounds, including two patients with venous leg ulcers, two trials in people with diabetic foot ulcers and single trials in infected post-operative wounds, pressure injuries, cutaneous Leishmaniasis and Fournier's gangrene. The remaining two trials recruited a mixed population of people with acute and chronic wounds. (90) The review concluded that it was difficult to draw overall conclusions on the effects of honey as a topical treatment for wounds. The reviewers allude to the heterogeneous nature of the patient populations and

comparators studied, and the mostly low quality of the evidence, which makes it hard to draw definite conclusions. However, the review identified that honey appears to heal partial thickness burns more quickly than conventional treatment, which includes polyurethane film, paraffin gauze, soframycin-impregnated gauze, sterile linen and leaving the burns exposed, and infected post-operative wounds more quickly than antiseptics and gauze. The reviewers also reported that the effects of honey relative to comparators were unclear for venous leg ulcers, minor acute wounds, diabetic foot ulcers, Leishmaniasis and mixed chronic wounds. (90)

Scrutinising the results of clinical studies that investigated the use of honey for the management of DFU and venous ulcers, chronic wound management as well as the Cochrane review findings, the scientific evidence in favour of honey is limited to that of healing partial thickness burns and infected post-operative wounds. In the management of DFU's and venous ulcers the evidence for the use of honey is contradictory. Robust RCT's must be done in-order to generate conclusive evidence that either supports or refutes the use of honey-based products in the care of DFU's and venous ulcers.

Honey and cellular research

The antimicrobial claims and wound healing properties of honey have encouraged researchers to investigate its activity at cellular level. In histological studies, honey exposed cells have been shown to have higher levels of antioxidants present and decreased numbers of inflammatory cells. (91) In cell cultures, honey has been shown to stimulate B and T lymphocytes and phagocytes and the release of modulator cytokines tumour necrosis factor-1 (TNF-1), IL, IL-1, and IL-6. Honey also provides macrophages with the essential glucose needed for energy and hydrogen peroxide production. (91-92) *In vitro* studies have demonstrated that honey may be able to modulate the activity of immuno- competent cells, such as monocytes. (79,93)

The role of universal stress protein A (UspA) and the inhibition of MRSA in Manuka honey treated cells were investigated by Jenkins and colleagues from Cardiff, UK. (94) The UspA super-family is found in many microorganisms, including bacteria as well as some higher organisms. UspA are normally induced in response to stress conditions such as temperature shock, starvation and the presence of agents that arrest cell growth. Mutant cells lacking UspA have been shown to be less fit by growing more slowly and dying prematurely during growth arrest. Down-regulation of UspA protein was confirmed by real-time polymerase chain reaction (PCR), which showed a 16-fold down-regulation in honey-treated cells compared with untreated samples. UspA protein is involved in the stress stamina response and its down-regulation according to the investigators could help to explain the inhibition of MRSA by Manuka honey. In addition, the investigators also observed a decreased expression of UspA in honey treated cells indicating that MRSA was unable to accommodate the stresses caused by exposure to Manuka honey. (94)

A review on the use of honey or wound healing indicated that honey seems to either reduce or activate the production of ROS from neutrophils, and honey-induced activation of monocytes and macrophages, leading to debridement of a wound and faster healing. Along with these factors, it is also claimed that human keratinocytes, fibroblasts, and endothelial cell responses such as cell migration and proliferation, collagen matrix production and chemotaxis are positively affected in the presence of honey, thus accelerating re-epithelisation and wound closure. (82)

Antibacterial properties of honey

The survival and growth of bacteria are influenced by nutrients in the environment such as hydrogen donors and acceptors, carbon source, nitrogen source, minerals such as sulphur and phosphorus, amino acids, purines, pyrimidines, vitamins and certain trace elements. Most pathogenic bacteria grow best in pH 7.2-7.4. The high acidity and sugar content of honey along with

its low water content present adverse conditions for the growth and the replication of micro-organisms.

The antibacterial nature of honey is dependent on various factors working either singularly or synergistically. (68-69) These include the concentration and the nature of the bacteria; the higher the concentration the greater its usefulness as an antibacterial agent. (95-96)

The antimicrobial activity of honey is also attributed to the enzymatic production of hydrogen peroxide. Levels of hydrogen peroxide in topically applied honey are estimated to be 1000 times lower than in medical rinse solutions. (59) While the peroxide action of honey is linked to antibacterial properties, investigators have also demonstrated significant antibacterial effects even when the hydrogen peroxide activity is blocked, for example in honey derived from *Leptospermum* trees. (75) Mandal asserts that this mechanism may be related to the low pH and high sugar content/high osmolarity of honey which is sufficient to hinder microbial growth. (75)

In vitro studies indicate that MGO is an effective antimicrobial agent against MRSA, and effectively acts in synergy with oxacillin in the treatment of otherwise oxacillin-resistant *S. aureus.* (64,97) Researchers have demonstrated that honey of varying types has broad-spectrum *in vitro* antibacterial activity against nearly 60 species of bacteria, including MRSA, vancomycin-resistant *enterococcus*, and *Pseudomonas aeruginosa*. (91,98-104) The high osmolarity, acidity, generation of hydrogen peroxide on dilution and insect derived antimicrobial peptides of honey are components that are attributed to antibacterial activity. (105) A number of investigators have demonstrated that honey reduces the numbers of MRSA in open wounds. (92,106-108)

According to Cooper, the high sugar and low water content as well as the marked acidity of all honeys provide an adverse environment for bacterial survival. This could explain why honey infrequently spoils at room temperature despite prolonged storage. (69)

A research team from Greece investigated the antimicrobial activity of various botanical origin honeys (n=60), against 16 pathogens and their respective

reference strains. In this study, the microbiological quality of honeys and the antibiotic susceptibility of the various isolates were also examined. All honey samples, whether coniferous, citrus, thyme or polyfloral had antibacterial activity against the pathogenic bacteria and their respective reference bacterial strains. The highest activity was showed by coniferous and thyme honeys with an average minimum dilution of 17.4% and 19.2% (w/v) followed by citrus and polyfloral honeys with 20.8% and 23.8%, respectively. Clinical isolates of *S. aureus*, *E. coli*, *Salmonella enterica* subsp. *Enterica*, *Streptococcus pyogenes*, *B. cereus* and *B. subtilis* were proven to be more resistant than their respective reference strains. These findings emphasize the variability in the antibacterial effects of honey. (109)

A bactericidal rather than bacteriostatic mode of action of honey has been demonstrated by various investigators. (98,101) It has also been identified that Gram positive bacteria are generally more susceptible than Gram negatives, and staphylococci seem to be the species most susceptible to honey. (69)

A research team investigated the mode of inhibition of Manuka honey against *S. aureus* 'NCTC 10017' by determining the MIC, minimum bactericidal concentration (MBC) and the effect of time on viability. (110) In this study, structural changes of cells were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of cells suspended in 0.05 mM Tris buffer containing 10% (w/v) Manuka honey. The structural changes observed were compared to cells in buffer alone or buffer containing 10% (w/v) artificial honey. Marked structural changes in honey-treated cells were seen only with TEM, where a statistically significant increase in the number of whole cells with completed septa compared to untreated cells were observed ($p<0.05$). Structural changes found with TEM suggest that honey-treated cells had failed to progress normally through the cell cycle and accumulated with fully formed septa at the point of cell division without separating. The elevated numbers of cells with entire septa were found to accumulate autolysins (also known as murein hydrolases) which according to the investigators are

implicated in the bactericidal effect. A bactericidal mode of inhibition for Manuka honey on *S. aureus* was thus demonstrated. (110) According to the investigators, the close proximity of the MIC and MBC values observed also indicates a bactericidal mode of action for Manuka honey with *S. aureus* NCTC 10017. The increased sensitivity of an autolysin (atl) mutant compared to its parental strain ($p<0.001$) suggests that the target site is associated with bacterial cell division. (110) The investigators claim that sugars are not implicated in the observed bactericidal effect.

In a related study, researchers observed an inability to complete the cell cycle in MRSA treated with Manuka honey despite an increased expression of the autolysin gene, where murein hydrolase activity was at undetectable levels. (111) Along with this, the failure to cleave peptidoglycan was thought to contribute to the persistence of the septa and to the failure to divide and complete the cell cycle. (111) Consequently, the investigators have concluded that the action of Manuka honey involves the cell division machinery. (111)

An *in vitro* study of four types of honey, three sourced from amateur bee keepers from Northern Ireland and one commercial honey from Suisse Normande, France, found that honey reduced the bacterial count of CA-MRSA isolates. (112) Similar findings were reported in a clinical study of medical-grade honey applied to chronic wounds. (113)

In time-kill studies, loss of bacterial viability was observed when bacteria were incubated in 10% (w/v) Manuka honey in nutrient broth and compared to untreated cells. In the presence of Manuka honey the mean time to achieve a 2 log reduction was 427 min. The investigators claim that failure to recover viable bacteria after 8 h confirmed that the bacterial inhibition was irreversible.

Eradication of pathogens from wounds using honey

In one of the early reports on the use of honey in clinical studies, rapid healing of an ulcer colonised with MRSA and its successful eradication from the ulcer was documented in 2001. (92) Later in 2007, investigators from Germany reported on the effects of medical honey on healing wounds. This report alluded to seven consecutive patients whose wounds were either colonised or infected with MRSA and which healed. (99) The authors state that despite the use of topical antiseptics and topical antibiotics in some patients, as well as the systemic use of vancomycin in three patients, MRSA had persisted in some of the seven patients for up to five years. Following the daily application of honey, MRSA was eradicated from all wounds and, in most cases, without the additional use of antimicrobial treatments.

The clinical observations from these two reports imply the potential benefit of using honey for the healing of wounds. However, direct causality was not established from observational studies, which necessitates RCTs.

A multi-centre RCT on the management of venous leg ulcers found that Manuka honey was found to be effective in eradicating MRSA from 70% (n=7 of 10) of chronic venous ulcers in comparison to hydrogel where no change on MRSA colonisation were identified (n=6). (113) A triple blind RCT concluded that honey was effective in healing Caesarean section incisions. (107) In 2015 a Cochrane review concluded that honey healed infected post-operative wounds more quickly than antiseptics and gauze. (90)

In 2007, the US Food and Drug Administration (FDA) approved the first honey-related wound product, a sterile, single-use wound care dressing impregnated with 95% honey and 5% sodium alginate. It was approved in the United States for use in minor traumatic or surgical cuts and burns, and on select ulcers. (114)

Honey and bacterial biofilms

Living organisms have evolved and developed mechanisms to adapt themselves to hostile environments and their survival. One of the mechanisms which bacteria have developed is to produce a slimy substrate or biofilm. Bacteria that attach to various surfaces often produce biofilm which serves as a protective covering render them harder to eradicate.

Many pathogenic bacteria colonising wounds produce biofilms. *Streptococcus pyogenes* (*S. pyogenes,)* group A Streptococcus (GAS) are some of the pathogens of clinical significance in wounds where it can initiate infection, destroy skin grafts and persist as a biofilm. Pseudomonas is another opportunistic pathogen that can cause persistent wound infections which is linked to its ability to form biofilms. The formation of biofilms on wounds depends on the adhesion of bacterial cells. It is thought that the most abundant sugar in honey namely fructose, prevents bacteria from binding to host-cell membrane receptor sites. (69) Without bacterial adhesion to wounds and other vulnerable surfaces, neither bacterial biofilms nor infection can develop.

The effect of Manuka honey on *S. pyogenes* (M28) *in vitro* with planktonic and biofilm cultures using MIC, MBC, microscopy and aggregation efficiency was studied. Bactericidal effects were found in both planktonic cultures and biofilms, although higher concentrations of Manuka honey were needed to inhibit biofilms. The prevention of adherence and intercellular aggregation were also noted. Manuka honey permeated 24 h established biofilms of *S. pyogenes*, resulting in significant cell death and dissociation of cells from the biofilm. (103)

An investigation on the impact of Manuka honey on wound pathogens *in vitro*, focusing on its anti-adhesive properties, found that Manuka honey effectively disrupted and caused extensive cell death in biofilms produced by *S. aureus*, *P. aeruginosa* and *S. pyogenes*. Sub-lethal doses of Manuka honey inhibited bacterial adhesion to fibronectin, fibrinogen and collagen. Manuka honey impaired adhesion of laboratory and clinical isolates of *S. aureus*, *P. aeruginosa* and *S. pyogenes* to human keratinocytes *in vitro*, and inhibited invasion by *S.*

pyogenes and vancomycin intermediate *S. aureus*. (115) Other investigators also have demonstrated that Manuka-type honeys showed significantly higher anti-biofilm activity than clover honey and an isotonic sugar solution in all *S. aureus* strains tested, including MRSA strains. In some cases honey was able to penetrate through the biofilm matrix and was able to kill the embedded cells. Based on these findings the investigators suggest that MGO requires other components that are present in Manuka-type honeys for the anti-biofilm activity observed. (116)

Honey and bacterial log reduction

Based on time kill studies, Henriques and colleagues calculated the mean time to achieve a 2 log reduction of viable bacterial populations of *S. aureus*, in the presence of 10% (w/v) Manuka honey which was 427 min. Using a similar approach the same investigation team assessed the time required to achieve a 3 log reductions for MRSA, and the mean estimated time was 770 min. (110)

Summary on honey for medicinal purposes

Honey has been used historically for medicinal purposes and in contemporary times a renewed interest on the use of honey for its healing purposes has invigorated researchers to investigate and validate such claims. The low pH of honey which ranges from 3.2 to 5.5 along with its high osmolarity renders honey inhospitable to microbial growth. The enzymatic action of hydrogen peroxide and the presence of phytochemical components i.e. MGO, the antimicrobial peptide; bee defensin-1 contributes to the antibacterial action of honey. An observed decreased expression of UspA protein in honey-treated cells implies that MRSA is unable to accommodate the stresses caused by exposure to Manuka honey. As a wound healing agent, honey acts both as an immunomodulator with both proinflammatory and anti-inflammatory properties, inducing or stimulating the release of TNF-α, IL-1β, and IL-6 from MM6 cells and peripheral blood monocytes. Emerging evidence suggest that other factors may also contribute to the beneficial effects of honey.

Evolution of the concept leading to the pilot study

The researcher, an infection prevention and control practitioner was inquisitive with regards to the antibacterial claims of licensed medical grade honey (MGH) preparations as well as MGH applications on the management of wounds and ulcers. The initial enquiry brought forth a wealth of evidence on the antibacterial properties of honey in *in vitro* studies and clinical studies on wounds and ulcers following the application of medical grade honey preparations. Various medical grade honey preparations that were used for the management of wounds with antibacterial claims were investigated. Consideration was given to an off-the-shelf *Conformité Européene* (CE) marked MGH preparation that was feasible to apply on the nasal passages, with antibacterial claims and which offered potential. In consultation with a Consultant Microbiologist in Beaumont Hospital (BH), a pilot study was planned. After Ethics (Medical Research) and Irish Medicines Board (currently Health Products Regulatory Authority - HPRA) approval, the 'Natural Honey for Eradication of nasal MRSA a pilot study' was carried out in 2012.

Pilot study

Medihoney™ Derma Cream, a standardised medical grade natural honey, licensed for application on the skin was used in the pilot study. Medihoney™ Derma cream contains 30% *Leptospermum* honey, which the manufacturer claims is antibacterial. Over a period of 20 weeks, seven patients were consented and were enrolled in the pilot study. All the seven patients were persistent MRSA colonisers i.e. had at least two courses of nasal and other non-nasal decolonisation using standard decolonisation regimen and had failed nasal eradication of MRSA. The standard MRSA decolonisation regimen employed in BH consists of three times a day application of mupirocin 2% for five consecutive days for nasal MRSA colonisation, and triclosan 1% (Skinsan®) daily wash and shampoo for at least two days over the five consecutive days for decolonisation of intact body sites. A second course of

decolonisation was applied in the event of persistent colonisation using mupirocin 2% and Skinsan®.

In the pilot study the following regimen was used for Medihoney™ Derma Cream. Medihoney™ Derma Cream was administered three times a day for five consecutive days along with Skinsan® wash daily and shampoo for at least two days over a period of five days. This was followed by two treatment free days. MRSA screening was performed according to the standard MRSA screening protocol, i.e. day seven or later after the commencement of the first course of Medihoney™ Derma Cream. Patients with persistent nasal colonisation after a course of decolonisation were administered a second course of Medihoney™ Derma Cream and Skinsan®, which was informed by the results of the patient screening.

Pilot study results

Of the seven patients who enrolled for the pilot study, two discontinued the study between 0-2 days after commencement. One patient completed one, and four patients' completed two full courses of Medihoney™ Derma Cream nasal application. Successful nasal MRSA decolonisation was achieved in three of the five cases and two had persistent nasal MRSA colonisation after two courses of Medihoney™ Derma Cream application. Of the two patients who discontinued Medihoney™ Derma Cream, one was due to clinical deterioration of the patients' chronic medical condition. The second patient experienced an unpleasant odour and taste which resulted in discontinuation from the study.

The pilot study also involved collating patient experience on the use of Medihoney™ Derma Cream, as well as patients' perception on the use of the natural agent for MRSA eradication. Six of the seven patients enrolled in the pilot study completed the structured statement questionnaire that used a Likert style response to collate patient experience on the use of the Medihoney™ Derma Cream. Most of the respondents were of the opinion that the investigative product was not sticky, did not cause any unpleasant sensation in the nose and was easy to apply to the nasal passages. Two of the six

participants felt that their nose was moist and runny after nasal application of the product. Of the six participants, five preferred the use of a natural product to an antibiotic, if they were given a choice, to eradicate MRSA from the nose.

Overall the pilot study results were encouraging which warranted further investigation. Considering the paucity of effective alternatives that are currently available along with increasing resistance to agents that are used for MRSA eradication, the investigating team proposed that medical grade honey was non-inferior to mupirocin 2%, i.e. the current standard of care.

A proposition must be tested to support or reject the informed hypothesis in order to be valid. The research team with a keen interest on prevention and control of MRSA developed the study protocol to conduct a clinical trial (CT). A well designed CT was essential in demonstrating the eradication of nasal MRSA using Medihoney™ Derma Cream in comparison to mupirocin 2%. Potential sources for funding for the CT were investigated.

In conclusion, nasal MRSA eradication with mupirocin 2% nasal ointment is suboptimal. Therefore, there arose a compelling need for evaluating alternative agents for the eradication of MRSA. The pilot study results using medical grade honey for nasal MRSA decolonisation offered potential. (Appendix Ⅰ Natural Honey for Nasal MRSA Pilot study report).

Justification on the use of honey in the NHNMRSA RCT

The research team, given past experience with the use of Manuka honey in pilot clinical studies, further investigated the potential for honey to eradicate MRSA from the nose. The research team were of the opinion that the antibacterial properties of honey along with its bactericidal mode of action identified by MIC, MBC and time kill studies merited a case for further studies in a clinical environment.

The idea conceived was further explored by the researcher for medical-grade honey preparations that were already available on market. The intended aim was to identify a possible 'off the shelf' CE marked honey product with a manufacturer's claim of antibacterial properties, that could be clinically evaluated. The search for a potential honey preparation narrowed the possibility to two products with varying concentrations of medical grade honey. Feedback was received from healthy volunteers who applied the selected honey products of varying concentrations on their nostrils. Based on antibacterial claims, volunteer feedback, ease of nasal application and resemblance to mupirocin 2% nasal ointment, the control product, a cream with 30% Leptospermum honey, Medihoney™ Derma Cream, was identified as the honey that could be used for a comparative investigation. The manufacturer's claim that Medihoney™ antibacterial honey is effective against *S. aureus* and MRSA at a dilution of 4%, supported by *in vitro* studies, and that Medihoney™ Derma Cream contains 30% Medihoney™ antibacterial honey.

The dose calculation of the investigative product used in the RCT, Medihoney™ Derma Cream was decided based on time kill studies, details of which will be discussed in detail in the Materials and Methods chapter.

1.7 Hypothesis

Medical grade honey is non-inferior to mupirocin 2% for nasal MRSA eradication.

1.8 Study Objectives

The overall aim of this project was to compare the efficacy of medical grade honey to eradicate nasal MRSA with that of mupirocin 2%.

In order to achieve the overall aim, five specific objectives were formulated, which are listed in order.

1. To conduct a systematic review of the literature on mupirocin resistance and alternatives for the decolonisation of MRSA in the nose.

2. To conduct a RCT comparing medical grade honey with mupirocin for nasal decolonisation of MRSA.

3. To identify rates of mupircoin resistance among MRSA isolates using routine laboratory methods i.e. MIC E test.

4. To investigate the phenotypic and genotypic characteristics of bacterial isolates; first time (historic, where available), baseline (at recruitment) and subsequent isolates using standard laboratory methods, and to investigate the MIC and minimum bactericidal concentration MBC of MGH product used.

5. To determine the perceptions and attitudes of patients on MRSA and the use of nasal decolonisation agents, mupirocin and Medihoney™ Derma Cream.

The methods used to achieve the objectives, results and discussion of the results are addressed as follows; chapter II describes the methodology applied to conduct the study to achieve each of the five stated objectives. Study results are collated in chapter III in the same order as the study methodology, which is followed by discussion, conclusion and recommendations in chapter IV. All appendices are listed in order which follows the bibliography.

Chapter II Materials and Methods

2.1 Literature review methodology

2.1.1 Introduction

This section summarises the methodology that was used in the search of existing literature on mupirocin resistance and alternatives for MRSA decolonisation.

2.1.2 Aim of the literature review

The aim of the review was to determine the prevalence of MR as well as measures employed to control MR. In addition, the review also ascertained the evidence supporting the use of new agents as potential therapeutic alternatives for MRSA decolonisation. The literature review was modified from what was initially conceived in the study, based on the initial literature search which we consider in the next section.

2.1.3 Literature search

The initial literature search sought to retrieve articles related to clinical use of honey for nasal MRSA decolonisation. No articles were retrieved in the initial search that addressed honey and nasal MRSA. Therefore the search was subsequently modified to MR and alternatives for MRSA decolonisation.

2.1.3.1 Information source and search strategy

The search was conducted using on-line data bases; PubMed, Cumulative Index of Nursing and Allied Health Literature (CINAHL), Scopus and Web of Science. The search strategy was initially developed for use in PubMed (using Medical Subject headings (MeSH) terms to identify alternative phrases for the key words) and was then modified for each data base, for example for Medline, Medline terminology (MH). Combinations of the following MeSH terms and combinations were used in the initial search. MRSA, methicillin-resistant *Staphylococcus aureus,* meticillin -resistant *Staphylococcus aureus,* MH

methicillin-resistant *Staphylococcus aureus,* MH methicillin-resistance, MH *Staphylococcus aureus, Staphylococcus aureus, S. aureus,* methicillin-resistant*, Meticillin-resistan*, nasal colonisation, nasal colonization, nasal, nose, MH nose, colonisation, colonization, decolonisation, decolonization, MH Honey OR honey, MH infection control OR prevention and control.

The modified search was done based on the revised literature review and included the following additional search terms: wounds and injuries OR wound infection OR wound healing OR surgical wound infection, ulcer, topical, administration topical, treatment or application, mupirocin, mupirocin, resistan*, Drug resistance OR drug resistance.

The search parameters were saved on the on-line data bases where the function was available and email updates of new publications were requested on new publications in the area. Records were kept of what data bases were searched and with what terms.

2.1.3.2 Study selection

Randomised controlled trials, case control studies, non controlled studies, observational studies, diagnostic / laboratory studies, surveillance reports, reviews, guidelines, expert opinions, consensus reports, qualitative reports, investigation and *in vitro* studies that focused on MRSA surveillance, decolonisation, mupirocin, mupirocin resistance and studies reporting alternative and novel agents for MRSA decolonisation were included. Studies from 1981 to May 2015 were included and the search was limited to English language and human studies. The searches were last updated in 31st January 2016.

2.1.3.3 Data extraction and analysis

The results of the searches were downloaded to Endnote file and reviewed for relevancy based on the article title and abstract. From the title and abstract review, those articles that were not relevant to the topic of interest or duplicates were deleted. Additional publications were identified by a manual search of the reference list of relevant articles. The full texts of the relevant articles were sourced either from the library, the web or from the author. Further articles were

eliminated following review of the full article. The studies selected were analysed qualitatively, findings collated under relevant sub-headings and the results summarised. From a total of 4636 titles that were initially found, 499 articles were shortlisted based on title and abstract review. After exclusion for reasons of not relevant or duplicates, 89 articles remained for inclusion including those identified from reference lists. The search process is illustrated in Figure I, appendix - II.

2.1.4 Conclusion

Undertaking a comprehensive literature review offers a unique learning experience. The researcher learned skills in carrying out searches in databases, the use of the scientific vocabulary, different expressions or terms to express similar meaning to broaden the search. The literature review enabled the researcher to submit a review to a scientific journal that was subsequently published with the title: Mupirocin resistance: Clinical Implications and potential alternatives for the eradication of MRSA, in the Journal of Antimicrobial Chemotherapy, October 2015 (appendix - Ⅲ copy of the article).

2.2. RCT methodology

2.2RCT Methodology

2.2.1 Introduction

The natural honey to eradicate nasal MRSA, a randomised controlled trial investigated the potential for eradication of MRSA from the nasal passages in humans with medical grade honey (MGH). The investigator hypothesised that MGH is non-inferior to mupirocin 2% for nasal MRSA eradication. This chapter outlines the methodology that were applied in the RCT, including the trial design, generation of sample size, randomisation and allocation, participants, patient data collection after consent, institutional/ethical submission and approval. The chapter concludes with a summary on the learning experience gained by the investigator.

2.2.2 Institutional / ethical submission and approval

The Beaumont Hospital Ethics (Medical Research) Committee reviews applications to undertake research in Beaumont Hospital (BH), and two other hospitals namely; St. Joseph's Hospital in Raheny, and St. Ita's Hospital in Portrane, Dublin. In March 2005, the committee was recognised by the Department of Health Ireland, to review applications for clinical trials of medicinal products (clinical drugs trials) for the whole of Ireland.

For a clinical trial to proceed in Ireland, both an Ethics Committee and the Health Products Regulatory Authority (HPRA), previously known as the Irish Medicines Board (IMB), must approve a clinical drugs trial. In addition, a drugs trial must have a European Clinical Trial number, called a EudraCT number, and adequate insurance must be in place. An indemnity form must be completed with the hospital for academic clinical trials before the study can commence.

Applications were submitted to the Beaumont Hospital Ethics (Medical Research) Committee in November 2013 to conduct the study, “Natural Honey to Eradicate Nasal MRSA A Randomised Controlled Trial, REC reference

number 13/103". REC approval was granted on 24th January 2014 (appendix – IV, copy of the REC approval)

The advice of the HPRA was sought in relation to the approval required to conduct this study at the pilot stage. Consequently, a clinical trial (CT) application for a medicinal product was made to what was then the IMB. Registration for CT was done in accordance with the European Union (EU) clinical trials registry requirements. European Clinical Trial - EudraCT is a database of all clinical trials which commenced in the EU from 1 May 2004, and also includes clinical trials linked to European paediatric drug development. On successful registration, a unique reference for the trial, a EudraCT number 2010-023408-28, was allocated to the NHNMRSA RCT (appendix V, a copy of the allocated EudraCT number).

Subsequent to the IMB review of the CT application, the researcher was advised to submit a revised application to the IMB for the study of a medical device. The investigative product, Medihoney™ Derma Cream is a CE marked licensed medical device bearing the 'CE number 0120'. The IMB subsequently issued the following statement on the review of the application for the study of a medical device;

> '*Further to the information you (researcher) have provided on your proposal please be informed that clinical research involving CE marked medical devices that is being conducted by academic / clinical investigators when there is no commercial intent for use of the data do not require review and approval by the IMB prior to commencement'.*

(appendix –VI copy of the IMB statement)

The study commenced following receipt of approval from the Beaumont Hospital Ethics (Research) Committee, and after insurance and indemnity arrangements with the hospital were confirmed.

2.2.3 Study settings and location

Study setting

The study setting, Beaumont Hospital is a tertiary referral centre and the national centre for neurosurgery, renal and pancreatic transplantation, and a regional treatment centre for ear, nose and throat, and gastroenterology patients. The 820 bed hospital incorporates a twelve-bedded general intensive care unit (ICU) and a ten-bedded neurosurgical ICU. The hospital serves a population of 1.2 million in North County Dublin and Fingal, (BH Annual report 2013) as well as patients referred from other parts of the country. In 2013 there were 24,634 admissions, during which 57,968 day cases and 227,958 bed days were used. The outpatient department had 181,389 patients attending its service in addition to 51,045 patient attendances in the Accident and Emergency Department.

Identification of eligible patients based on laboratory surveillance

Microbiology specimens collected from patients for clinical and screening purposes are processed in the BH microbiology laboratory. Test results were reported according to the routine reporting protocol and filed electronically in the laboratory system 'Patient Information Profile Explorer (PIPE)'. The Irish National Accreditation Board (INAB) accredited diagnostic laboratory in BH and the microbiology laboratory utilises systems and processes that safeguard user data and that comply with the Data Protection Act 1998 and subsequent Data Protection (Amendment) Act 2003.

Positive MRSA results were routinely notified to the IPC personnel, and this data was made available to the researcher. Potential candidates for recruitment to the NHNMRSA RCT were initially identified by the researcher from the routine clinical microbiological surveillance data. Individual patient test results were also available to the clinical supervisor who had access to the password protected hospital information technology (IT) system. A preliminary screening for eligibility was performed by the researcher for the patients who were confirmed with nasal MRSA colonisation, based on the patient's current location

of stay and the episode / health care point of contact where the relevant microbiological investigations were ordered.

Target Population

The target population for the RCT was the adult patient population of the hospital. This accounts for a total population of 1.2 million in North County Dublin as well as patients referred to BH from the rest of Ireland. The entrants for the NHNMRSA study originate from the target population.

Source and eligible population

Due to confines of this study resources, the source population, a selected group based on MRSA screening outcomes was a necessity. In identifying the source population, the following criteria were applied. Inpatients or outpatients in BH, who are colonised with MRSA on their nose and living with the geographical catchment area of BH at a distance not more than 30 kilometres, for patient follow up purposes, were considered suitable for recruitment. In addition, it was essential that each patient met the specific inclusion and exclusion criteria. For the purpose of eligibility in the NHNMRSA study, a detailed inclusion and exclusion criterion was developed and presented in the section that describes the Participants, Sec 2.2.4

Study entrants

Study entrants were defined as those patients who consent to and enrol in a study. The study entrants at the outset originate from the target population. Subsequently the criteria for initial screening and follow up of patients were applied to the source population, and thus the eligible population was identified. Using this approach the study entrants were identified from the eligible population.

2.2.4 Study participants

Patients were recruited for the RCT from BH only. The eligibility criteria were patients who were laboratory confirmed with MRSA in their nasal passages, on a prospective basis and otherwise suitable according to inclusion and exclusion criteria.

Inclusion criteria

- Age at recruitment, over 18 years.
- In-patient in Beaumont Hospital at the time of MRSA detection.
- Able to understand the nature and purpose of the study and provide informed consent.

Exclusion criteria

- Participating in another clinical trial.
- Uncontrolled diabetes mellitus.
- Known hypersensitivity to, or intolerance of, honey or mupirocin.
- Known to be colonised with strains of *S. aureus* that were mupirocin resistant.
- Likely to be difficult to follow up due to geographic location; i.e. place of residence 30 km or more from BH (see below).
- Bloodstream infection or active infection in other body site/s.
- Undergoing acute or elective surgery that involved placement of implants.
- In an intensive care unit.

Justification of exclusion of a segment of the population

If the patient's normal residence was over 30 kilometres away from BH, they were excluded from the study due to restrictions on the time and resources available to the researcher for adequate follow up of patients on their discharge.

2.2.5 Study outcomes

Primary outcome

The primary end point of the RCT was 'the proportion of patients decolonised of nasal MRSA after a second course of mupirocin or the nasal honey product, regardless of whether re-colonisation occurred later'.

The primary outcome was assessed based on the microbiology laboratory screening of each patient who completed the study. The microbiology laboratory screening and reporting were according to established protocols specified in BH Laboratory Protocol; LP-MIC-Screens-Revision 4-2013 (appendix-VII).

Case definition

A current documented microbiological finding of MRSA in the nasal passages of a patient, reported by the BH microbiology laboratory, were defined as a case of nasal MRSA in the NHNMRSA study.

2.2.6 Available sample

In order to estimate the overall prevalence of MRSA among the source population, the researcher considered the routinely collected annual surveillance data on MRSA at BH for two consecutive years. In 2014, 18,318 swabs for screening test were processed in BH microbiology laboratory, i.e. for MRSA detection, resulting in 274 new cases of MRSA being identified. During 2013, 13,726 swabs for screening test were processed with 275 new MRSA cases detected.

In addition to new cases of MRSA, previously known patients who were colonised with MRSA were also screened on subsequent admissions or for other clinical purposes. Of the 544 unique patient records identified in 2014 with a new or known MRSA status, 137 patients were classified external cases, i.e. the patients were not in-patients but were in nursing homes or in the community. Of the remaining 407 patients, 274 were classified as new and 133

were known cases of MRSA. In 2013, of the 275 new cases of MRSA, 196 were BH inpatients or outpatients and 78 were external cases.

Based on the microbiology laboratory surveillance data, approximately 500 cases of MRSA were detected annually of which approximately 30% of cases were ineligible i.e. patients not admitted to the hospital. Having also excluded those patients with only non-nasal colonisation, i.e. about 30% of MRSA patients, the potentially eligible number of patients therefore was 115-120 in a calendar year.

Sampling strategy

In BH, screening of patients for MRSA was based on the hospital 'Guidelines on the control and prevention of MRSA' (2007), which are based on the National Guideline 'The Control and Prevention of MRSA in Hospitals and in the Community' (2005). (25) In practice, no constraints were applied in BH on the screening of patients who otherwise fit the criteria for MRSA screening. Swabs were processed according to established laboratory protocols and results were reported on an ongoing basis.

A simple random strategy for sampling patients, where patients had an equal chance for allocation into one of the study groups, provided a patient had documented nasal MRSA colonisation, was deemed the most appropriate sampling approach for the study.

2.2.6.1 Sample size estimation

The most important consideration in estimating the sample size for a study is that, the study should have adequate power to detect a statistical significant difference between intervention and control groups, with reference to a defined end point. In order to estimate the sample size for the NHNMRSA RCT, the researcher investigated studies that evaluated the efficacy of mupirocin for nasal decolonisation as well as RCTs that employed honey based products. Other comparable studies were also examined for sample size determination. In

consultation with an expert methodologist who was part of the RCT team, data from the following studies were reviewed to estimate the sample size.

In a multicentre trial in care homes of 127 residents, intranasal mupirocin ointment was compared with a placebo among persistent carriers of *S. aureus* and MRSA with a follow-up period of 6 months. Mupirocin initially eradicated *S. aureus*, including MRSA in 94% (60/64) compared with 86% (54/63) in the placebo group, but after 90 days recolonisation occurred in 39% (p=0.003) of the mupirocin group. (35)

In another study of 87 patients, a double-blind, randomized, placebo-controlled trial in a tertiary healthcare facility evaluated the efficacy of mupirocin in eradicating nasal carriage of MRSA along with body washes using chlorhexidine soap for non-nasal sites. At four weeks from the initiation of decolonisation, 19/43 (44%) who received mupirocin were free of nasal MRSA compared with 11/44 (25%) (p=0.40) in the control group. (24)

An RCT of 108 patients that employed honey based dressings (n=54) in comparison to hydrogel (n=54) for vascular leg ulcers, identified that ulcers colonised with MRSA treated with honey based dressings (10) had a 70% decolonisation rate in comparison to hydrogel (6) with 16% decolonisation.

An *in vitro* study of six CA-MRSA clinical isolates found that all isolates were susceptible to natural honey, and bacterial counts were at undetectable levels at 24 h. (117)

Based on the scientific literature review, the efficacy of nasal MRSA decolonisation with mupirocin was estimated to be 40-60%. Overall, the research team therefore concluded that on the upper side a 60% success rate was achievable with mupirocin 2% nasal ointment. Therefore an estimated success rate of 60% was used as the basis for sample size calculation.

2.2.6.2 Power and sample size

From previous literature it was estimated that the decolonisation rate with mupirocin 2% nasal ointment was approximately 60%. Estimation of the sample

size in the study was based on the achievement of an 80% decolonisation rate (a 33% relative difference), with 80% power at significance level of 0.05%. A sample size of 188 would be required to achieve this, i.e. 94 in each group.

2.2.7 Randomisation

The ability to randomise subjects to treatments that distinguishes experiments from surveys is termed randomisation. Randomisation is the method of removing selection bias between two groups of patients. Randomisation is essential in experiments such as clinical trials as it minimises the chance of a biased result. Pocock is of the opinion that randomisation is the best method of removing selection bias between two groups of patients. (118) Randomisation is therefore fundamental in designing good experiments as it substantially reduces the chance of bias. (119)

Methods of randomisation

For randomisation of subjects, various methods were employed by investigators. In practice, computer assisted generation of random numbers and random sequences were used for randomisation. Simple and block randomisation were the two methods commonly used for randomisation of subjects.

In simple randomisation, each patient's treatment is determined at random independently with no constraints applied. Consequently, there is an equal chance of allocation to two treatment groups, which is equivalent to tossing a coin. According to Hewitt, simple randomisation is a safe approach as it is completely unpredictable and is less prone to technical errors. (120)

Block randomisation, also called restricted randomisation is another approach that keeps the subject numbers in each group very close at all times. The generation of randomisation sequence and securing random sequence using the block randomisation method is elaborated in the forthcoming section.

In the NHNMRSA RCT, block randomisation and remote allocation method was used which will be elaborated in the following section.

2.2.7.1 Generation of randomisation sequence

In the NHNMRSA study, the generation of random sequences was done using the block randomisation method. For the purpose of equal block randomisation, pre-sealed envelopes and pre-printed labels were used. Pre-printed labels entitled, mupirocin 2% and Medihoney™ Derma Cream, were placed in blank opaque envelopes and sealed and held in two separate packs. Eight envelopes, four with mupirocin and four with Medihoney™ Derma Cream labels, were mixed and bundled into a block, and each block of eight envelopes were serially numbered in total 24, (8 x 24) blocks.

2.2.7.2 Securing random sequence

The 24 blocks of eight envelopes each were securely stored in the IPC nursing office. Access to the random sequence blocks of envelopes was limited to an administrator who was not involved in the study. The researcher did not have access to the random sequence blocks. The benefit of the block randomisation method used in the study was that, in the event of patient enrolment being less than estimated, equal block randomisation approach offered optimal comparative analysis of patients in both the investigative and control groups.

2.2.7.3 Allocation concealment

Once a patient was consented, she/he must be allocated to one of the treatment groups; investigative or control. This must be based on the random sequence that was generated and held remotely for study rigor and an unbiased approach.

In randomised studies, the process of allocation can be compromised such that allocation results in biased groups of patients. Allocation bias may occur when,

for example, the investigator holds the random sequence, if there is tampering of the envelopes holding the allocation before patient consent, or a number of envelopes are opened at the same time. Unconcealed randomisation can lead to tampering of envelopes thereby leading to potential bias in allocation. Therefore, various methods have been employed by investigators to minimise, and where possible, eliminate bias in allocation. One of the most common methods employed is the sealed opaque envelope system. (121) In this approach, only after a patient is consented to enter a trial is the pre-sealed opaque envelope opened and then the patient is allocated to the treatment regimen as specified in random allocation obtained from the sealed envelope.

In this study, once a patient was consented, the researcher telephoned the IPC nursing office for the next allocation and the unique study number for the study participant. The study number and allocation were recorded by the researcher on the case report form (CRF), as well as the proforma study enrolment sticker, which were then affixed to the patient's medical record specifying the date and time of enrolment. Access to the pre-sealed block envelopes were restricted to one administrator who were not involved in the study. The researcher did not have access to the random sequence, i.e. the opaque pre-sealed envelopes holding the random sequence. In addition, the researcher was based at a remote location from the IPC office during the study period.

2.2.7.4 Blinding

After allocation, due to the nature of the interventions in the study, both the patient and the researcher knew the group that each participant was assigned to. As both mupirocin 2% (Bactroban® 2%) nasal ointment and Medihoney™ Derma Cream were distinguishable due to packing, the participant and researcher were able to recognise the specific product that a patient was allocated. Therefore, blinding of the participant or researcher was not achievable in the RCT.

However laboratory analyses were completed without knowledge of treatment allocation thus minimising the potential for ascertainment bias. The laboratory

personnel in the clinical microbiology department of BH were not able to distinguish screening swabs from the study or from other patients screened for MRSA. This approach eliminated detection bias in reporting the MRSA screening results.

Statistical analysis of the data was undertaken after completion of the RCT. The data of both treatment and control groups were masked, thus blinding the statistician.

2.2.8 Patient recruitment

2.2.8.1 Recruitment process

For patients admitted to the study hospital and confirmed that they were colonised with MRSA based on laboratory results, the researcher liaised with a senior nursing staff responsible for the patient and evaluated the patient's suitability for recruitment based on their clinical condition, expected prognosis and the criteria for inclusion and exclusion. Prior to taking patient consent, an individual patient's eligibility to enrol in the study was formally assessed, i.e. considering each of the inclusion and exclusion criteria by the researcher. Patients who fulfil the inclusion criteria were deemed eligible for enrolment. Thereafter, a patient who was considered suitable for participation in the RCT was approached by the researcher. At the first instance the patient's awareness of their MRSA colonisation and or specimen collected for screening tests were assessed. The researcher then discussed with the patient the results of their MRSA screening tests and where appropriate, the outcome of past decolonisation attempts. The researcher then provided information about the NHNMRSA study in detail as listed in the Patient Information leaflet (PIL) (appendix - VIII copy of PIL version 3). Patients were encouraged to ask questions or clarifications during the subject - investigator interaction. The researcher also summarised the key aspects of the RCT to the patients recruited, especially the random allocation to either group after consent. Patients were advised to consider the study information at the first instance and encouraged to make an informed decision on their participation in the study.

After sufficient time, usually twenty-four hours from the first contact by the investigator, the researcher made a follow-up visit to the patient to address queries and provide any clarifications as required. During the follow up visit, the patient's study enrolment and consent was considered. A patient's consent was obtained in triplicate; a copy for the patient, medical record and for the researcher. Each patient consented and enrolled in the study was allocated a unique case number, which was provided by an administrator through remote allocation.

Non-nasal MRSA decolonisation

The routine practice in BH in the decolonisation of patients who are colonised with MRSA at non-nasal sites is concurrent decolonisation using skin antiseptics while undergoing nasal decolonisation. This practice continued during the RCT for both patient groups. A concurrent nasal and non-nasal decolonisation course consisted of daily showering or washing with chlorhexidine shampoo for at least two days during the five consecutive days of decolonisation and nasal decolonisation. Aqueous chlorhexidine 4% w/v or triclosan 2% (Skinsan™) was used for body washing and showering.

2.2.8.2 Patient Recruitment challenges

The ability of patients to consent, apply the study product, and their geographic location, i.e. normal residence, were factors likely to impact upon potential patient recruitment. Past experience of the researcher as a specialist IPC practitioner and from patient surveys, as well as audits of patient communication, indicated that there would be significant challenges in patient recruitment. In addition, effective patient communication is another challenge that the researcher was cognisant of. A patient awareness survey (2012) among inpatients in BH who were colonised with MRSA confirmed that only 35% of patients were able to consent for an interview or a medical procedure. Therefore, the researcher anticipated that no more than one third of all new and known cases of MRSA, i.e. approximately 115 of 350 patients per year would be eligible for recruitment.

Notifying participants of individual results

Each patient enrolled and completing the study was informed by the researcher of their final MRSA screening results. Where feasible, this interaction was a face-to-face contact with the patient or by telephone call. A note with the date of communication to the patient of their final study outcome was also documented in the individual CRF.

2.2.8.3 Dissemination plan

The results of the RCT will be communicated to the general public and to special interest groups such as MRSA and Families, the Irish Patients Association as well as North Dublin GP newsletter. National newspapers and healthcare periodicals will also be approached to highlight the study outcome. The BH Newsletter as well as RCSI outlets will also be contacted for communicating the study outcomes to the general healthcare audience.

The results of the RCT will be disseminated at the conclusion of the study through abstracts at professional and scientific meetings as well as through articles in peer reviewed professional journals. Scientific meetings such as journal clubs, IPC team meetings, national MRSA study days, microbiology and IPC conferences, will also be accessed to publicise the results. In addition the findings of the study will be presented at internal hospital meetings and a short summary of the findings and conclusions will be prepared for the public. The researcher will also provide periodic briefings to BH clinical, nursing and management team meetings on the study progress as well as the final outcome of the study. The findings will also be reported in a thesis that will be submitted as part fulfilment of PhD, registered with the RCSI.

2.2.8.4 Study protocol

The ICH defines a protocol as 'a document that describes the objective(s), design, methodology, statistical considerations and organisation of a trial'. The study protocol explains the purpose of the study as well as how to carry out the study from initiation to the closure of the study.

The detailed protocol that was applied in the RCT will be listed in the following section.

The NHNMRSA RCT Protocol

Decolonisation regimen

- Mupirocin 2% (Bactroban® 2% nasal ointment) and Medihoney™ Derma Cream were procured and stored in the host facility's pharmacy in accordance with protocols on storage of trial medicinal products.
- The labelling of mupirocin 2% (Bactroban® 2% nasal ointment) and Medihoney™ Derma Cream was done according to good clinical practice (GCP) guidelines in the host facility pharmacy.
- The researcher identified patients colonised with MRSA of the nasal passages, from the BH daily microbiology surveillance reports.
- Potential patients identified from the microbiology surveillance reports were then evaluated by the researcher liaising with a senior nurse responsible for the care of the patient, to see at the outset if they meet inclusion and exclusion criteria.
- The researcher then discussed the study with potential eligible patients including the benefits and adverse events if any, and how they would be addressed. A detailed PIL on the NHNMRSA study was provided to the patient. The eligibility criteria for each patient's participation in the proposed study were evaluated during the initial patient contact.

- Participation of all patients in the study were on a voluntary basis. No monetary or other rewards or inducements were offered to participate in the study.

- After approximately twenty-four hours, i.e. the following day, the researcher followed up with the patient to address any queries or concerns about the study. The patient's decision to take part, or decline the study were noted during this interaction.

- Explicit written consent was obtained in triplicate from those patients who expressed an interest in enrolling in the study. A copy of the signed consent form was given to the patient, a copy was held by the researcher and a copy was filed in the patient's medical record.

- Thereafter allocation of the consented patient was done remotely. The researcher initiated a telephone call to the IPC administrator for this purpose. A unique case number was allocated by the IPC administrator and the researcher recorded this in the CRF, as well as the product allocated namely, mupirocin 2% (Bactroban® 2%) or Medihoney™Derma Cream. The researcher then completed the study initiation documentation and commenced patient data collection which were recorded onto the CRF.

- Mupirocin 2% (Bactroban® 2%) and or Medihoney™ Derma Cream were prescribed for patients according to the allocation. A proforma sticker was applied onto the patient's drug Kardex specifying the dose, start and end dates.

- Each study participant was provided by the researcher with a unit of mupirocin 2% (Bactroban® 2%) or Medihoney™ Derma Cream. Each of these products were a single patient application provided to the patient according to his/her allocation.

- After patient consent, allocation and completion of study initiation documentation, the researcher collected the named product from the host hospital pharmacy and delivered it to each participant. The dispensing and labelling of the prescribed product were done as

specified in the pharmacy documentation. (Refer to the pharmacy SOP for further details, appendix - IX).

- The researcher demonstrated how to self administer the product to the patient, and or next-of-kin as appropriate. A repeat demonstration was provided where appropriate to ensure the patient was able to administer the product correctly.
- The researcher then assisted the patient to self administer the first dose of nasal application to both nostrils. Thereafter the application of mupirocin 2% (Bactroban® 2%) or Medihoney™ Derma Cream was documented in the relevant notes.
- The procedure for nasal application was as follows: approximately 2 cm (one segment of the little finger OR pea size / head of cotton bud) of mupirocin 2% (Bactroban® 2%) or Medihoney™ Derma Cream were placed on a disposable sterile cotton bud, and applied on the middle part of each nostril.
- After application to both nostrils, the patient was instructed to pinch and rub the nose with two fingers for 10-15 seconds to ensure spread and extensive coverage of the nasal epithelium. Following this step, five minutes of rest with the head up was advised to facilitate dispersion of the ointment / cream into the rear of the nostrils.
- A printed record sheet was provided to mark the application of the prescribed product with the time and the date of application to confirm consistent and effective application. For patients in the control group, i.e. mupirocin 2% (Bactroban® 2%) the record with the title 'Directions for application of Bactroban® 2% were given. This record is in two pages copied onto one sheet, front and back. The directions list the step-by-step application guide which forms one side of the printed record. In addition, a document named 'Patient record of administration' which includes the day of the week and date, time of application and stop date for nasal application, which enables the patient to record self-

administration of each dose, was provided to patients. This forms the second side of the printed record.

For patients assigned to the investigative group, i.e. allocated Medihoney™ Derma Cream, 'Directions for application of Medihoney™ Derma Cream', were given. This record is in two pages copied onto one sheet. The Directions of application of Medihoney™ Derma Cream lists the step-by-step application. A table with date and times of application of each dose of Medihoney™ Derma Cream is printed on the form which allows the patient to record each dose after administration.

The directions for application of mupirocin 2% (Bactroban® 2%) / Medihoney™ Derma Cream were developed in consultation with the chief pharmacist, an expert in the field and who is also supervising the pharmacy component of clinical trials of medicinal products in BH.

- The registered nurse(s) responsible for the patient on the relevant ward administered the prescribed product for those patients who were unable to apply it themselves, and they recorded it in the drug Kardex as is standard practice.

- The directions and record sheets had information specified on the date for discontinuing the product, date for collecting of follow up screening swabs, contact telephone number for the researcher, to enhance patient safety and confidence.

- The dose and route of application of the products were as follows. Medihoney™ Derma Cream or mupirocin 2% (Bactroban® 2%), according to allocation, were to be applied to both the nasal passages three times a day, for five consecutive days. A flow chart depicting the sequence of events is attached as an appendix. (A copy of the screening and follow up flowchart appendix –X.)

- Patients were instructed on how to safely store the closed tube (ointment / cream) in its packaging.

- Participants were advised to perform hand hygiene, either washing hands with soap and water or using alcohol hand gel based on availability and accessibility, before and after nasal application.
- Patients who were colonised in non-nasal intact body sites, i.e. groin/perineum, were instructed to follow the standard decolonisation procedure using body wash and shampoo with triclosan 1% (Skinsan^{TM}), as per hospital policy. This involves five days of daily washing/showering after application, and shampoo for at least two days. This applied to patients in both the control and investigative groups.
- On completion of five days of application, either of the nasal application products and or triclosan 1% (Skinsan^{TM}), body wash, the treatment was discontinued for two days.
- Nasal and other body site screening was done two or more days after discontinuing the study products, as per the MRSA screening protocol.
- Swabs were sent to the BH microbiology laboratory and processed according to standard testing protocols.
- Bacterial isolates (MRSA) from the study participants were collected and stored by the researcher according to the microbiology laboratory SOP; LP-MIC Screens Rev 4 May 2013. (SOP LP MIC Screens is outlined in the laboratory aspects methodology chapter), (appendix-VII).
- Screening test results were reviewed by the researcher and further action; either to rescreen the patient or to start a second course of the allocated product took place based on these results.
- Patients who were negative for MRSA in the nose were rescreened between three to seven days after a negative nasal screening result.
- Patients who were negative for MRSA in the nose, but positive for groin/perineum were started on triclosan 1% (Skinsan^{TM}), body wash for five days as per the decolonisation protocol. Patients in this category were rescreened, both nasal and groin, two or more days after

discontinuing the decolonisation products to determine colonisation status.

- Patients who remained positive for MRSA in their nose after one course of the nasal application of the allocated product were commenced on a second course of the allocated product, which was based on their original allocation. A proforma sticker was applied on the drug Kardex specifying the dose, start and end dates. Mupirocin 2% (Bactroban® 2%) or Medihoney™ Derma Cream were supplied by the researcher to the respective patient on commencing a second course of decolonisation. Patients in this category were rescreened, both nasal and groin, two or more days after discontinuing the products to determine colonisation status.

- A second and third set of nasal and body sites screening were done on patients negative for MRSA to verify persistent decolonisation.

- Patients who were positive for MRSA in the nose after two courses of the decolonisation regimen were deemed persistently colonised with MRSA, and no further eradication efforts were attempted. The following exception applied for first time MRSA cases.

  If a patient who had MRSA identified for the first time in BH was enrolled, and allocated to the investigative study group, had two courses of Medihoney™ Derma Cream, and had persistent MRSA colonisation, then the patient was offered one course of mupirocin 2% (Bactroban® 2%) nasal ointment. The researcher arranged dispensing of the product to the patient.

- A negative MRSA status was confirmed after three consecutive negative MRSA screening samples were obtained after the decolonisation intervention.

Administration of study questionnaires

- On completion of one course of mupirocin 2% (Bactroban® 2%) or Medihoney™ Derma Cream, patients were provided with two

questionnaires. The questionnaire development and administration methodology is discussed comprehensively in a separate section, Chapter II section 2.5.

- The product experience questionnaire (PEQ) was administered in the first instance to record patients' perceptions on the use of mupirocin 2% (Bactroban® 2%), or Medihoney™ Derma Cream, based on their allocation, (appendix - XI a, product experience questionnaire Medihoney™ Derma Cream version 2, and product experience questionnaire mupirocin version 2, appendix - XI b).

- A second questionnaire on the perception of being MRSA positive was also administered to patients. Both questionnaires were given to the patient with a pre-labelled envelope addressed to the researcher.

Study completion

- On completion of the study, each patient was informed of the results of their MRSA screening, and all study documentation was completed by the researcher. This included affixing a proforma sticker in the patient's medical record stating study completion.

- The relevant laboratory results were recorded in the CRF.

- Any unused pharmaceutical products were disposed of according to the procedure specified in the Pharmacy SOP.

2.2.9 Data collection and management

2.2.9.1 Variables collected in the NHNMRSA RCT

Based on evidence based guidelines on the prevention and control of MRSA, the following variables were attributed to MRSA colonisation and impinge on decolonisation methods, and were decided by the research team as appropriate for collecting/recording. This included data on prior hospitalisation, patient's age at enrolment, receipt of antibiotics active against MRSA, history of MRSA

colonisation, multisite colonisation, presence of skin wounds, higher assistance required with activities of daily living (ADL) (i.e. carer assistance) and the presence of invasive medical devices, patients' compliance with the decolonisation protocol, isolation and healthcare worker practice of standard and transmission-based precautions (contact), healthcare contact frequency, co morbidity and concomitant carriage of drug resistant pathogens. (45,122) Each of the identified variables were coded for recording and data analysis.

2.2.9.2 Patient data collection tools

The following study specific tools were developed and used throughout the study: a CRF, a questionnaire on the product experience, and a questionnaire on patient perception of MRSA colonisation. A data dictionary was developed which served as a reference point for each of the variables collected and also served as the source for systematic coding of data. The data dictionary provides the definition for each variable identified in the study, abbreviations and the code used to identify the study participants and the database, (appendix - XIII data dictionary).

2.2.9.3 Patient data collection

The researcher was primarily responsible for the collection of individual patient data. Data were collected and recorded on a CRF that was assigned to each study participant at the first instance (appendix XII). After quality checks, data were entered onto an electronic data base. Primary data were collected by the researcher from direct patient interactions in addition to data obtained from the interventions over the course of patient participation in the RCT. Data (electronic and hard copy) from secondary sources such as the patients' medical records were also collected as appropriate by the researcher. The administration of the questionnaires and data collection are addressed in the questionnaire methodology, see Chapter II, section 2.5.

2.2.9.4 Data management

Data cleaning was completed by the researcher through cross checking each patient's data as entered in the CRF with the database entry, and verified as correct. Logical consistency among all parts of a record was validated by cross checking the data. There was no alteration or transformation of data and laboratory results as reported were captured in the CRF. Coded data from the CRF were entered on to a Microsoft (MS) EXCEL data base by the researcher for preliminary review and data cleaning. The CRF and the corresponding database entry of a random number of patients (10%) were cross checked and verified correct by an administrator external to the study.

For statistical analysis the software package 'SPSS' version 20 was utilised. An expert methodologist from the *Centre for Support and Training in Analysis and Research* (CSTAR) at University College Dublin (UCD) contributed to the methodological aspects of the study. Data analysis was also facilitated and supervised by the expert methodologist.

2.2.9.5 Quality control

In order to minimise bias in data collection, and to ensure conformity between source data and the RCT record, a random number of CRFs were audited. Conformity to source data and source verification was performed by an external auditor. The RCT documents were also audited and adjudged compliant to CT standards by a Quality and Regulatory Manager in the RCSI. The audit of compliance to CT standards was not originally planned by the research team. However, based on the expert advice of the Project Advisory Group (PAG), an approach was made to RCSI Clinical Research Centre, where Quality and Regulatory Managerial expertise on CTs were available. The results of audits were reported to the PAG.

2.2.10 Data analysis plan

Statistical methods

Statistical data analyses were conducted using SPSS version 20. Descriptive analysis was performed and comparable data were presented in descriptive, tabular and graphical forms. The clinical presentation of patients at the time of enrolment and outcome on completion of the study i.e. nasal and/or other body site MRSA colonisation were summarised using proportions, means and standard deviations (SD), or medians and interquartile ranges (IQR), as appropriate. Patients were classified as responders (negative nasal MRSA), or non-responders (positive nasal MRSA decolonisation), according to the screening test results.

For comparisons between groups, appropriate inferential statistical tests were used. Factors associated with decolonisation response were identified using Chi-squared tests or t-tests/Mann-Whitney U tests. Factors considered for all analyses included demographic and healthcare factors, age, gender, hospitalisation, co-morbidities, healthcare contacts, previous MRSA status etc. Both univariate and multi-factorial modelling were conducted to include possible confounders in determining the outcome. To assess homogeneity of distribution and to assess any association between the intervention and control groups, on specific variables such as gender, age, co morbidities and other IPC aspects, Chi-square (χ^2) and significance tests were done, the results form part of the Univariate analysis.

Intention to treat analysis (ITT) of all study participants in the study or control group they were originally allocated to was done. ITT analysis maintains the original comparability of study and control groups with respect to potential confounding factors achieved after randomisation of the study participants. Per protocol (PP) analysis was also undertaken and included in the results.

Multivariate logistic regression

Logistic regression (LG), a statistical analytical method, is used to predict the odds of being a case based on the values of the independent predictors, i.e. variables. The 'odds' is defined as the probability that a particular outcome is a case divided by the probability that is not a case.

For the purposes of LG, binary data collected in the study had to be re-coded in a way that enables seamless data analysis. Variables with multi-level coding options had to be grouped into two groups, where an apriori decision was taken to apply the code for a variable that denotes risk=1 or no / limited risk=0. Clinical IPC knowledge and practices were applied to group the multi-level coding options, which are discussed later in this section. The limited number of patients in the RCT also necessitated limiting the number of variables that can be applied for multivariate regression. Logistic regression necessitates apriori decisions by the researcher on the variables that are known or potentially contributing to risk or a particular outcome, i.e. in this study failure of the intervention leading to persistent nasal MRSA colonisation.

Re-coding of variables for logistic regression analysis

Gender

In the RCT data base, participants gender was coded as male=1 and female=2, which was re-coded to female=0, and male=1.

Age category

Participant's age in number of years was calculated as date at enrolment, from the date of birth of each participant. For LG, age was categorised and coded as below 65, y=0, and over 65, y=1. In clinical practice, over 65 y of age is considered a high risk category for illness and infections.

Skin integrity

Five categories were used to code for the skin condition of the participants namely; healthy=1, poor (paper skin) =2, wound=3, ulcer=4, and stoma=5. For

LG, healthy and poor category grouped into a single category and coded=0. The following categories namely wound, ulcer and stoma were grouped into a single group, 'altered skin integrity' and coded=1. The limited number of values in each category necessitated the collapse of the variable 'skin integrity' into two groups; where intact skin was considered less risky compared to altered skin integrity which was considered at risk.

Invasive and indwelling devices

Five categories were also used to code for the presence of invasive and indwelling devices; CVC's/PICC's=1, PVC's=2, urinary catheter=3, more than one device=4, and participants with no device=9. The categories were coded for LG as participants with no device=0 and with any device=1. Similar to skin integrity, the device variable had to be grouped in a way that made clinical sense. Therefore presence of a device was considered a risk when compared with no device.

Residence during the RCT

The participant's residence during the intervention period was categorised into four; inpatient=1, outpatient/home=2, nursing home=3, and other healthcare facility=4. The categories were collapsed into two groups namely; outpatient/home, code=0; inpatient, nursing home and other healthcare facility into one category, 'resident healthcare facilty' code=1. Patients at their home in comparison to those who are resident or admitted to healthcare facilities are unlikely to have frequent HCW contact. HCW contact is considered a risk factor for transmission of MRSA as well as other MDROs. Therefore residence at a healthcare facility was considered a risk and patient's stay at home was not.

Isolation of participants

Isolation of participants during the intervention period in the RCT were categorised into four types; single room with CP=1, cohort with CP=2, not isolated=3, and not applicable (home) =4. The categories were collapsed into two groups; single room with CP, cohort with CP and not applicable into one group and applied code=0; and not isolated=1. Isolation of patients with CP is recommended for the control and prevention of transmission of MRSA. Single

room, cohort isolation and patient stay at home were considered a form of isolation for the purposes of LG. The rationale was, if HCW contact occurred CP were deemed to be applied, and if at home HCW contact was unlikely, therefore the benefit of isolation from other patients reduced the risk for MRSA acquisition and transmission, compared to patients colonised with MRSA not isolated.

Dependency

The NDS dependency score of the participants were coded in five categories; independent=1, minimal assistance=2, moderate assistance=3, dependent=4, and specialised nursing care=5. For LG, the dependency categories were collapsed into two groups; independent, minimal assistance and moderate assistance to a single group, independent to moderate care, code=0; dependent and specialised nursing care to a second group, dependent care, code=1. Higher dependency of care commands for increased HCW contact with sicker patients, which is a recognised risk for acquisition of MRSA and other transmissible MDROs.

History of mupirocin 2% (Bactroban® 2%) use

The number of courses of past mupirocin use were classified into four groups, no previous use=0, one=1, two=2, more than two courses of the antibiotic=3, and unknown=9. For LG, three groups were created, one of which included participants with one or two courses of mupirocin, code=0, those who had more than two courses, code=1, and participants no previous mupirocin use or unknown, code=9, i.e. missing data. In clinical practice, the number of mupirocin courses is usually limited to two courses, considering increasing MR.

Multisite colonisation

Participants who were identified with non-nasal MRSA colonisation on study completion, one or more sites, were categorised as multisite colonisation=1, MRSA negative cases=2, and if this information was unknown=9. For LG, participants who had no documented MRSA colonisation on non-nasal sites or if unknown were coded=0; and those with multisite colonisation were coded=1. Multisite colonisation is a recognised risk for persistent MRSA carriage.

2.2.11. Handling of unexpected or adverse events and emergency care

Definitions

Adverse Reaction: An adverse reaction is defined in Article 2(n) of Directive 2001/20/EC as: 'all untoward and unintended responses to an investigational medicinal product (IMP) related to any dose administered'. (123) The definition implies a reasonable possibility of a causal relationship between the event and the IMP.

Unexpected Adverse Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information, e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product. (123)

Adverse Event (AE). Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. (International Conference on Harmonisation Good Clinical Practice (ICH GCP) 4.11.2). (124)

Serious Adverse Event (SAE): Any untoward medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect (ICH GCP 4.11.1). (124)

Monitoring of untoward events

Patients enrolled in the study were informed that they would be monitored for any unexpected or adverse events. Adverse events or SAE were considered unlikely, however RCT participants were monitored for AE or SAE. While no adverse consequences after the use of honey for medicinal purposes have been reported in the healthcare literature, patients were informed that there may be a possibility that the honey product used were inferior to the antibiotic used to decolonise nasal MRSA. Study entrants were also informed that there were no adverse reactions when we conducted our pilot study using the honey product, Medihoney™ Derma Cream. However, as honey has not been

extensively used for medicinal purposes, study entrants were informed that it was possible that there may be other risks which were not known at this time. The research team carefully monitored for any unintended effects of honey during the course of the study. It was expected that participants receiving Medihoney™ Derma Cream might feel a runny nose based on experience from the pilot study. After application of either the control or investigative products, patients were monitored for any allergic reactions, itching or rash on the nose or face immediately or during the five day course. Study entrants who continued to be inpatients were advised to inform the nursing staff assigned for his/her care or a doctor who was available at the first instance. Any unexpected event information were also elicited from patients by the researcher on follow up visits or via telephone contacts. Outpatients were advised to contact the emergency care services available in the community at the first instance in the event of an unexpected or adverse event. Patients were also provided with a mobile telephone contact number to contact the researcher as required.

In the event of any unpleasant or unexpected patient experience after using either control or investigative products, information from the affected patient were collected and documented in the AE/SAE record. The clinical team were notified and the consultant team continued to manage the patient's medical care. The clinical supervisor was promptly informed of any unexpected or adverse events, if they occurred. A record of any unexpected or adverse reactions of each patient was maintained by the researcher in the study file and was noted in the patient's CRF. Continuation of the study by a study participant in the event of an unexpected or adverse event was carefully considered, and the study was discontinued if a patient was unable to continue with the assigned protocol.

On study enrolment, study entrants were informed by the researcher that the study was indemnified by the state healthcare insurance applicable for public healthcare providers in Ireland, in the very unlikely event that a patient was harmed in any way as a direct result of this study.

2.2.12. Procurement and storage of pharmacy products

The investigative product for the experimental decolonisation arm in the NHNMRSA study was natural honey, Medihoney™ Derma Cream 50gm in a pre-filled tube, manufactured by Derma Sciences Europe Limited. The standard product for the control group was mupirocin 2% (20 mg/g) 3gm in a pre-filled tube, (Bactroban® 2% (20 mg/g) nasal ointment) manufactured by GlaxoSmithKline (GSK), Ireland Limited.

Medihoney™ Derma Cream and Bactroban® were purchased and stored at controlled room temperature according to the product information in the Pharmacy Department, Beaumont Hospital. Medihoney™ Derma Cream as recommended was stored at room temperature, at 15 - 30 ºC (59-86 F). Bactroban® ointment was kept at room temperature, 15 - 30 ºC (59-86 F).

Temperature logs with daily minimum and maximum temperature readings were kept by pharmacy personnel to monitor the storage temperature of the study products. The temperature monitoring device included routine calibration records, which are managed by the Pharmacy Department. Temperature logs were kept in the pharmacy and made available for inspection upon request.

On receipt of Medihoney™ Derma Cream and mupirocin 2% (Bactroban® 2%) in the Pharmacy, the standard practice was to clearly label the items by pharmacy personnel and place the stock in the clinical trials area of the pharmacy. The study products and documentation were kept in an area with restricted access to authorised site personnel only. The researcher dispensed the study products in the original packing after completing dispensing details. Dispensing details were documented by the researcher in the Product Inventory Log for each item dispensed. Each product was dispensed to a named patient after printing the relevant patient details onto the pre-approved proforma label stickers affixed to the product's outer cover and product tube, as specified in the pharmacy standard operating procedure.

2.2.13. Study documents

In order to conform to good research practice, a number of documents were developed and used systematically in the RCT; these are listed below (appendix - XIV copies of the documents)

- Patient information leaflet. Version 3.
- Patient consent form. Version 1.
- Proforma clinical notes stickers. Version 1.
- Case report form. Version 2.
- Data dictionary.
- Questionnaire; Version 2.
    - Product Experience Questionnaire mupirocin 2% nasal ointment.
    - Product Experience Questionnaire Medihoney™ Derma Cream.
- Questionnaire. On MRSA perception; MRSAPQ. Version 1.
- Standard operating procedure: LP MIC Screens; MRSA screening, collection and storage of isolates from colonized patients Lab Protocol. Version 1.
- Standard operating procedures for pharmacy, Version 1.
- Directions for application of Medihoney™ Derma Cream and patient record of application. Version 1.
- Directions for application of mupirocin 2% (Bactroban® 2%) nasal ointment and patient record of application. Version 1.
- Letter to consultant doctor. Version 1.
- Letter to family doctor. Version 1.

2.2.14. Summary

Estimating the number of potential participants for a study from the source population requires careful consideration by investigators. At the same time, for the outcome of a study to be meaningful, the results should be applicable to the target population. In the NHNMRSA study the researcher had the advantage of having access to microbiology surveillance data for many years before conducting the study, in addition to being part of the IPC team in BH involved in MRSA prevention and control. Despite this, estimating the potential number of participants that could be enrolled in the RCT was challenging. While surveillance data can help estimate the eligible population, inclusion and exclusion criteria substantially reduces the available and accessible population for recruitment. A thorough, well defined step-by-step study protocol was essential for the conduct and success of the study. Its development was a significant and useful learning experience for the researcher. The guidance of a project team with specialist and subject matter expertise involved in drafting and finalising the study protocol was vital. Methodological rigor of protocol development cannot be underestimated. The expertise of a methodologist, and in the NHNMRSA RCT a reputed methodologist from CSTAR, significantly contributed to and complimented the researcher's learning experience.

2.3 Mupirocin susceptibility test methodology

2.3 Mupirocin susceptibility test methodology

2.3.1 Introduction

The overall aim of the NHNMRSA RCT was to investigate the eradication of MRSA from the nasal passages in humans with medical grade honey (MGH). Two of the five study objectives were pertinent to the laboratory investigation of isolates. In this section the methodology that was used to determine mupirocin susceptibility of isolates, which includes the collection and storage of bacterial isolates as well as the controls that were used, is enumerated. The data generated was used to determine the outcome of decolonisation for individual patients, as well as to ascertain the prevalence of mupirocin resistance.

2.3.2 Collection of MRSA isolates

In accordance with routine clinical practice and the BH 'Guideline for the control and prevention of MRSA' (2007), patients are screened in BH for MRSA and specimens are processed through the BH diagnostic microbiology laboratory. Nasal MRSA isolates from consenting patients were collected after enrolment to the RCT. Collection and storage of MRSA isolates commenced in February 2014 and continued until the last patient was enrolled on 31st March 2016. The sequence of patient screening was as follows: following the first decolonisation course, and where applicable the second course, nasal and other body sites were screened. Specimens were collected at least 48 h after the last dose of the nasal ointment or cream and Skinsan® application using invasive sterile collection swabs with transport media (EUROTUBO®, Deltalab, Spain, supplied by Premier Scientific Ltd, Belfast).

Arising from testing during the RCT, positive MRSA isolates from nasal specimens were collected and stored at -20^{o}C on Microbank™ beads (Pro-Lab Diagnostics., USA) For previously 'known MRSA positive' patients recruited to the RCT, the 'first time detected MRSA' isolate (historic isolate) was also collected if available from stored slope cultures. A sample from stored slope cultures (Nutrient agar) was grown overnight at 37^{o}C on Columbia Blood Agar

(CBA) and single isolated colonies were selected and stored at $^{-}20^{o}C$ on Microbank™ beads. A patient enrolled in the study may therefore have had up-to three consecutive isolates; historic, baseline and end of study, if nasal decolonisation failed, or only one isolate if only the baseline isolate was available and the patient had successful decolonisation. All the baseline and end of study isolates were of nasal origin, but the first time isolates included nasal as well as those from non-nasal sites.

2.3.3 Bacterial Controls

MRSA ATCC 43300 was the reference strain used. The reference strain was obtained from the BH diagnostic microbiology laboratory.

2.3.4 Media and growth conditions

MRSA isolates were routinely grown on CBA (Fannin Ltd, Galway, Ireland) overnight at $37^{o}C$ in a static incubator (Gallenkamp, Leicestershire, UK). For antibiotic susceptibility testing, isolates were grown on Mueller Hinton (MH) (Fannin Ltd, Galway, Ireland) agar. For DNA extraction, single colonies from CBA were grown overnight in 5 ml of Brain Heart Infusion (BHI) liquid medium in an orbital shaking incubator (Gallenkamp, Leicestershire, UK) at $37^{o}C$ and 150 rpm.

2.3.5 Buffers and solutions

Milli-Q water (Millipore Ireland, Cork, Ireland) was used for making buffers, solutions and agarose gels. Molecular biology reagent water from Sigma-Aldrich was used in all PCR reactions, DNA elutions and dilutions. Tris-borate/EDTA (TBE) was used at 0.5X concentration as a buffer for agarose gel electrophoresis. This was diluted from a 10X stock solution supplied by Sigma-Aldrich, UK.

2.3.6 Sterilisation techniques

BHI broth and agar media were prepared under aseptic conditions and were autoclaved at 121^{o}C for 15 min. All biological laboratory waste was sterilised at 115^{o}C for 30 min before appropriate disposal.

2.3.7 Mupirocin susceptibility testing

The susceptibility of MRSA isolates were investigated in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoints, Bacteria (v3.1) February 2013.

The MIC was determined using mupirocin E-test strips, (bioMerieux®, UK) according to the manufacturer's instructions. Briefly, a suspension was prepared in saline from isolated colonies from an overnight culture and adjusted to the density of a 0.5 McFarland standard using the Vitek DensiCHEK™ Plus densitometer (bioMerieux®, USA). The suspension was used within 15 min of preparation. Using a sterile cotton swab the suspension was inoculated onto MH agar in three directions, rotating the plate at 60 degrees each time to ensure an even distribution of inoculum. After air drying for 15 min, a mupirocin E-test strip was placed centrally on the agar surface. Plates were incubated for 16-20 h at 37^{o}C. The MIC was interpreted as the point of intersection of the zone of inhibition with the E-strip. Phenotypically, mupirocin susceptibility is determined according to MIC breakpoints, with susceptible being ≤4 mg/L. MIC's of 8-256 mg/L are classified as low level mupirocin resistance (LLMR) and MIC's above >512 mg/L are classified as high level mupirocin resistance (HLMR). (46,47) The MIC breakpoints were consistently applied in determining susceptibility of MRSA isolates. The data were then used to determine the outcome of decolonisation as well as to evaluate bacterial resistance to mupirocin. MRSA isolates that were tested for mupirocin susceptibility are listed in appendix - XV.

2.4 Methodology of Laboratory Investigations

2.4 Methodology of Laboratory investigations

2.4.1. Analysis of bacterial isolates

2.4.1.1 Introduction

The investigation of the MRSA isolates such as the phenotypic and genotypic characteristics enables to distinguish the attributes that may potentially contribute to the outcome of decolonisation. In this section the methodology that was applied in the isolation of bacterial genomic DNA, *spa* typing and *mupA* polymerase chain reaction (PCR) is catalogued. The MIC and minimum bactericidal concentration (MBC) of Medihoney™ Derma Cream were also determined as it was considered important.

2.4.1.2 Bacterial molecular genetics techniques

The identification of bacterial genetic characteristics facilitates the differentiation of MRSA colonising strains. Comparison of the *Staphylococcus* protein A (*spa)* type from a individual patients at multiple time points such as historic, baseline and end of study differentiates the colonising *spa* type, i.e. persistent colonisation with same strain or re-colonisation with a different strain. The data generated is important to evaluate the outcome of decolonisation attempts for an individual patient. Molecular genetics studies helps to classify the representativeness of the MRSA isolates with that of local and national surveillance data. The data generated was used to determine the colonising strains and the outcome of decolonisation.

2.4.1.3 Isolation of bacterial genomic DNA

Genomic DNA was isolated from overnight cultures of bacteria using the Wizard® Genomic DNA Purification Kit (Promega, USA) according to the

manufacturer's instructions. Prior to DNA extraction, cells were pre-treated with 10µl of lysostaphin (1 mg/ml) (Ambi-Products Ltd., USA) in 100µl 50 mM EDTA (Sigma-Aldrich., MO, USA. All centrifugation steps were carried out in an Eppendorf Centrifuge (5804 R, Hamburg, Germany).

DNA was eluted with 50 µl DNA rehydration solution (Promega, WI, USA) for 1 h or overnight at 4^{o}C and stored at $^{-}$20^{o}C. The concentration of DNA in each sample was determined using a Nanodrop 8000 V2.0.0 Spectrometer (Thermo Scientific, USA).

2.4.1.4 *spa* typing

spa typing is a discriminatory method which distinguishes between strains of *S. aureus* and can be used to infer sequence types (ST). The MRSA isolates were prepared for *spa* typing by the sequencing of a polymorphic 24 base-pair (bp) variable number tandem repeat (VNTR) region within the 3' end of the *spa* gene. The primers used were *spa*113F (5'-TAAAGACGATCCTTCGGTGAGC-3') and *spa*1514R (5'-CAGCAGTAGTGCCCGTTTGCTT-3'). PCR reactions were carried out in a Peltier Thermal Cycler (PTC-200, MJ Research, Bio-rad, USA) and contained 25µl of goTAQ green master mix (Promega, WI, USA), 2µl of DNA template, 2 µl each of forward and reverse primers (10 pmol/µl), in a final volume of 22 µl of sterile water. PCR conditions were: 80^{o}C for 5 min, 35 cycles of 94^{o}C for 45 s, 60^{o}C for 45 s, and 72^{o}C for 90 s with a final extension step for 10 min at 72^{o}C.

PCR products were cleaned-up using the Promega PCR Clean-Up Kit (Promega, WI, USA) according to the manufacturer's instructions. DNA was subsequently prepared for sequencing with the following; 5 µl of DNA mixed with 5 µl of primers and both forward and reverse primers in separate tubes sent for sequencing to a commercial service provider in labelled tubes. LIGHTrun™ sequencing was performed by GATC-biotec, Germany. The *spa*-sequence analysis was carried out using the Ridom® StaphType software, version 1.3 (Ridom GmbH, Wurzburg, Germany) to identify the *spa* type. The ST of isolates was inferred from the *spa* type. The ST and *spa* type of available

sequential isolates from individual patients were compared and analysed to generate meaningful results.

2.4.1.5 *mupA* PCR

The identification of the presence of *mupA* in the bacterial genomic DNA is a recognised method of confirming mupirocin resistant MRSA. The presence of *mupA* in MRSA isolates were investigated using the PCR method which is now discussed.

DNA was extracted from historic as well as MRSA isolates on study enrolment to investigate for the presence of *mupA* gene by PCR, using *ileS* forward and *ileS* reverse Primers as previously described by Roth Perez et al.(125) The primers used were *mupA*-F (5'-TATATTATGCGATGGAAG-3') and *mupA*-R (5'-AATAAAATCAGCTGGAAA-3'). The PCR reactions were carried out in a Peltier Thermal Cycler and contained 2µl of DNA template, 1 µl each of forward and reverse primers (20 pmol/µl), 3 µl of magnesium chloride (25 mM), all in a final volume of 18 µl of sterile water. PCR conditions were: 95^{o}C for 5 min, 35 cycles of 95^{o}C for 30 s, 60^{o}C for 30 s, and 72^{o}C for 30 s with a final extension step for 10 min at 72^{o}C. The *mupA* PCR result of each isolate was listed in order. The *mupA* PCR results of available sequential isolates from individual patients were compared and analysed.

2.4.1.6 Agarose gel electrophoresis (AGE)

Performing AGE detects the presence or absence of bacterial DNA fragments in comparison to a representative DNA ladder, such as 1kb ladder, under ultraviolet light (UV). Electrophoresis of DNA extracted and PCR products were carried out using horizontal 0.8% (w/v) agarose gel containing 10 mg/ml ethidium bromide (Promega, WI, USA). Agarose gels were prepared and electrophoresed in 0.5X TBE buffer. A 3-6 µL sample of the DNA or PCR product containing loading dye was loaded into the test wells. A 1 kb DNA ladder (Promega, WI, USA) was used and 6 µL of the ladder was loaded into

the reference well. Horizontal agarose gel tanks (SCIE-PLAS, UK) and Sub-Cell®, (Bio-rad, USA) were used and electrophoresis performed at 100 - 140 volts (V) for 30 – 45 min. DNA fragments were visualised by illumination with an ultraviolet light source (Ingenius^3, SYNGENE., USA) and the images were analysed by comparing with the 1 kb DNA ladder, positive control and 'no-template' control.

2.4.2 Determination of MIC and MBC of 30% Medical Grade Honey

2.4.2 Determination of MIC and MBC of 30% Medical Grade Honey

2.4.2.1 Introduction

The NHNMRSA study investigated the efficacy of 30% MGH (Medihoney™ Derma Cream) the investigative agent, for nasal MRSA decolonisation in comparison to mupirocin 2% nasal ointment (control) in a RCT. In this section the methodology used to ascertain the antimicrobial properties of Medihoney™ Derma Cream is described.

Bacterial Strains

Eight clinical MRSA isolates (nasal) collected from participants in the RCT, and one reference strain, MRSA ATCC 43300, were used. The clinical isolates were identified by routine laboratory methods; i.e. detection of staphylocoagulase and clumping factor (Staphaurex Plus, Remel, U.K.), and oxacillin resistance (by determining oxacillin minimum inhibitory concentrations (MIC)) using the MIC Evaluators system (Oxoid, U.K.) and verified MRSA. The reference strain, which is routinely used for quality control were obtained from the diagnostic microbiology laboratory, BH.

Components of the investigative product

Table 2.4.2.1 presents the composition of Medihoney™ Derma Cream as provided by the manufacturer. The finished product pH is of the range 3.5-5.

Table 2.4.2.1 NHNMRSA RCT composition of the investigative product Medihoney™ Derma Cream

Composition	Function
Manuka honey (Leptospermum honey, 30%, medical grade)	Antibacterial component
Rodi Water (USP)	Medium-dilution-filler
Coconut oil	Moisturiser and softener
natural Triglyceride	Prevent loss of water from skin
Natural wax	Barrier function
Evening Primrose oil	Hydrating dry skin
Aloe Babadenisis (USP)	Skin conditioner
Sodium Benzoate	Preservative
Chamomile	Cell regeneration
Tochenol Acetate	Topical nutrients
Vitamin E	Nutrient

2.4.2.2 Dilutions

Medihoney™ Derma Cream, 50 gm in its original sealed packing were stored at room temperature prior to testing and honey dilutions were prepared fresh prior to testing. Serial dilutions of Medihoney™ Derma Cream (30% to 0.16% v/v in nutrient broth (Oxoid, Fannin, Ireland)) were prepared aseptically for use both the agar well diffusion and broth microdilution assays.From the 30% (v/v) Medihoney™ Derma Cream, 11 serial dilutions were made, resulting in final concentrations of; 30%, 25%, 20%, 15%, 12.5%, 10%, 5%, 2.5%, 1.25%, 0.62%, 0.31% and, 0.16%. Hereafter, these solutions are referred to as ‘test honey’.

2.4.2.3 Agar well diffusion assay

A previously described screening assay using agar well diffusion were carried out with some minor modifications for MIC determination. (126) Bacterial suspension were prepared in sterile saline from isolated colonies from an overnight culture and adjusted to the density of a 0.5 McFarland standard using the Vitek DensiCHEK™ Plus densitometer (bioMerieux®, USA). The

suspensions were used within 15 min of preparation. Using a sterile cotton swab the suspension was inoculated onto Mueller Hinton (MH) agar in three directions rotating the plate at 60 degrees each time to ensure an even distribution of the inoculum. After air drying for 15 min, 5 mm diameter wells were cut into the surface of the agar using a sterile bore former. Sixty micro-litres of test honey, at each of the concentrations stated above, were added to each well. Plates were incubated at 37℃ for 24 h. A diffusion control of methylene blue was used. (127) Zones of inhibition were measured using a Vernier calliper (Draper). The diameter of zones, including the diameter of the well was recorded. Each assay was carried out in triplicate, and mean values obtained. The MIC was defined as the lowest concentration of test material which resulted in a measurable inhibition of growth of the test organism based on the diameter of the inhibition zone.

2.4.2.4 Determination of MBC of test honey by broth micro-dilution assay

To establish whether the antibacterial activities of the test honey were bacteriostatic or bactericidal, a method previously described were used. (128)

Ten µl of 0.5 McFarland standardised culture were added to 190 µl of each test honey concentration, in the wells of a 96 well plate and incubated in the dark at 37℃ with shaking at 150 rpm for 24 h. Control wells contained nutrient broth only (sterility control), or bacteria and nutrient broth (positive control). Following overnight incubation, 10 µl from each well was added to 90 µl sterile saline and spread on CBA plates (Oxoid) and incubated overnight. Plates with no visible colonies after 24 h growth were recorded as representing bactericidal honey activity.

2.5 Questionnaire development methodology

2.5 Methodology; Development of questionnaires

2.5.1 Product Experience Questionnaire

The rationale for a Product Experience Questionnaire

The aim of the NHNMRSA RCT was to compare MGH with mupirocin for nasal decolonisation of MRSA. Mupirocin 2% w/w (Bactroban® 2.0% w/w) nasal ointment, a licensed medicinal product was the control, and Medihoney™ Derma Cream, a licensed medical device, was the investigative product that was used in the study.

Mupirocin 2% nasal ointment has been employed for nasal MRSA decolonisation since early 1970's; however, there is paucity of information on patient experience on using the nasal ointment. (51) In the NHNMRSA RCT, it was considered important to assess patient experience on application of Medihoney™ Derma Cream on nasal passages, as it is a novel approach in the nasal eradication of MRSA. Patient compliance for a product prescribed for topical application such as an ointment or cream, is potentially influenced by patients experience on its use. Therefore, collating patient experience using a simple tool that enables the analysis and generation of meaningful information was considered important and were incorporated in the study.

At the first instance, while considering a tool to collate patient experience on use of the control and investigative products in the study, an investigation on known factors that may contribute to user experience were done.

What are the factors that affect user experience?

A literature search on assessment criteria for usability identified three general themes, namely effectiveness, efficiency and satisfaction.(129-130) Another key aspect that was identified in the search was that a participant's reaction in any assessment is just one measure of usability. Therefore, in order to get the

complete usability picture, investigators should consider effectiveness and efficiency. (131) These two dimensions of usability, i.e. effectiveness and efficiency, stem from the International Standard Organisation (ISO), ISO 9241-11 standard, which defines usability as: 'the extent to which a product can be used by specified users to achieve specified goals with effectiveness, efficiency and satisfaction in a specified context of use'. (130) The ISO definition reiterates that user satisfaction is just one important dimension of usability. People may be well disposed to a system but fail to complete critical tasks with it, or in the case of a product, they may complete the tasks specified in a roundabout way, or for a period or frequency less than specified.

Based on the outcome of the literature search and the ISO definition, the three measures of usability can be deduced as effectiveness, efficiency and satisfaction. Effectiveness is the accuracy and completeness with which users achieve certain goals. Indicators of effectiveness include quality of solution and error rates. Efficiency is the relationship between the accuracy and completeness with which users achieve certain goals, and the resources expended in achieving them. Indicators of efficiency include task completion time and learning time. Satisfaction is the user's comfort with, and positive attitudes towards, the use of the system. User satisfaction can be measured by attitude rating scales. (129)

Investigators are of the opinion that the three measures of usability; effectiveness, efficiency and satisfaction are independent, and an investigator should measure all three elements to get a rounded measure of usability. (129,131) While measuring usability, investigators are alerted on a frequent mistake which is to measure satisfaction by using a questionnaire only, either at the end of the session or on completion of each task. It is also described that once an investigator invites participants to assign a number to their experience, their experience suddenly becomes better than it actually was. (131)

Frokjaer, Hertzum and Hornbaek are of the opinion that while it is tempting to assume simple, general correlations between effectiveness, efficiency, and satisfaction, any relationship between these factors seems to depend on a range of issues such as application domain, context of use, user experience,

and task complexity. (129) For routine tasks, Frokjaer is of the opinion that good performance depends on the efficient, well-trained execution of a sequence of actions which is known to yield stable, high quality results. For such tasks, high-quality results are routinely achieved, and task completion time may therefore be used as an indicator of overall usability. (129)

Tools to measure the user's experience of the product

Investigators who have appraised user experience have commented that user experience is not only a snapshot of the present usage a product has. It is the entire impression a product makes on the user. Furthermore, the user's judgement starts before touching and using a new product. In addition, the change of impression carries on during and after the usage of a particular product. (132)

User Experience Questionnaire (UEQ) is one of the tools that is regularly quoted by investigators, that is used to assess user experience. (132) The UEQ contains six scales; each one of the scales is listed and described in brief. The first scale is attractiveness. It is the general impression towards the product. The second is efficiency, i.e. it conveys a judgment, is it possible to use the product quickly and efficiently. The third is perspicuity, which conveys the view of the user; is it easy to understand how to use the product. The fourth is dependability, which elicits users view, does the user feels in control of the interaction. Fifth is stimulation. It is the user judgement that conveys, whether it is interesting and exciting to use the product. The final one is novelty, which is an impression that communicates to the user that the design of the product is innovative and creative.

Survey method to measure user experience

Of the many alternatives that are considered useful to measure user experience, survey is one of the methods that are frequently employed by investigators. Survey methods are broadly classified into two; the first method utilises a personal approach, which can consist of face to face structured interviews as well as telephone interviews. The second method is a self administered approach; which can be through a paper and pencil survey, online as well as mail surveys. The benefits of the traditional survey administration method, using the paper and pencil survey is that it is ideal for respondents who are not computer literate or do not have access to the internet. The limitations of this method are; the paper and pencil self administered technique usually requires the researcher to be present during the administration; it also necessitates doing the expensive reproduction of survey questionnaires and the tiring manual distribution of the questionnaires to the respondents. Typically, a questionnaire is a paper and pencil instrument that is administered to the respondents.

Response rates to questionnaire surveys

The response rates to surveys can be influenced by the method that is utilised. As outlined in the earlier section, each type of survey method has its advantages and disadvantages. However, there are various ways by which the researcher can encourage participants to respond and complete the survey, thereby improving response rates. One of the approaches is compensation; to compensate the participant's effort by means of providing an incentive. Another approach is by ensuring confidentiality and anonymity, (if it applies) of the participant. This can be an assurance to the participants that all their answers will be kept confidential and their responses will only be used for the purpose of the survey. The third approach is by following the KISS principle; Keep It Short and Simple. (133) Higher response and completion rates to questionnaire surveys are associated with concise, simple, and easy to answer survey questionnaires. (133) By portraying the tool as professional, courteous and

polite, an investigator can influence the return rates. Saying please and thank you as well as guiding the respondent politely, are also helpful in motivating the participant to complete the survey. Finally, participants are more likely to cooperate if the researcher practices professionalism, whether in appearance or their behaviour.

How to address potential bias in a survey questionnaire

A well developed questionnaire is imperative to gather the most appropriate data on the subject matter that is investigated. Therefore, in developing a good questionnaire there are many issues that investigators should consider. The basic tenant while developing questions or statements are the use of neutral wording when introducing questions and avoiding leading questions. Along with this one should make the questions as simple as possible, avoid too many open questions, and ask general questions before specific questions.

While developing user experience questionnaires investigators should avoid introduction of potential bias, this includes ‘acquiescence bias’, i.e. the fact that people are more likely to agree with a statement than disagree with it, stated by Cronbach 70 years ago, applies even today. (134) This means that one needs to balance positively phrased statements, such as ‘I found this interface easy to use’ with negative ones, for example ‘I found this interface difficult to navigate’. From questionnaires that are positively phrased, the results are more likely biased towards positive respondence and vice versa.

Questionnaires that avoid acquiescence bias often suffer from other sources of bias such as reliability and validity. Reliability bias means that the same questionnaire may yield different results at different times. This can be checked by measuring the questionnaire's test-retest reliability. The third is validity bias. If validity is poor, there is no guarantee that the questionnaire actually measures what it is intended to measure, i.e. user satisfaction.

A good positive user experience is central for the success of investigative as well as novel and interactive products. To improve a product’s quality aspects, it

is important to be able to measure user experience in an efficient and reliable way. But measuring user experience is not sufficient in itself.

How the user is able to record his/her most appropriate response to a statement or question, and the method to be employed, was the next consideration by the researcher while developing the questionnaire survey tool. The next section will consider the tools that were used to record user response, for example rating scales.

Rating scales for survey questionnaires

A response format to a statement or questions in assesment tools can vary in form to suit the investigator requirements; however, it should be user friendly. The response formats used in surveys vary depending on the type of question being asked. Responses can be as simple as a choice between yes and no or choosing an answer among several response options, which is complex and may puzzle users. In a survey the response options for each question may include a dichotomous, a three, five, seven point scale or more, or a semantic differential scale. Each of these response scales has its own advantages and disadvantages. The rule of thumb is that the best response scale to use is the one which can be easily understood by respondents and interpreted by the researcher. (133)

A dichotomous scale is a two-point scale which presents options that are the opposite of each other, for e.g. yes or no. This type of response scale does not give the respondent an opportunity to be neutral on his/her answer in a question. A semantic differential scale is generally used in specialist surveys in order to gather and interpret data based on the connotative (the emotions and associations connected) meaning of the respondent's answer. A semantic differential scale uses a pair of clearly opposite words, and can either be marked or unmarked. A rating scale that provides more than two options, in which the respondent can answer in neutrality over a question being asked, e.g. good, fair, poor in a three point scale, or in a five point scale the options for a

response may range from strongly agree, agree, neutral i.e. neither agree or disagree, disagree, or strongly disagree. (133)

Designing rating scales

Rating scales are used for making an appropriate response to a statement or a question by the respondent. In devising rating scales the following four factors are considered important for a good outcome. Firstly, to use scales with equal options for positive and negative replies, e.g. five point scales should have two positive and two negative options with a neutral middle choice. Secondly, to use scales whose end points are equally positive and negative, e.g. scales going from very good to very poor or from good to poor, but not from very good to poor. Thirdly, to use scales that measure only one thing at a time i.e. a specific question or statement; and the fourth, to give people a do not apply or 'N/A' option if that is appropriate.

Likert scale rating is a rating scale frequently discussed by investigators who employ survey questionnaires. Likert scale question itself was invented by the educator and psychologist Rensis Likert. (129) Likert scaling is a bipolar scaling method, measuring either positive or negative response to a statement. In a good Likert scale, the scale is balanced on both sides of a neutral option, creating a less biased measurement. For example, scales from positive to negative with five point scales; strongly agree, agree, neither agree or disagree, disagree and strongly disagree. The actual scale labels, as well as the numeric scale itself, may vary. Sometimes an even-point scale is used, where the middle option, neither agree nor disagree is not available. Likert scale is an ordinal scale, not an interval or ratio scale. A survey using a Likert scale rating is designed to measure an underlying construct of interest, so a numeric value may not be applied to the rating scale, in addition if a numeric rating scale is applied it is unlikely to represent a correct of the construct appraised.

Development of the NHNMRSA Product Experience Questionnaire

For the purpose of constructing the product experience questionnaire (PEQ) in the NHNMRSA study, the researcher considered the six UEQ scales and the suitability of each of the scales in the PEQ. Of the six UEQ scales, attractiveness, dependability, stimulation and novelty scales were not considered of primary importance while considering user experience on the application of Medihoney™ Derma Cream, or mupirocin 2% w/w (Bactroban® 2%), in the study. The logic was that, a medical device or a medicinal product is used in general for a medicinal purpose, prescribed by a practitioner, where, a necessity rather than a choice that determines the need to use a medicine, or a medical device. In order to assess user experience on a product, it is also important to determine that a user is able to comprehend how to use the product. Therefore, perspicuity was identified as the scale that was most applicable in the study.

A self administered survey questionnaire method using paper and pencil to record user experience was considered optimal for use in the NHNMRSA study. The PEQ was developed through an iterative process involving supervisors for face and content validity. A statement or a question's face and content validity can influence a respondent in making a correct response. Face validity describes the degree to which an assessment measures what it appears to measure. Content or logical validity refers to how accurately an assessment or measurement tool taps into the various aspects of the specific construct in question i.e. are the responses by the person answering the questions influenced by other factors. Four statements were developed by the researcher that elicited elements of perspicuity. These four statements were neutral statements that elaborated on perspicuity, that the user experienced on application of the allocated product. Each of the four statements measured only one aspect of the user experience. The fifth statement was a personal preference question, to record patients' own preferences, where an option was given to patients to choose.

A Likert scale rating was used in the PEQ. Scaling of four of the Likert statements in the questionnaire were with equal options, scales from positive to

negative with five point scales; which ranged from strongly agree, agree, neither agree or disagree, disagree and strongly disagree. A do not apply option was not used in the PEQ, as it was not appropiate. The fifth item in the PEQ, to record patients' own preference where an option was available to the patient to choose between an antibiotic and a natural product, to eradicate MRSA from the nose. The fifth statement therefore had a scale with two options, to record the choice of the product. The PEQ incorporated a comment section which offered users with an option to place a free text if they wished, in addition to the response to five Likert statements.

The PEQ was initially administered to 10 patients during the pilot study to evaluate its suitability for patients and their ease of registering a response. Completed questionnaires from 10 participants' and their responses were evaluated to ascertain user encounter with the PEQ.

While the PEQ was developed initially to evaluate the user experience of the investigative product, i.e. Medihoney™ Derma Cream, the research project team considered the merit of administering the PEQ to all study entrants, i.e. in both arms of the RCT. This was based on the evidence of limited scientific literature on patient experience on use of mupirocin 2% (Bactroban® 2%), nasal ointment for MRSA decolonisation. The research team deliberated on the potential merit of generating comparable data from control and study groups by administering the PEQ to all eligible study entrants. The decision to administer the PEQ to all study entrants necessitated modification of the questionnaire title to reflect the product allocated to the patient. The title of the PEQ for patients in the investigative group was labelled 'Experience with Medihoney™ Derma Cream'. In addition, where the product name was specified in the questionnaire, the term 'Derma Cream' was used consistently. The title of the PEQ for patients allocated in the control group was labelled 'Experience with Mupirocin nasal ointment'. In the PEQ for the investigative group where the product name was specified, the term mupirocin was consistently used.

The amended PEQ was then administered to a group of ten patients, five in each arm who were enrolled in the RCT to evaluate their responses and ease of

use. The informal feedback received was satisfactory and the questionnaires were completed in full.

PEQ amendments

A NHNMRSA RCT project governance body 'Project Advisory Committee' (PAC) was set up to advice on the research study. The PAC at its first meeting (held in May 2014) considered the study instruments including the PEQ. The PAC were of the opinion that the wording of the PEQ statement five, '*If given a choice between an antibiotic and a natural product to eradicate MRSA from the nose, I would prefer the natural product'* may influence a patients opinion in favour of the natural product thus generating a potential bias. Based on the suggestion of the PAC the wording of the statement was revised to reflect statement neutrality. The revised statement which the PAC agreed with is as follows; 'If given a choice between an antibiotic and a natural product to eradicate MRSA from the nose, which would you prefer'. The amendment to the PEQ necessitated a change of rating scale to record patients' response to the specific statement, as well as approval by the Ethics (Medical Research), Committee (ERC), Beaumont Hospital. For the revised statement in the PEQ, a scale was not applicable and the option was not positive or negative rating, but to record patients' choice of the product. Therefore the option provided was to choose 'antibiotic' or 'natural product'. A submission was made by the researcher to the ERC, Beaumont hospital seeking the committee's approval for the amendment to the wording of the PEQ. The ERC approved the proposed amendment of the wording in the PEQ approval date 23/05/2014. The revised version (V2) of the PEQ was used in the study thereafter (appendix - XIV).

2.5.2 Development of the MRSA Perception Questionnaire

Background

In the past, the researcher was instrumental in developing and implementing a 'patient communication service on MRSA' in Beaumont hospital. The provision of this service involved the following; a specialist nurse in infection prevention and control (IPC) providing detailed information to patients colonised or infected with MRSA. This began with a process of IPC written notification of MRSA colonisation in the patient's medical record. Subsequently the respective clinical team provided initial information to the patient about the microbiological finding. This service was audited at planned intervals, (appendix - XIV, proforma sticker patient notification of alert organisms). The insight gained from implementing and auditing the patient communication service encouraged the researcher to investigate patients' illness perception on MRSA colonisation while taking part in the RCT. With this aim, the researcher carried out literature reviews and electronic searches for pre-existing validated questionnaires that might be considered for use in the RCT.

Assessing Illness Perception of patients

Illness affects a person in a multitude of ways. The impact of an illness and coping with the illness of each individual can vary. An understanding of the patient's perception of an illness, and how it impacts them psychologically and manifests in their behaviour, may assist healthcare practitioners and social therapy professionals to inform and develop appropriate responses to patients' needs. (135)

The illness perception approach can be best understood in the context of wider changes in psychology. Since the emergence of contemporary cognitive psychology during the mid 19th century, the focus on cognition and cognitive approaches has dominated all areas of psychological research and theory. At the core of the cognitive approach is the view that individuals construct models,

internal representations, or schema, which reflect their pooled understanding of previous experiences and are used for interpreting new ones and planning their behaviour. (135)

Studies by Leventhal showed that patients' emotional responses to changes in tumour size following chemotherapy for lymphoma were a function of their own personal cognitive model of the illness. From this and other studies, Leventhal developed a self-regulatory model whereby patients construct their own representations or models which help them make sense of their experience and provide a basis for their own coping responses. (135) The self regulatory model developed by Leventhal contains core components, beliefs about the etiology of the illness, its symptoms and label, the personal consequences of the illness, how long it will last, and the extent to which the illness is amenable to control or cure. These components show logical interrelationships. For example, a strong belief that the illness can be cured or controlled is typically associated with shortly perceived illness duration and relatively minor consequences.

Until the early 1990's, the assessment of illness perceptions has been by open-ended interviews designed to encourage patients to elaborate their own ideas about their illness. Weinman and colleagues has developed a new scale called the Illness Perception Questionnaire (IPQ) that can be used in a variety of physical illnesses which according to the architects of the IPQ should make patient assessment more efficient for researchers. (136)

Weinman is of the opinion that in healthcare, cognitive approach offers an opportunity for researchers to identify the critical factors in patients' adaptation to illness. (136) Furthermore, this approach can facilitate the development of interventions that modify or take account of specific patient cognitions such as beliefs about the cause or potential for control/cure of an illness. Cognitive approach in healthcare influenced the development of a collaborative relationship with patients in which their beliefs and expectations are acknowledged in consultations and treatment, thereby patients are encouraged to take a more active and informed role. Early exploration and identification of patients' perceptions also offers the opportunity of minimising or avoiding later

difficulties such as non-adherence to treatment or recommended behaviour changes. (136)

The Illness Perception Questionnaire

The IPQ is a widely used multifactorial pencil and paper questionnaire which assesses cognitive illness representations of patients on a five-point Likert scale. (136) There are five dimensions of cognitive representation of illness in the IPQ. The first is identity, i.e. the label the person uses to describe the illness and the symptoms they view as being part of the disease. The second is consequences, the expected effects and outcome of the illness. The third is cause, the personal ideas about the cause of the illness. The fourth is timeline, i.e. how long the patient believes the illness will last, and the fifth is cure or control, the extent to which the patient believes that they can recover from or control the illness. The emotional representation in the IPQ incorporates negative reactions such as fear, anger, and distress. The importance of illness representations to patient behaviour has been demonstrated by research over four decades. (137) Changing patients' illness perceptions has been shown to improve recovery following myocardial infarction (MI) (138), and other self-regulatory interventions in illnesses as diverse as diabetes and AIDS, have also improved patient outcomes. (139)

A revised version of the IPQ scale, the Illness Perception Questionnaire–Revised (IPQ-R), extended the original scale by adding more items, splitting the control dimension into personal control and treatment control, and incorporating a cyclical timeline dimension, an overall comprehension of illness factor, as well as an emotional representation. (140) Based on the IPQ, a single-item scale approach to assess perceptions on a continuous linear scale was constructed by the research team who constructed the IPQ. This tool allowed the recording of short and simple measures of illness perceptions, as well as measuring with an alternative format such as the multifactorial Likert scale approach used in the IPQ and IPQ-R. The Brief Illness Perception Questionnaire (BIPQ) is copyrighted. (141) (Appendix - XVI) The BIPQ incorporates nine items, each

item assesses one dimension of illness perceptions such as; consequences, time line, personal control, treatment control, identity, coherence, emotional representation, concern and the final aspect is a casual item which asks patients to list the three most important causal factors that caused their illness. The BIPQ has been used for studies of conditions such as asthma, renal disease, diabetes, myocardial infarction and a group of patients with chest pain undergoing stress exercise testing prior to diagnosis. The BIPQ authors claim that the tool is a valid and reliable measure of illness perceptions in a variety of illness groups. The evaluation of BIPQ in the above mentioned conditions report good test–retest reliability and concurrent validity with relevant measures. The results also indicate that there are moderate to good associations between the BIPQ and the IPQ-R on all of the equivalent dimensions. The main advantage offered by the BIPQ is the brevity and speed of completion for patients, as well as the easy interpretation of scores. The BIPQ authors assert that the tool is most useful for ill and elderly populations who would find completion of a long questionnaire difficult. It also offers advantages when researchers are already using a number of other pencil and paper measures, but wish to also include an assessment of illness perceptions over a relatively short period, to reduce the burden on research participants. The results from the scale can be easily scored and are readily interpretable by researchers and clinicians.

Development of the patient MRSA Perception Questionnaire

In order to gather patients' perception on their MRSA colonisation, the researcher considered the merits of using a validated IPQ. With this purpose the BIPQ was assessed for its advantages such as the ease and speed of completion for users, brevity, as well as the easy interpretation of scores. For BIPQ to be employed in the NHNMRSA study, it was deduced that the terminology required modification for contextualisation and describing the condition evaluated i.e. MRSA colonisation. With the permission of the copyright holder (lizbroadbent@clear.net.nz) the BIPQ was adapted to suit the NHNMRSA study. The researcher amended the BIPQ, with the term illness changed to MRSA colonisation in the adapted questionnaire, MRSA perception

questionnaire (MRSA PQ). Similar to the BIPQ, the MRSA PQ incorporates all the nine items as in the BIPQ, with each item assessing one dimension of illness perceptions. The MRSA PQ also follows the same sequence of items as in the BIPQ; starting with consequences, time line, personal control, treatment control, identity, coherence, emotional representation and concern. The final item in the MRSA PQ is a casual item which invites patients to list the three most important causal factors that caused their MRSA colonisation.

The MRSA PQ rating scale

The original BIPQ response scale consists of an 11 point scale, from 0 to 10, the scale 0 representing a neutral or positive view of the item to the maximum score of 10 that corresponds to the individual patients' views. The MRSA PQ followed the same11 point scale from 0-11in each of the eight Likert statements. The ninth, an open question was to list in rank order, the three most important factors that the patient believed caused his/her MRSA colonisation. The MRSA PQ was initially administered to healthcare volunteers. The purpose of which was to evaluate the user's ability to understand the questions and response scores listed in the adapted questionnaire, i.e. MRSA PQ. Based on feedback, the format of the MRSA PQ was changed from portrait to landscape, without any change of content.

Administration of the PEQ and MRSA PQ

The NHNMRSA RCT research team considered the best possible opportunity to administer the questionnaires to study entrants for optimal return, to obtain an informed and correct opinion on the product experience as well as their perceptions on MRSA. The PEQ was administered to study entrants based on their allocation. The investigative group was administered with the PEQ titled 'Experience with Medihoney™ Derma Cream' and the control group was administered the PEQ titled 'Experience with mupirocin nasal ointment'. The PEQ and the MRSA PQ were printed without any respondent identifiers and held with the researcher. Patients were handed a copy of the PEQ according to

the group each patient was allocated to, in addition to a copy of the MRSA PQ. Patients were provided with both the questionnaires, i.e. the PEQ and the MRSA PQ on the last day of the first course of Medihoney™ Derma Cream or mupriorcin 2% nasal application, or a at a later date. The researcher guided each patient who was provided with the questionnaires on how to complete each of the questionnaires, in addition to how and where to record their responses. The study participants were encouraged to complete the questionnaires and the anonymous nature of the tool and responses were reiterated. A pre-labelled envelope addressed to the researcher was also provided to patients along with the questionnaires to facilitate return of completed questionnaires. The returned questionnaires were stored in a secure location in the host facility for analysis.

Chapter III Results

3.1 Literature review

3.1.1 Introduction

The aim of the literature review was to determine the prevalence of MR and its clinical consequences, as well as measures to control MR. By undertaking the review the researcher also aimed to gather the evidence if any, that supports the use of new agents as potential therapeutic alternatives for the prevention and control of MRSA.

3.1.2 Results

3.1.2.1 Types and prevalence of mupirocin resistance

Phenotypically, mupirocin susceptibility is determined according to minimum inhibition concentration (MIC) breakpoints with susceptible being ≤4 mg/L, LLMR 8–256 mg/L and HLMR >512 mg/L. (142,47) Mupirocin MICs of 8–64 mg/L are usually due to non-synonymous changes in the native isoleucyl-tRNA synthetase gene. *S. aureus* isolates with an MIC of 128 or 256 mg/L are uncommon but are considered to demonstrate LLMR; these isolates have acquired base changes in the native isoleucyl RNA synthetase gene, *ileS*. (39,142,51) MICs of ≥512 mg/L reflect HLMR and this is mediated by the acquisition of a conjugative plasmid containing *mupA* (*ileS2*), which encodes an alternative isoleucyl-tRNA synthetase. (47,48) Although *ileS2* does not encode resistance to other antibiotics, the presence of *ileS2*-carrying plasmids has been associated with resistance to antibiotics such as clindamycin, tetracycline, erythromycin and levofloxacin. (143)

Among *S. aureus* isolates MR ranges from 1% - 81%. (39,144) In a Canadian hospital MR increased from 2.7% to 65%, between the beginning of the first year of the epidemic (1990) and the end of the third year. (145) In Brazil (1996)

in two tertiary care university hospitals, in one of which there was extensive use of mupirocin, 62/114 (63%) of isolates were MR. (43)

In Shanghai and Wenzhou (China), during a three year surveillance program (2005 – 2008), HLMR was identified in 53/803 (6.6%) isolates that were MR MRSA. (146) In Singapore, HLMR was reported from 34/307 (11%) isolates; 14% from screening isolates and 10% from clinical isolates during 2009 - 2010. (147) In Korea in 2011, HLMR was also reported from a neonatal ICU where 101/223 (45%) of admissions were MRSA positive; of these, 70% had isolates that were MR. (148)

In the USA, in a multicentre study in care homes over 30 months (2008 - 2011), MR was detected in 101 (12%) isolates; HLMR in 78 (9%) isolates and LLMR in 23 (3%) isolates. (122) In Taiwan, in a review of 240 MRSA isolates recovered between 1990 and 2005, from patients who had failed decolonisation, MR was identified in 63% of the isolates. (149) In a nested case-control study conducted in an acute hospital in Switzerland (2011) MR ranged 9% - 81%. (144)

Amongst staphylococcal isolates generally MR ranges from as low as 10.3% to as high as 97%. (150,151) In a French study in 2011 of staphylococcal isolates a MR rate of 10.3% was reported amongst 708 isolates of CoNS, mainly HLMR (5.6%). (150) In a Dutch study of 238 CoNS BSI isolates, *S. epidermidis* was most prevalent, i.e. 150 isolates (63%) and it was also the most common species expressing HLMR isolates, i.e. 25 isolates (78%). In another study from the Netherlands in 2015 of 607 CoNS isolates collected from 469 patients after decolonisation with mupirocin, 588 (97%) were HLMR. *S. epidermidis* was most prevalent species with HLMR, i.e. 568 (94%). (151)

3.1.2.2 Chlorhexidine resistance

Bacterial resistance to chlorhexidine was initially reported in 1995. (38) Resistance to chlorhexidine is conferred by two gene families, *qacA/B* and *smr*. (152) The plasmid mediated *qacA/B* genes encode proton-dependent multidrug efflux pumps, expression of which results in high-level resistance to antiseptics, whereas the *smr* gene confers low-level resistance. (144,38,153)

Chlorhexidine resistance, i.e. *qacA/B* and *smr* ranges from 0.6% - 91%. (122,144) In Taiwan where chlorhexidine has been used for 20 years for hand hygiene, the proportion of MRSA isolates with a chlorhexidine MIC of ≥4 mg/L increased from 1.7% in 1990 to 50% in 1995 to 40% in 2000 and then to 46.7% in 2005. Among these isolates, 46/83 (55.4%) carried the *qacA/B* gene. In addition, *qacA* and/or *qacB* were identified in 91% of MRSA isolates from patients who had failed decolonisation. (149)

In Korea among MR MRSA isolates collected between 2006 and 2009, the *qacA/B* and *smr* genes were detected in 65% of isolates. (154) In a nested case-control study of MRSA decolonisation from Switzerland, *qacA/B* was very prevalent among 68/75 cases (91%) and 51/75 of controls (68%). (144) The same study also found that combined LLMR and the presence of chlorhexidine resistance significantly increased the risk of persistent MRSA carriage. (144) It appears that chlorhexidine resistance is increasingly reported and the presence of *qacA* and/or *qacB* is associated with persistent MRSA colonisation.

3.1.2.3 Controlling mupirocin resistance

Three approaches were proposed by Patel *et al.* in controlling MR. (142) First, additional studies are needed to quantify the efficacy and unintended consequences of mupirocin use as a prevention strategy. Second, a strategy for monitoring the prevalence of resistance should be in place whenever mupirocin is routinely used. Third, monitoring should not only focus on MR itself, but also should help determine whether mupirocin use might amplify the spread of other MDR via its linkage to other resistance determinants. (142)

Incorporating MR surveillance as part of ongoing surveillance programmes such as EARSS-Net, which monitors antibiotic resistance amongst invasive isolates of MRSA, i.e. in BSI, may be beneficial as these are representative of isolates responsible for serious infection from a population in which many have had or will be undergoing MRSA decolonisation.

For persistent MRSA carriers, mupirocin MIC testing should be repeated to assist in informed decision making and provide the potential opportunity to impact on the control of resistance. Point prevalence surveillance is also indicated in centres where mupirocin is widely used and / or resistance is reported.

Control of mupirocin use, i.e. targeted decolonisation in selected patients based on risk assessment rather than the decolonisation of all MRSA-positive patients, has proved an effective strategy to combat MR. (44) There was a precipitous decline in the number of isolates with HLMR (from 31% to 4%) and also LLMR (from 26% to 10%) after measures were introduced to control or limit the use of mupirocin over 2 years (1996 - 1998), in a mixed healthcare setting that included acute, domiciliary and nursing homecare.

In the Netherlands reductions in MR following the control of mupirocin use were reported from a neonatal unit in 1992 where the routine application of mupirocin to central vascular catheter insertion sites was discontinued. (155) In Western Australia, restricted mupirocin use for nasal decolonisation led to reductions in MR from 6.4% (n=16) in 1994 to 0.3% (n=3) in 1997. (156)

3.1.2.4 Alternative agents for decolonisation

The alternative agents investigated by researchers for MRSA decolonisation include other antimicrobials, non-antimicrobials as well as natural agents. The review findings on the use of various alternative agents for decolonisation are now discussed.

Antimicrobial agents

Bacitracin ointment, usually in combination with polymyxin B and neosporin (e.g. polysporin), has been studied as a potential MRSA decolonisation agent. In an RCT that compared mupirocin with polysporin and daily chlorhexidine gluconate (CHG) washes, only 15/49 (30.6%) patients in the polysporin arm were MRSA negative at all body sites at 48 h, compared with 35/54 (64.8%) of those treated with mupirocin. (157)

Retapamulin is a pleuromutilin and a new class of antibiotic that exhibits activity against various skin bacteria including MSSA and MRSA. An *in vitro* study reported that retapamulin had good activity against 15/16 (94%) of MR isolates. (158) However, a double-blind RCT concluded that the clinical success rate in the treatment of secondarily infected traumatic lesions amongst patients with MRSA was significantly lower with retapamulin compared with linezolid. (159) The antimicrobial options such as polysporin and retapamulin appear less effective when compared with mupriocin for decolonisation.

Octenidine dihydrochloride

In the decolonisation of extra-nasal sites in a study using octenidine dihydrochloride body washes a successful outcome was recorded in 18/32 (56.3%) of patients. (160) In another study where CHG or povidone-iodine was contraindicated, success with vaginal MRSA decolonisation has also been reported using octenidine solution. (161)

Polyhexanide

In a study that retrospectively evaluated polyhexanide (Prontoderm) Gel Light nasal ointment, body foam and mouthwash for decolonisation, persistent MRSA was identified among 51/72 (71%) of those who underwent the Prontoderm regimen. Pooled cultures of nasal, perineal and throat samples were used, which limits conclusions on the components that resulted in an inferior rate of decolonisation. (162) A double blind placebo controlled RCT that evaluated a single 10 day course of polyhexanide nasal application has reported sub-optimal success in nasal MRSA decolonisation. (163) Polyhexanide therefore compares unfavourably with mupirocin nasal application for MRSA decolonisation.

Ethanol

The nasal application of 70% ethanol combined with emollients and a preservative (Nozin Nasal Sanitizer), resulted in a 98.8% reduction in colony counts at the end of the normal (10 h) workday. (164) My search did not retrieve other studies that used ethanol for nasal decolonisation. Therefore, further studies are needed before this approach can be safely recommended.

Sodium hypochlorite

An RCT comparing three decolonisation regimens using mupirocin, chlorhexidine and bleach on patients with community-based skin and soft tissue infections and multisite *S. aureus* colonisation revealed that the highest rate of successful *S. aureus* eradication (71%) occurred in patients with a combination of nasal mupirocin and daily bleach baths. (57) In skin conditions there may be role for concurrent nasal and skin/soft tissue decolonisation/treatment using sodium hypochlorite, however, there is paucity of information on its use in routine practice.

Lysostaphin

Lysostaphin is a glycylglycine endopeptidase that cleaves the cross-linking pentaglycine bridges in the cell walls of staphylococci. In an animal model, a single application of 0.5% lysostaphin cream eradicated MSSA and MRSA from

the nares of animals more effectively than mupirocin. (165) In 24 h time–kill studies, lysostaphin has also been found to be superior to mupirocin and tea tree oil. (166)

Omiganan pentahydrochloride

Omiganan pentahydrochloride is a novel topical cationic peptide active against a broad spectrum of bacteria and yeast. An *in vitro* study has demonstrated potent activity against *S. aureus* regardless of the underlying resistance mechanism, at a concentration significantly below the concentration that would probably be used in clinical practice (1% gel). (167)

Natural honey

Honey is of interest to healthcare practitioners involved with wound management and reductions in the number of MRSA colony counts in open wounds have been demonstrated. (92,106-108,113) An *in vitro* study of four types of honey (botanic origin), three sourced from Northern Ireland and one from Suisse Normande, France, found that they reduced the bacterial count of community acquired MRSA isolates. (117) Similar findings on MRSA eradication are reported elsewhere when medical-grade honey was applied to chronic wounds. (113,99)

Tea tree oil

A study of a tea tree (Melaleuca alternifolia) oil-based regimen was compared with standard treatment consisting of mupirocin, chlorhexidine or silver sulfadiazine. (54) Of the patients who received the tea tree oil regimen, 46/110 (42%) were decolonised in comparison to 56/114(49%) the patients who received standard treatment, 14 days after a five day course. Mupirocin was significantly more effective at clearing nasal carriage than the tea tree oil preparation (78% versus 47%; P=0.0001). A RCT in two ICUs evaluated the effect of daily washing with tea tree oil (Novabac 5% skin wash) compared with standard care with a baby soft wash (Johnson's Baby Softwash) on the incidence of MRSA colonisation and found that there was no statistical difference between the two approaches. (55)

Silver

The successful topical application of silver agents (Acticoat 7w®, Smith & Nephew) in treating patients with MRSA surgical site infection (n=2) without systemic antibiotics as well as with gentian violet (0.5%) for skin lesions (n=28) and for the eradication of nasal carriage (n=9) has been described. (168) Silver impregnated dressings are indicated in the management of certain type of wounds.

Bacteriophages

Bacteriophage therapy is an alternative to antibiotics for the treatment of chronic MRSA infections, as success has been reported both in treating infections (n=6) as well as in the eradication of MRSA carrier status in a healthcare worker. (169) The potential for an engineered staphylococcal-specific phage lysin (ClyS) to be used for topical decolonisation was investigated in a mouse model. (170) ClyS eradicated a significantly greater number of MSSA and MRSA with a 3 log reduction compared with a 2 log reduction with mupirocin. (170)

Another agent, P128 a chimeric protein that combines the lethal activity of two enzymes, consists of a phage tail-associated muralytic enzyme of phage K and the staphylococcal cell wall-targeting domain (*SH3b*) of lysostaphin. In time–kill assays, P128 reduced cfu by 99.99% within 1 h and inhibited growth for up to 24 h. (171) Evidence that phages can effectively combat experimentally induced *S. aureus* infections in animals, warrants further study in clinical trials. (172)

3.1.3 Discussion

Bacterial resistance to mupirocin initially identified in *S. aureus,* in recent years has also been identified amongst other staphylococci. Increasing MR among *S. aureus* as well as other staphylococcal isolates, either alone or combined with chlorhexidine resistance means that, ongoing monitoring of resistance is necessary. Surveillance of MR and chlorhexidine resistance is especially important where there is widespread and even indiscriminate use of decolonisation regimens using mupirocin and chlorhexidine.

The emergence of HLMR amongst CoNS isolates indicates an expanding reservoir of plasmids encoding MR, which can be transferred to other CoNS strains as well as to *S. aureus* including MRSA. (173) HLMR and resistance to other antibiotics amongst CoNS may result in a reduction in oral antibiotic options for prolonged treatment of prosthetic infections with CoNS.

Resistance to mupirocin and chlorhexidine, the commonly used decolonising agents limits the options for patients who may benefit from MRSA suppression or decolonisation therapy. Before application, LLMR is significantly associated with persistent MRSA carriage. In addition, there is a strong association between previous mupirocin exposure and both LLMR and HLMR. An association exists between HLMR (*mupA* carriage) and multidrug resistance (MDR). The presence of *qacA* and/or *qacB* and MR is another factor associated with failed decolonisation. (174)

Cross-sectional studies have found that chlorhexidine resistance alone did not predict persistent carriage, suggesting that the combination of LLMR and chlorhexidine resistance may be necessary for clinical failure, i.e. persistent colonisation. (144) Therefore, among persistent colonisers it is logical to investigate for MR and chlorhexidine resistance before attempting additional courses of suppression or decolonisation therapy if ongoing monitoring of resistance is not performed. Therapeutic trials of alternative agents such as honey and bacteriophages show some promise but need to be further evaluated in clinical trials.

3.1.4 Summary

- Among *S. aureus* isolates, MR ranges from 1% - 81%.
- Among other staphylococcal isolates, MR ranges from 10.3% - 97%, mostly in *S. epidermidis*.
- The emergence of HLMR amongst CoNS isolates indicates an expanding reservoir of plasmids encoding MR.
- Among MRSA isolates, chlorhexidine resistance ranges from 0.6% - 91%.

- The genetic determinants of MR either alone or combined with chlorhexidine resistance, i.e. *qacA/B* or *smr*, presents a significant challenge for the control and prevention of MRSA.
- Alternate antimicrobial agents such as polysporin and retapamulin are less effective in comparison to mupirocin for MRSA decolonisation.
- Agents such as octenidine dihydrochloride, polyhexanide, ethanol (70%), sodium hypochlorite, lysostaphin, omiganon pentahydrochloride, natural honey, tea tree oil, silver and bacteriophages have been investigated with varying success for MRSA decolonisation.

3.2. Results of the Randomised Control Trial

3.2 Patient characteristics and RCT outcome

3. 2.1 Introduction

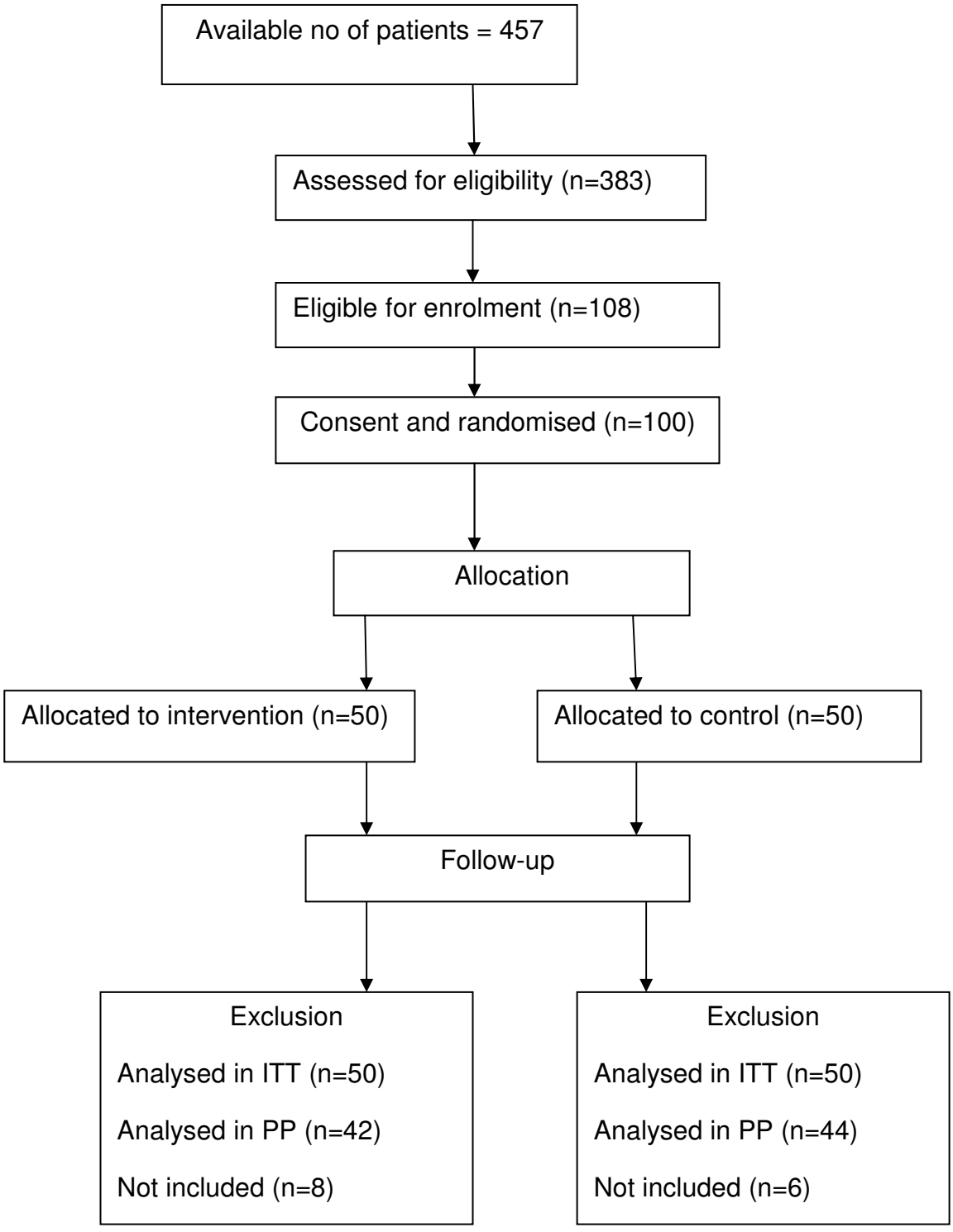
MRSA colonise different body sites, nasal colonisation being very common. In healthcare, MRSA screening programs usually incorporate nasal screening in addition to other sites such as groin/perineum, wounds, ulcers, device insertion sites as well as the throat. Decolonisation of the nasal passages of MRSA is attempted as part of prevention of infection, as well as control of transmission. Nasal as well as non-nasal decolonisation efforts may be influenced by many factors, of which patient risk factors are significant. The overall aim of the NHNMRSA study was to determine the comparative efficacy of 30% MGH (Medihoney™ Derma Cream) the investigative product, to mupirocin 2% (Bactroban® 2%) nasal ointment (control), to decolonise the nasal passages of MRSA, in a RCT. In executing the RCT, a number of patient characteristics (hereafter termed variables) were collected to determine the impact if any, on the outcome of nasal MRSA decolonisation. The patient variables and the study outcome is enumerated in detail.

3.2.2 Results; Participants and baseline characteristics

Participants

During the study period (1st March 2014 to 31st March 2016) there were 457 patients with nasal MRSA positive swab that were reported from BH microbiology laboratory. Of the 457 patients, 74 swabs were from external patient specimens, and where therefore not eligible for recruitment to the study. All the remaining 383 patients were assessed for eligibility. Following the preliminary assessment and application of the exclusion criteria, 108 (28%) were eligible and 100 (93%) patients consented to participate in the study, and were then allocated randomly to intervention and control groups. All the 100 participants had a positive MRSA nasal swab at baseline. The study groups and reason for non-inclusion are shown in Figure 3.2.1.

Figure 3.2.1 Natural honey for nasal MRSA RCT enrolment flow diagram



Demographic, clinical and microbiologic characteristics

Statistical analysis

The characteristics of the study participants are described using frequencies, percentages, measures of central tendencies and measures of dispersions as appropriate. The homogeneity of the intervention and control groups such as the basic socio-demographic and clinical characteristics was assessed using Chi-square (χ^2) tests. In the analysis of data if any cell had a frequency less than 5, then Fishers Exact test values in the 2 x 2 contingency table were used to assess for any significant difference between the intervention and control group.

As a rule of thumb for statistical analysis and reporting of results, χ^2 value is used as the preferred option (where a 2X2 table was available), and if the value is less than five in any column, then likely hood ratio is used instead of the χ^2 value. In addition, the *p*-value and significance is recorded.

Baseline characteristics

The baseline characteristics of the study participants and comparisons between the intervention and control groups are presented in Table 3.2.1

Table 3.2.1 Natural honey for nasal MRSA RCT comparison of the baseline characteristics of the intervention and control groups

	Intervention n=50		Control n=50			
Age at enrolment						
Mean (Sd)[a]	71.7	(13.2)	74.7	(11.0)		
Gender	n	%	n	%	*p*-value	*Sig*[b]
Male	36	(72.0)	28	(56.0)	0.096	ns[c]
Female	14	(28.0)	22	(44.0)		
Medical Specialty						
Medicine	34	(68.0)	36	(72.0)	0.866	ns
Surgery	9	(18.0)	7	(14.0)		
Renal	6	(12.0)	5	(10.0)		
Neuroscience	1	(2.0)	2	(4.0)		
Comorbidity						
Diabetes mellitus	16	(39.0)	13	(33.3)	0.597	ns
Prosthesis	0	(0)	2	(5.6)	0.151	ns
Liver disease	0	(0)	1	(2.7)	0.321	ns
COPD[#]	13	(32.5)	18	(45.0)	0.251	ns
Malignancy	4	(10.5)	4	(10.5)	1	ns
Vascular disease	17	(44.7)	15	(35.7)	0.411	ns
Immuno- suppression	10	(27.0)	14	(38.9)	0.281	ns
Renal disease	8	(20.0)	11	(28.2)	0.394	ns
Heart disease	11	(26.2)	11	(24.4)	0.851	ns
Normal residence						
Nursing home	2	(15.4)	3	(18.8)	0.523	ns
Other HCF[*]	1	(7.7)	0	(0)		ns
Unknown	10	(76.9)	13	(81.2)		ns
Health care contact pre enrolment						
High (>6/ year)	41	(82.0)	37	(74.0)	0.87	ns
Medium (3-5/ year)	5	(10.0)	12	(24.0)		
Low (<2/ year)	4	(8.0)	1	(2.0)		
Recruitment location						
Inpatient	42	(84.0)	44	(88.0)	0.564	ns
Outpatient	8	(16.0)	6	(12.0)		

a- Standard deviation, b- Significance, c-Not significant, #COPD - Chronic obstructive pulmonary disease, [*] HCF- Health care facility

The mean age and standard deviation (Sd) of the study entrants was 73.2 y (12.1). In the intervention group the mean age of the participants was 71.7 y, and in the control group 74.7 y. The age ranged from 34.7 years to 91.6 years.

A preponderance of males was observed in the intervention group, however, in the control group the distribution of both genders were comparable. The distribution of patients according to their medical specialty was also comparable in the intervention and control groups, refer Table 3.2.1.

Various co-morbidities were identified in our study participants, refer Table 3.2.1. Among the 100 study participants the most prevalent pre-existing conditions was diabetes mellitus (DM) 39%, vascular disease 32%, chronic obstructive pulmonary disease (COPD) 31%, immunodeficiency 24%, chronic heart disease (CHD) 22% and end stage renal disease (ESRD) 22%.

Of the 100 patients, 5% were nursing home residents, one patient was a communal facility resident and the remaining 94% patients lived at home prior to the RCT enrolment. The distribution of patients based on their location of residence prior to study enrolment was comparable between the two groups. In a similar manner, the location of recruitment was also comparable between the intervention and control groups.

Overall, there were no significant differences between the intervention and control groups at baseline, as presented in Table 3.2.1.

Risk factors

In Table 3.2.2 the baseline risk factors of the study participants collected such as the acuity of care recorded, using the nursing dependency information system (NIS), their skin condition, and the presence of invasive and indwelling devices, are presented.

Table 3.2.2 Natural honey for nasal MRSA RCT, comparison of baseline risk factors between the intervention and control groups

Variable	Intervention n=50		Control n=50		Univariate analysis *P*-value	*Sig*[a]
Nursing dependency (NIS)*	n	%	n	%		
Low-medium	37	(74.0)	36	(72.0)	0.822	ns[b]
High-specialised care	13	(26.0)	14	(28.0)		
Skin integrity						
Skin - Healthy	25	(50.0)	28	(56.0)	0.816	ns
Wound	14	(28.0)	13	(26.0)		
Ulcer	11	(22.0)	9	(18.0)		
Device in situ						
CVC**/PICC#	5	(13.9)	5	(15.6)	0.411	ns
PVC$	19	(52.8)	21	(65.6)		
Urine catheter	3	(8.3)	3	(9.4)		
More than 1 device	9	(25.0)	3	(9.4)		

a -Significance, b- not significant, * - Nursing dependency score, ** CVC - Central vascular catheter, #-Peripheral inserted central catheter, $-PVC - Peripheral vascular catheter

The NIS score is a weighted cumulative score that is applied following nursing assessment and is updated regularly. NIS is composed of five elements; personal care, feeding, mobility, nursing attention and the fifth item is other; which includes involuntary drainage, major intervention and specialist intervention. Three levels of care apply to each of the first four NIS elements. A weighted score applies for each of the five elements which generate a cumulative NIS score, range 1-5. The NIS score of each of the participants in the study was transcribed from the NIS system to the database.

Of the 100 participants, 19% had a NIS score of 5, that signifies they needed specialist nursing care, 8% had a NIS score=4, which means they were nursing dependent, 19% had a NIS score=3, who required moderate assistance, 53% had a NIS score=2, who only required minimal assistance, and one participant was independent. For statistical analysis, independent, low and medium NIS scores (scores 1-3) were grouped into a single group as low-medium, and the remaining two categories high dependency and specialised care (scores 4-5) into high-specialised care. Overall 27% of the participants were of the high dependent and specialised care category (NIS scores=4-5), which denotes a high level of healthcare contact among such patients.

Of the 100 participants 47% had altered skin integrity of which 24% participants had wounds, 20% ulcers and 3% stoma.

Overall, 68 participants had an invasive device *in situ*. Of the 100 participants,10% had a central vascular catheter (CVC) or a peripherally inserted central catheter (PICC), 40% had a peripheral vascular catheter (PVC), 6% had a urinary catheter (UC) and 12% had more than one invasive device during their participation in the study.

The distribution of participants in the intervention and control groups was comparable for their acuity of care, skin condition; i.e. integrity, wounds and ulcers, as well as the presence of invasive devices. There were no statistically significant differences between the intervention and control groups in terms of their nursing dependency, skin condition and the presence of invasive and indwelling devices.

Baseline microbiological characteristics

Microbiological characteristics such as MRSA status; whether first time identified in BH or previously known and past decolonisation attempts were data of clinical significance in the study. In Table 3.2.3 the baseline microbiological characteristics of the study participants including MRSA status on study enrolment, colonisation with multi drug resistant organisms (MDRO) *Clostridium difficile* Infection (CDI) and past decolonisation attempts are presented.

Table 3.2.3 Natural honey for nasal MRSA RCT comparison of the baseline microbiological characteristics of the intervention and control groups

Variable	Intervention		Control		Univariate analysis	
		n=50		n=50	*P*-value	*Sig*[a]
MRSA history	n	%	n	%		
First time identified	4	(8.0)	3	(6.0)	0.695	ns[b]
Previously known	46	(92.0)	47	(94.0)		
MDRO* CDI status						
VRE"	8	(80.0)	11	(84.6)	0.772	ns
C difficile#	2	(20.0)	4	(80.0)	0.793	ns
ESBL[£]	6	(75.0)	7	(100)	0.155	ns
CRE[$]	2	(100)	1	(100)		
MRSA decolonisation history						
H/o[€] decolonisation	45	(90.0)	44	(88.0)	0.749	ns
Past mupirocin nasal use						
Yes	43	(86.0)	44	(88.0)	0.766	ns
Mupirocin number of courses						
1-2	26	(60.4)	27	(61.3)	0.869	ns
3& >	17	(39.5)	17	(38.6)		
Past non-nasal decolonisation						
Yes	45	(90.0)	43	(87.8)	0.722	ns

a- Significance, b-Not significant, *- multi drug resistant organisms, CDI - *Clostridium difficile* infection, " VRE - vancomycin resistant *enterococci*, [#] *Clostridium difficile*, [£]ESBL - Extended spectrum *beta lactamase* organisms, [$]CRE - Carbapenem resistant *enterobacteriacae,* ~NA – Not applicable, € - History of.

Of the 100 participants, 93% were previously identified with MRSA colonisation / infection, i.e. known cases. The remaining 7% were first time identified MRSA in BH, during their current episode of admission to the hospital.

Cumulatively, 89% (89/100) of patients had a history of attempted nasal colonisation, and 88% (88/100) had non-nasal decolonisation. Among the patients who had attempted nasal decolonisation, 60% (53/89) had up to two courses of mupirocin, 38% (34/89) three or more courses of nasal mupirocin2% ointment before study enrolment.

Ten patients (10%) had a laboratory confirmed *Clostridium difficile* infection in the current episode of hospital admission, 19% (19) had history of VRE colonisation and 13% (13) ESBL colonisation, refer Table 3.2.3.

The distribution of participants in the intervention and control groups were comparable for their MRSA status, colonisation with MDROs CDI, past history of decolonisation, both nasal and non-nasal body sites, as well as the use of mupirocin for nasal decolonisation of MRSA, refer Table 3.2.3. There were no significant statistical differences in their MRSA status, colonisation with MDROs CDI as well as past decolonisation attempts between the two groups.

Infection prevention and control characteristics

Decolonisation of patients of their MRSA carriage is attempted while in hospital settings, and isolation of patients along with practice of CP is recommended and practised where feasible and appropriate. Multisite colonisation, i.e. MRSA colonisation of non-nasal sites is also of clinical significance and affects nasal decolonisation attempts.

In Table 3.2.4 the comparison of the concomitant IPC characteristics that were relevant to the study, such as place of residence of the study participants along with the application of CP and patient isolation where appropriate, along with MRSA colonised sites, are presented.

Table 3.2.4 Natural honey for nasal MRSA RCT concomitant infection prevention and control (IPC) characteristics in the intervention and control groups

Variable	Intervention		Control		Univariate analysis	
	n	%	n	%	*p*-value	*Sig*[b]
Residence during the study	50	(100)	50	(100)		
Inpatient	42	(84.0)	44	(88.0)	0.564	ns[a]
Out patient	8	(16.0)	6	(12.0)		
Isolation of patients						
Single room CP[*]	22	(44.0)	25	(50.0)	0.747	ns
Cohort CP	6	(12.0)	4	(8.0)		
Not isolated	10	(20.0)	13	(26.0)		
Not applicable	8	(16.0)	6	(12.0)		
MRMRSA** on enrolment						
Yes	3	(6.0)	0	(0)	0.079	ns
MRSA positive sites on enrolment						
Nasal	50	(100)	50	(100)		
Groin	19	(38.0)	20	(40.0)	0.904	ns
Urine	0	(0)	1	(2..0)	0.248	ns
Respiratory	1	(2.0)	0	(0)	0.157	ns
Wound	13	(26.0)	5	(10.0)	0.844	ns
Ulcer	5	(10.0)	4	(8.0)		
Other site/s	3	(6.0)	1	(2.0)		

a -not significant, b -significance [*]Contact precautions, ** Mupirocin resistant MRSA

On commencement of the study, 86% of the participants were inpatients and 57% of the patients were isolated, either in a single room or cohort with CP.

Current nasal colonisation was an essential prerequisite for RCT enrolment and all of the 100 participants had documented MRSA nasal colonisation at study enrolment. Non-nasal colonisation was identified cumulatively at one or more of the sites screened among 72% of the participants. Groin/perineum was the most common site colonised 39%, followed by wounds 18% and ulcers 9%.

The distribution of participants in the intervention and control groups were comparable for their location of residence and IPC practices such as isolation and CP during study commencement, refer Table 3.2.4. MRSA colonisation of non-nasal sites was also comparable between the two groups. There were no significant statistical difference in the location of residence and IPC practices, as well as MRSA colonised sites between the intervention and control groups.

3.2.3 RCT outcome, nasal MRSA screening

In Table 3.2.5 the microbiological outcome of the attempted nasal MRSA decolonisation, on an intention to treat (ITT) of all the 100 consented and randomised patients, irrespective of whether a final outcome was available, is presented. This includes patients who deviated from the allocated protocol, were lost to follow-up as well as withdrew from the study, 14 (14%) in total; eight (8%) in the intervention and six (6%) in the control group. Per protocol analysis of participants who completed the study according to the allocated protocol is also presented. Data on MRSA colonisation of non-nasal sites where available, at the end of the study, were also compared between the two groups.

Table 3.2.5 Natural honey for nasal MRSA RCT microbiological outcome in the intervention and control groups

MRSA	Intervention		Control		Univariate analysis		
	n	%	n	%	Chi-square	*p*-value	Sig[a]
Nasal negative (ITT basis)	18	(36.0)	25	(50.0)	1.999	0.157	ns[b]
Nasal negative (PP basis)	18	(42.9)	25	(56.8)	1.675	0.196	ns
Positive on any non-nasal site/s	34	(85.0)	34	(79.1)	0.492	0.483	ns

a - Significance, b - not significant

On an ITT analysis, of the 100 participants, 18 (36%) in the intervention group and 25 (50%) in the control group were decolonised of nasal MRSA by the end of the study. A χ^2 test was performed to assess the difference in the rate of decolonisation of MRSA between the intervention and control group. There was no statistically significant difference between the two groups (χ^2=1.999, df=1, *p*=0.157), Table 3.2.5. On a PP analysis, the final outcome i.e. nasal screening results were available for 86 (86%) of the participants; 42 (84%) from the intervention group and 44 (88%), from the control group. In the intervention group 18 (43%) participants and in the control group 25 (57%) participants were decolonised of their nasal MRSA, however, a χ^2- test showed no significant difference (χ^2=1.675, *p*=0.196).

MRSA colonisation was identified at one or more non-nasal sites in 68% (68) of the participants on study completion.

The outcome of the intervention and control groups were comparable on their outcome, nasal MRSA, refer Table 3.2.5. In a similar way, outcome of non-nasal sites MRSA screening was also comparable for both groups. No significant statistical association or significance was identified based on the nasal screening outcome between the intervention and control groups, as for non-nasal screening results.

Univariate analysis of the data confirms robust comparability of the study participants in the intervention and control groups. In addition, none of the study variables were of statistical significance on the outcome, i.e. nasal MRSA. Based on the ITT and PP analysis, there was no statistical significance in the outcome of nasal MRSA decolonisation, between the intervention and control groups. Therefore, the NHNMRSA RCT null hypothesis is not rejected. However, a particular concern is the impact of the less than anticipated number of patients enrolled in the study on the power to detect significant differences between the intervention and control groups.

Multivariate logistic regression

For logistic regression analysis binary data were re-coded, details itemised in the RCT methodology section, and multivariate logistic regression analysis undertaken. In order to perform multivariate analysis, from a statistical point of view a rule of thumb is that 10 subjects is the minimum number required for each dependent variable. Therefore, based on elements of clinical risk and importance previously described by others, and variables with a *p*- value of up to 0.2 in univariate analysis, were included in the model constructed for sensitivity analysis.

The best fitting model for multivariate sensitivity testing was developed based on the convention that the lowest -2log likelihood and Nagelkarke R^2 (NR^2) scores, whereby the lowest -2log likelihood and NR^2 highest explains the best fit of the model used.

Table 3.2.6 presents the results of the multivariate logistic regression analysis. Eight variables were initially considered for the logistic regression analysis; age, gender, skin integrity, presence of invasive devices, location of patient residence during the RCT, patient isolation, nursing dependency, past mupirocin use and MRSA colonisation of non-nasal sites. Table 3.2.7 that

follows presents the Hosmer and Lemeshow test results, the model that was used to perform logistic regression analysis.

Table 3.2.6 Natural honey for nasal MRSA RCT multivariate logistic regression analysis on the outcome of nasal MRSA decolonisation

Variable	*p*	OR	95% CI L	95% CI U	Sig[a]
Age category >65 y	0.433	1.805	0.413	7.888	ns[b]
Gender, Male	0.776	1.177	0.382	3.627	ns
Skin, non-intact	0.115	0.391	0.122	1.256	ns
Invasive device present	0.884	0.915	0.280	2.989	ns
Residence, HCF[c]	0.451	0.531	0.102	2.755	ns
Isolation, HCF	0.349	1.908	0.493	7.382	ns
NDSs dependent care	0.529	1.507	0.420	5.410	ns
Mupirocin >2 course	0.101	2.625	0.830	8.308	ns
Multisite MRSA colonisation	0.008	4.707	1.496	14.815	s[d]

a - significance, b - not significant, c - Health care facility, d- significant

The test model summary is further explained using the model coefficients and the significance (*p*) values derived, in Table 3.2.7

Table 3.2.7 Natural honey for nasal MRSA RCT LG results of the model using the Hosmer and Lemeshow test

Model summary			Hosmer and Lemeshow Test		
-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square	Chi-square	df	*p*
85.398	0.216	0.288	7.241	8	0.511

Multisite i.e. concomitant non-nasal MRSA colonisation, was significantly associated, (χ^2=7.241, *p*=0.008) with persistent nasal MRSA colonisation.

Table 3.2.8 presents the results of logistic regression analysis of the outcome of the intervention, compared to four variables that displayed significance or close association, and Table 3.2.9 displays the test model summary results.

Table 3.2.8 NHNMRSA RCT results of logistic regression (LG) of four variables of association.

Variable	*p*	OR	95% CI L	95% CI U
Skin non-intact	0.070	0.354	0.116	1.087
Isolation, HCF[a]	0.305	1.946	0.545	6.952
Mupirocin >2 course	0.081	2.652	0.886	7.935
Multisite MRSA colonisation	0.003	5.186	1.736	15.489

a - Health care facility

Table 3.2.9 Natural honey for nasal MRSA RCT LG analysis, model summary using Hosmer and Lemeshow test, on nasal MRSA decolonisation outcome

Model Summary			Hosmer and Lemeshow Test		
-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square	Chi-square	df	*p*
86.859	0.2	0.268	2.391	6	0.88

Logistic regression analysis using four variables that potentially showed relevance indicates that non-nasal MRSA colonisation was significantly associated with persistent nasal MRSA colonisation, p=0.003. Altered skin integrity and the application of more than two courses of mupirocin 2% nasal ointment prior to RCT enrolment were also associated with persistent nasal carriage of MRSA.

3.2.4 Summary

- Univariate analysis confirms the homogenous allocation of study participants between the intervention and control groups on their baseline demographics, risk factors, microbiologic characteristics, as well as concomitant IPC practices.
- Most study participants (94%) lived at home prior to the study enrolment.
- The dependency of care were of the high and specialised care category for 27 (27%) of the participants. A high level of healthcare contact to increased the potential risk of transmission and colonisation with MDRO's.

- One half of the participants had altered skin integrity, 47 (47%), of which 27 (27%) had wounds/ stoma and 20 (20%) chronic ulcers.
- Of the 100 participants who had invasive devices, PVC's were most common, 40%.
- Most of the patients who enrolled in the study were previously known to be MRSA colonised 93 (93%).
- Previous decolonisation attempts were recorded for 87 (87%) of the study participants, both nasal as well as non-nasal decolonisation.
- On study enrolment, 57 (57%) of the patients were isolated, either in a single room or cohort with CP.
- A high prevalence of non-nasal MRSA colonisation was evident, cumulatively 72 (72%) among study participants, of which groin/perineum 39% (30) was the most common colonised site.
- On an ITT analysis, a negative nasal MRSA screen was identified among 18 (36%) of the participants in the intervention group and 25 (50%) in the control group. However, using a PP approach, negative nasal screening was evident among 18 (43%) of the participants in the intervention group and 25 (57%) in the control group.
- Logistic regression analysis confirms that non-nasal MRSA colonisation was significantly associated with failure of nasal MRSA decolonisation.
- There was an association with failed nasal MRSA decolonisation and altered skin integrity; wounds/stoma/ulcer were identified by LG analysis.
- On LG analysis, the application of more than two courses of mupricoin was associated with failure to eradicate nasal MRSA.
- The RCT null hypothesis is not rejected based on the statistical test results.

3.3 Mupirocin susceptibility test results

3.3.1Introduction

MRSA isolates can be classified according to mupirocin susceptibility. The aim of determining mupirocin susceptibility (MS) is to determine the susceptibility of isolates to mupirocin, which is used for nasal MRSA decolonisation. Two methods were employed to determine MS, namely the antibiotic disc diffusion test and the minimum inhibitory concentration (MIC) using the E test method. In our study the E test method was used. The MIC is defined as the lowest concentration inhibiting bacterial growth.

In clinical practice, MS of MRSA isolates is usually determined on the first time MRSA is recovered from clinical or screening specimens of a patient. Determination of MS in previously known MRSA colonised / infected patient varies. Comparison of MS from multiple isolates from the same patient over time facilitates determining when MR was acquired, and to estimate the prevalence of MR.

Prior mupirocin exposure is identified as a potential risk factor for the acquisition of MR. The use of the MIC E test to characterise MRSA isolates is discussed in this chapter and test results are compared for each patient at sequential time points.

3.3.1.1 Methods used to collect isolates

MRSA isolates from the RCT participants were collected prospectively. Purity cultures were prepared from the chromogenic agar plates (MRSAid®; Basingstoke, U.K.), which is the current method employed in BH microbiology laboratory for MRSA identification. The isolates were frozen on beads and stored for future tests. The susceptibility of the following clinical isolates were assessed; historic (first time identified in BH) where available, baseline on study enrolment and isolates from persistent colonisers at the end of the study. Purity plates were prepared from specimens processed in BH, isolates sloped and archived.

3.3.1.2 Prognostic variable; prior mupirocin use

A history of prior mupirocin exposure was enumerated from each participant at study enrolment. Mupirocin exposure is associated with potential acquisition of MR. In clinical practice mupirocin 2% nasal application is limited to two consecutive courses at a given time period. However, practice on the use of mupirocin 2% varies, from target to universal decolonisation such as in ICU patients in some countries. (56)

3.3.2. Category of bacterial isolates investigated

Historic, baseline and final isolates

Of the 100 study participants, historic isolates were obtained from archived isolates for 66 cases. These isolates includes clinical as well as isolates derived from screening specimens. The source of collection of the 66 historic isolates are as follows; nose 34, and the remaining from wound/skin soft tissue, groin, sputum/bronchial lavage/respiratory , ulcer, urine and from blood. Baseline isolates (nasal) were available from all the 100 participants; 50 each from the investigative and control groups, and 43 isolates from persistent colonisers (nasal), on study completion. Of the 43 persistent colonisers, 24 isolates were from the investigative group and 19 from the control group.

3.3.3 Results

3.3.3.1 Comparison of mupirocin susceptibility

The susceptibility of MRSA isolates to mupirocin was investigated using the phenotypic method i.e. MIC E test, test kits supplied by bioMerieux®, Basingstoke, UK. MS was determined according to MIC breakpoints with susceptible being ≤4 mg/L, LLMR 8–256 mg/L and HLMR ≥512 mg/L. (142) The MS results for the historic, baseline and final isolates were categorised according to the respective MIC breakpoints and are tabulated in Table 3.3.1.

Table 3.3.1 NHNMRSA study mupirocin susceptibility of historic, baseline and final isolates

MRSA isolates	Susceptibility range - MIC			
	≤4 mg/L %	8 - 256 mg/L %	≥ 512 mg/L %	Total No of isolates
Historic	91	3	6	66
Baseline	88	4	8	100
Final	77	2	21	43

Of the 66 historic isolates, 65 (91%) were MS, two (3%) were LLMR and four (6%) HLMR. Of the 100 baseline isolates, 88 (88%) were MS, four (4%) LLMR and eight (8%) HLMR. Of the 86 participants who completed the study and where a final outcome was available, 43 (50%) participants had persistent nasal MRSA colonisation. Of the 43 persistent colonisers, 33 (77%) were MS, 23% MR; of which one (2%) was LLMR and nine (21%) HLMR.

3.3.3.2 Prevalence of mupirocin resistance

In the RCT, of the 100 patients enrolled 11 (11%) had no documented evidence of prior mupirocin use or could not confirm use of nasal mupirocin. Of the remaining 89 patients, 13 (13%) had one course, 41 (41%) two courses and 35 (35%) had more than two courses of intra nasal mupirocin.

At baseline, eight cases were HLMR but for none of these were MS results available on enrolment. Of these eight isolates with HLMR at baseline, MS was unchanged subsequently for six, one became susceptible and one participant discontinued from the study. Table 3.3.2 displays the MIC's of the HLMR cases at baseline and on completion of study.

Table 3.3.2 NHNMRSA RCT, prevalence of high level mupirocin resistance at baseline and the corresponding MIC for final isolates

Isolate no	Susceptibility - MIC mg/L	
	Baseline MIC	Final isolate MIC
xx36	>1024	>1024
xx97	>1024	>1024
xx79	>1024	0
xx54	>1024	>1024
xx57	>1024	0
xx61	>1024	>1024
xx62	>1024	>1024
xx66	>1024	>1024

A comparison of the MIC's of the historic, baseline and final isolates of the patients who acquired MR was undertaken. Four patients acquired MR; three HLMR and one case of LLMR. Of the four acquired MR cases an historic isolate was available for three and a final isolate was available for all four. The acquisition of MR; LLMR and HLMR is displayed in Table 3.3.3.

Table 3.3.3 NHNMRSA RCT, mupirocin resistance amongst historic, baseline and final isolates

Isolate no	Susceptibility range - MIC mg/L		
	Historic	Baseline	Final
xx22	NA	0.19	>1024
xx84	0.38	32	>1024
xx95	0.25	0.75	>1024
xx08	0.38	0.5	32

NA – Isolate not available

3.3.3.3 Outcome of intervention of MR cases

The final outcome i.e. nasal MRSA screening results of 86 participants are available. Among the participants who had MR identified on study enrolment, three patients cleared MR MRSA carriage. One participant each in the investigative and control groups with LLMR were decolonised, as well one with HLMR in the investigative group. The successful decolonisation outcome of the MR cases is provided in Table 3.3.4.

Table 3.3.4 NHNMRSA RCT, MICs amongst mupirocin resistant MRSA cases who were cleared of MRSA

	Susceptibility range - MIC mg/L		
Isolate no	Historic	Baseline	Final
xx28	16	32	0
xx77	NA	16	0
xx57	>1024	>1024	0

NA – Isolate not available

3.3.4 Discussion

Mupirocin susceptibility of the isolates investigated

A comparison of MS of isolates from the same patient at sequential time points enabled us to evaluate the MS of isolates and the probable time of MR acquisition. Most of the isolates were MS; 91% historic, 88% at baseline and 77% at the end of the study. However, MR progressively increased from the point of first time identification, from 8% to 23% at the end of study.

A comparison of MS at baseline and at the end of the study, between the Medihoney™ Derma Cream (investigative) and mupirocin 2% (control) groups demonstrates that, in the control group the rate of MR acquisition was 10% (4); HLMR 8% and LLMR 2%. MS was unchanged for all the persistent colonisers in the investigative group, i.e. for the baseline and final isolates.

The incidence of MR acquired is similar to that reported elsewhere. The rate of HLMR was 6% following an intensive MRSA nasal eradication program. (34) In an RCT that compared different eradication regimens for MRSA decolonisation, the rate of MR was 5%. (175) Another study found that MR increased exponentially from 2.7% (1990) to 65% (1993) during a three year period where mupirocin was used extensively. (145)

Prevalence and acquisition of mupirocin resistance comparing historic, baseline and final isolates

Overall the prevalence of MR progressively increased from the time initially identified to the end of the study, i.e. MR was 8% on first time identification, 12% at study enrolment and 23% at the end of study. Of all the cases who newly acquired MR, 75% were HLMR.

The prevalence of MR identified in the RCT is comparable with findings previously described. A national prevalence survey from France reported 2.2% MR (2013) (150), a surveillance study from Singapore, 11% MR (42), an RCT from Switzerland, 23% (1999) (24), a surgical ICU surveillance program from USA, 13.2% (2007) (176), a four year surveillance program from China, 6.6% (2010) (146), a 10 year surveillance program from Canada, 4% (2007) (39), and finally in an observational study from USA, 47.5% of isolates were reported as resistant (2003). (36)

Decolonisation of MR MRSA cases

Three cases of MR were decolonised; two cases had LLMR and one had HLMR at baseline.

3.3.5 Summary

- Of the historic, baseline and final isolates, MS was 91%, 88% and 77%, respectively.
- Among historic MRSA clinical isolates, MR was 8%.
- At baseline, the proportion of nasal isolates that were MR was 12%.
- On completion of the study the proportion of nasal isolates that were MR was 23%.
- New acquisition of MR amongst RCT participants was 10%.

3.4 Results of Laboratory investigations

3.4.1 Genotypic investigation of MRSA isolates

3.4.1 Introduction

Genotyping of bacterial isolates is undertaken to differentiate and characterise isolates and can reveal molecular similarities and differences between isolates that can support epidemiological studies. The genotypic characterisation of the clinical MRSA isolates was undertaken here to investigate stability of the MRSA genotypes before, during and after nasal decolonisation.

3.4.1.1 Results *spa* typing

Bacterial molecular genetic analysis

Table 3.4.1 presents the genotypic characterisation of the MRSA isolates based on *spa* types. The ST of the isolates, where available, was inferred from the *spa* type. At baseline 100, and on study completion 43, nasal MRSA isolates were available from 100 study participants for *spa* typing.

Table 3.4.1 Natural honey for nasal MRSA study comparison of *spa* types at baseline and after attempted decolonisation from nasal MRSA isolates collected from 100 study entrants

	MRSA isolates	
***spa* type**	**Baseline**	**Final**
t008	0	0
t020	5	2
t022[#]	4	0
t032[#]	40	19
t040	1	0
t045[$]	1	0
t084	0	0
t1214	0	0
t127*	10	7
t1370	1	0
t1499	1	0
t15373[@]	1	1
t15959[~]	1	0
t1612	3	3
t190[£]	1	0
t223[#]	0	0
t2436	2	1
t379	1	0
t4599[#]	9	1
t515[#]	12	7
t557[#]	0	0
t578	1	0
t6764	1	0
t7636	1	1
t8046	1	0
t9570	1	0
Failed spa test	1	0
Unknown	1	1
Total	100	43

#ST22, *ST1, £ST8, $ST5, [@] new spa type - a, [~] new spa type - b

From the 143 MRSA isolates tested, 26 different *spa* types were identified. The common *spa* types were; t032, 59 (41%), t515, 19 (13%), t127, 17 (11.8%) and t4599, 10 (7%). A *spa* type could not be assigned for two isolates. Two new *spa* types were identified and *spa* type t15373 and t15959 were assigned by *spa* type curator, website (http://www.seqnet.org).

Based on the *spa* types, a ST could be inferred from 110 (77%) isolates. The common STs were ST22, 91 (63.6%) followed by ST1, 17 (11.8%), and one isolate each of the ST5 and ST8 types.

Historic MRSA isolates

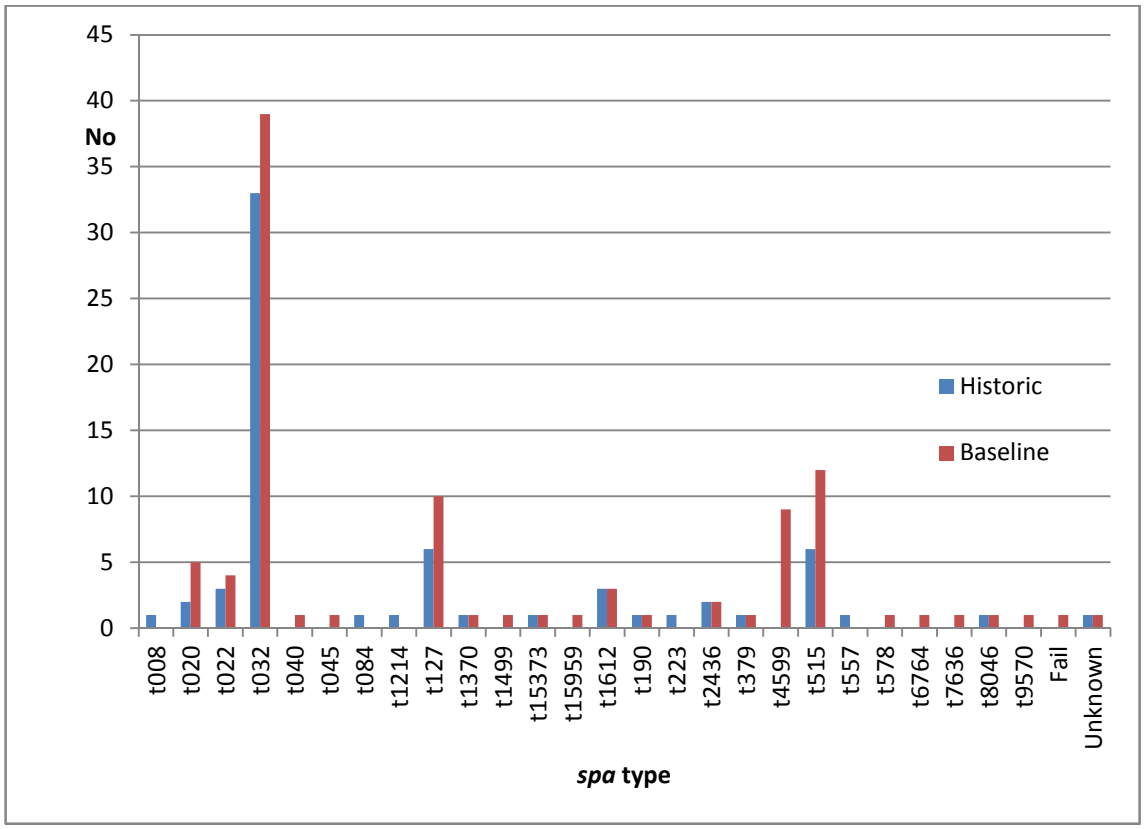
Of the 100 study participants, historic isolates were available from 66 participants and they were investigated for their *spa* type. Table 3.4.2 and Figure 3.4.1 present the *spa* types for the historic and baseline isolates from the 66 participants.

Table 3.4.2 Natural honey for nasal MRSA study *spa* types of the historic and baseline nasal MRSA isolates collected from 66 study participants

	MRSA isolates	
***spa* type**	**Historic**	**Baseline**
t008	1	0
t020	2	5
t022#	3	4
t032#	33	40
t040	0	1
t045$	0	1
t084	1	0
t1214	1	0
t127*	6	10
t1370	1	1
t1499	0	1
t15373@	1	1
t15959~	0	1
t1612	3	3
t190£	1	1
t223#	1	0
t2436	2	2
t379	1	1
t4599#	0	9
t515#	6	12
t557#	1	0
t578	0	1
t6764	0	1
t7636	0	1
t8046	1	1
t9570	0	1
Failed spa test	0	1
Unknown	1	1
Total	66	100

#ST22, *ST1, £ST8, $ST5, @ -new spa type - a, ~ new spa type - b

Figure 3.4.1 Natural honey for nasal MRSA RCT *spa* types of the historic and baseline isolates from the 66 participants



Of the 166 historic and baseline MRSA isolates, the common *spa* types were; t032, 73 (44%), t515, 18 (11%), and t127, 16 (10%). The most prevalent ST type was ST22 109 (66%), followed by ST1, 16 (10%).

Comparison of historic, baseline and final isolates based on spa type

Table 3.4.3 presents the comparison of the *spa* types from all of the study participants where available, at multiple time points; historic and baseline for 66 participants, and baseline and on study completion for 43 persistently colonised participants.

Table 3.4.3 Natural honey for nasal MRSA study comparison of *spa* types of 66 historic and baseline isolates and 43 baseline and final MRSA isolates

spa type	Historic and baseline isolates n=66 (%)	Baseline and final isolates n=43 (%)
Indistinguishable	45 (68)	39 (91)
Distinguishable	21 (32)	4 (9)

The *spa* types of the 100 study participants, historic, baseline and on study completion are listed in appendix - XV.

Duration of MRSA carriage

Table 3.4.4 outlines the duration of carriage for the 100 study participants since the first time they were confirmed as MRSA positive until RCT enrolment.

Table 3.4.4 Natural honey for nasal MRSA RCT duration of MRSA colonisation in years prior to RCT enrolment

Duration of MRSA colonisation	No of cases
0 <1 Yr	40
1-2 Yr	17
3-5 Yr	27
6-10 Yr	10
11-15 Yr	6

Of the 100 study participants, 40% were colonised for less than one year, 44% between1-5 years and the remaining 16% between 6 and 15 years.

Duration of MRSA colonisation and comparison of spa types

A comparison of the duration of MRSA colonisation of the 66 study participants, where the historic and baseline isolates, were available was done. Figure 3.4.3 shows the duration of MRSA carriage since first identification of MRSA to RCT enrolment of the 45 participants where the *spa* type was indistinguishable. Figure 3.4.4 illustrates the duration of MRSA carriage of the 21 cases where the *spa* type was distinguishable.

Figure 3.4.3 Natural honey for nasal MRSA RCT duration of MRSA carriage of 45 participants who had an indistinguishable *spa* type, both historic and at baseline

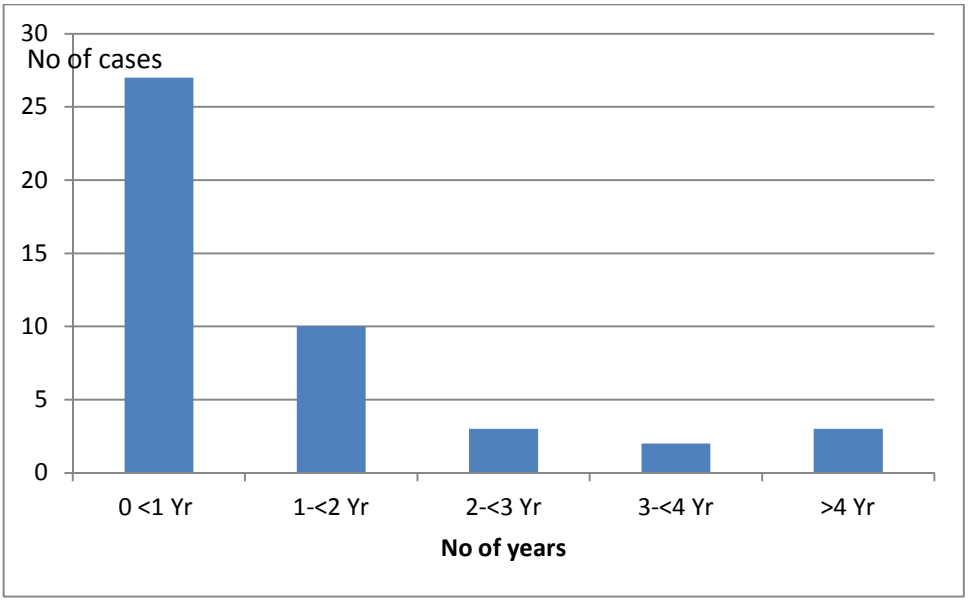
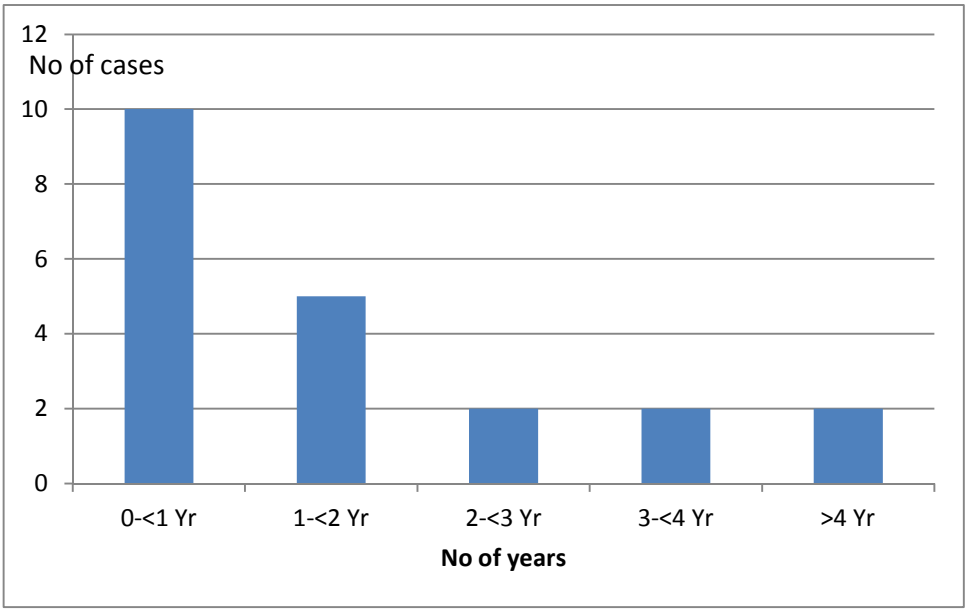


Figure 3.4.4 NHNMRSA RCT duration of MRSA carriage of 21 participants where a distinguishable *spa* type was evident



Of the 66 study participants where a historic and baseline isolate was available for *spa* type comparison, 45 (68%) had an indistinguishable *spa* type on first identification and on RCT enrolment. For 21 (32%) participants the *spa* type was distinguishable at two time points. Of the 21 cases where a distinguishable

spa type was evident, the duration of MRSA colonisation was less than one year for 14 (67%) cases, refer Figure 3.4.3.

Table 3.4.5 presents the RCT outcome comparison of the 43 participants who had successful nasal MRSA decolonisation, intervention and control groups, and their baseline *spa* types. The percentage success is calculated based on the 43 (100%) patients who were decolonised, and compared between the intervention and control groups.

Table 3.4.5 Natural honey for nasal MRSA RCT comparative analysis of the baseline *spa* types of the 43 participants who had successful nasal decolonisation

	Baseline MRSA isolates	**Successful decolonisation**		**Intervention group**		**Control group**	
***spa* type**	**n**	**n**	**%**	**n**	**%**	**n**	**%**
t008	0	0		0		0	
t020	5	1	20.0	0		1	
t022#	4	2	50.0	1	50.0	1	50.0
t032#	40	19	48.0	7	37.0	12	63.0
t040	1	1	100	1	100	0	
t045$	1	0		0		0	
t084	0	0		0		0	
t1214	0	0		0		0	
t127*	10	3	30.0	2	67.0	1	33.0
t1370	1	1	100	0		1	100
t1499	1	1	100	0		1	100
t15373@	1	0		0		0	
t15959~	1	1	100	1		0	100
t1612	3	0		0		0	
t190£	1	1	100	0		1	100
t223#	0	0		0		0	
t2436	2	1	100	0		1	100
t379	1	1	100	1	100	0	
t4599#	9	5	56.0	3	60.0	2	40.0
t515#	12	3	25.0	2	67.0	1	33.0
t557#	0	0		0		0	
t578	1	1	100	0		1	100
t6764	1	1	100	0		1	100
t7636	1	0		0		0	
t8046	1	0		0		0	
t9570	1	0		0		0	
Failed spa test	1	1	100	0		1	100
Unknown	1	0		0		0	
Total	100	43		18		25	

#ST22, *ST1,£ST8, $ST5, @new spa type – a. ~new spa type - b

The *spa* type of the carriage isolates did not appear to influence the outcome of the nasal decolonisation. Persistent colonisation with the same *spa* type was evident even over relatively long time-spans. However, replacement of the colonising *spa* type was also identified. In summary, no pattern according to the *spa* type/s was evident that influenced the nasal decolonisation outcome among the intervention and control groups.

3.4.1.2 *mupA* PCR test results

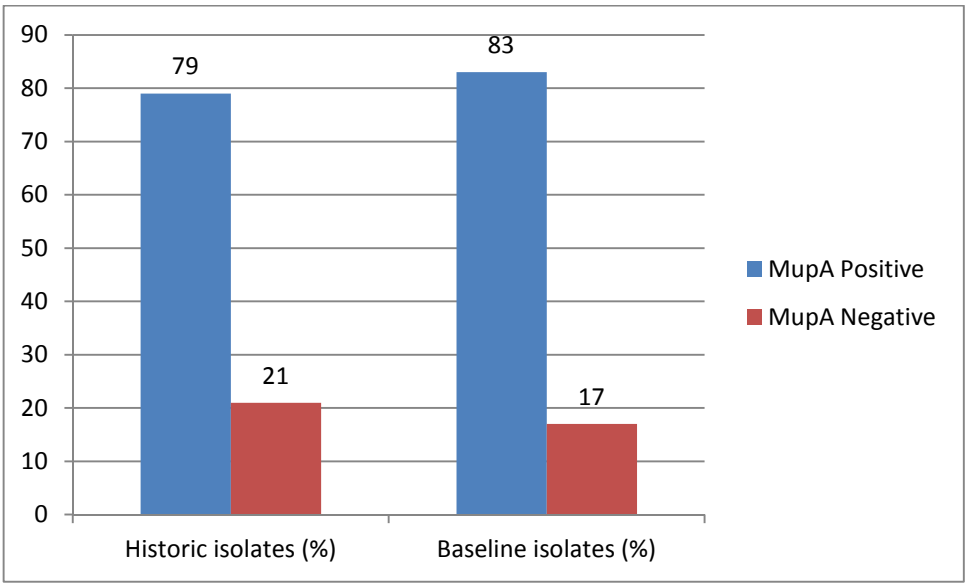
Table 3.4.6 presents the cumulative *mupA* PCR test results of the 100 baseline and 66 historic MRSA isolates investigated.

Table 3.4.6 Natural honey for nasal MRSA RCT, *mupA* PCR test results of the 100 baseline and 66 historic isolates.

mupA	Historic isolates n=66 (%)	Baseline isolates n=100 (%)
Positive	52 (79)	83 (83)
Negative	14 (21)	17 (17)

Figure 3.4.5 shows the percentage comparison of the of *mupA* PCR test results of the 166 historic and baseline MRSA isolates from 100 study participants.

Figure 3.4.5 Natural honey for nasal MRSA RCT *mupA* PCR test results of the 66 historic and 100 baseline MRSA isolates



Determining the presence of *mupA* gene using PCR is a standard method employed for ascertaining genotypic mupirocin resistance amongst *S. aureus* isolates. When amplifying a 310 bp fragment of the *mupA* gene by PCR followed by agarose gel electrophoresis and DNA band visualisation under UV light, 17 (17%) of baseline and 14 (21%) of historic MRSA isolates were *mupA* negative. This suggests that these MRSA isolates do not harbour the *mupA* genes that confer low (*ileS-1*) and high level mupirocin resistance (*ileS*-2). However, this does not exclude the potential for non-*ileS-1 or ileS*-2 genes that confer mupirocin resistance previously described by others. (49,46,50)

3.1.4.3 Summary

The genotypic investigation demonstrates extensive diversity of the MRSA isolates with 26 *spa* types. The findings are similar to the diversity of MRSA isolates reported by the others nationally in Ireland, according to *spa* type. (177-178)

3.4.2 Results of the MIC and MBC determination of 30% Medical Grade Honey

3.4.2 Results of the MIC and MBC determination of 30% Medical Grade Honey

3.4.2.1 Introduction

In order to demonstrate the antibacterial property of the investigative product, the MIC and MBC of 30% MGH were performed. The results of the agar well diffusion assay for MIC, and MBC by broth culture and micro-dilution are presented.

3.4.2.2 MIC by agar well diffusion assay

In table 3.4.2.1 the agar well diffusion assay results are presented. The zone of inhibition measured in mm, which is attributed to the antibacterial activity of the test honey, is also displayed in figure 3.4.2.1.

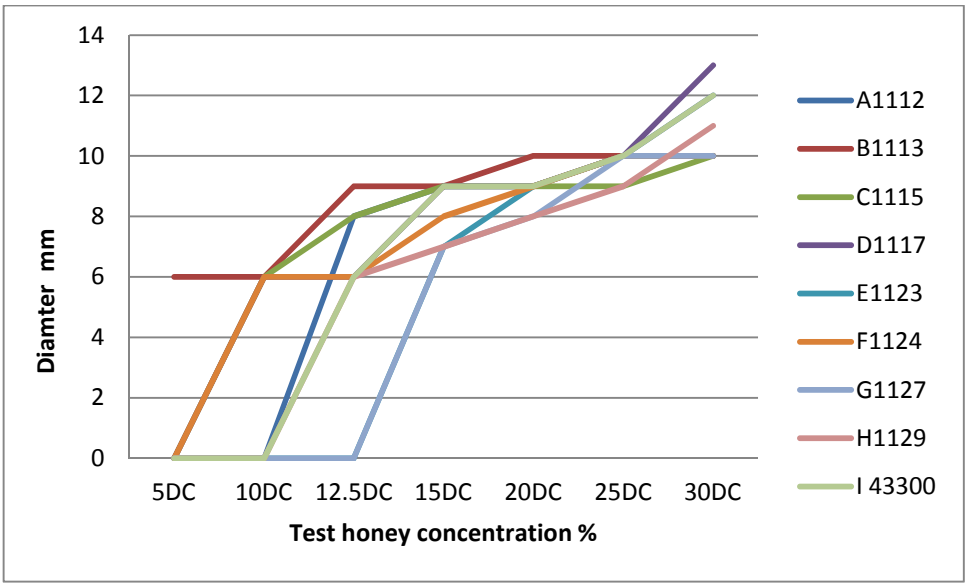
Table 3.4.2.1 Mean zones of inhibition* to determine antibacterial activity of test honey

MRSA	**Test honey concentration**						
Isolate No	30%	25%	20%	15%	12.5%	10%	5%
	mm	mm	mm	mm	mm	mm	mm
ATCC43300	12	10	9	9	6	0	0
1112	12	10	9	9	8	0	0
1113	10	10	10	8	9	6	6
1115	10	9	9	9	8	6	0
1117	13	10	9	9	6	6	0
1123	10	10	9	7	0	0	0
1124	10	10	9	8	6	6	0
1127	10	10	8	7	0	0	0
1129	11	9	8	7	6	0	0

*Including well diameter 5 mm

A zone of inhibition was observed from 15% of the test honey against all eight clinical isolates and the reference MRSA isolate, demonstrating antibacterial activity, refer Table 3.4.2.1.

Figure 3.4.2.1 Mean zones of inhibition* to determine antibacterial activity of test honey



A clear zone of bacterial inhibition was observed for test honey concentrations starting from 5% for the nine isolates investigated, as displayed in Table 3.4.2.1 and Figure 3.4.2.1.

3.4.2.3 MBC by broth micro-dilution method

In Table 3.4.2.2 the results of MBC of test honey to clinical and reference MRSA isolates are presented.

Table 3.4.2.2 MBC of test honey and determination of antibacterial activity

Test honey	Isolate number								
	1112	**1113**	**1115**	**1117**	**1123**	**1124**	**1127**	**1129**	**43300**
5%	TNTC[a]	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
10%	1[b]	33	0	0	0	0	0	0	0
12.50%	0	0	0	0	0	0	0	0	0
15%	0	0	0	0	1	0	0	0	0
20%	0	0	0	0	0	0	0	0	0
25%	0	0	0	0	0	0	0	0	0
30%	0	0	0	0	0	0	0	0	0
Positive Control	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Negative Control	NG[c]	NG	NG	NG	NG	NG	NG	NG	NG

a - TNTC - Too numerous to count, b - colony forming units (cfu), c- NG - No growth

No bacterial growth was observed after 24 h from test honey of concentrations of 12.5% and higher for all the eight clinical and one reference MRSA isolates. The MBC values correlates with MIC values, therefore providing supporting evidence of bactericidal rather than bacteriastatic action of test honey.

Comparison of MIC and MBC of test honey

In Table 3.4.2.3 the observed MIC and MBC of test honey for the eight clinical and one reference MRSA isolates tested are compared. The bactericidal concentration was in concordance with the bacteristatic concentration for most isolates.

Table 4.3.2.3 Comparison of MIC and MBC of test honey

MRSA isolate No	Test honey conc:	MIC (mm)	MBC* (honey conc:)	Bacterial growth
ATCC 43300	12.5%	6	12.5%	NG[a]
1112	12.5%	8	12.5%	NG
1113	12.5%	9	12.5%	NG
1115	12.5%	8	12.5%	NG
1117	12.5%	6	12.5%	NG
1123	15%	7	15%	NG
1124	12.5%	6	12.5%	NG
1127	15%	7	12.5%	NG
1129	12.5%	6	12.5%	NG

*The MBC of test honey to inhibit 100% of microbial growth *in vitro,* expressed in % v/v solution for clinical isolates (n = 8) and reference (n=1) , ATCC 43300 isolate. a -no growth

3.4.2.4 Conclusion

Antibacterial activity of test honey was demonstrated using the agar well diffusion assay and broth micro-dilution methods using clinical and reference MRSA isolates. The MIC using the agar well diffusion method, demonstrated antibacterial activity of test honey at 5% and higher concentrations. Using the broth micro-dilution method to differentiate bactericidal from bacteriostatic action, it was established that the MBC of the test honey was 12.5%. From a comparison of MIC and MBC values, it can therefore be deduced that the MIC and MBC of the test honey is in the range of 12.5% to 15% for the MRSA

isolates tested. Other investigators have reported MICs of 4% and higher to antibacterial honey. (70,179) The bactericidal action of test honey observed is in concordance with findings reported by others. (110,117)

3.5 Questionnaire survey results

3.5.1 The Brief Illness Perception Questionnaire

3.5.1.1 Introduction

In the RCT, the participants beliefs and perceptions on MRSA colonisation (illness) was collated using the modified BIPQ; MRSAPQ. The MRSAPQ was administered to participants on completion of a course of mupirocin 2% or Medihoney™ Derma Cream nasal application. The response rate was 87.5% (n=35). The BIPQ questionnaire survey results are discussed.

3.5.1.2 Results

The Likert style rating questionnaire (MRSAPQ) used an ordered continuum of response categories, with a balanced number of positive and negative options, a neutral option and each category was labelled. This facilitated balanced response options as well as assigning an even number scale for analytical purposes. Each item of the modified BIPQ items assessed one dimension of illness perception. Eight dimensions and a causal item on causation of MRSA were gathered using the questionnaire. Individual scores relative to each item were distributed throughout a full scale range (0-10), 11 scales. The numeric values assigned to the response categories were consistently applied for all questionnaires received and analysed. The results of each of the dimensions are listed in the same order, and cumulatively displayed in Table 3.5.1.

Consequence

Of the 35 respondents, for 23 (66%) MRSA colonisation had little or no affect on their daily lives. However, for nine (26%), MRSA colonisation had a moderate impact on their daily lives. For three (8.5%) participants, it did not have an impact at all. Therefore it can be construed that MRSA colonisation had limited or no impact for 26 (74%) participants.

Timeline

A clear majority 29 (83%) of the participants believed that their MRSA colonisation would continue forever, three (8.5%) participants believed that it would continue for a short duration, while another three (8.5%) participants believed that it would be for a medium time period.

Personal control

Most participants 33 (94%) deemed that they did not have any control on their MRSA colonisation. The remaining two (6%) felt they had some control over it.

Treatment control

All the participants 35 (100%), were in agreement that decolonisation of MRSA will be extremely helpful.

Identity

Of the 35 participants, eight (23%) experienced moderate or major symptoms related to MRSA. However, the overwhelming number of participants 27 (77%) did not experience any symptoms they attributed to MRSA colonisation.

Concern

This item reflects a combination of emotional and cognitive representation of participants. Of the 35 participants, a significant proportion, 28 (80%), were concerned about their MRSA colonisation but for seven (20%), this was not a matter of concern.

Coherence

While 20 (57%), of participants stated that they had moderate to good understanding of MRSA, 15 (43%) stated that they had only minimal or limited information on MRSA.

Emotional representation

Of the 35 participants, 27 (77%), were emotionally detached from their colonisation status. However, for eight (23%), participants MRSA colonisation had an impact on their emotional wellbeing.

Casual item

The participants attributed the following causes or factors as being responsible for their positive MRSA status; hospitalisation 16 (34%), surgery 10 (21%), poor hospital hygiene eight (17%), chronic illness six (13%), use of antibiotics five (11%) and contact with health care workers two (4%).

Table 3.5.1 NHNMRSA RCT results of the eight BIPQ dimensions.

BIPQ items	Mean (Std Dev)	Median (IQR)	Mode
Consequences	2.94 (3.5)	1 (0)	0
Timeline	8.69 (2.32)	10 (8.5)	10
Personal control	0.77 (2.37)	0 (0)	0
Treatment control	9.69 (0.9)	10 (0)	10
Identity	2.09 (3.05)	0 (0)	0
Concern	6.83 (2.91)	7 (5)	7
Coherence	4.77 (2.21)	5 (3)	5
Emotional representation	2.74 (2.39)	2 (1)	0

3.4.1.3 Discussion

Descriptive statistics pertinent to the eight BIPQ items showed that, in general, the participants tended to view their MRSA carriage as a chronic condition (timeline mean score = 8.69), and perceived that if cleared with appropriate treatment (treatment control mean score = 9.09), it was significantly beneficial. However, participants were firmly convinced that they had limited or no control over MRSA colonisation (personal control mean score = 0.77). Most participants perceived that their MRSA colonisation did not have serious consequences or negative impact on their lives (consequences mean score = 2.94). However, participants were deeply concerned (concern mean score =6.83) due to their colonisation. Participants in general were emotionally detached (emotional response mean score = 2.74) from the condition. Most participants did not have MRSA-related symptoms (identity mean score =2.09), but participants understanding of MRSA was moderate to low (coherence mean score = 4.77).

Consistently low scores for consequences; 26% standard deviation (Sd) 2.94, identity; 23% (Sd 2.09) and emotional response; 23% (Sd 2.74) are associated with good psychological, social and physical functioning which implies good illness outcomes. (180) However, a high score for concern 71% (Sd 6.83) is associated with poor psychological functioning which contradicts the earlier assumption based on consequences, identity and emotional responses. Low personal control 6% (Sd 0.77) and very high treatment control 100% (Sd 9.69) are associated with suboptimal psycho-social and physical functioning which implies poor illness outcome. A longer perceived time line 83% (Sd 8.69) with MRSA colonisation is associated with poor outcome and quality of life. Most of the study participants were chronic carriers of MRSA and the survey results should be interpreted in this context.

3.4.1.4 Summary

- Participants perceived that MRSA colonisation was a chronic condition and that it did not have serious consequences on their daily lives.
- Participants were in general emotionally detached from the condition and few had MRSA related symptoms.
- Most participants felt they had limited or no control over carriage, but decolonisation was considered beneficial, indicating the importance attached to treatment/control of MRSA.

Understanding of the disease can lead to more favourable health behaviours and disease outcomes. A sub-optimal understanding of MRSA among 40% of patients necessitates measures to target improving knowledge about MRSA. This should enable patients to understand MRSA acquisition and transmission as well as adherence to treatment/decolonisation potentially leading to better outcome.

3.5.2. Product experience questionnaire

Patients experience on the use of medical grade honey and mupirocin

3.5.2.1 Introduction

The NHNMRSA RCT employed a PEQ to collate patient's experience on the use of the investigative and control products. The PEQ was developed by the researcher through an iterative process. A Likert scale type rating was used in the PEQ for four of five questions. The PEQ scaling of four of the Likert statements ranged from strongly agree, agree, neither agree or disagree, disagree and strongly disagree. The fifth item was to record the patients' own preference where an option was offered to the patient; to choose between an antibiotic and a natural product, to eradicate MRSA from the nose. This fifth statement therefore had a scale with two options, i.e. to record the product choice. Patients in the investigative group were provided with the PEQ labelled 'Experience with Medihoney™ Derma Cream' and the control group with the PEQ labelled 'Experience with mupirocin nasal ointment'. The PEQ was administered to study participants on completion of a course of Medihoney™ Derma Cream (investigative) or mupirocin 2% (control) nasal application. The cumulative response rate was 87.5%, details are now considered.

3.5.2.2 Results

Analysis of the Likert style PEQ was done assigning numeric values to each of the five point scale. Numeric values were assigned as follows; strongly agree =1, agree =2, neither agrees or disagree =3, disagree =4 and strongly disagree =5. The fifth item; a choice of an antibiotic or natural product was analysed as recorded by respondents. The results of each of the items are listed in the same order. For the investigative group, the cumulative results are displayed in Table 3.5.2, followed by the statistical analysis in Table 3.5.3. For the control group the results are displayed in Table 3.5.4, followed by statistical analysis for the same group in Table 3.5.5.

Of the 35 respondents, 20 PEQ's were returned from the investigative group and 15 from the control group.

Medihoney™ Derma Cream

Of the 20 respondents who had Medihoney™ Derma Cream nasal application, 19 (95%) agreed that it was easy to apply to their nasal passages, and one (5%) neither agreed nor disagreed. Most, 18 (90%) agreed that Derma Cream was not sticky to the nasal passages but one (5%) found it was sticky, and the remaining one respondent neither agreed nor disagreed to this statement. Likewise 17 (85%) respondents did not experience a runny nose after Derma Cream application, one (5%) did experience runny nose and two (10%) neither agreed nor disagreed to the statement. Almost all, 19 (95%) did not experience any unpleasant sensation but one (5%) patient did state that they experienced an unpleasant sensation following Medihoney™ Derma Cream nasal application.

If a choice was available for patients, most of the respondents 17 (85%) in the investigative group preferred a natural product an antibiotic in one (5%) and two (10%) had no preference. One of the respondent stated that the aroma of Medihoney™ Derma Cream was similar to that of 'Heather' - a plant that grows in acid soil and abundant in mountains of Ireland.

Table 3.5.2 NHNMRSA RCT patient's experience on the use of Medihoney™ Derma Cream

Abbreviated Statement	Agree / strongly agree n (%)	Neither agrees / disagree n (%)	Disagree / strongly disagree n (%)
Ease of application	19 (95)	1 (5)	0
Sticky on nose	1 (5)	1 (5)	18 (90)
Runny nose on application	1 (5)	2 (10)	17 (85)
Unpleasant nasal sensation	1 (5)	0	19 (95)
	Antibiotic	No preference	Natural product
Patients preferred option if a choice was available	1 (5)	2 (10)	17 (85)

Table 3.5.3 NHNMRSA RCT c results of statistical analysis of the PEQ, Medihoney™ Derma Cream

PEQ items	Mean (Std Dev)	Median (IQR)	Mode
Ease of application	1.5 (0.6)	1 (1)	1
Nasal stickiness	4 (0.6)	4 (4)	4
Runny nose	3.9 (0.5)	4 (4)	4
Unpleasant nasal sensation	4 (0.7)	4 (4)	4

Note: Numeric values: 1= strongly agree, 2= agree, 3= not agree or disagree, 4= disagree, 5 = strongly disagree.

Mupirocin 2% (Bactroban® 2%) group

Of the 15 respondents who had mupirocin 2% nasal application, 13 (87%) agreed that it was easy to apply the ointment to their nasal passages, two (13%) neither agreed nor disagreed. In response to the statement on nasal stickiness, 10 (67%) responded that mupirocin was not sticky but three (20%) found it was sticky, and the remaining two respondents neither agreed nor disagreed. Many patients, five (33%) experienced a runny nose after mupirocin 2% application but nine (60%) did not experience this, and the rest one (7%) neither agreed nor disagreed. Almost all, 14 (93%) respondents did not experience any unpleasant sensation following mupirocin 2% application but one (7%) patient did state that they experienced an unpleasant sensation following the nasal application.

If a choice was available for patients, the antibiotic option was preferred by four (27%), a natural product by eight (67%) and three (20%) had no preference.

Table 3.5.4 NHNMRSA RCT patient experience on the use of mupirocin 2% nasal ointment

Abbreviated Statement	Agree / strongly agree, n (%)	Neither agrees / disagree, n (%)	Disagree / strongly disagree, n (%)
Ease of application	13 (87)	2 (13)	0
Sticky on nose	3 (20)	2 (13)	10 (67)
Runny nose on application	5 (33)	1 (7)	9 (60)
Unpleasant nasal sensation	1 (7)	0	14 (93)
	Antibiotic	No preference	Natural product
Patients preferred option if a choice was available	4 (27)	3 (20)	8 (53)

Table 3.5.5 NHNMRSA RCT results of statistical analysis of the PEQ, mupirocin 2%

PEQ items	Mean n, (Std Dev)	Median n. (IQR)	Mode, n
Ease of application	1.6 (0.7)	1 (1)	1
Nasal stickiness	3.6 (0.9)	4 (3)	4
Runny nose	3.3 (1)	4 (2)	4
Unpleasant nasal sensation	4 (0.9)	4 (4)	4

Note: Numeric values assigned: 1= strongly agree, 2= agree, 3= not agree or disagree, 4= disagree, 5 = strongly disagree

3.5.2.3 Discussion

PEQ Medihoney™ Derma Cream

Most patients (95%) concurred that Medihoney™ Derma Cream was easy to apply, not sticky (90%), did not lead to a runny nose (85%) and they did not experience an unpleasant sensation (95%). Based on the survey results it can therefore be inferred that Medihoney™ Derma Cream is safe to apply to the nasal passages, with minimal undesirable effects. Medihoney™ Derma Cream is a CE marked medical device, and the manufactures claim is a sophisticated emollient for skin application. Considering the user experience and observations from the RCT, Medihoney™ Derma Cream may be used judiciously for nasal application.

PEQ mupirocin2% (Bactroban®2%)

Although mupirocin 2% nasal ointment has been used for nasal MRSA decolonisation for over four decades, there is limited information on patients' experience of its use. In our study, most respondents (87%) concurred that mupirocin 2% nasal ointment was easy to apply, but it was sticky (20%), and a runny nose was experienced by 33% of respondents. Almost all (95%) respondents agreed that they did not experience any unpleasant sensation following its application.

3.5.2.4 Summary

- Medihoney™ Derma Cream may be applied to the human nasal passages with minimal undesirable effects.
- The application of Medihoney™ Derma Cream and mupirocin 2% nasal ointment by participants to their nasal passages was easy.
- A runny nose may be experienced by some patients following mupirocin 2% nasal application.

  Patients' preferred choice is a natural alternative to an antibiotic, if that was available for MRSA decolonisation.

Chapter IV Discussion, conclusions, reflections and future research

Discussion, conclusions, reflections and future research

In the final chapter the researcher aims to bring the essence of the study to a meaningful conclusion. The discussions in this chapter are condensed under the themes which follow the introduction; study rationale, results, how it correlates, as well as contrasts, with the findings of others. The crucial discussion revolves around the clinical and economic implications which will also consider the advantages and limitations of the study, and concludes with recommendations for future work.

4.1 Introduction

Why did I take this path? To conduct a clinical trial with no previous hands on experience is a demanding and challenging project? Having reflected on this point in depth the following quote strikes me as appropriate '*It is not so important what you do, but, be the best in what you do*'. I believe that this neatly summarises why I chose this path and what contribution it has made.

Now let me tell my story. I remember a patient who I looked after during my nursing training, which was an experience which deeply affected me, and instilled in me the importance of *preventing infection*. A moment of weakness left a young woman with more than 70% burns. Twenty five years ago in a resource constrained health system, there was little hope that she would survive. Then I saw in her eyes a plea to live. Was it possible, we could have done something better? A unique bond developed between the trainee nurse and the patient, until she succumbed to infection and was freed from all her pains. This had a profound impact on me and my future professional career. There were many other incidents which I recall, about preventable infection that left a mark on me.

Why did I do this RCT? Reflecting on a little over two decades of my professional IPC career, what I value is the basics, which if we can get right, we

can prevent many infections and save lives. I lived through many phases of MRSA prevention and control. From the 1980s when MRSA was not so common to the 2000s when it became endemic, and in epidemic proportions in some health systems. I recall the change from search and destroy, to horizontal prevention, and the dramatic fall in MRSA BSI when basic IPC practices were aggressively implemented.

However, our approach to patient decolonisation of MRSA using antibiotics and antiseptics remains a battle. When pathogens are adapting for survival we need to think outside the box and to look to mother nature. Is there an answer in nature? Perhaps there is, however, we need to search with an open eye and a curious mind. Scientifically it has been proven that bacteria survive and multiply where there is water. How then, does honey, derived from bees, stay stale-free for 1000s of years? Over the centuries many traditional health systems used honey for many ailments, and as late as 2015, modern medicine has scientifically proven that honey treated burns and infected wounds heal quicker than with antiseptic and antibiotic based treatment.

Can honey eradicate MRSA? Before we can come to a conclusion, one must enquire with an inquisitive mind. One may find an answer, and disprove or generate more questions during the journey. That is what I have done and largely answers why I embarked on this research.

4.2 Study findings

Literature review

Undertaking the review of the literature on MR and alternatives for the eradication of MRSA was a unique learning experience. The review findings confirmed the necessity for alternative MRSA decolonisation options other than the conventional antibiotic route, thereby affirming the principle and the basis for the NHNMRSA RCT. The review presented a composite picture on MR and CHZ resistance, such as a strong association between previous mupirocin exposure and both low-level and HLMR as high as 81%. (174) Widespread acquisition among CoNS of MR, especially HLMR from surveys in Europe,

following nasal decolonisation with mupirocin, have been as high as 97% (588/607), which is alarming. (181,151) Along with MR and CHZ resistance, HLMR (*mupA* carriage) is also linked to MDR, which signifies the extent of the antimicrobial resistance challenge faced by modern health systems. The review also showed that among MRSA isolates, the presence of the *qacA* and/or *qacB* genes encoding resistance to chlorhexidine, ranges from 65% to 91%, which along with MR is associated with failed decolonisation. Therefore, for safe clinical practice the optimal use of mupirocin and CHZ for MRSA decolonisation must be deduced from up to-date scientific evidence. This point will be further elaborated in the discussion of the RCT results.

Randomised Clinical Trial

Considering the body of work involved in obtaining regulatory and ethical approval for the conduct of the RCT, competing for research funding was probably the less challenging part in the entire project. The study opened many avenues of learning, which the researcher, prior to commencement of the study was not so knowledgeable about. Laboratory based learning such as bacterial molecular investigation methods, MIC and MBC assays were complemented with scientific communication techniques, scientific leadership skills, research methods and statistical analysis, with the overall management of the project as the lead investigator and administrator.

The regulatory requirements necessitated the researcher to select a product that met the criteria for use in humans for medicinal purposes. The researcher investigated any licensed off-the-shelf honey based product/s if that was available that was antibacterial, considering the challenges in locally formulating a MGH product. The enquiry ultimately narrowed to Medihoney™ Derma Cream, a medical device, for which the manufacturers claim antibacterial activity with 30% MGH. The RCT results substantiate the potential for MGH as a viable alternative for MRSA decolonisation. However, studies must be done using higher concentrations of MGH. In clinical practice, honey based dressings at concentrations of 50% and higher are used for the management of infected wounds and ulcers. The dilution effects of a cream or ointment when applied

topically such as in moist nasal passages, could potentially be overcome by the application of higher concentrations of MGH. The MIC and MBC investigations demonstrated antibacterial properties of the MGH product at 5% and higher, i.e. MIC, and MBC at 12.5% and higher, after 24 h at 37^0 C. In theory, higher concentrations of MGH should demonstrate higher antibacterial properties. Investigators have reported that the antibiotic mupirocin demonstrated a slow bactericidal action in time-kill tests, resulting in 90 to 99% killing after 24 h at 37^0 C when tests of bactericidal activity were performed. (173) In another study that investigated the concentration of mupirocin, sterile cultures were obtained when *S. aureus* strains were exposed to 1.0 mg/l or more of mupirocin for 120 h. The investigators concluded that mupirocin has slow bactericidal activity against *S. aureus* and that 2% mupirocin may well be effective for topical treatment of skin infections. (182) Similarly, dose concentration investigations of MGH must be performed to determine the optimal bactericidal concentration for safe clinical use.

I concluded that at best 30% of the eligible participants could be enrolled in the RCT, whereas in actuality 28% of the eligible patients consented for the study. A 10% drop out rate was predicted for various reasons, and on study completion, a final outcome was not available for 14% of patients due to loss of follow up, protocol violation and attrition from the study. I followed up patients up in their residential home or other HCF, which was easier for some patients as they said 'it was less demanding'. Moreover, the IPC expertise of the research team and the academic nature of the study encouraged patients and families to take part. Overall, the strategies employed by the researcher benefitted in optimal patient recruitment. Researchers may consider the successful strategies the NHNMRSA team employed while planning further similar studies.

Sub-optimal patient compliance with medications has been previously reported. Although Medihoney™ Derma Cream and mupirocin 2% (Bactroban® 2%) was prescribed in the Drug Kardex, in some instances I saw poor medication compliance on the administration of prescribed medication. Various strategies were employed to ascertain patient medication administration. A simple count of the remaining cotton buds in the pack, direct patient interaction enquiring about

smell or patient discomfort, prompted me to investigate this vital aspect further. A note of self-administration in the Drug Kardex was another challenge to address. For inpatients during their study period, the researcher visited them at least once a day to administer the prescribed product. This opportunity was also used to interact with the staff nurse assigned and or nurse manager, and to reiterate administration of the intervention product as prescribed. Patients at home or in another HCF were followed up by telephone and supported on optimal administration. Clear directions on the application and recording of Medihoney™ Derma Cream or mupirocin 2% (Bactroban® 2%) were provided to participants. Patients were requested to mark a tick ✓after each dose in the appropriate box in the sheet provided, which to some extent benefitted in enhancing compliance.

Many patients who required minimal assistance for self care and who were assessed as competent for self administration of medications, in practice experienced nasal application as challenging. The demand on nursing staff to prioritise available time for dependent patients may also have influenced their decision to direct patients to self administer and record accordingly in the drug Kardex. Both these were also probable factors that influenced medication compliance. In future, consideration must be given to the form of medication such as a nasal ointment/cream, which might be preferred. While compliance on injectable and oral medications may be addressed by the HCW and patient education, for local application of medications, user versatility is an important factor that may potentially influence its administration. The MRSAPQ results confirm patients' perception of MRSA as a chronic condition and that treatment control is optimal. Therefore before enrolling any patient for a decolonisation program, there must be active engagement with the patient and family to augment compliance with the protocol. During the decolonisation course, good hygiene practices are paramount especially daily washing and showering along with the use of clean personal clothing for its success. All these measures must be communicated and patients should be assessed on their ability to actively engage with the protocol. If not, we may inadvertently contribute to decolonisation failure as well as anti-microbial resistance.

Patients with non-nasal MRSA colonisation should be carefully evaluated before enrolling in a decolonisation program. As evident from the statistical analysis, non-nasal MRSA colonisation was significantly associated with failed nasal MRSA decolonisation. Therefore where appropriate, non-nasal MRSA decolonisation such as wounds and ulcers should be addressed first, followed by nasal decolonisation. However, if suppression therapy is planned such as in the case of ITU patients or before high risk surgery, consideration should be given on the potential merit of this strategy before a decision to repeat courses of nasal mupirocin is made.

Other formulations such as single use pre-soaked sponge/swabs or aerosols are another option for ease of medication administration. In recent years certain anti-tuberculosis medications are used in the aerosolised form which provides optimal target drug delivery as well as limited adverse effects. (184) The potential for an aerosolised preparation of both MGH and mupirocin is worth investigating for decolonisation of the respiratory passages including nasal MRSA decolonisation. In the case of patients with COPD and other pulmonary conditions, respiratory MRSA colonisation is difficult to eradicate, therefore an aerosol may offer the potential for pulmonary and nasal decolonisation. In such patients attempting nasal MRSA decolonisation alone is not sufficient as re-colonisation from the patients' airways is inevitable.

Mupirocin susceptibility

I collected MRSA isolates at sequential time points where possible, i.e. historic, baseline on recruitment and from persistent nasal MRSA carriers, at the end of the study. The baseline and final isolates were collected prospectively and historic isolates from archived isolates, where available. A comparative analysis of mupirocin susceptibility at multiple time points provided a robust picture of MR among the clinical isolates, historic as well as new acquisition. This approach enabled me to make meaningful conclusions on the risks of the administration of multiple courses of mupirocin.

The comparative analysis of MS between the clinical isolates from the same patient at sequential time points facilitated the evaluation of the probable time of acquisition of MR. While most of the isolates were MS; 92% historic, 88% at baseline and 77% at the end of the study, MR progressively increased from the point of first time identification, from 8% to 23% at the end of the study. The incidence and the acquisition of MR is comparable to that reported by others, such as HLMR of 6% following an intensive MRSA nasal eradication program and 5% in an RCT that compared different eradication regimens for MRSA decolonisation. (34,175) The increasing incidence of MR, 23%, when multiple courses of mupirocin were administered identified in this study, that compares with the surveillance findings reported by others, for example in Canada where an exponential increase in MR from 2.7% to 65% (1990-1993) was recorded during a three year period where mupirocin was used extensively. (145) In Taiwan (149), a case control study also confirmed that previous mupirocin exposure was an independent predictor for LLMR and HLMR. (41) The mounting evidence underlines the necessity for stewardship measures to conserve this valuable antibiotic option for nasal MRSA decolonisation, to prevent infection, and in clinical situations such as prosthetic device implants for optimal patient benefit.

Phenotypic and genotypic analysis of the isolates

Initially I acquired competence in performing these laboratory tests, which in itself was exciting. The complexities involved in the identification and classification of the isolates along with the presence of resistance genes helped me to better understand the clinical aspects of IPC in MRSA control.

All of the 209 clinical isolates collected in the RCT were investigated. A *spa* type was assigned for 205 isolates. In total, 26 *spa* types and an unknown type were identified. The predominant *spa*-types were t032, t515, t127 and t4559. Two new *spa* types which had a unique DNA-sequence, not previously reported, were identified; t15373 and t15959 were assigned to these two *spa* types by the SeqNet curator. The predominant ST was ST22 (65%), followed by ST1 (11%). Of the 66 patients where a historic and recruitment isolate were available, 45

(68%) had an indistinguishable *spa* type on both occasions. Among persistent MRSA carriers (n=46/100), 42 patients had an indistinguishable *spa* type on recruitment and at the end of study. In decreasing order, hospitalisation, surgery, poor hospital hygiene, chronic illness, antibiotic use and contact with health care workers, are amongst the factors probably causing MRSA acquisition.

The comparative genotypic analysis demonstrated the diversity of MRSA colonising strains, thereby emphasising the necessity of surveillance and molecular analysis to identify new and resistant strains. Among persistent carriers where MRSA decolonisation is indicated such as during ITU admission and before high risk surgery, molecular and genetic analysis of the isolates directs the optimal decolonisation choice as well as treatment if necessitated

Identification of an indistinguishable *spa* type among two thirds of persistent MRSA carriers supports the widely held view that most persistent carriers are colonised with the same strain, rather than with new strains. This informs the interpretation of molecular and genotypic analysis of isolates from at risk patients when there is a considerable time lapse from when the patient was confirmed MRSA positive.

Patient MRSA perception questionnaire

A dearth of pre-existing IPQs necessitated the development of an instrument from pre-existing IPQs. This led to the modification of the BIPQ, an instrument that is tested for its content and logical validity, to the MRSAPQ, which was used to record patients' perceptions on their MRSA carriage.

The literature review on IPQs provided a new learning experience as well as insights into the psychological domain that impacts illness perception and patient outcome. This experience enabled me to acknowledge that an understanding of the disease can lead to more favourable health behaviours and disease outcomes. The analysis of the completed MRSAPQ shows that the study participants perceived MRSA colonisation a chronic condition and that it did not have serious consequences on their daily lives. In addition, participants

were in general emotionally detached from the condition and few had MRSA related symptoms. Interestingly, most participants felt they had limited or no control over carriage, but decolonisation was considered beneficial, indicating the importance attached to treatment/control of MRSA.

Most of the study participants, 93%, were previously known to be colonised with MRSA, and had experienced previous decolonisation attempts, some with success and subsequent re-colonisation, and others were not decolonised at all. The results must be considered in the context that most participants were known MRSA carriers, which would have influenced the participants' responses. A sub-optimal understanding of MRSA among nearly one half of the patients necessitates measures to improve knowledge about MRSA among patients and the public. Such measures should engage patients and their care givers so that patients attain a robust understanding of MRSA, acquisition and transmission, as well as the necessity for adherence to treatment/decolonisation, potentially leading to better patient outcomes.

Product experience questionnaire

A Likert style response PEQ, developed through an iterative process, was used to record patient perception on nasal application of Medihoney™ Derma Cream, and mupirocin 2% (Bactroban® 2%).

The results of the PEQ showed that patients perceived the nasal application of the MGH as pleasant, 19 (95%), not sticky on their nasal passages 18 (90%) and 17 (85%) did not have a runny nose. Concurrently, the control group, 13 (87%), were of the opinion that mupirocin 2% was easy to apply. However, 5 (33%) experienced a runny nose sensation after its application. This compares with unpleasant user experience of 39% previously reported, subsequent to nasal mupirocin use. (183)

In the intervention group, if a choice were available for patients, most of the respondents 17 (85%) preferred a natural product to an antibiotic. In the control group, if a choice was available, the antibiotic option was preferred only by 4 (27%), and a natural product was the preferred option for 8 (67%). Patients'

perception on the use of a non-antibiotic alternative in both the groups provides further ammunition to look for alternative treatment options.

4.3 Strengths and limitations of the study

Strengths

There are some strengths which facilitated the successful conduct of the NHNMRSA study. Firstly, the investigator's familiarity with the study setting as an IPC practitioner, and the investigating team's working relationship with clinicians as well as other stakeholders, provided a buy-in which contributed enormously to the success of the study. Investigator rapport with the IPC team, nurse managers and frontline staff facilitated optimal patient recruitment. Patients were recruited from a heterogenous population in a real world clinical setting. Scientifically sound randomisation and patient allocation principles were applied and patients were followed up as planned. The RCT results were reported in conformity to the consolidated standards for reporting of clinical trials (CONSORT) statement.

The findings substantiate previous findings supporting the antibacterial properties of honey and its potential for clinical use. For the first time in a RCT, honey was investigated for nasal MRSA eradication with a large sample size, well designed and meeting Cochrane and CONSORT reporting criteria in addition to independent statistical analysis.

Limitations

Clinical setting – tertiary acute/referral

Patients were recruited from a tertiary care hospital which is also a national referral centre. The wide geographic origin of patients in the study population limited recruitment to those the investigator could follow up for home/care centre visit. Therefore, many patients were excluded from recruitment, especially in the renal and neuroscience specialities.

Sample size

The number of study participants recruited was less than what was anticipated before study commencement. A sample size of 88 patients in each group was planned, but 50 in each group were enrolled within the time and resources that were available for the study. The less than planned sample size enrolled impacted on the statistical significance of the findings.

Single centre, time and resources

The study was limited to a single centre due to time and resources issues. In future, larger studies involving multiple centres should be conducted to confirm the results in order to recommend or dissuade the use of MGH as a valid choice for nasal MRSA decolonisation.

Concealment of products

One of the limitations of the study specifically is that the NHNMRSA was an open label study. After remote allocation the patient and the researcher were aware of the product allocated and the group the patient was assigned to. Therefore performance bias may have occurred. However, within the constraints of the study, allocation concealment and avoidance of detection bias, were the best that could be achieved in the RCT.

Generalisability of the RCT results

Limited options for decolonisation of MRSA necessitate the investigation of potential alternatives that demonstrate clinical benefit. The bactericidal action of the investigative product, i.e. 30% MGH, was established *in vivo.* The outcome of *in vitro* nasal MRSA decolonisation among a heterogenous population showed no statistical difference between the investigation and control groups. However, this may be due to the sample size, which was less than anticipated. Therefore, the results lack statistical power, even though the clinical outcomes do not show wide variation.

4.4 Clinical implications

The limited alternate options for nasal MRSA decolonisation place mupirocin at the cornerstone for most eradication programs to date, despite MR as well as suboptimal rates of successful decolonisation. Decolonisation approaches vary from search and destroy to universal decolonisation, as well as the management of individual clinical cases. The extent of MR in the wider context of antimicrobial stewardship has been well explored and presented in this thesis. The researcher will now consider the long-term impact of MR on three areas of mupirocin use: clinical cases, screen and treat and universal decolonisation, to estimate the relative transmissibility of MR and MSSA strains, and MRSA control policies.

A simulation model that evaluated the total prevalence of MR in colonised and infected MRSA patients in two London hospitals found that after 5 years of simulation MR was 9.1%, with the screen and treat mupirocin policy but this increased to 21.3% (95% CI 20.9%–21.7%) with universal mupirocin use. The modelling provides evidence derived from a real clinical setting of a fitness cost associated with MR in MRSA strains. Even under conservative estimates of relative transmissibility, the investigators predict long-term increases in the prevalence of MR where universal decolonisation policies apply. (185) What does this therefore mean? Should we limit MRSA prevention to screen and treat only? If so, what about persistent MRSA carriers. I will now consider these two scenarios and how the RCT results may provide direction in translating the evidence into practice.

MRSA screening strategies in many health systems now focus on targeted rather than universal screening of patients, such as in at risk patients, for example ITU admissions as well as before high risk surgery. Where MRSA decolonisation is ultimately unsuccessful, suppression therapy may be of short term benefit, if the patient becomes initially MRSA negative as it reduces the microbial load and thus potentially reduces the risk of infection. Is it safe to administer multiple courses of mupirocin? Stewardship as well as evidence of increasing MR does not support this approach. However molecular diagnosis and genotypic data on AMR resistance genes and or gene expression may help

direct therapeutic decisions. In certain patients, additional decolonisation courses or suppression therapy may be beneficial if bacterial susceptibilities are known, especially among persistent MRSA carriers. Increasing AMR and the prevalence of MDRO particularly in the high risk patient population, necessitates clinicians to target and therefore limit antibiotic use, i.e. conserve the antibiotic armoury to treat infections that are susceptible to that antibiotic. If a non-antibiotic choice is available it is a win-win situation for patients and society as the needs of the patient are met and antibiotics are preserved for society's use. The BIPQ results confirm user preference for non-antibiotic options, if that is clinically beneficial. However additional work is required before MGH can be safely recommended for MRSA decolonisation, which is further explored in the recommendations that follow.

4.5 Economic implications

The fitness cost model explored in the clinical implications section (Sec 4.4) neatly summarises the long-term impact of MR where universal decolonisation policies are applied. (185)

In Ireland (2012) the overall hospital acquired infection (HAI) prevalence was 5.2% (95% CI 4.7-5.6), highest in tertiary hospitals 7.5%, and in the host hospital it was 10.9%. Of the microorganisms that were implicated in HAI, *S. aureus* was the second most frequent pathogen detected (n=46; 15%), of which 37% were MRSA. (186) In Ireland in 2012, the average length of stay (LOS) for a hospital inpatient was 5.7 days, compared with an estimated LOS of 22 days when the HAI was contracted. The Health Service Executive (HSE) published figures for the cost of a day of stay in a public hospital (2010) as €899/day. An additional 16 days of inpatient stay translates to an extra €14,384.00. This does not include all other costs, such as productivity loss, as well as the pain and distress caused to the patient and family.

An independent review on AMR commissioned by the UK (2014), supported by the Wellcome Trust, commissioned RAND Europe (a not for profit research institute whose mission is to help improve policy and decision making through

research and analysis) to examine the likely global cost of AMR by the year 2050, if the AMR problem is not tackled. (187) Using a theoretical model called 'a dynamic general equilibrium model' the research team explored different global scenarios for AMR from now until 2050, to see what effect they would produce on the global economy. Five AMR organisms were considered in the model namely; MRSA, resistant *Escherichia coli*, *Klebsiella pneumoniae*, multi drug resistant-tuberculosis and human immuno-deficiency virus (HIV), from published data. The team considered the effects that AMR has on the economy through disruption of the supply of labour, by estimating the impact that an increase in resistance would have on the labour force through two mechanisms: increased morbidity that reduce the size of the working age population, and increased mortality that temporarily reduces the size of the workforce, and in severe cases to permanent reductions in labour productivity.

Failing to tackle AMR will mean that by 2050 the world population will be between 11 million to 444 million lower than it would otherwise be in the absence of AMR. The reduction in population and the morbidity impact would also reduce the level of world gross domestic product (GDP). That means by 2050 the world economy would be smaller by between 0.06% and 3.1%, a cumulative loss that ranges between $2.1 trillion and $124.5 trillion in the best and worst case scenarios. By 2050, failing to tackle AMR is expected to cause 10 million deaths attributable to AMR every year compared to other major causes of death, including cancer.

Delaying the development of widespread resistance by just 10 years could save 65 trillion US$ of the world's output between now and 2050, as estimated by the researchers. (187) Therefore the independent review calls policy and decision makers to look carefully at how to conserve the world's existing antibiotics and those developed in the future.

Sir Alexander Fleming in his address on receiving the coveted Nobel prize in 1945, reminded the world;

The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily under-dose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant'. (188)

Methicillin resistance was first reported in 1961. Was the common man able to buy penicillin in shops in the 1960s? Was it under dosing or broad antimicrobial cover that led to resistance? There may be many plausible answers, however, the logical and simple answer directs one to the reasons for the abuse of the antibiotic and non-lethal concentrations. Survival and the evolution of bacteria to adapt to the most challenging environments made them potentially the most successful living organisms. Bacteria adapt to external threats and have survived for long periods of time, and that will continue. Humanity must accept the fact that for bacteria, antibiotic exposure is no different to other threats which makes these living organisms able to adapt and survive. Therefore the principle of conservation and the conservative use antimicrobials should be the fundamental principle in our approach to containing resistance. Where alternatives are available, they should be used in the prevention and treatment of health conditions, and society must invest in research and development.

4.6 Reflections

The complexities involved in the conduct of an RCT opened many avenues of learning for me. Approvals and permissions such as those from the HPRA, Ethics Committee and indemnity provided administrative learning experience, which was complimented by that from setting up and conducting the RCT, patient interaction, data collection and data management aspects, enabled me to develop skill in niche areas. The skills development was complimented by academic learning in research methods and statistical analysis, scientific writing and leadership, publication, along with presenting results to scientific as well as other audiences. In comparison with multi centre RCTs the single centre NHNMRSA study offered me the opportunity to conceive, setup and conduct all aspects of the study. On reflection it was a challenging long journey that I walked through patiently, generously supported by the supervisors and many others who were experts in their own field.

To gain a comprehensive understanding on the antibiotic susceptibility testing methodology and the reporting of susceptibility, I wanted hands-on laboratory experience, i.e. to test mupirocin susceptibility, MIC and MBC assays. The

laboratory experience provided me with an opportunity to comprehend the complexities involved in specimen processing and results reporting. Performing these tests under the supervision of senior microbiology scientists using approved laboratory protocols was an invaluable learning experience, especially as such opportunities are seldom available for non-scientists outside of the research settings. The controls employed for media, storage, buffers, solutions, reference bacterial strains as well as in the interpretation of results provided a unique insight into the quality assurance methods utilised in diagnostic laboratories. The skill gained in performing these tests and the knowledge acquired undertaking the scientific study have enabled me to attain a profound understanding of the multifaceted aspects of MRSA prevention and control, and to correlate this with clinical scenarios.

In this journey, on reflection I recognise that every difficult situation was a learning experience, which reaffirmed me that the light is near and near, and to stick to the fight and not to quit.

4.7 Recommendations

The NHNMRSA RCT was ground breaking. To date, there are no studies published where MGH was used to investigate its potential for nasal MRSA decolonisation. I believed in the medicinal properties of honey and pursued the idea using the best possible approach, an RCT, the gold standard for clinical studies. To overcome the limitations outlined in this thesis, in future, larger studies involving multi-centres, acute hospitals as well as non-acute care settings, should be conducted, to replicate the results as well as to enrol a large heterogenous population so that the results can be applied to a wider population.

The potential for an aerosolised preparation for MGH and mupirocin administration should also be further investigated, in addition to further *in vitro* studies. Pharmaceutical and industry engagement is worth investigating for the aerosolised product development. This approach may offer future potential, both for pulmonary and nasal decolonisation.

Chapter V Bibliography

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Mupirocin resistance: clinical implications and potential alternatives for the eradication of MRSA

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Mupirocin 2% ointment is used either alone or with skin antiseptics as part of a comprehensive MRSA decolonization strategy. Increased mupirocin use predisposes to mupirocin resistance, which is significantly associated with persistent MRSA carriage. Mupirocin resistance as high as 81% has been reported. There is a strong association between previous mupirocin exposure and both low-level and high-level mupirocin resistance. High-level mupirocin resistance (*mupA* carriage) is also linked to MDR. Among MRSA isolates, the presence of the *qacA* and/or *qacB* gene, encoding resistance to chlorhexidine, ranges from 65% to 91%, which, along with mupirocin resistance, is associated with failed decolonization. This is of significant concern for patient care and infection prevention and control strategies as both these agents are used concurrently for decolonization. Increasing bacterial resistance necessitates the discovery or development of new antimicrobial therapies. These include, for example, polyhexanide, lysostaphin, ethanol, omiganan pentahydrochloride, tea tree oil, probiotics, bacteriophages and honey. However, few of these have been evaluated fully or extensively tested in clinical trials and this is required to in part address the implications of mupirocin resistance.

Background

Staphylococcus aureus is a leading cause of healthcare-associated infections worldwide and is associated with increased morbidity, mortality and higher healthcare costs, including infections caused by MSSA and MRSA.[1] Colonization with MRSA increases the risk of adverse health outcomes and it is estimated that 10%–30% of carriers subsequently develop infection.[2] The nose as well as extranasal sites such as the throat and perineum, skin ulcers and skin lesions are frequently colonized.[3–5] A meta-analysis concluded that MRSA colonization conferred a 4-fold increased risk of infection as compared with MSSA colonization.[6] Eradication of MRSA carriage from the nose and other body sites forms an integral part of strategies to prevent and control MRSA in many countries.[7–9] Mupirocin is an important component in MRSA prevention and specifically for the eradication of nasal MRSA. However, reports of increasing mupirocin resistance (MR) are of serious concern.

This review aims to determine the prevalence of MR and its clinical consequences as well as measures to control MR. It also reviews the evidence supporting the use of new agents as potential therapeutic alternatives for the prevention and management of MRSA.

Search strategy

The following databases were searched (date of last search: 30 March 2015): PubMed, CINAHL, Scopus and Web of Science. The search was limited to humans and English language publications from 1985 to March 2015. Search terms included multiple variants of the following terms: "*Staphylococcus aureus*, nose/nasal, colonisation/colonization, honey, infection control or prevention and control, wound, ulcer, surgical wound infection, topical, treatment, chlorhexidine, mupirocin and drug resistance" alone or in combination and/or 'infection'. Medical subject headings (MeSH) terms where available were used. Additionally, the reference lists of retrieved articles were scanned to identify any further studies. The titles and abstracts identified were screened for relevance by one author. The list of potential articles was reviewed to remove duplicates and full-text versions were obtained. Further articles were eliminated following review. The original articles were obtained and assessed in detail for inclusion. Articles included in this review are those that addressed mupirocin, i.e. infections associated with *S. aureus*, MRSA, decolonization, resistance, surveillance reports, systematic reviews or meta-analyses where the search terms appeared in the article title or abstract. From a total of 499 articles initially found, after exclusion

for reasons of unsuitability or duplicates, 88 articles remained for inclusion, including those identified from reference lists. The search process is illustrated in Figure 1.

Mupirocin use

The antibiotic mupirocin (pseudomonic acid A) is produced by the bacterium *Pseudomonas fluorescens*. Mupirocin calcium ointment was clinically introduced in the late 1980s and has proved to be one of the most successful topical antibiotics for the clearance of nasal *S. aureus*, both MSSA as well as MRSA.[5,10–12] Mupirocin is a competitive inhibitor of bacterial isoleucyl-tRNA synthetase and is active against most 'Gram-positive' and some 'Gram-negative' bacilli. Mupirocin-mediated inhibition of isoleucyl-tRNA synthetase impedes protein and RNA synthesis, ultimately leading to bacterial death. There is very little systemic absorption following the topical application of mupirocin. After systemic administration, mupirocin has a short half-life (15 min) and is rapidly converted into inactive monic acid, which is excreted principally through the kidneys.

The therapeutic indication for mupirocin is the elimination of nasal carriage of staphylococci, including MRSA. The method of application is nasal ointment, usually 2%, applied to the anterior nares two to three times daily. Nasal carriage is then normally cleared within 5–7 days of commencing treatment.[12,13] A systematic review that included 23 trials concluded that mupirocin applied two or three times daily for 4–7 days to both nostrils showed excellent efficacy and eradicated *S. aureus* in 90% of patients as assessed 1 week after treatment.[13] A meta-analysis in 2008 concluded that mupirocin appears to be cost-effective only in those patients who are proven nasal carriers, where a significant and strong reduction in *S. aureus* infection was confirmed.[12]

A significant limitation to the use of mupirocin is resistance, which reportedly ranges from 1% to 81%.[14–21] Mupirocin is also used by some clinicians for the treatment of local skin and soft

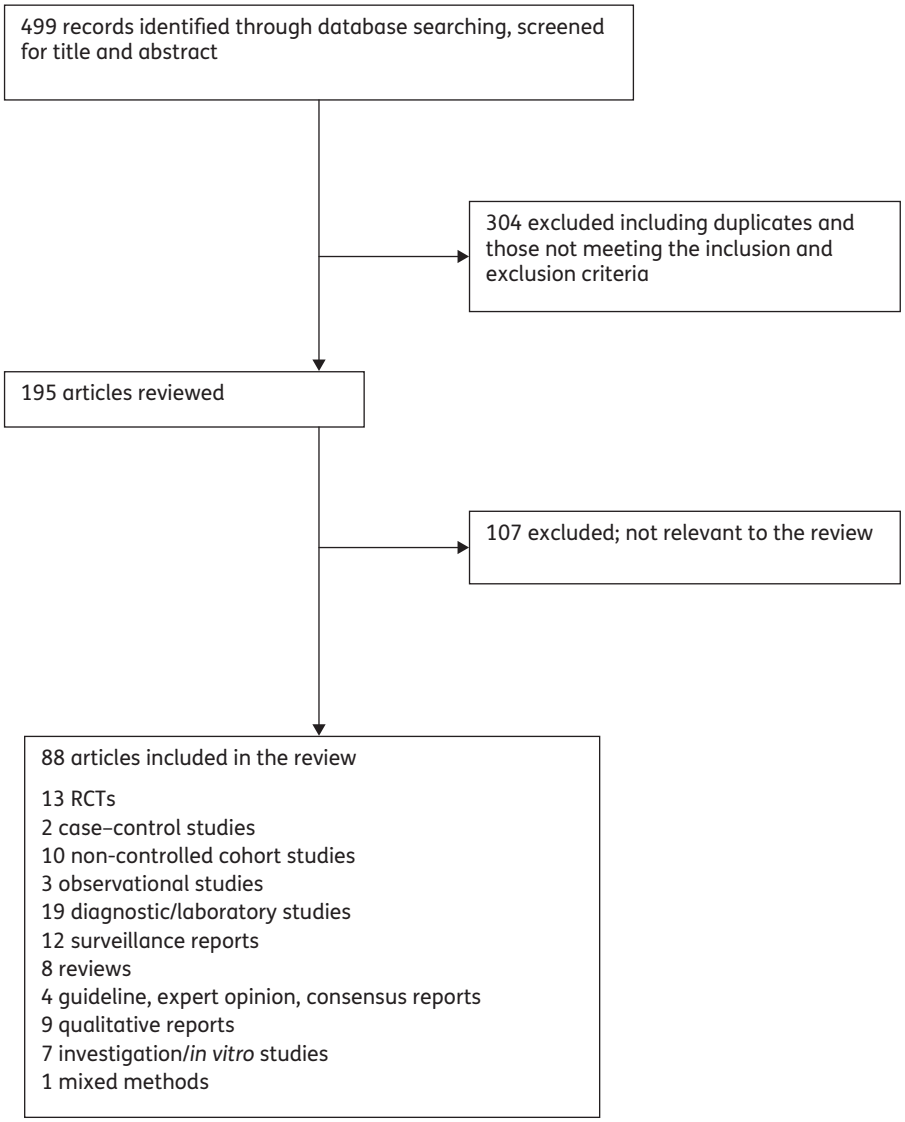

Figure 1. Search process and the number of relevant references.

tissue infections caused by *S. aureus* and streptococcal species, which also contributes to MR.[22]

Mupirocin 2% ointment is used for nasal decolonization alone or as part of a comprehensive MRSA decolonization strategy along with skin antiseptics such as chlorhexidine. The impact of the application of mupirocin to the nose has been investigated by various researchers with varying success, in terms of immediate as well as medium- to long-term sustained nasal MRSA decolonization.[5,23–25] In a multicentre trial in care homes, intranasal mupirocin ointment was compared with a placebo among persistent carriers of *S. aureus* and MRSA ($n=127$) with a follow-up period of 6 months. Mupirocin initially eradicated *S. aureus*, including MRSA in 60/64 (94%) compared with 54/63 (86%) in the placebo group, but after 90 days recolonization occurred in 39% of the mupirocin group.[24] In a study of 40 hospitalized patients, it was found that MRSA clearance was more common amongst patients with mupirocin-susceptible isolates (91%) than in those patients colonized with low-level MR (LLMR) and high-level MR (HLMR).[25] A double-blind, randomized, placebo-controlled trial in a tertiary healthcare facility evaluated the efficacy of mupirocin in eradicating nasal carriage of MRSA with body washing using chlorhexidine soap for other sites. At the end of follow-up, i.e. 4 weeks from the initiation of decolonization, 19/43 (44%) who received mupirocin were free of nasal MRSA compared with 11/44 (25%) in the control group.[5]

Mupirocin resistance

MR is very important for infection prevention and control personnel who are engaged in MRSA control efforts and also in the management of individual patients such as before major surgery to minimize post-operative MRSA infection, as the presence of resistance significantly reduces the likelihood of MRSA eradication.

Mechanisms

Phenotypically, MR is determined according to MIC breakpoints with susceptible being $\leq$4 mg/L, LLMR 8–256 mg/L and HLMR >512 mg/L.[21,26] Mupirocin MICs of 8–64 mg/L are usually due to non-synonymous changes in the native isoleucyl-tRNA synthetase gene. *S. aureus* isolates with an MIC of 128 or 256 mg/L are uncommon but are considered to demonstrate LLMR; these isolates have acquired base changes in the native isoleucyl RNA synthetase gene, *ileS*.[14,21,27] MICs of $\geq$512 mg/L reflect HLMR and this is mediated by the acquisition of a conjugative plasmid containing *mupA* (*ileS2*), which encodes an alternative isoleucyl-tRNA synthetase.[26,28] Plasmid-mediated HLMR can spread clonally and horizontally, even between different staphylococcal species.[29] In addition to the *mupA* gene, another mechanism of HLMR, mediated by a novel locus (*mupB*), has been reported.[30] The *mupB* gene (3102 bp) shares 65.5% sequence identity with *mupA*, but only 45.5% with *ileS*. The resultant MupB protein shares 72.3% and 41.8% similarity with MupA and IleS, respectively. These findings support the presence of non-*mupA*-mediated HLMR as reported by others.[21,30,31] Molecular studies of MR in *S. aureus* populations indicate that nearly all *S. aureus* isolates with HLMR have the *mupA* gene.[27] However, low or non-expression of the *ileS2* gene has been described amongst LLMR isolates.[32] Although *ileS2* does not encode resistance to other antibiotics, the presence of *ileS2*-carrying plasmids has been associated with resistance to antibiotics such as clindamycin, tetracycline, erythromycin and levofloxacin.[33] A recent review of the presence of *ileS2* in CoNS bloodstream infection (BSI) isolates found that the increase in the percentage of CoNS isolates carrying *ileS2* (8% in 2006 to 22% in 2011; $P=0.01$) was correlated with increased mupirocin use in each of the corresponding years (3.6 kg/year in 2006 to 13.3 kg/year in 2010).[34] Widespread acquisition of MR following nasal decolonization with mupirocin among CoNS is reported from the Netherlands and higher MR among CoNS is reported from a prevalence survey in France.[35]

Prevalence

Increased mupirocin use predisposes to both LLMR and HLMR.[14,17,18,35–43] Some of the larger studies are outlined in Table 1. In a Canadian study, the proportion of MRSA strains with HLMR increased from 1.6% in the first 5 years of surveillance (1995–99) to 7.0% (2000–04). The pattern of mupirocin use during the study periods is not described, but the investigators acknowledge the widespread use of mupirocin in their institution.[14] Another study in a tertiary care facility in the USA over 18 months reported MR amongst positive MRSA patients on hospital admission in 20/591 (3.4%); HLMR occurred in 0.62% and LLMR in 2.9%.[36]

A surveillance programme carried out over 2 years in a 24 bed surgical ICU between December 2002 and December 2004 in Missouri, USA, with a low level of mupirocin use, detected MRSA in 13.6% ($n=338/2840$); 13.2% of 302 isolates were MR, 8.6% being HLMR and 4.6% LLMR.[37] A nationwide prospective study of MR amongst staphylococcal isolates in France between October 2011 and February 2012 reported a resistance rate of 10.3% amongst 708 isolates of CoNS, mainly HLMR (5.6%). Among the MRSA isolates, 2.2% ($n=8$) were MR, of which 0.8% were HLMR and carried the *mupA* gene.[35] Another study compared MR during two time periods in a 500 bed tertiary hospital in Brazil. In the first period (1990–95), when mupirocin was used extensively including application to any skin wound comprising <20% of body surface, 28/43 (65%) MRSA infections were caused by MR isolates, which decreased to 15% when mupirocin use was restricted to only patients colonized in the nose (1996–2000).[38] The effect of mupirocin ointment for nasal decolonization along with other infection prevention and control measures was evaluated in a study during an MRSA epidemic in a Canadian hospital. There was a significant increase in MR, from 2.7% to 65%, between the beginning of the first year at the onset of the epidemic (1990) and the end of the third year.[39] Similar findings have been reported from another study in Brazil in two tertiary care university hospitals, in one of which there was extensive use of mupirocin and where 72/114 (63%) of isolates were MR, compared with the second hospital in which mupirocin use was controlled and where only 3/49 (6.1%) were MR.[18]

The emergence of HLMR MRSA following the use of mupirocin for prophylaxis at intravenous exit sites to prevent local infection and BSI was reported in 3% of patients on a peritoneal dialysis unit after a 4 year period of continuous use.[40] In a screening programme in Shanghai and Wenzhou (China), 53/803 (6.6%) isolates that were MR MRSA over a 3 year period were HLMR with the *mupA* gene detected by PCR.[41] In a prevalence study in a tertiary care hospital in Singapore, HLMR was reported from 34/307 (11%) isolates; 14% from screening isolates and 10% from clinical

Table 1. Larger studies on the prevalence of MR

Author	Year	Country	Study description	Patient population	No. of patients/no. of isolates	Molecular methods used	Prevalence (%)		
							LLMR	HLMR	overall
Miller *et al.*[39]	1990–93	Canada	LS	hospital	231/310	NA	NA	NA	2.7–65
Vivoni *et al.*[38]	1990–95	Brazil	LS	hospital	43/43	PCR, PFGE	NA	NA	65
Vivoni *et al.*[38]	1996–2000	Brazil	LS	hospital	89/108	PCR, PFGE	9	6	15
dos Santos *et al.*[18]	1995–2004	Brazil	PS	hospital	62/114	NA	NA	NA	6.1–63
Simor *et al.*[14]	1995–99[a]	Canada	LS	hospital	NA/4980	PCR	6.4	1.6	NA[a]
	2000–04[b]						10	7	NA[b]
Jones *et al.*[37]	2002–04	USA	PS	hospital, SICU	338/302	PCR	4.6	8.6	13.2
Liu *et al.*[41]	2005–07	China	LS	hospital	NA/803	PCR	0	6.6	6.6
Babu *et al.*[36]	2008	USA	PS	hospital	948/591	NA	2.9	0.6	3.4
Choudhury *et al.*[17]	2010	Singapore	PS	hospital	NA/307	PCR	0	11	11
Park *et al.*[42]	2011	Korea	PS	hospital, NICU	101/101	PFGE	0	47 CA/79 HA	73
Walker *et al.*[19]	2004	USA	LS, 5 eras	hospital, mixed population	50–100 isolates per era, random	PCR	28	31	67
Caffrey *et al.*[16]	2010	USA	retrospective case–control	hospital	310/40 cases MR and 270 controls mupirocin susceptible	NA	NA	NA	NA
Cadilla *et al.*[15]	2011	USA	LS	hospital	837 isolates (191 MDR and 646 non-MDR)	PCR	0	6.8 MDR	NA
Lee *et al.*[20]	2011	Switzerland	nested case–control	hospital, acute	150/75 cases and 75 controls; HLMR was excluded from the study	Etest, PCR	NA	NA	9–81
Desroches *et al.*[35]	2013	France	PS	hospital, national surveillance	NA/367	PCR, PFGE, microarray	1.4	0.8	2.2

LS, laboratory surveillance; NA, not available; PS, prospective surveillance; SICU, surgical ICU; NICU, neonatal ICU; CA, community acquired; HA, hospital acquired.
[a]Period 1.
[b]Period 2.

isolates during 2009–2010.[17] HLMR was also reported from a neonatal ICU in Korea where 101/223 (45%) of admissions were MRSA positive; of these, 79% of heathcare-associated MRSA isolates and 47% of community-acquired MRSA were HLMR.[42]

A multicentre study in care homes involving 3806 residents in the USA over 30 months detected MR in 101 (12%) isolates; HLMR in 78 (9%) isolates and LLMR in 23 (3%) isolates.[43] In a review of 240 MRSA isolates recovered over 20 years from patients who had failed decolonization, MR was identified in 63% of the isolates.[44]

In a matched case–control study of 40 cases with MR MRSA and 270 controls without MR MRSA during 2004–08, prior exposure to mupirocin in the preceding year was the most significant independent predictor for both LLMR and HLMR.[16]

In the Netherlands, widespread acquisition among CoNS of MR following nasal decolonization with mupirocin has been reported.[34,45] In the first study (2012), among the 238 CoNS BSI isolates, *Staphylococcus epidermidis* was most prevalent [150 isolates (63%)] and it was also the most common species amongst HLMR isolates, i.e. 25 isolates. In the latter report, a nasal decolonization study (2015), among the 607 CoNS isolates collected from 469 patients post-decolonization with mupirocin, 588 (97%) were HLMR. *S. epidermidis* was most prevalent with 568 isolates (94%).[45] A review of the clinical implications of MR among *S. aureus* suggests that unrestricted over-the-counter use and treatment of wounds and pressure sores with mupirocin are strongly associated with resistance.[22]

Associated chlorhexidine resistance

In most MRSA infection prevention programmes, chlorhexidine is a major component and is often used in various forms as part of oral care, skin antisepsis prior to intravascular device placement, before surgical procedures, during patient bathing and as a component of some antimicrobial-impregnated catheters and dressings. As with any antimicrobial or antiseptic agent, increased use raises concerns about emerging chlorhexidine resistance and its implications for MRSA decolonization strategies. Chlorhexidine is a biguanide cationic bactericidal agent that is rapidly taken up by *S. aureus*.[46,47] Chlorhexidine gluconate (CHG) is a topical antimicrobial agent with broad-spectrum activity, including against *S. aureus*. At low concentrations it disrupts the integrity of the cell wall and membranes, resulting in leakage of the intracellular contents; at high concentrations, chlorhexidine causes coagulation of the intracellular contents. Significant reductions in central line-associated BSI were observed when CHG was used for procedural skin preparation.[48] Bacterial resistance to chlorhexidine was initially reported in 1995.[47] Resistance to chlorhexidine is conferred by two gene families, *qacA/B* and *smr*.[49] These plasmid-mediated *qacA/B* genes encode proton-dependent multidrug efflux pumps, expression of which results in high-level resistance to antiseptics, whereas the *smr* gene confers low-level resistance.[20,47,50] MRSA isolates carrying the *qacA/B* gene initially belonged to a single clone, but the *qacA/B* gene has been detected in MRSA isolates belonging to seven different clones from different countries.[20,43,44,51–53]

Concomitant resistance to other antiseptics and systemic antibacterial agents presents additional challenges in terms of decolonization strategies. For example, a strong association has been reported between HLMR and resistance to at least four non-β-lactam antimicrobial classes.[15] In that study, the investigators also identified that *mupA* was significantly more likely to be carried by isolates resistant to gentamicin, rifampicin or trimethoprim/sulfamethoxazole ($P<0.0001$) in comparison with erythromycin-, clindamycin- or ciprofloxacin-resistant isolates ($P=1.00$, $P=0.30$ and $P=0.07$), respectively.

Very high prevalence of *qacA* and/or *qacB* MRSA isolates has been reported from Taiwan where chlorhexidine has been used for >20 years for hand hygiene; of 240 isolates obtained during 1990–2005, the proportion of MRSA isolates with a chlorhexidine MIC of ≥4 mg/L was 1.7% in 1990, 50% in 1995, 40% in 2000 and 46.7% in 2005. Among these isolates, 46/83 (55.4%) carried the *qacA/B* gene. In addition, *qacA* and/or *qacB* were identified in 91% of MRSA isolates from patients who had failed decolonization.[44] Similar findings were reported from a secondary and tertiary hospital in Korea over 4 years among the MR MRSA isolates; the *qacA/B* and *smr* genes were detected in 65% of isolates.[51] A nested case–control study of MRSA decolonization found that combined LLMR and the presence of chlorhexidine resistance significantly increased the risk of persistent MRSA carriage.[20] However, the investigators reported that chlorhexidine resistance alone did not predict persistent carriage, suggesting that the combination of LLMR and chlorhexidine resistance may be necessary for clinical failure, i.e. persistent colonization. In practice, both these agents (mupirocin and CHG) are often administered concurrently as part of MRSA decolonization regimens. Studies evaluating chlorhexidine resistance and MRSA and the clinical significance are outlined in Table 2.

Controlling MR

In controlling MR, Patel *et al.*[21] proposed three approaches. First, additional studies are needed to quantify the efficacy and unintended consequences of mupirocin use as a prevention strategy. Second, a strategy for monitoring the prevalence of resistance should be developed and implemented whenever mupirocin is routinely used. Third, monitoring should not only focus on MR itself, but also should help determine whether mupirocin use might amplify the spread of other MDR via its linkage to other resistance determinants.[21] There may be a benefit in incorporating MR surveillance as part of ongoing surveillance programmes such as EARSS-Net, which monitors antibiotic resistance amongst invasive isolates of MRSA, i.e. in BSI. While these do not represent isolates from the nose or other carriage sites, they are representative of isolates responsible for serious infection throughout Europe and from a population in which many have had or will be undergoing MRSA decolonization.

The assessment of mupirocin susceptibility amongst isolates of MRSA varies. While most centres determine and report mupirocin susceptibility when MRSA is initially isolated from an individual patient, the ongoing testing of repeat isolates from the same patient varies. For persistent MRSA carriers, mupirocin MIC testing should be repeated to assist in informed decision making and provide the potential opportunity to impact on the control of resistance. Point prevalence surveillance in centres where mupirocin is widely used and/or resistance is reported is also indicated.

Control of mupirocin use, i.e. targeted decolonization in selected patients based on risk assessment rather than the decolonization of all MRSA-positive patients, has proved an effective strategy to combat MR. For example, in the ICU there may be little point in attempting to eradicate upper respiratory tract

Table 2. Studies evaluating MR and chlorhexidine resistance and their clinical significance

Author	Year	Country	Study design	Patient population	MRSA/ MSSA	No. of patients/ isolates	Molecular method	Follow-up	Prevalence of MR	Prevalence of *qacA/B*, *smr*	Comments
Wang *et al.*[44]	2008	Taiwan	longitudinal analysis	hospital	MRSA	240 BSI and clinical isolates	MLST	NAP	NA	1.7% (1990), 46.7% (2005)	83/240 had high CHX MIC >4 mg/L, 55.4% carried *qacA/B*
Vali *et al.*[53]	2008	UK (Scotland)	longitudinal analysis	hospital	MRSA	120 clinical isolates	PCR	NAP	NA	8.3% ($n=10$) *qacA/B*, 44.2% ($n=53$) *smr*	high resistance to AMX, GEN, OXA, CTX, CXM and CIP; low resistance to TET; none resistant to VAN
Lee *et al.*[20]	2011	Switzerland	nested case–control	hospital	MRSA	150: 75 cases and 75 controls	PCR	2 years	LLMR present in all *qacA/B*-positive isolates	91% cases ($n=68$), 68% controls ($n=51$) *qacA/B*	HLMR excluded
Longtin *et al.*[46]	2011	Canada	longitudinal analysis	hospital ICU	MRSA	234 isolates	PCR	NAP	NA	2% ($n=7$) *qacA/B*, 7% ($n=23$) *smr*	
McDanel *et al.*[43]	2013	USA	longitudinal analysis	nursing homes ($n=26$)	MRSA	3806 patients, 829 MRSA isolates	PCR	NAP	3% ($n=23$) LLMR, 9% ($n=78$) HLMR	0.6% ($n=5$) *qacA/B*	all five were resistant to CLI, ERY, LVX, TET, SXT and GEN
Lee *et al.*[51]	2013	Korea	longitudinal analysis	hospital ($n=2$)	MRSA	456 isolates	MLST, PCR	NAP	12% ($n=53$) LLMR, 2% ($n=9$) HLMR	65% ($n=40$) *qacA/B*, 71% ($n=44$) *smr*	all MR isolates resistant to ERY, CLI, GEN and CIP; none resistant to VAN
Fritz *et al.*[52]	2013	USA	longitudinal analysis	hospital	MSSA and MRSA	1089 patients/ 696 isolates	PCR	NAP	2.1% ($n=23$) at baseline to 4.5% ($n=31$)	0.9% ($n=10$) at baseline to 1.6% ($n=11$) *qacA/B*	isolates resistant to CLI were more likely to be MR compared with CLI-susceptible isolates; CHX resistance was not associated with resistance to other systemic antibiotics

NAP, not applicable; NA, not available; CHX, chlorhexidine; AMX, amoxicillin; GEN, gentamicin; OXA, oxacillin; CTX, cefotaxime; CXM, cefuroxime; CIP, ciprofloxacin; TET, tetracycline; VAN, vancomycin; CLI, clindamycin; ERY, erythromycin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole.

colonization, including nasal carriage, in a patient who is intubated and ventilated given the presence of an endotracheal tube or a tracheostomy to which the bacteria will adhere and form a biofilm. Targeted decolonization therefore involves an antimicrobial stewardship programme, healthcare worker education and refresher training, surveillance, feedback and electronic alerts.[19] There was a precipitous decline in the number of isolates with HLMR (from 31% to 4%) and also LLMR (from 26% to 10%) after measures were introduced to control or limit the use of mupirocin over 2 years in a mixed healthcare setting that included acute, domiciliary and nursing homecare.

Similar reductions in MR following the control of mupirocin use were reported from a neonatal unit in the Netherlands by Zakrzewska-Bode *et al.*[54] where the routine application of mupirocin to central vascular catheter insertion sites was discontinued. Finally, in Western Australia, susceptibility testing of *S. aureus* isolates was mandated from 1993 and restricted mupirocin use led to similar reductions, where MR decreased from 6.4% ($n=16$) in 1994 to 0.3% ($n=3$) in 1997.[55]

Recent years have seen an emphasis on horizontal infection prevention and control approaches, i.e. applying measures to a whole population rather than to those at risk. An example of this is the use of mupirocin and chlorhexidine applied to all patients in an ICU compared with their use in those patients screened and found to be positive for MRSA. The case for the universal decolonization approach in ICU settings may inevitably contribute to higher MR as well as resistance to chlorhexidine.[56] The independent effect of mupirocin could not be distinguished from the combined mupirocin/chlorhexidine effect in the same study. The downside of universal decontamination is the unnecessary use of mupirocin in 70%–80% of the patients not carrying *S. aureus*, potentially enhancing resistance in CoNS and creating a reservoir of MR for *S. aureus*.[22] A systematic review on chlorhexidine body washing reports that evidence is lacking that it reduces carriage or infections with antimicrobial-resistant bacteria.[57] Consequently, if this practice becomes more widespread, it will be essential to monitor for the emergence and spread of both MR and chlorhexidine resistance.

In a multicentre, cluster-randomized, non-blinded crossover trial, the effect of daily bathing with chlorhexidine-impregnated washcloths on the acquisition of MDR organisms and the incidence of hospital-acquired BSI was evaluated.[58] The overall rate of hospital-acquired BSI was 4.78 cases per 1000 patient-days with chlorhexidine bathing versus 6.60 cases per 1000 patient-days with non-antimicrobial washcloths ($P=0.007$), a 28% lower rate with chlorhexidine-impregnated washcloths. However, when analysed by individual organism, there were no significant reductions in MRSA acquisition or *S. aureus* BSI. In a similar trial, paediatric ICU patients demonstrated a 36% reduction in BSI with 2% CHG bathing, which failed to achieve significance in the ITT analysis. In that study, the incidence of BSI was lower in patients receiving CHG bathing compared with standard practices.[59]

Other antimicrobial agents

Bacitracin ointment, usually in combination with polymyxin B and neosporin (e.g. polysporin), has been studied as a potential decolonization strategy for MRSA and the results have not been as encouraging as those for mupirocin. In a double-blind, randomized controlled trial (RCT), bacitracin was compared with mupirocin and all the patients received daily CHG body washes. Only 15/49 (30.6%) patients in the polysporin arm were MRSA negative at all sites at 48 h, compared with 35/54 (64.8%) of those given mupirocin.[60]

Retapamulin is a pleuromutilin (a new class of antibiotic) that exhibits activity against various skin bacteria including MSSA and MRSA and is used for the treatment of impetigo. An *in vitro* study assessed susceptibilities amongst various MSSA and MRSA strains from acute and chronic wounds to commonly used topical antimicrobial agents. The investigators found that mupirocin treatment was the most effective antimicrobial, with areas of inhibition ranging from 30.34 to 61.70 cm^2 ($P<0.05$), as compared with the next most effective, retapamulin, with areas of inhibition ranging from 11.97 to 23.54 cm^2.[61] Another study reported that retapamulin had good activity against 15/16 (94%) of MR isolates.[62] A recent double-blind RCT concluded that the clinical success rate in the treatment of secondarily infected traumatic lesions amongst patients with MRSA was significantly lower with retapamulin compared with linezolid.[63]

Alternative approaches to decolonization

The increasing prevalence of MR and associated chlorhexidine resistance means that alternative agents to decolonize patients with MRSA need to be considered. In 2009, Coates *et al.*[12] discussed alternatives that were in various stages of development with a diversity of mechanisms, but had yet to be proved efficacious in clinical trials. While considering the alternatives, the investigators were of the opinion that a more bactericidal antibiotic than mupirocin is needed, on the grounds that it might reduce the relapse rate and so clear the patient of MRSA for a longer period of time than mupirocin.[12] Oral antimicrobials for decolonization of MRSA carriage may be considered in certain populations (e.g. multiple sites of colonization) or under specific circumstances (e.g. prior to surgery); however, the risk of resistance to oral therapy or systemic side effects must be carefully considered. This is beyond the scope of this review and below we focus on emerging promising topical agents.

Octenidine dihydrochloride

Decolonization of the nose and other body sites has been investigated using octenidine dihydrochloride body wash along with the intranasal application of 2% mupirocin. The efficacy was highest in the nose, where decolonization was successful in 28/32 (87.5%), and in the decolonization of extranasal sites it was successful in 18/32 (56.3%) of patients.[64]

Polyhexanide

The efficacy of polyhexanide (Prontoderm®) Gel Light nasal ointment, body foam and mouthwash was retrospectively compared with the success rate achieved with a chlorhexidine and mupirocin regimen. Persistent MRSA was identified among 51/72 (71%) of those who underwent the Prontoderm® regimen compared with 20/44 (45%) of those who underwent the chlorhexidine and mupirocin regimen.[65]

Ethanol

The bactericidal activity of 70% ethanol combined with emollients and a preservative (Nozin Nasal Sanitizer), when applied to the nasal vestibules of *S. aureus*-colonized volunteers, was compared with a placebo. The nasal application was performed at 0, 4 and 8 h during the course of a normal workday. The researchers reported a significant reduction in nasal vestibular carriage of both *S. aureus* and other cultivable bacteria in the antiseptic group. The reductions were very consistent, with a median decrease in the antiseptic-treated group of 98.8% at the end of the normal (10 h) workday. The investigators claim that the ethanol-based antiseptic provides a unique opportunity for regular daily use over prolonged periods by patients and staff in long-term care environments as it is unlikely to contribute to bacterial resistance.[66]

Sodium hypochlorite

Sodium hypochlorite (bleach) was originally described in 1915 by Dakin and has since been used extensively as a topical antimicrobial for the treatment of wounds and burns. IDSA guidelines recommend nasal mupirocin and dilute bleach baths for 15 min twice weekly for 3 months as treatment for patients with refractory MRSA skin and soft tissue infections.[67] An RCT comparing various decolonization regimens using mupirocin, chlorhexidine and bleach on patients with community-based skin and soft tissue infections and multisite *S. aureus* colonization revealed that the highest rate of successful *S. aureus* eradication (71%) in patients occurred with a combination of nasal mupirocin and daily bleach baths.[68]

Lysostaphin

Lysostaphin is a glycylglycine endopeptidase that cleaves the cross-linking pentaglycine bridges in the cell walls of staphylococci. In an animal model, a single application of 0.5% lysostaphin cream eradicated MSSA and MRSA from the nares of animals more effectively than mupirocin.[69] In 24 h time–kill studies, lysostaphin has also been found to be superior to mupirocin and tea tree oil.[70] However, to date, there have been no studies in humans and its potential remains to be confirmed.

Omiganan pentahydrochloride

Omiganan pentahydrochloride is a novel topical cationic peptide active against a broad spectrum of bacteria and yeast. An *in vitro* study has demonstrated potent activity against *S. aureus* regardless of the underlying resistance mechanism. The observation that omiganan remains equally active against all isolates of *S. aureus* at a level significantly below the clinical formulation concentration (1% gel) is promising and warrants further studies.[71]

Natural honey

Honey is of interest to healthcare practitioners involved with wound management and reduces the numbers of MRSA in open wounds.[72–76] An *in vitro* study of four types of honey, three sourced from Northern Ireland and one from Suisse Normande, France, found that they reduced the bacterial count of community-acquired MRSA isolates.[77] Similar findings are reported elsewhere when medical-grade honey was applied to chronic wounds.[76,78] The antibacterial properties of honey vary between different geographic regions and floral species.[79]

Tea tree oil

Tea tree (*Melaleuca alternifolia*) oil has also been investigated as an antimicrobial and anti-inflammatory agent. Edmondson *et al.*[80] investigated the efficacy of tea tree oil for the decolonization of wounds positive for MRSA in 12 patients and concluded that although wounds in most cases showed signs of healing, they remained persistently colonized with MRSA. In another study, a tea tree oil-based regimen was compared with standard treatment consisting of mupirocin, chlorhexidine or silver sulfadiazine.[81] Of the patients who received standard treatment, 56/114 (49%) were cleared of MRSA carriage. Of the patients who received the tea tree oil regimen, 46/110 (42%) were cleared. Mupirocin was significantly more effective at clearing nasal carriage than tea tree cream (78% versus 47%; $P=0.0001$). However, tea tree oil treatment was more effective than chlorhexidine or silver sulfadiazine in clearing superficial skin sites and skin lesions of MRSA. A Phase 2/3 RCT in two ICUs evaluated the effect of daily washing with tea tree oil (Novabac 5% skin wash) compared with standard care with a baby soft wash (Johnson's Baby Softwash) on the incidence of MRSA colonization. There was no statistical difference between the two approaches. The investigators therefore did not recommend tea tree oil as an effective means of MRSA decolonization.[82] Tea tree oil has been reported to cause allergic dermatitis in addition to gynaecomastia, probably owing to its oestrogenic and antiandrogenic properties, and should therefore be used with caution.[71]

Probiotics

The potential of probiotics as agents for MRSA decolonization was investigated by Sikorska *et al.*,[83] who reported that many strains of lactobacilli and bifidobacteria isolated from a variety of sources inhibited *in vitro* the growth of *S. aureus* including clinical isolates of MRSA, suggesting that further research is warranted including clinical trials.

Silver

The successful topical application of silver agents (Acticoat 7®, Smith & Nephew) in treating MRSA surgical site infection ($n=2$) without systemic antibiotics as well as with gentian violet (0.5%) for skin lesions ($n=28$) and for the eradication of nasal carriage ($n=9$) has been described.[84]

Bacteriophages

Bacteriophage therapy could also be an alternative to antibiotics for the treatment of chronic MRSA infections, as success has been reported both in treating infections ($n=6$) as well as eradication of MRSA carrier status in a healthcare worker.[85] The potential for an engineered *Staphylococcus*-specific phage lysin (ClyS) to be used for topical decolonization was investigated in a mouse model.[86] ClyS eradicated a significantly greater number of MSSA and MRSA with a 3 log reduction compared with a 2 log reduction

with mupirocin. The use of ClyS also demonstrated a decreased potential for the development of resistance amongst MRSA and MSSA compared with mupirocin *in vitro*. Another agent, P128, a chimeric protein that combines the lethal activity of two enzymes, consists of a phage tail-associated muralytic enzyme of phage K and the staphylococcal cell wall-targeting domain (SH3b) of lysostaphin. Using the broth microdilution method, the investigators found that P128 was active against *S. aureus* clinical strains including MRSA, MSSA and MR MRSA. MBCs and MICs of P128 (1–64 mg/L) were similar across the 32 *S. aureus* strains tested, demonstrating its bactericidal nature. In time–kill assays, P128 reduced cfu by 99.99% within 1 h and inhibited growth up to 24 h.[87] Evidence that phages can effectively combat experimentally induced *S. aureus* infections in animals warrants further study in clinical trials.[88]

Overall, there is a paucity of studies on alternative agents for eradication of MRSA, such as alcohol-based agents, omiganan pentahydrochloride, lysostaphin, honey, bacteriophages and other agents. Clinical trials are warranted to confirm their potential before such agents can be routinely used for MRSA decolonization.

Conclusions

Nasal carriage of MRSA is a recognized risk factor and a precursor for invasive infection. Clinical trials report that of all the various topical treatments used for the eradication of MRSA from the nose, mupirocin is the most effective. Increasing MR, either alone or combined with chlorhexidine resistance, means that ongoing monitoring of resistance is necessary, especially where there is widespread and even indiscriminate use of decolonization regimens. Before application, LLMR is significantly associated with persistent MRSA carriage and in addition there is a strong association between previous mupirocin exposure and both LLMR and HLMR. An association exists between HLMR (*mupA* carriage) and MDR. The presence of *qacA* and/or *qacB* and MR is another factor associated with failed decolonization.

The emergence of HLMR in CoNS isolates indicates an expanding reservoir of plasmids encoding MR, which can be transferred to other CoNS strains as well as to *S. aureus* including MRSA. HLMR and resistance to other antibiotics amongst CoNS may result in a reduction in oral antibiotic options for prolonged treatment of prosthetic infections with CoNS. Mupirocin should be used with caution if at all in patients with chronic extranasal colonization and should be limited to one or two 5 day courses of nasal application to reduce the emergence of resistance. However, the outcome following repeated courses of mupirocin application in the same patient is not explicit in the studies evaluated. We may eventually reach a point, or have done so already in some centres, where the benefits from mupirocin use are restricted to a minority of patients. Persistent colonization in the setting of MR may still be associated with reduced numbers of colonizing bacteria after attempts at decolonization, thus helping to reduce the risk of infection compared with patients colonized with MRSA who have not undergone decolonization of any kind. New antimicrobial therapies either on their own or in combination with alternative therapies are needed. There are very few antibacterial agents with new mechanisms of action under development to meet the challenge of MDR. The EU identifies a widening gap between the burden of infections due to multidrug antibacterial resistant organisms and the development of new systemic agents as a key priority.[89] This also applies to topical agents used in decolonization. Tea tree oil, medical-grade honey, bacteriophages and other natural agents with antiseptic and antibacterial properties show promise but need to be further evaluated. Many of these have been initially developed in the academic sector or by small commercial enterprises, neither of which usually has the resources to develop the agents further and to carry out the necessary clinical trials to confirm or not their usefulness in the clinical arena. Consequently, there is a need for national and international agencies to sponsor further studies and evaluations.

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The beliefs and emotional response of patients colonised with MRSA

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RCSI DEVELOPING HEALTHCARE LEADERS WHO MAKE A DIFFERENCE WORLDWIDE

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Background

Understanding an illness (MRSA) can lead to more favourable health behaviours and disease outcomes. The Brief Illness Perception Questionnaire (BIPQ) is a simple and rapid assessment of illness perceptions. It includes eight dimensions; perceived consequences, timeline, personal control, treatment control, identity and concern, coherence, emotional representation and an open ended question about cause, rated on an 11 point scale to assess the perceived importance.

Aims

- To assess patients beliefs and perceptions on MRSA carriage.
- To assess patients beliefs on the source of acquisition of MRSA.

Methods

Questionnaire. A modified BIPQ was administered to patients participating in a RCT of two approaches to nasal MRSA decolonisation. Ethical approval for the study was granted by a Recognised Ethics (Medical Research) Committee.

Results

Results -1

- The survey response rate was 87.5% (n=35).
- Mean age 73.3 y & 35% were females.
- The ranked order of importance the participants assigned to the 11 point scale is displayed in table 1 and figure 1.
- The mean, median and inter-quartile range (IQR) of each item scores are displayed in table 2.
- Hospitalisation, surgery and poor hospital hygiene were mostly associated as the cause of MRSA acquisition, as shown in figure 2.

BIPQ elements	Rank of importance		
	Less important (0-4)	Mid score (5)	Most important (6-10)
Consequences	66%	8.00%	26.00%
Timeline	8.50%	8.50%	83.00%
Personal control	94.00%	0	6.00%
Treatment control	0	0	100%
Identity	77.00%	6.00%	17.00%
Concern	20%	9.00%	71.00%
Coherence	43.00%	23.00%	34.00%
Emotional representation	77.00%	11.50%	11.50%

Table 1: Ranked order of importance

Most patients (94%) believed that they did not have any control of their MRSA, and all respondents assigned the maximum score denoting the importance of treatment/control.

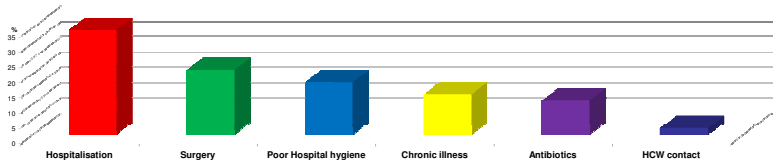

Figure 2: Ranked order of MRSA Acquisition

In decreasing order, hospitalisation, surgery, poor hospital hygiene, chronic illness, antibiotic use and contact with HCW, were felt to be responsible for MRSA acquisition.

Results - 2

- MRSA colonisation had little or no consequence for 74% (26) patients.
- 83% (29) believed that colonisation would continue forever.
- Most (94%) believed that they did not have any control of their MRSA.
- All respondents assigned the maximum score for treatment/control.
- Only 23% (8) patients experienced symptoms they related to MRSA.
- MRSA was a matter of concern for 80% (28).
- More than one half, 57% (20) had a good understanding of MRSA.
- Most patients 77% (27) were not emotionally affected by MRSA carriage.

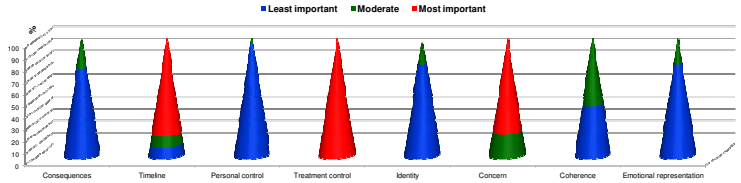

Figure 1: Beliefs and perceptions on MRSA carriage

BIPQ elements	Mean	Median	IQR
Consequences	2.94	1	0-5.5
Timeline	8.69	10	8.5-10
Personal control	0.77	0	0
Treatment control	9.69	10	10
Identity	2.09	0	0-4
Concern	6.83	7	5-10
Coherence	4.77	5	3-6
Emotional representation	2.74	2	1-4

Table 2: BIPQ mean, median and inter quarter range scores

Conclusions

- Patients consider MRSA carriage as chronic condition and that decolonisation is beneficial.
- Most patients believed they had no control over MRSA, but it had no serious consequence on their lives.
- Only few patients had any MRSA related symptoms.
- Patients were generally emotionally detached from their colonisation of MRSA.
- Almost 50% of patients did not have a reasonable understanding of MRSA, which highlights necessity for continued patient education.

Acknowledgements

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Mupirocin and medical grade honey to eradicate nasal MRSA. Efficacy and molecular epidemiology of isolates

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BACKGROUND

MRSA

- *Staphylococcus aureus (S.aureus)* most frequently colonise in the anterior nares, although other body sites such as throat and perineum are also colonised.
- Nasal carriers of *S.aureus* also have an increased risk of acquiring an infection with this pathogen[1]. The widespread use of antibiotics has led to the emergence of resistance and meticillin-resistant *Staphylococcus aureus* (MRSA) is the term used to describe these resistant isolates.
- Up to 70% of invasive *S.aureus* infections are drug-resistant in the European Union (EU)[2].
- Eradicating MRSA from the nose is important in preventing MRSA infection. An association between previous mupirocin exposure and subsequent mupirocin resistance in MRSA is reported, which restricts the use of repeated courses of intra-nasal mupirocin.
- Higher rates of mupirocin resistance have been reported with widespread use or abuse of mupirocin[3].
- There is also a strong association between high level mupirocin resistance (HLMR) and resistance to ≥4 non-β-lactam antimicrobial classes[4].
- Lack of evidence based alternate options for nasal MRSA decolonisation lead the researchers to investigate for viable alternatives.

PURPOSE

To determine if medical grade honey (MGH) is an alternative to mupirocin for nasal decolonisation of MRSA by conducting a randomised control trial (RCT).

The purpose of the interim review was;

- To characterise study isolates using *spa* typing.
- To determine and evaluate mupirocin susceptibility of MRSA isolates from individual patients at specific time periods.

MATERIALS AND METHODS

Recruitment and decolonisation

- MRSA colonised patients were identified from prospective laboratory reports.
- Nasal MRSA colonised patients were evaluated for suitability based on predetermined criteria.
- Consenting patients were recruited and randomised to control and study groups.
- Control group received upto two courses of mupirocin2% nasal ointment, three times a day for five days.
- Study group received upto two courses of nasal MGH, three times a day for five days.
- Triclosan1% was used for non nasal decolonisation i.e. body wash and shampoo for both groups.
- MRSA screening was done at least 48 hours after the last dose of the nasal application.
- Screening results guided the necessity for a second course of either product and follow up.

Characterisation of isolates and susceptibility testing

- First time MRSA isolates (historical) and baseline isolates (recruitment) were investigated for mupircoin susceptibility and compared.
- MRSA isolates; historical and baseline isolates were genetically characterised using *spa* typing. The sequence type (ST) was inferred from the *spa* type.

RESULTS

Nasal MRSA decolonisation

Interim review of 20 patients enrolled shows that:

- 67% (6) patients were successfully decolonised with mupirocin.
- 40% (4) patients were successfully decolonised with MGH.
- 11% (1) patient acquired high level mupirocin resistance (MIC >512µg/ml) .
- Mupirocin susceptibility among persistent MRSA carriers in study group were unchanged.

***Spa* types**

- The predominant *spa*-types were t022, t032, t515, t557 and t4559.
- The predominant sequence type was ST22.
- 75% of the historical isolates were identical to the pre-decolonisation isolate.

Table 1. Results

Group	Results		
	Nasal decolonisation	Persistent colonisation	Mupirocin susceptibility
Control = 9	6	3	1HLMR*
Intervention = 10	4	6	No change

* HLMR – High level mupirocin resistance

Table 2. Sequence Type

Sequence Type	Historic Isolates	Baseline Isolates
ST22	**14**	**14**
ST8	**1**	**1**
ST1	**1**	**0**
Unknown Sequence	1	4
Missing / failed	3	1
Total	20	20

CONCLUSIONS

- Successful nasal decolonisation may be possible with MGH. The final result of the RCT should inform and guide further investigation.
- The laboratory evidence confirms that MRSA colonising strains do not vary over time among persistent carriers. The findings supports evidence of persistent colonisation rather than re-colonisation with a different MRSA isolate among such carriers.

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Appendices

Appendix - I

Natural honey to eradicate nasal methicillin-resistant *Staphylococcus aureus* (MRSA)

Results of the pilot study.

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Background

Staphylococcus aureus is a frequent coloniser of normal skin and is a common cause of bloodstream infection. The widespread use of antibiotics has led to the emergence of resistance and **m**ethicillin-**r**esistant **S***taphylococcus* ***a****ureus* (MRSA) is the term used to describe these resistant isolates. A study reported by European Disease Surveillance Centre (ECDC) confirms that MRSA was the most common, multidrug-resistant bacterium in the EU.[1] Around 3% of the public, rising to 6–7% of those admitted to hospital, are carriers of MRSA.[2] In 2009, 1,309 cases of *S. aureus* bloodstream infection (BSI) were reported in Ireland; MRSA accounted for 355 (27.1%) of 1,309 *S. aureus* isolates.[3]

Nasal colonisation of MRSA is often a precursor of invasive infections such as BSI , surgical site or wound, device and implant infections. Nasal decolonisation with mupirocin is reported to be sub-optimal but potential abuse has resulted in increasing levels of resistance to mupirocin. Rossney et al report increasing high-level mupirocin resistance (MpR) among MRSA BSI isolates between 1999 (1.4% to 2.9%) and 2007.[4] Surveillance of new MRSA isolates of MRSA in Beaumont Hospital found that 7.3% of isolates were resistant to mupirocin in 2009. Therefore, there is a need for developing alternative approaches that are safe and cost-effective.

The ability of natural honey as a potential beneficial therapeutic agent is widely reported in the published literature.[5,6,7,8] Honey-based dressings are now used in the treatment of ulcers. The specific antimicrobial action of honey includes the ability to bind to proteins[9] the inherent non-peroxide antibacterial activity generated by methylglyoxal (MGO)[10, 11, 12] and the high osmotic pressure which render an adverse environment for bacterial survival, all of which represent factors that inhibit bacterial survival when exposed to honey preparations. In addition it is reported that resistance to honey cannot be induced under conditions that rapidly induce resistance to antibiotics.[13] A bactericidal mode of action using manuka honey against *S.aureus* is also reported in the scientific literature.[14] We propose that the properties of natural honey, in particular the bactericidal action, could be beneficial in the decolonisation of nasal MRSA and undertook a preliminary study to later inform a larger clinical trial.

Methods

Pilot study design
An open label pilot study was conducted in Beaumont Hospital, an acute tertiary referral, between March and September 2012 to assess the effectiveness of natural honey (Dermacream®) in eradicating nasal carriage of MRSA. Ethical approval for the study was obtained from Beaumont Hospital and the trial was conducted in accordance with good clinical practice (GCP) guidelines.

The investigative product chosen for the study, Dermacream®50gm tube Medihoney (Europe) Ltd, was purchased and stocked in the hospital pharmacy in accordance with protocols on storage of trial medicinal products. Labelling of the honey product was done according to GCP guidelines in the Beaumont Hospital's pharmacy (Appendix 1 - sample label). This was to ensure traceability of the study products supplied to study participants and to comply with clinical trial requirements. A log of product dispensing was maintained during the pilot study. No monetary or other rewards were offered to patients to participate in the study, which was entirely voluntary.

Inclusion and exclusion criteria
Patients over 18 years, who were capable of providing consent, were invited to participate. (Appendix 2- consent form). Laboratory confirmation of nasal MRSA colonisation was a prerequisite for inclusion in the study. Patients were excluded if they were participating in another study, had uncontrolled diabetes mellitus, or were unable to provide written consent.

Protocol
Study subjects agreed to the nasal application of Dermacream®50gm tube (Appendix 3), three times a day for five consecutive days. If the study participants were colonised at non-nasal body sites i.e. groin/perineum, then the standard decolonisation regimen using a body wash and shampoo with 1% triclosan (Skinsan) was commenced according to Beaumont Hospital policy (Appendix 4). The screening of nasal and other body site/s was done as per the hospital's MRSA screening protocol. A second course, three times a day, for five days with Dermacream® was commenced if screening identified persistent MRSA colonisation. Invasive sterile collection swabs (EUROTUBO) were used for specimen collection.

Up to two five day courses of nasal Dermacream® were administered to study participants. Successful MRSA decolonisation was confirmed if three follow-up MRSA screens taken 48 hours apart, i.e. nasal, groin and other relevant body sites, were negative for MRSA. The screening swabs were processed in the CPA accredited Beaumont hospital laboratory using chromogenic medium plate (BIORAD®) followed by coagulase testing to confirm the identification of the organism, i.e. MRSA with antibiotic susceptibilities undertake according to routine methods (PHOENIX®).

The study participants' perception of using a non-antibiotic product for nasal MRSA decolonisation, as well as their experience of the application of the investigative product were collected during the study. This information was collected using structured statements (Appendix 5).

Results

Seven patients consented for inclusion, all of whom were persistent carriers of MRSA, i.e. had nasal and other body site decolonisation, despite the standard decolonisation regimen, i.e. nasal mupirocin and Triclosan 1% body wash, on two or more occasions. Therefore all

had failed MRSA decolonisation attempts before recruitment to this pilot study. One of the patients enrolled was colonised with mupirocin-resistant MRSA who had had one course of Naseptin® for ten days but MRSA colonisation persisted. Nasal and other body site/s screening were done as per the hospital MRSA screening protocol.

Two of the seven patients discontinued the study agent up to two days after commencement. Therefore five patients completed one or two full course of the nasal honey application. Successful nasal MSA decolonisation was confirmed in three cases, including the successful decolonisation of the patient with mupirocin resistant MRSA. Two patients had persistent nasal MRSA colonisation after two courses of nasal honey application.

Table 1 Details of the patients recruited and the outcome.

No	MRSA first detected	Previous decolonisation attempts	Number of Dermacream® course/s	Outcome
1	September 2009	Two or more	<1	Discontinued on day 2 due overall clinical deterioration with end-stage renal disease and gastrointestinal haemorrhage
2	June 2011	Two or more	<1	Discontinued day 2 due to unpleasant and intolerable taste
3	Jan 2011	Two or more	02	**Successful decolonisation**
4	Nov 2009	Two or more	02	**Successful decolonisation**
5	Nov 2010	Two or more	02	Failed nasal decolonisation
6	Dec 2006	Two or more	02	Failed nasal decolonisation but could not comply with protocol due to chronic obstructive disease
7	Dec 2008	Two or more	02	**Successful decolonisation**

The study also sought to document patients' perception and experience of using the trial product. Six of the seven patients recruited responded using the structured statement questionnaire and their experiences were:.

- All six found that the trial product was not sticky, did not cause any unpleasant sensation in the nose and was easy to apply to the nasal passage.
- Two of the six felt that their nose was moist and runny after nasal application of the product.
- Given a choice, five of the six participants preferred the use of a natural product to an antibiotic to eradicate MRSA from the nose.

Discussion and recommendations

The potential beneficial therapeutic agent of honey is described in the published literature with most of the data originating in the last 20 years. Honey-based dressings are used in the treatment of ulcers. The high osmotic pressure of honey and the bactericidal mode of action, in addition to other beneficial factors, renders honey a potential therapeutic agent. This approach could also be utilised in the decolonisation of nasal MRSA as identified in this pilot study. If successful, this approach may provide an alternative approach to the current MRSA decolonisation strategies with a key advantage of not inducing antimicrobial resistance. However, controlled trials are essential before the clinical efficacy of natural honey can be fully confirmed and established in comparison to other products that are currently recommended for nasal MRSA decolonisation.

A systematic review of the literature on the use of honey for nasal MRSA should be undertaken. Following this a randomised controlled trial comparing honey versus mupirocin for nasal decolonisation of MRSA with sufficient power should be carried out. Both would expand our knowledge of the clinical effectiveness of natural honey for nasal decolonisation of MRSA.

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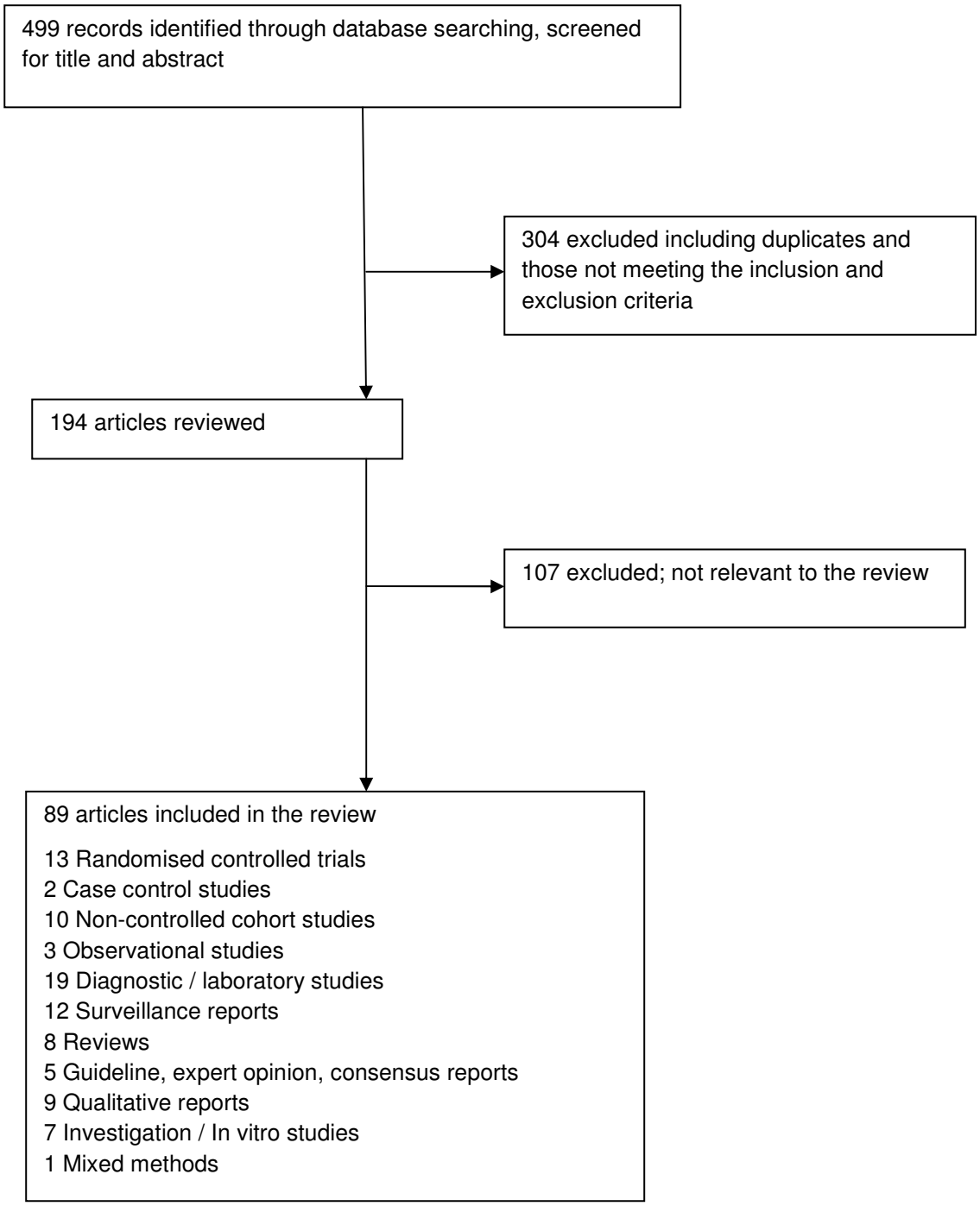
Appendix -II

Figure 1.Literature review search process



Appendix -III

Mupirocin resistance: Clinical implications and potential alternatives, JAC July 2015 (a full copy of the article in publications)

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Mupirocin resistance: clinical implications and potential alternatives for the eradication of MRSA

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Mupirocin 2% ointment is used either alone or with skin antiseptics as part of a comprehensive MRSA decolonization strategy. Increased mupirocin use predisposes to mupirocin resistance, which is significantly associated with persistent MRSA carriage. Mupirocin resistance as high as 81% has been reported. There is a strong association between previous mupirocin exposure and both low-level and high-level mupirocin resistance. High-level mupirocin resistance (*mupA* carriage) is also linked to MDR. Among MRSA isolates, the presence of the *qacA* and/or *qacB* gene, encoding resistance to chlorhexidine, ranges from 65% to 91%, which, along with mupirocin resistance, is associated with failed decolonization. This is of significant concern for patient care and infection prevention and control strategies as both these agents are used concurrently for decolonization. Increasing bacterial resistance necessitates the discovery or development of new antimicrobial therapies. These include, for example, polyhexanide, lysostaphin, ethanol, origanan pentahydrochloride, tea tree oil, probiotics, bacteriophages and honey. However, few of these have been evaluated fully or extensively tested in clinical trials and this is required to in part address the implications of mupirocin resistance.

Background

Staphylococcus aureus is a leading cause of healthcare-associated infections worldwide and is associated with increased morbidity, mortality and higher healthcare costs, including infections caused by MSSA and MRSA.[1] Colonization with MRSA increases the risk of adverse health outcomes and it is estimated that 10%–30% of carriers subsequently develop infection.[2] The nose as well as extranasal sites such as the throat and perineum, skin ulcers and skin lesions are frequently colonized.[3–5] A meta-analysis concluded that MRSA colonization conferred a 4-fold increased risk of infection as compared with MSSA colonization.[6] Eradication of MRSA carriage from the nose and other body sites forms an integral part of strategies to prevent and control MRSA in many countries.[7–9] Mupirocin is an important component in MRSA prevention and specifically for the eradication of nasal MRSA. However, reports of increasing mupirocin resistance (MR) are of serious concern.

This review aims to determine the prevalence of MR and its clinical consequences as well as measures to control MR. It also reviews the evidence supporting the use of new agents as potential therapeutic alternatives for the prevention and management of MRSA.

Search strategy

The following databases were searched (date of last search: 30 March 2015): PubMed, CINAHL, Scopus and Web of Science. The search was limited to humans and English language publications from 1985 to March 2015. Search terms included multiple variants of the following terms: "*Staphylococcus aureus*, nose/nasal, colonisation/colonization, honey, infection control or prevention and control, wound, ulcer, surgical wound infection, topical, treatment, chlorhexidine, mupirocin and drug resistance" alone or in combination and/or 'infection'. Medical subject headings (MeSH) terms where available were used. Additionally, the reference lists of retrieved articles were scanned to identify any further studies. The titles and abstracts identified were screened for relevance by one author. The list of potential articles was reviewed to remove duplicates and full-text versions were obtained. Further articles were eliminated following review. The original articles were obtained and assessed in detail for inclusion. Articles included in this review are those that addressed mupirocin, i.e. infections associated with *S. aureus*, MRSA, decolonization, resistance, surveillance reports, systematic reviews or meta-analyses where the search terms appeared in the article title or abstract. From a total of 499 articles initially found, after exclusion

Appendix -IV

Approval – Recognised Ethics Committee_24th January 2014

Ethics (Medical Research) Committee - Beaumont Hospital
Notification of ERC/IRB Approval

Principal Investigator: Mr. Toney Thomas (Research Fellow/Beaumont Hospital)

Consultant co-investigator: Prof. H. Humphreys

REC reference: 13/103

Protocol Title: Natural Honey to Eradicate Nasal MRSA, A Randomised Control Trial

Ethics Committee Meeting Date: 13th December 2013

Final Approval Date: 24th January 2014

From: Ethics (Medical Research) Committee - Beaumont Hospital, Beaumont, Dublin 9

Documents Reviewed

Document and Date	Date Reviewed	Approved
Application Form, signed T. Thomas, 26/11/13	24/1/14	Yes
Study Protocol, V1, 11/13	24/1/14	Yes
Patient Information Leaflet, V2, 2014	24/1/14	Yes
Consent Form, V1, 2013	24/1/14	Yes
Patient Questionnaire, Medicare Derma Cream, V1, 2013	24/1/14	Yes
Patient Questionnaire, Mupirocin, V1, 2013	24/1/14	Yes
GP Letter, V1, 11/13	24/1/14	Yes
Consultant Letter, V1, 11/13	24/1/14	Yes
Study Flowchart, V1, 26/11/13	24/1/14	Yes
Email, Irish Medicines Board, 10/11/13 (REC REF: 11/78)	24/1/14	Noted
Results (Pilot Study: 11/78)	24/1/14	Noted
Product Information, DermaCream	24/1/14	Noted
Directions for Use of Medihoney Dermacream and Patient Record, V1, 2013	24/1/14	Noted

Letter to REC. re. standard of care in Beaumont Hospital, 26/11/13 (enclosing standard of care protocol)	**24/1/14**	**Noted**
Grant Contract, HRB, 24/10/13	**24/1/14**	**Noted**
Lay Summary, V1, 2013	**24/1/14**	**Noted**
CV: T. Thomas	**24/1/14**	**Noted**

Prof. Alice Stanton
ERC/IRB Convenor's Signature
Approval # 1, dated 24th January 2014

Terms of Approval

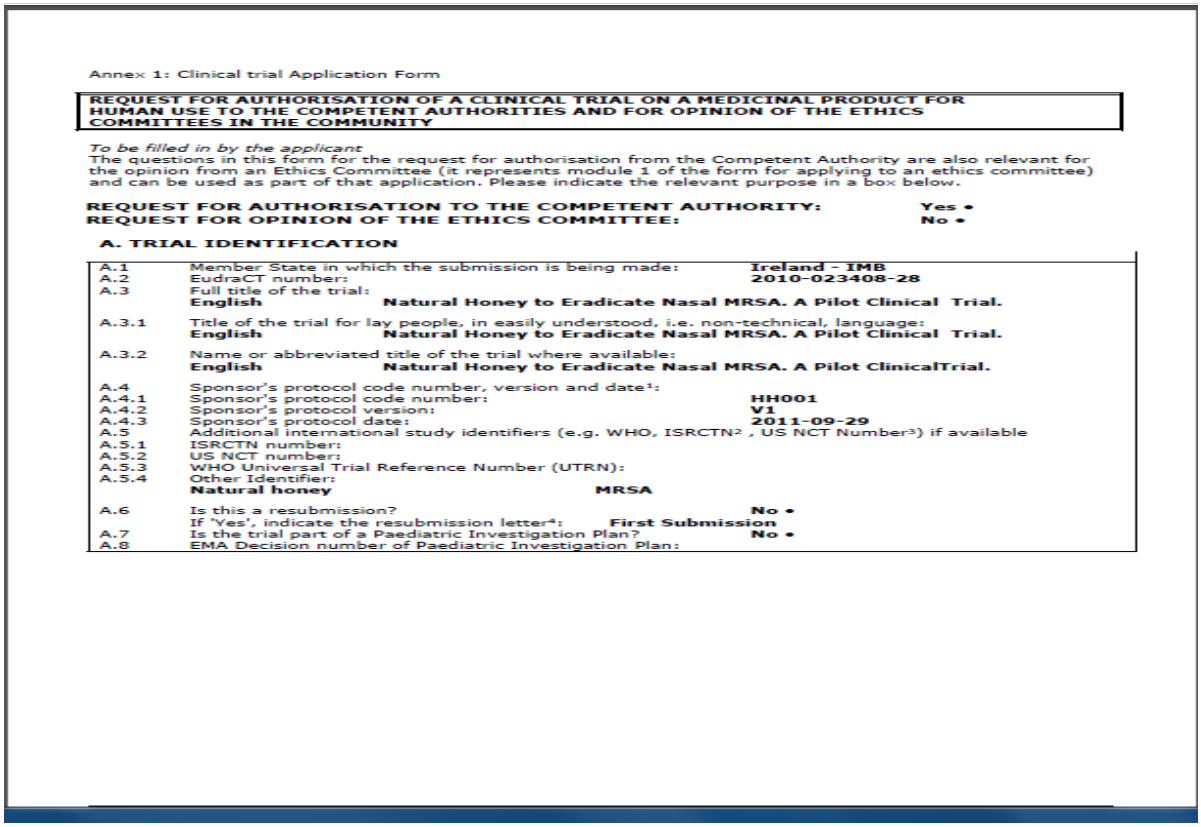
- The protocol and research must comply with all relevant Irish legislative requirements and the researchers must abide by the ethical principles outlined in the Declaration of Helsinki and Good Clinical Practice.
- Prior approval from the Ethics Committee must be sought for any proposed changes/amendments to this protocol and research.
- Annual Progress Reports and a Final report must be supplied to the Ethics Committee.

FIRST REPORT DUE: JANUARY 2015

- All relevant information about serious adverse reactions and new events likely to affect the safety of the subjects must be reported to the Ethics (Medical Research) Committee in writing.

Appendix - V

EUDRACT Registration and EUDRACT number



Annex 1: Clinical trial Application Form

REQUEST FOR AUTHORISATION OF A CLINICAL TRIAL ON A MEDICINAL PRODUCT FOR HUMAN USE TO THE COMPETENT AUTHORITIES AND FOR OPINION OF THE ETHICS COMMITTEES IN THE COMMUNITY

To be filled in by the applicant
The questions in this form for the request for authorisation from the Competent Authority are also relevant for the opinion from an Ethics Committee (it represents module 1 of the form for applying to an ethics committee) and can be used as part of that application. Please indicate the relevant purpose in a box below.

REQUEST FOR AUTHORISATION TO THE COMPETENT AUTHORITY: **Yes •**
REQUEST FOR OPINION OF THE ETHICS COMMITTEE: **No •**

A. TRIAL IDENTIFICATION

A.1	Member State in which the submission is being made:	**Ireland - IMB**
A.2	EudraCT number:	**2010-023408-28**
A.3	Full title of the trial:	
	English	**Natural Honey to Eradicate Nasal MRSA. A Pilot Clinical Trial.**
A.3.1	Title of the trial for lay people, in easily understood, i.e. non-technical, language:	
	English	**Natural Honey to Eradicate Nasal MRSA. A Pilot Clinical Trial.**
A.3.2	Name or abbreviated title of the trial where available:	
	English	**Natural Honey to Eradicate Nasal MRSA. A Pilot ClinicalTrial.**
A.4	Sponsor's protocol code number, version and date[1]:	
A.4.1	Sponsor's protocol code number:	**HH001**
A.4.2	Sponsor's protocol version:	**V1**
A.4.3	Sponsor's protocol date:	**2011-09-29**
A.5	Additional international study identifiers (e.g. WHO, ISRCTN[2] , US NCT Number[3]) if available	
A.5.1	ISRCTN number:	
A.5.2	US NCT number:	
A.5.3	WHO Universal Trial Reference Number (UTRN):	
A.5.4	Other Identifier:	
	Natural honey	**MRSA**
A.6	Is this a resubmission?	**No •**
	If 'Yes', indicate the resubmission letter[4]:	**First Submission**
A.7	Is the trial part of a Paediatric Investigation Plan?	**No •**
A.8	EMA Decision number of Paediatric Investigation Plan:	

Application for EudraCT Number Page 1 of 1

From EudraCT@eudra.org
Subject Application for EudraCT Number
Date Tue, October 12, 2010 1:13 pm
To toneythomas@beaumont.ie

The EudraCT number 2010-023408-28 has been issued for your Sponsor's Protocol Cod Number HH001.

THIS IS AN AUTOMATED EMAIL - PLEASE DO NOT REPLY AS EMAILS RECEIVED AT THIS ADDR WILL BE AUTOMATICALLY DELETED.

Download this as a file

Appendix - VI

Response from Irish Medicines Board for conduct of the study

Manuka Honey study

Catherine McHugh [catherine.mchugh@imb.ie]
Sent: 10 November 2011 9:50 AM
To: Toney Thomas
Cc: Elaine Breslin [Elaine.Breslin@imb.ie]; Niall MacAleenan [niall.macaleenan@imb.ie]; Muireann Lydon [muireann.lydon@imb.ie]; Agnieszka Przybyszewska [Agnieszka.przybyszewska@imb.ie]; IMB Clinical Trials [Clinical.Trials@imb.ie]
Importance: High

Dear Dr. Thomas,

Further to the information you have provided on your proposal please be informed that clinical research involving CE marked medical devices that is being conducted by academic / clinical investigators when there is no commercial intent for use of the data do not require review and approval by the IMB prior to commencement.

We wish you well with your study.

Regards,
Catherine

Catherine McHugh B.Sc.(Pharm), Ph.D., MRPharmS, MPSI
Executive Pharmaceutical Assessor
Irish Medicines Board | Bord Leigheasra na hÉireann
Kevin O'Malley House, Earlsfort Centre, Earlsfort Terrace, Dublin 2.
Tel: +353 1 6764971
Fax: +353 1 6767836
Catherine.McHugh@imb.ie
www.imb.ie

Appendix - VII

Laboratory protocol: LP-MIC-Screens Revision 4-2013

Clinical Directorate of Laboratory Medicine, Beaumont Hospital				
Doc No:	LP-MIC-Screens	Revision:	4	Active Date: 8th May 2013
Title: Screens – MRSA, VRE, CRE, Environmental				

SCREENS – MRSA, VRE, CRE, ENVIRONMENTAL

Microbiology Laboratory,
Beaumont Hospital,
Beaumont Road,
Dublin 9.
Ireland.

Authorised By: Dr. Karen Burns

7. PROCEDURE

7.1 Specimen receipt

Specimens are received on the BHIS (see LP-MIC-COMPUTER)

7.2 Culture

- Containment Level 2
- All work which is likely to generate aerosols should be performed in a microbiological safety cabinet.
  (See fig. 1)

Refer to LP-MIC-SPEC.PUT-UP for instructions on specimen put up.

7.2.1 Reading primary MRSA Plates DAY 1

Read primary plates after exactly 24 hours (i.e., at 9am unless otherwise indicated). Plates read before or after this time may give erroneous screening results and lead to unnecessary work and delays in reporting results.

- Check control plate and ensure that growth is as expected
  - Positive (*S. aureus* ATCC 43300) control: Deep pink colonies

- Negative (*S. aureus* ATCC 25923) control: Scanty and faint pale pink hazy growth

- Examine test plates for the presence of pink colonies
- Follow the algorithm outlined in the Appendix1
- Report all previously known and MRSAs once confirmed by primary identification tests (see Table 3).
- Prepare purity plates on blood agar of all query new MRSAs and those too scanty for full controlled Pastorex Staph-Plus
- Incubate for referral to the Phoenix next day
- Record query new MRSA on the BHIS only after full controlled Pastorex Staph-Plus has been performed.
- Check on PIPE that the patient is a query new MRSA by checking their risk group (RG) status (see Appendix 2)
- Record and report results as outlined in the Computer Reporting table

Note: Any growth, even one or two colonies, is significant and should be followed up. For very scanty cultures, it may be necessary to plate out for purity and perform primary screening tests the following day. This should be recorded in the notes field of the workcard.

Table 2: MRSA Reading and Storage Guidelines

Organism	Primary Identification Tests	Susceptibility Testing	Storage of Isolates	Recording of Isolates	Organism Referral
MRSA	Pastorex, Catalase, Gram Stain	All new isolates: Order PM67sensitivity panel and submit organism on Blood Agar purity plate for testing	All new isolates on Nutrient Agar slopes held at room temp.	All new isolates in the MRSA book	Outbreaks or where epidemiologically indicated. On direction of CMT

7.2.2 MRSA DAY 2

Follow-on of subbed purity plates

- Perform confirmatory tests (eg. Pastorex Staph-Plus) on scanty growths from the previous day
- Check all purity plates are acceptable for the Phoenix
- Order Gram Positive Phoenix panels on the BHIS for all cultures sent to Phoenix

- Record the isolate number, QMRSA and all tests carried out on the purity plate
- Transfer plates to the Phoenix as soon as possible to avoid delay in set-up

7.2.3 MRSA DAY 3

Phoenix Follow on

- Slope and record all confirmed isolates returned from the Phoenix

Appendix - VIII

Patient Information Leaflet - VERSION 3

Protocol Title: Honey to Eradicate Nasal MRSA, A Randomised Control Trial

Principal Investigator: Toney Thomas, HRB Research Fellow, Beaumont Hospital.
Telephone No: 01 8093133.
Co-investigator: Prof. Hilary Humphrey's, Consultant Microbiologist, Beaumont Hospital. **Telephone No***:* 01 8093312.

You are being invited to take part in a pilot clinical research study carried out at Beaumont Hospital. Before you decide whether or not you wish to take part, you should carefully read the information provided below and, if you wish, discuss it with your family, friends or GP (family doctor). Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.

You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as 'Informed Consent'.

You don't have to take part in this study if you prefer not to. If you decide not to take part it won't affect your future medical care.

You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, you can be assured that this decision won't affect the quality of treatment you get in the future.

WHY IS THIS STUDY BEING DONE?

The presence of meticillin resistant *Staphylococcus aureus* (MRSA) in the nose without symptoms or evidence of infection is called, "MRSA carriage". Such carriage may predispose to infection and therefore in hospital, efforts are made to eradicate MRSA from the nose, i.e. MRSA decolonisation. However, most patients carrying

MRSA are well and are merely carrying the MRSA, i.e. carriage. The medication normally used to decolonise or remove MRSA from the nose is an antibiotic, mupirocin, but its use is restricted to a maximum of two courses, to prevent the emergence of resistance.

There is some evidence to suggest that honey may have a beneficial effect on MRSA decolonisation. Studies done on wounds and ulcers have reported effective MRSA decolonisation or eradication in addition to the healing of the wound or ulcer. A pilot study was done in Beaumont Hospital recently. The results of the study show evidence of successful nasal MRSA decolonisation in patients after nasal honey application, similar to or higher than what would be normally expected from using nasal mupirocin. However, a larger study is required to confirm that before the widespread use of nasal honey. It is not fully understood how honey eradicates MRSA from the nose. If we could understand this process more clearly, it might help us develop new medications to eradicate MRSA.

In order to investigate the effects of honey, we plan to use honey to eradicate MRSA in those patients who would normally be tested for the presence of MRSA in the nose and who would normally receive mupirocin. The honey product we will be using is called Medihoney “Derma cream”.

In order to scientifically demonstrate that nasal decolonisation of MRSA using nasal honey is of the same or better than using mupirocin, a clinical study that involves investigating the outcome among patients who receive the honey product and mupirocin in two separate groups using a random controlled trial is required. Your participation in the study means that you may be allocated to either group that is nasal honey or nasal mupirocin. If you are in the study group and received nasal honey and nasal decolonisation of MRSA is not achieved with Medihoney ® “Derma cream” even after two courses, you will subsequently be offered mupirocin for nasal application.

WHO IS ORGANISING AND FUNDING THIS STUDY?
The study is being carried out by the Infection Prevention and Control Department and the Microbiology Department in Beaumont Hospital.

Mr Toney Thomas, Health Research Board (HRB) Research Fellow, Beaumont Hospital is the Principal Investigator in the study and the Co-Investigator is Prof. Hilary Humphreys, Consultant Microbiologist, Beaumont Hospital. Both are full-time employees of Beaumont Hospital/RCSI.

This academic study is funded by the HRB by means of a research grant. The investigator will also pursue a doctoral degree conducting the research. No other funding from any other sources will be utilised for the study. The investigators are not paid to enrol patients to this study.

WHAT AM I BEING ASKED TO PARTICIPATE IN?
You are being asked to take part in the study because the laboratory has confirmed that you have MRSA in the nose. Normally in such a situation a patient admitted to the hospital (and in some cases seen in the outpatients) would be offered decolonisation or attempted removal of MRSA followed by screening of the nose at intervals to confirm the presence or absence of MRSA afterwards. The presence of MRSA in the nose is not unusual i.e. carriage, and most patients are not ill as a result. However, in many patients it is best to try and remove the MRSA from the nose, i.e. decolonisation.

HOW WILL THE STUDY BE CARRIED OUT?
This study will be carried out by the Infection Prevention and Control Department and the Microbiology Department in Beaumont Hospital over a period of 24 months. Approximately 200 patients will be recruited to this study. Patients in the study will be provided with nasal honey or nasal mupirocin followed by MRSA swabs tested in Beaumont Hospital, that includes nasal, groin and other relevant body sites such as wounds/ulcer, during the period of study all free of charge.

WHAT WILL HAPPEN TO ME IF I AGREE TO TAKE PART?
If you agree to participate in this study, you will receive a 50gm tube of Medihoney Derma cream or a 3 gm tube of mupirocin depending on the group you are allocated to. You will be shown how to apply either of the products to the nose. A course of nasal application will involve its use three times a day for five consecutive days. You will also receive one unit of Skinsan® which is the routine hospital- approved antiseptic body wash and shampoo for application to the rest of the body for five consecutive days irrespective of which group you are allocated to.
The study involves the taking of swabs from the nose and groin and from other relevant body sites on three separate occasions at least two days apart after finishing a five day course. This approach is the same as when using mupirocin to eradicate MRSA from the nose. These repeat swabs are required to confirm effective MRSA decolonisation. The first set of samples will be taken two days after finishing the five day course of one of the products and Skinsan. A second course of nasal Derma cream or mupirocin and Skinsan application may be required if MRSA persists in the nose and the groin. In this case the follow up screening will be similar i.e. three separate screens at least two days apart on completion of the second five day course. Three sets of negative results are required to confirm effective MRSA decolonisation.

If you continue to be an inpatient your swabs will be collected while in the hospital. If you are discharged during the course of the study, the collection of swabs and or the provision of Derma cream or mupirocin will be arranged either through your GP or through a home visit, which will be pre arranged by the Primary Investigator.

The study is expected to take from two to four weeks.

You will be asked to complete a short anonymous questionnaire on your experience using the product and your perception on MRSA.

Only the study team and the regulatory authorities will have direct access to your personal medical records. This will ensure that the study is performed according to the approved protocol and that the coded data is correctly recorded. All study team representatives and other healthcare staff are ethically and professionally obliged to maintain patient confidentiality at all times.

WHAT OTHER TREATMENTS ARE AVAILABLE TO ME?
For nasal MRSA decolonisation the current recommended product is mupirocin. Patients in the control group will be given mupirocin. You will be offered mupirocin if you decide not to take part in the study. Your decision to take part or not to take part in the study will not result in any change to your medical care in Beaumont Hospital. You will continue to be followed up by your doctors in Beaumont Hospital as usual.

WHAT ARE THE BENEFITS?
The main reason for carrying out this study is to improve our knowledge about the effectiveness and ease of the use of honey to eradicate MRSA from the nose and to compare it with nasal mupirocin. You are likely to benefit from participating in the study by the eradication of MRSA from your nose. Your participation may also benefit others as our ultimate study aim is to scientifically demonstrate that nasal decolonisation of MRSA using nasal honey is as good as or better than mupirocin. If it is as good or better, this would mean that we have an alternative choice to eradicate MRSA from the nose. Your participation may also help reduce antimicrobial resistance by the use of a product rather than an antibiotic.

WHAT ARE THE RISKS?
No adverse consequences after the use of honey for medicinal purposes have been reported in the healthcare literature. There may be a possibility that the honey product is inferior to the antibiotic that is used to decolonise nasal MRSA. There were no adverse reactions when we conducted our pilot study using honey. However, to date honey has not been extensively used and it is possible that there may be other risks which are not known at this time. A “runny nose” may occur based on experience from the pilot study. However, the research team will carefully monitor any unintended effects of honey used during the course of the study. Honey is generally well-tolerated when swallowed, when applied to the skin, to ulcers and to chronic wounds such as when it is included in wound dressings.

WHAT IF SOMETHING GOES WRONG?
The researchers in this study are covered by state healthcare insurance (indemnity), which includes Beaumont Hospital as well in the very unlikely event that you are harmed in any way as a direct result of this study.

WILL IT COST ME ANYTHING TO TAKE PART?
No. You will be provided with the products free of charge in addition to the follow up tests that will be done during the study. If you are discharged from the hospital during the study follow up period, swabs will be organised either through your GP or through a home visit which will be arranged by the Primary Investigator.

IS THE STUDY CONFIDENTIAL?
Your hospital consultant will be notified of your participation in the study which is essential, and your GP if you are agreeable to this.

Your medical record will be accessed by the Primary Investigator who is a registered nurse, and fully trained in Infection Prevention and Control. Your MRSA screening results will be electronically filed in your health record. The results from your screening will be discussed with you. Unless you specifically request, the screening results will not be provided to your GP. The MRSA bacteria from the screening tests may be stored in the Microbiology Department in Beaumont Hospital for the purpose of scientific research. The coded results of this study may be used in the future to identify better methods to eradicate MRSA from the nose and from other body sites. However, such data **will not** include your name or any other identifiable details.

Data will be kept in “coded” form – this means that a number, rather than your name, will appear beside any information about you, thereby maintaining your anonymity. The coded results from the research may be published in scientific journals and conferences at a later date.

WHERE CAN I GET FURTHER INFORMATION?
If you have any further questions about the study or if you want to opt out of the study, you can be rest assured that it won't affect the quality of any treatment you receive in the future. If you need any further information now or at any time in the future, please contact:
Mr. Toney Thomas to discuss the nature of the study in more detail. You are, of course, also entitled to seek an opinion from a separate source if you so wish.

Name: Mr. Toney Thomas
Address: Infection Prevention and Control Department,
Beaumont Hospital, Beaumont Road, Dublin 9
Phone No: 01-8093133. Please note that this number is only answered during normal office hours.

Appendix - IX

Natural honey for nasal MRSA eradication Study

Standard Operating Procedures for Pharmacy

Version 1 dated 17th February 2014

Beaumont Hospital
Department of Infection Prevention and Control,
Post Box 1297, Dublin 9.
Dublin, Republic of Ireland
Telephone 353 1 8093133 www.beaumont.ie

Contents

Purpose

The purpose of this document is to describe the pharmacy procedures for the conduct of the Natural honey for nasal MRSA eradication research project at Beaumont Hospital pharmacy.

Overview of the Protocol

Overview

Natural honey for nasal MRSA is an open label, randomised controlled trial of 188 patients comparing the effect of Natural honey Medihoney® Derma Cream to mupirocin on patients with nasal MRSA colonisation. Patients will be recruited based on prospective laboratory MRSA screening results confirming nasal MRSA colonisation. Consent for participation will be obtained from individual patients prior to randomisation. Eligible patients will be randomized to receive either Medihoney® Derma Cream or mupirocin nasal application, daily three times for five consecutive days, for up to maximum of two courses.

The primary outcome is: proportion of patients with sustained nasal MRSA decolonisation after one or two courses of Medihoney® Derma Cream or mupirocin, followed by three consecutive negative nasal MRSA screens at least 48 hours apart.

Serious adverse events will be monitored from commencement to completion of the study, approximately one month after randomisation.

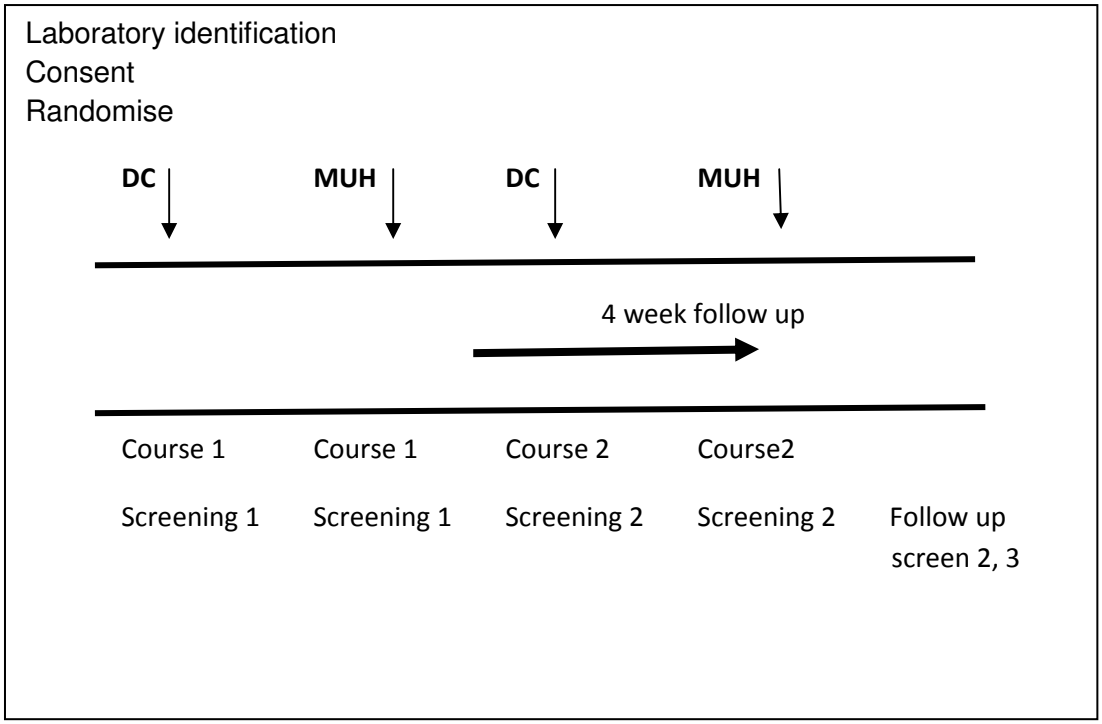
MRSA screening

Routine MRSA screening involves collection of swabs from both nasal passages and groin or perineum in addition to other body sites that are clinically relevant such as wounds, ulcers, catheter specimen of urine, sputum and device insertion sites. Patients with history of MRSA colonisation or infection as well as who fit criteria for MRSA screening as per hospital MRSA guidelines are screened on admission to the hospital or during clinic visits.

Patients enrolled for the study will receive either nasal application of Derma cream or

mupirocin three times a day for five consecutive days, followed by two intervention free days. Thereafter patients will undergo MRSA screening; nasal and groin in addition to relevant body sites. Up to two courses of Derma cream or mupirocin may be administered to each patient depending up on the outcome of MRSA screening.

Diagram of Study Procedure



Pharmacy Procedures

Study Product

The study product for the experimental decolonisation arm is natural honey 30% 50gm in pre-filled tube, Medihoney® Derma Cream manufactured by Derma Sciences Europe Limited.

The standard product for the control decolonisation arm is mupirocin 2% (20 mg/g) 3gm in pre-filled tube.

Approximately 2 cm of Derma cream OR 0.5cm (match head size) of mupirocin will be administered by nasal application into both nostrils three times a day for five days, and if required followed by a second course; three times of nasal application for five days. This will

be based on the nasal screening tests.

Study Product Supply and Storage

Upon receipt of the Medihoney® Derma Cream, the product will be stored at controlled room temperature according to product information in the Pharmacy Department.
Mupirocin will be supplied by the site Pharmacy Department and stored at controlled room temperature according to product information. The ointment is recommended to be kept at room temperature, 15-30 C (59-86 F).
Temperature logs with daily minimum and maximum temperature readings will be kept by the pharmacy personnel to monitor the storage temperature of the study drugs.
The temperature monitoring device for recording the storage temperatures should have routine calibration records, which will be managed by the pharmacy department.

Temperature logs will be kept on pharmacy file and made available for inspection upon request.
Products will be labelled by Pharmacy and stored in the clinical trials area of the pharmacy.

Study products and study documentation will be kept in an area with restricted access to authorised site personnel only.

The PI will dispense the study product in the original packing and will have the following labelling:

Clinical Trial Protocol: NATURAL HONEY FOR NASAL MRSA
Patient Name and Hospital Identification
Number / Case number
Date of dispensing

Dispensing procedure

The Primary investigator (PI) will (a) randomize the eligible patient after (b) consent. A pre-printed sticker indicating the correct product will be affixed on the (c) Kardex which will be (d) signed by a registered medical practitioner.

For patients in experiment group will receive:

One unit of Medihoney® Derma Cream 50gm tube labelled with patient details

For patients in control group will receive:

One unit of mupirocin 2% w/v 3gm tube labelled with patient details.

Picture: mupirocin 3gm tube

Pharmacy label for tube: mupirocin 2%w/v

Mupirocin 2%w/v nasal ointment 3g
NHNMRSA study
Apply three times a day for 5 days
Patient initials: Date of Birth:
Study No:
Investigator T Thomas
Disp date .../.../.....
Clinical Trial use only

Pharmacy Dept Beaumont Hospital
Beaumont Road Dublin 9

Picture: Derma cream 50gm tube

Pharmacy label for Derma cream tube

Derma cream 50gm
NHNMRSA study
Apply three times a day for 5 days
Patient initials: Date of Birth:
Study No:
Investigator T Thomas
Disp date .../.../.....
Clinical Trial use only

Pharmacy Dept Beaumont Hospital
Beaumont Road Dublin 9

The dispensed study product should be documented on the Product Inventory Log.
If the product is not given for any reason, the product should be returned to the PI and the returned product should be documented on the Inventory Log.

Administration by investigator

Randomization must be performed and patient allocated to experiment or control group after consent. Thereafter the experimental or control product must be prescribed by a medical practitioner for each patient. The experimental or control product will be then issued by the primary Investigator, who will then offer the product to the named patient.
On administration of the first dose of the prescribed product, the PI will sign off the product administration, and or Medication order chart (Kardex - in patients) as appropriate. Subsequent doses on self administration will be marked off on the product administration sheet by the patient, or if nurse assisted will be signed (Kardex - in patients) by the nurse administering the dose.
The product is to be stored in its packaging after applying the cap and held with the patient.

Destruction of products

Any expired Medihoney® Derma Cream or mupirocin can be destroyed on site after documentation on the product Inventory Log.
Expired product will be destroyed according to Beaumont hospital standard operating procedures.

Purchasing Program

Both Medihoney® Derma Cream and mupirocin will be purchased by the Beaumont Hospital Pharmacy department

Written request will be provided via a letter to purchase the products specifying quantities signed by the primary investigator to the Chief or Chief 11 Pharmacist.

The PI will monitor stock for a minimum of 5 units of Medihoney® Derma Cream and mupirocin for the study, during the period specified.

Site Signature Log

A Signature and Responsibility Log will be kept and the Primary Investigator. PI will be trained by the lead clinical trials pharmacist who will sign off certifying that the Primary Investigator is suitably qualified and trained.

The lead Pharmacist will sign the Signature and Responsibility Log located in the Investigator Site File.

Protocol Deviations

Please enter a note stating deviation if any on the comment box on the Dispensing Form in the event of a protocol deviation.

Additional units of mupirocin 3gm or Medihoney® Derma cream 50gm tubes if required for a patient enrolled in the study will be issued on prescription of the product on Kardex using pre-printed sticker and signed by a registered medical practitioner.

Regulatory Requirements for the Study

1. Ethics (Medical Research) Committee Approval letter.

References

Appendix 1: **Pharmacy Request to <u>Purchase Form 1</u>**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Pharmacy Request to Purchase Medihoney® **Derma cream 50gm tube**

Instructions:

- Complete this Form.
- Send the completed Form and the Medihoney® Inventory Log to Beaumont hospital pharmacy.
- File all purchase approval forms in Pharmacy Manual
- Allow 2 working days for approval to purchase

Site Name:	Beaumont Hospital
Site Number:	
Investigators Name:	Toney Thomas
Date of Request:	
Requested By: Telephone: Fax: Email:	(Print name)
Quantity to be purchased: number of Medihoney® Derma cream 50gm tubes	
Date Stock required on site:	

Approved by: _____(print name)

Signature _____

Date: _____

Appendix 2: **Pharmacy Request to <u>Purchase Form 2</u>**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Pharmacy Request to Purchase Bactroban® **(mupirocin2%w/v) 3gm tube**

Instructions:

- Complete this Form.
- Send the completed Form and the Bactroban® (mupirocin) Inventory Log to Beaumont hospital pharmacy.
- File all purchase approval forms in Pharmacy Manual
- Allow 2 working days for approval to purchase

Site Name:	Beaumont Hospital
Site Number:	
Investigators Name:	Toney Thomas
Date of Request:	
Requested By: Telephone: Fax: Email:	(Print name)
Quantity to be purchased: number of Bactroban® (mupirocin) **3**gm tubes	
Date Stock required on site:	

Approved by: _____(print name)

Signature _____

Date: _____

Appendix 3: **Inventory Log 1. Medihoney® Derma cream 50gm tubes**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Site: Beaumont Hospital
Inventory Log

Medihoney® Derma cream 50gm tubes

Lot Number: _____ Expiry Date: _____

Date	Patient Study Number	Patient initial	Quantity Received	Quantity Dispensed	Balance No	Signature	Comments

Appendix 4: **Inventory Log 2. Bactroban® (mupirocin) 3gm tube**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Site: Beaumont Hospital
Inventory Log

Bactroban® (mupirocin 2%w/v) 3gm tube

Lot Number: _____ Expiry Date: _____

Date	Patient Study Number	Patient initial	Quantity Received	Quantity Dispensed	Balance No	Signature	Comments

Appendix 5: **Temperature Log**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Site: Beaumont Hospital

Temperature Log

Medihoney® Derma cream 50gm tubes and

Bactroban® (mupirocin 2%w/v) 3gm tubes

Store at room temperature 15-30 degree Celsius

Instructions:

- For the purposes of the study the pharmacy's current temperature monitoring chart that complies with the requirements of the study will be used.

Date	Minimum temperature	Maximum temperature	Signature

Note: For the purpose of this study, Beaumont Hospital Pharmacy Department temperature monitoring log held on file is agreed to be used and available on request.

Appendix 6: **Product Dispensing Log**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Site: Beaumont Hospital

Principal Investigator: Toney Thomas

Product Dispensing Log

Patient Name	Patient ID (case number)	Date of supply	Dispensing initials	Date of return	Comments
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	
	MDC____	__/__/____		__/__/____	

Appendix 7: **Site Signature Log**

NATURAL HONEY FOR NASAL MRSA

Protocol NHNMRSA /BH2014

Site: Beaumont Hospital

Site Signature Log

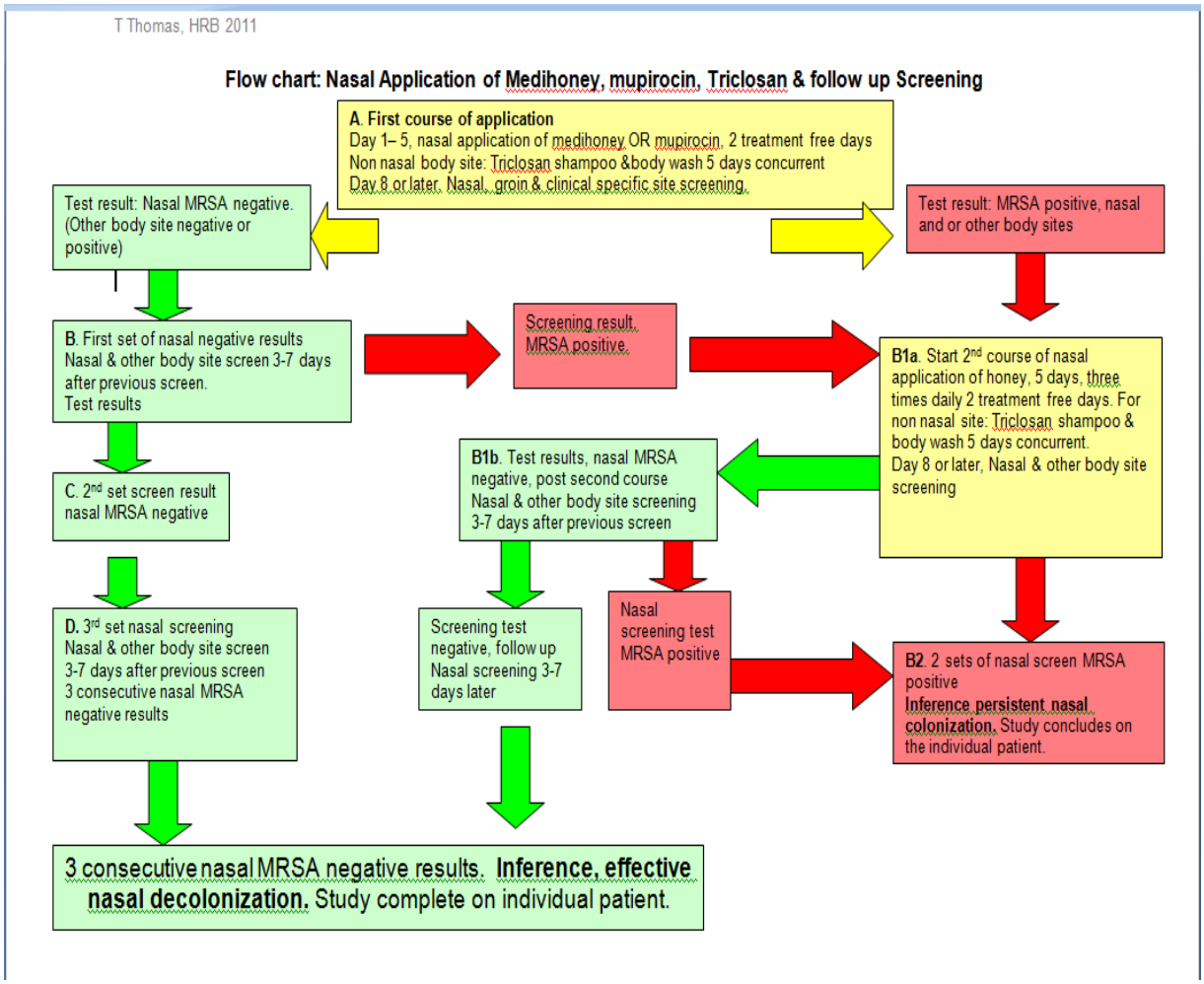
Instructions:

- The Primary Investigator is responsible for the study will sign the Site Signature & Responsibilities Log.
- The lead pharmacist will be responsible for training of the Primary Investigator.
- The lead pharmacist's signature certifies that the Primary Investigator is qualified and has received training to perform the study tasks.

Name	Initials	Signature	Date from	Date to	Primary Investigators signature

Original to Natural honey for nasal MRSA Eradication study Site File. Copy filed in Pharmacy Manual.

Appendix - X: NHNMRSA RCT sequence of actions flow chart



Appendix - XI a: Product experience questionnaire_Version 2_Medihoney™ Derma Cream

Honey to Eradicate Nasal MRSA

Patient Questionnaire – Experience with Medihoney Derma cream.

Please circle below the option in the grid that best fits your opinion about the statements below

		Strongly agree	Agree	Neither agree or disagree	Disagree	Strongly disagree
1	*I found it easy to apply Derma cream to my nose.*					
2	*My nose was sticky after applying Derma cream.*					
3	*My nose was "runny" after applying the Derma cream.*					
4	*I experienced an unpleasant sensation in my nose immediately after or during application of the Derma Cream*					
5	*If given a choice between an antibiotic or a natural product to eradicate MRSA from the nose, which would you prefer.*	Antibiotic			Natural product	

*Comment*_____

_____

Thank you for taking time for completing the questionnaire. Please return the questionnaire in the envelope addressed to the investigator.

Appendix - XI b: Product experience questionnaire_Version 2_mupirocin 2% (Bactroban® 2%)

Honey to Eradicate Nasal MRSA

Patient Questionnaire – Experience with Mupirocin nasal ointment.

Please circle below the option in the grid that best fits your opinion about the statements below

		Strongly agree	Agree	Neither agree or disagree	Disagree	Strongly disagree
1	*I found it easy to apply mupirocin to my nose.*					
2	*My nose was sticky after applying mupirocin*.					
3	*My nose was "runny" after applying mupirocin.*					
4	*I experienced an unpleasant sensation in my nose immediately after or during application of mupirocin*					
5	*If given a choice between an antibiotic or a natural product to eradicate MRSA from the nose, which would you prefer.*	Antibiotic			Natural product	

*Comment*_____
_____

Thank you for taking time for completing the questionnaire. Please return the questionnaire in the envelope addressed to the investigator.

Appendix - XII: NHNMRSA RCT: Case Report Form_Version_2

Natural Honey for Nasal MRSA: Case Report Form_V2

Case number_____(4 digit)

Contact No 1_____

Contact No 2_____

Label / addressograph

Date of enrolment	Date of study completion	Lab: sp no NS-0

Date of Admn	Date of Birth	Sex	Date of Disch	Pt: type	Loc ward

Risk -a

DIAB	PROSTH	C LIV DS	C PULM	MALIG	VASC	ORGTR	IMUSP	REN	CHRTD

Risk –b; Skin condition

HLTY	POOR	WND	ULC	STOM	UNK

Risk –c; Device insitu

CVC	PVC	URC	UNK

Risk-d; Communal stay

NHOM	OTHCF	UNK

Location of recruitment

IP	OPD	NHOM	OTHCF	UNK

Residence during study C1 / C2

IP	OPD	NHOM	OTHCF	UNK

Isolation during study C1 / C2

	SRCP	CRCP	NOTIS	UNK	NAPP
C1					
C2					

Frequency of contact

HIGH	MED	LOW	UNK

MRSA history

NMRSA	KMRSA	UNK	Date first ID

Nursing Dependency

NDS

HCAI

VRE	CDF	ESBL	CRE	UNK

Contact PI: Tel 3133 NHNMRSA/CRF_V2_Mar2014

Natural Honey for Nasal MRSA: Case Report Form_V2

Decolonisation History and supply of study products

Description	Yes	No	Unknown
H/o Decolonisation			
Mup nasal			

Mup no courses	One	Two	>2	Unknown
Non nasal decolonisation				
MupR MRSA				

	Yes	No	Unknown	Prod Return
C1 Mupirocin				
C1 Derma cream				
C1 Triclosan				
Second course				
C2 Mupirocin				
C2 Derma cream				
C2 Triclosan				

MRSA screening; Positive=Yes / No / Not App / Unknown

Site	Screen-0	SCN-1	SCN-2	SCN-3	Scn-4/F
Nose					
Groin					
Urine					
Resp					
Wound					
Ulcer					
Other					
Res inf pt					
Date inf pt					

Contact PI: Tel 3133 NHNMRSA/CRF_V2_Mar2014

Address , & Next of Kin		GP details

Summary

No of mup courses	One	Two	Unknown
No of DC courses	One	Two	Unknown
	Yes	No	Unknown
Pers NS MRSA			
MUH indicated			
MUH prsc & gvn			
Wdraw in 1 course			
Drop U Contact			
Wdraw Cl detero			
Wdraw form complted	Yes	No	Not applicable
MRSA other sites OC			

Questionnaire

	1=Issued. 2=not issued. 3=not applicable. 9=Unknown
Questionnaire 1- Product experience	
Questionnaire 2- MRSA perception	
	1=Returned. 2=non return. 3=not applicable. 9=Unknown
Q1 - Completed and returned	
Q2 - Completed and returned	

1. Information leaflet given □
2. Written consent X 3 copies □ 1 each medical record, PI, patient.
3. Letter to GP, letter to Hospital Consultant □
4. Written prescription – Mupirocin / Medihoney Derma cream □
5. Mupirocin / Medihoney labelled & dispensed to patient □
6. File dispensing log details completed □
7. Demonstration of application to patient □
8. Witness self administration by patient □
9. How to apply mupirocin / Medihoney information sheet □
10. Product administration record sheet (held with patient) □

Natural Honey for Nasal MRSA: Case Report Form_V2

Notes

Appendix - XIII: NHNMRSA RCT Data dictionary_Version_1_2014

Natural Honey for Nasal MRSA A RCT: Data Dictionary

Column	Abbreviation	Full name of the variable	Coding option	Definition	Source	Type of variable Quantitative / Qualitative
A	CAN	Case number allocated	Number; four digits	Self explanatory	Primary investigator	Quantitative
B	ENO	Episode no	Number; nine digits	Self explanatory	Medical record	Quantitative
C	DOA	Date of Admission	yyyy/mm/dd	Date of admission to Beaumont hospital	Medical record	Quantitative
D	DOD	Discharge Date	yyyy/mm/dd	Date of discharge from Beaumont hospital	Medical record	Quantitative
E	DOB	Date of Birth	yyyy/mm/dd	Self explanatory	Medical record	Quantitative
F	PTY	Patient Type	1= Inpatient. 2= Out patient. 9=Unknown	Self explanatory	Beaumont Hospital information system (BHIS)	Quantitative
G	LOW	Location Ward		Name of the ward in Beaumont hsopital	BHIS	
H	SPC	Speciality	1= Medicine. 2= Surgery. 3= Renal. 4= Neuroscience. 9=Unknown	The name of the speciality patient listed	BHIS	Quantitative
I	SEX	Sex	1=Male 2=Female 9=Unknown	Self explanatory	Medical record	Quantitative
J	SNO	Specimen number of the 1st study isolate		Laboratory number assigned	BHIS	Quantitative

Appendix – XIV: NHNMRSA RCT documents not listed else where

XIV a. Patient Consent Form

STUDY TITLE: **HONEY TO ERADICATE NASAL MRSA** STUDY

I have read and understood the **Information Leaflet** about this research project. The information has been fully **explained** to me and I have been able to ask questions, all of which have been answered to my satisfaction.	**Yes**	**No**
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	**Yes**	**No**
I am aware of the potential risks of this research study.	**Yes**	**No**
I give permission for researchers to look at my medical records to get information. I have been assured that information about me will be kept private and confidential.	**Yes**	**No**
I have been given a copy of the Information Leaflet and this completed consent form for my records.	**Yes**	**No**
I consent to take swabs from nose and groin for the purpose of microbiological testing during my participation in the study.	**Yes**	**No**
Storage and future use of information: I give my permission for information collected about me to be stored or electronically processed for the purpose of scientific research and to be used in <u>related studies or other studies in the future</u> but only if the research is approved by a Research Ethics Committee.	**Yes**	**No**

Patient Name (Block Capitals)	Patient Signature	Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

Name (Block Capitals)	Qualifications	Signature	Date

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

XIV b: Proforma NHNMRSA trial Sticker for placement on Clinical Notes

Proforma NHNMRSA trial Sticker for placement on Clinical Notes

Sticker version

NHNMRSA Study Enrolment visit Case No.....

Inclusion Criteria Date..... Time.....

Nasal MRSA colonisation - Yes, No, Informed consent obtained - Yes, No, Consent form V_1

Exclusion Criteria

Participating in another trial - Yes, No, Uncontrolled Diabetes Mellitus - Yes, No
Hypersensitivity to, or intolerance of, honey or mupirocin - Yes, No.
Unable to provide consent - Yes, No.
Ability to understand or comply with the requirements of the study - Yes, No.
Difficult to follow up due to normal place of stay - Yes, No.

The MRSA Nasal Decolonisation Trial was discussed with this patient. The patient meets the inclusion criteria and has provided informed consent. A copy of the information leaflet and signed consent form is filed in the medical record, a copy given to patient. He/She is now enrolled in MRSA nasal decolonisation trial. Randomisation done and patient is allocated to .....group. Letter to hospital consultant - Yes, No, family doctor - Yes No, done, after verbal consent. For additional details please contact Investigator, Toney Thomas. Tel: 3133.

Name: _____Signature:_____Date:_____

NHNMRSA Study Follow up Visit No..... Case No..... Date.....

Reason for visit: MRSA screening - Yes No, MRSA screening done - Yes No

OR post screen visit - Yes No. Adverse Event - Yes No

Nasal MRSA colonisation - Yes, No, Action: Second course of decolonisation applies - Yes, No,

If Yes, to commence on second course of.....

If No, state reason / comments.....

For additional details please contact Investigator, Toney Thomas. Tel: 3133.

Name: _____Signature:_____Date:_____

NHNMRSA Study End of study Visit Case No..... Date.....

Results of MRSA screening informed to patient- Yes No

Additional course of MRSA decolonisation applies – nasal mupirocin - Yes No / non nasal site decolonisation - Yes No.

Any other action / comments .....

.....

For additional details please contact Investigator, Toney Thomas. Tel: 3133.

Name: _____Signature:_____Date:_____

XIV c: MRSA illness perception questionnaire

The Brief MRSA Perception Questionnaire_V1_2014

For the following questions, please circle the number that best corresponds to your views:

How much does being MRSA positive affect your life?	0 No affect at all	1	2	3	4	5	6	7	8	9	10 severely affects my life
How long do you think being MRSA positive will last?	0 a very short time	1	2	3	4	5	6	7	8	9	10 forever
How much control do you feel you have over being MRSA positive?	0 absolutely no control	1	2	3	4	5	6	7	8	9	10 extreme amount of control
How much do you think undergoing the treatment to remove MRSA from your nose or skin will help you?	0 not at all	1	2	3	4	5	6	7	8	9	10 extremely helpful
Do you have any symptoms from MRSA?	0 no symptoms at all	1	2	3	4	5	6	7	8	9	10 many severe symptoms
How concerned are you about being MRSA positive?	0 not at all concerned	1	2	3	4	5	6	7	8	9	10 extremely concerned
How well do you feel you understand MRSA?	0 don't understand at all	1	2	3	4	5	6	7	8	9	10 understand very clearly
Are you affected emotionally (e.g. does it make you angry, scared, upset or depressed?) by being MRSA positive?	0 not at all affected emotionally	1	2	3	4	5	6	7	8	9	10 extremely affected emotionally

Please list in rank-order the three most important factors that you believe caused you acquire MRSA. *The most important causes for me:-*

1. _____
2. _____
3. _____

Thank you for taking time to complete the questionnaire. Please return the completed questionnaire in the pre-addressed envelope to the investigator.

T Thomas, Natural Honey for Nasal MRSA Eradication, BIPQ_V1_2014

XIV d: Directions for application of Medihoney® Derma Cream_ VERSION 1

Study name: *Natural Honey for Nasal MRSA Eradication Randomised Control Trial* **(NHNMRSA trial)**

Three times a day application for five consecutive days.

1. **Wash hands. Verify the product with the label with details of the study and your name written on the label.**
2. **Remove the product from packing and check the label.**
3. **Open the cap of the tube and squeeze approximately 2 centimetre of the cream into the applicator. This is about the size of last digit of your little finger.**
4. **Apply the cream into one of the nostril at a time.**
5. **Repeat step 3, i.e. squeeze 2 centimetre of the cream into the applicator and apply it into the other nostril.**
6. **Pinch the nose with two or more fingers and rub for 10-15 seconds. Wash your hands with soap and water.**
7. **Close the cap and place the cream in its packing.**
8. **Lay on your back with head up preferably raised on a pillow for at least five minutes.**
9. **If you experience wet nose, wipe nose with a disposable paper towel or cloth. Avoid force blowing off the nose if possible immediately after the application of the cream.**

WHAT SHOULD I DO IN CASE OF OVERDOSE?

If an overdose or too much honey is applied to the nose, use a disposable tissue paper and blow the nose to remove excessive Derma cream. Seek medical advice if you wish.

WHAT DO I DO IF I MISS A DOSE?

If you miss a dose of Derma cream, apply it as soon as you remember. Do not apply Derma cream to the nose more than three times a day. If it has been more than 6 hours since you missed the last dose wait and apply the next dose at the regular time.

Principal Investigator: Toney Thomas, Research Fellow, Infection Prevention & Control, Beaumont Hospital. **Telephone No**: 01 8093133.
Co-investigator: Prof. Hilary Humphrey's, Consultant Microbiologist, Beaumont Hospital. **Telephone No*:*** 01 8093133.

Directions for application of Medihoney® Derma Cream

Patient record

Study name: *Natural Honey for Nasal MRSA Eradication Randomised Control Trial* **(NHNMRSA trial)**

Three times a day application for five consecutive days.

√ Tick the time box after you have applied the cream into your nose.

First course commencing date----------

Day & date	Time	Time	Time	Comments if any
1				
2				
3				
4				
5				
No treatment	-	-	-	
No treatment	-	-	-	
Nasal screening				
Second course commencing date----------				
1				
2				
3				
4				
5				
No treatment	-	-	-	
No treatment	-	-	-	
Nasal screening				

Principal Investigator: Toney Thomas, Research Fellow, Infection Prevention & Control, Beaumont Hospital. **Telephone No**: 01 8093133.
Co-investigator: Prof. Hilary Humphrey's, Consultant Microbiologist, Beaumont Hospital. **Telephone No*:*** 01 8093312.

XIV e: Directions for application of Bactroban® 2% mupirocin _VERSION 1

Study name: *Natural Honey for Nasal MRSA Eradication Randomised Control Trial* **(NHNMRSA trial)**

Three times a day application for five consecutive days.

1. **Wash hands. Verify the product with the label with details of the study and your name written on the label.**
2. **Remove the product from packing and check the label.**
3. **Open the cap of the tube and squeeze approximately 0.5cm of the ointment into the applicator. This is about the size of a "match stick head".**
4. **Apply the ointment into one of the nostril at a time.**
5. **Repeat step 3, i.e. squeeze 2 centimetre of the ointment into the applicator and apply it into the other nostril.**
6. **Pinch the nose with two or more fingers and rub for 10-15 seconds. Wash your hands with soap and water.**
7. **Close the cap and place the ointment in its packing.**
8. **Lay on your back with head up preferably raised on a pillow for at least five minutes.**
9. **If you experience wet nose, wipe nose with a disposable paper towel or cloth. Avoid force blowing off the nose if possible immediately after the nasal application.**

WHAT SHOULD I DO IN CASE OF OVERDOSE?

If an overdose or too much mupirocin is applied to the nose, use a disposable tissue paper and blow the nose to remove excessive ointment. Seek medical advice if you wish.

WHAT DO I DO IF I MISS A DOSE?

If you miss a dose of mupirocin, apply it as soon as you remember. Do not apply mupirocin to the nose more than three times a day. If it has been more than 6 hours since you missed the last dose wait and apply the next dose at the regular time.

Principal Investigator: Toney Thomas, Research Fellow, Infection Prevention & Control, Beaumont Hospital. **Telephone No**: 01 8093133.
Co-investigator: Prof. Hilary Humphrey's, Consultant Microbiologist, Beaumont Hospital. **Telephone No***:* 01 8093133.

Directions for application of Bactroban® 2% mupirocin

Patient record

Study name: *Natural Honey for Nasal MRSA Eradication Randomised Control Trial* **(NHNMRSA trial)**

Three times a day application for five consecutive days.

√ Tick the time box after you have applied the cream into your nose.

First course commencing date----------

Day & date	Time	Time	Time	Comments if any
1				
2				
3				
4				
5				
No treatment	-	-	-	
No treatment	-	-	-	
Nasal screening				
Second course commencing date----------				
1				
2				
3				
4				
5				
No treatment	-	-	-	
No treatment	-	-	-	
Nasal screening				

Principal Investigator: Toney Thomas, Research Fellow, Infection Prevention & Control, Beaumont Hospital. **Telephone No**: 01 8093133.
Co-investigator: Prof. Hilary Humphrey's, Consultant Microbiologist, Beaumont Hospital. **Telephone No*:*** 01 8093312.

XIV f: Letter to consultant doctor

Consultant's Name and Address

Patient's Name and Address
Date

Dear Dr.

I am writing to inform you that your patient, XXXXX ,has agreed to take part in a research study in Beaumont Hospital to assess the effectiveness of a honey preparation to eradicate MRSA from the nose compared to mupirocin, the standard treatment. This is a randomised control study to evaluate eradication of MRSA from this site, following a pilot study recently carried out in Beaumont Hospital. The study is being funded by the Health Research Board.

As you may be aware, meticillin-resistant *Staphylococcus aureus* (MRSA) colonisation of the nose predisposes to infection. Current guidelines recommend nasal decolonisation of MRSA using mupirocin for a maximum of two courses. Decolonization is especially recommended in patients, who are due to undergo an elective operative procedure, who have a prosthesis *in situ,* or who are in a clinical area where there is a high risk of colonisation leading to invasive infection. There is some evidence to suggest that natural honey may have a beneficial effect on MRSA decolonisation and this is increasingly important as many MRSA strains are resistant to mupirocin..

As part of a randomised controlled study being conducted with Professor Hilary Humphreys, we plan to recruit patients with recently confirmed nasal colonisation of MRSA or who have completed two courses of mupirocin to eradicate MRSA from the nose but without success. Patients will receive either a nasal application of Medihoney Derma Cream© or Bactroban© (mupirocin) nasal ointment depending on the group the patient is randomly allocated to. Both treatments will be applied three times a day for five days and nasal screening two days after completion of a five day treatment will be undertaken to assess if the MRSA has been eradicated or persists. The course of Medihoney or mupirocin may be repeated for a second time if the follow-up samples after the first course remain positive for MRSA. MRSA eradication will be confirmed when three sets of nasal swabs at least two days apart are negative for MRSA.

Patients will be recruited from Beaumont Hospital and follow up screening will be done to confirm decolonisation or failure to eradicate MRSA. The conduct of this study will not affect any other aspects of this patient's management.

I am happy to forward a copy of the protocol as required. Feel free to contact me if you have any queries on extension 01-8093133 or on my mobile 087-1957264. A similar letter has been forwarded to the patient's general practitioner.

Yours sincerely,

_____

Toney Thomas,
Health Research Board Research Fellow
Beaumont Hospital.

XIV g: Letter to family doctor

GP's name and Address

Patient's Name and Address

Date

Dear Dr.

I am writing to inform you that your patient, XXXXX ,has agreed to take part in a research study in Beaumont Hospital to assess the effectiveness of a honey preparation to eradicate MRSA from the nose compared to mupirocin, the standard treatment. This is a randomised control study to evaluate eradication of MRSA from this site, following a pilot study recently carried out in Beaumont Hospital. The study is being funded by the Health Research Board.

As you may be aware, meticillin-resistant *Staphylococcus aureus* (MRSA) colonisation of the nose predisposes to infection. Current guidelines recommend nasal decolonisation of MRSA using mupirocin for a maximum of two courses. Decolonization is especially recommended in patients, who are due to undergo an elective operative procedure, who have a prosthesis *in situ,* or who are in a clinical area where there is a high risk of colonisation leading to invasive infection. There is some evidence to suggest that natural honey may have a beneficial effect on MRSA decolonisation and this is increasingly important as many MRSA strains are resistant to mupirocin..

As part of a randomised controlled study being conducted with Professor Hilary Humphreys, we plan to recruit patients with recently confirmed nasal colonisation of MRSA or who have completed two courses of mupirocin to eradicate MRSA from the nose but without success. Patients will receive either a nasal application of Medihoney Derma Cream© or Bactroban© (mupirocin) nasal ointment depending on the group the patient is randomly allocated to. Both treatments will be applied three times a day for five days and nasal screening two days after completion of a five day treatment will be undertaken to assess if the MRSA has been eradicated or persists. The course of Medihoney or mupirocin may be repeated for a second time if the follow-up samples after the first course remain positive for MRSA. MRSA eradication will be confirmed when three sets of nasal swabs at least two days apart are negative for MRSA.

Patients will be recruited from Beaumont Hospital and follow up screening will be done to confirm decolonisation or failure to eradicate MRSA. The conduct of this study will not affect any other aspects of this patient's management.

I am happy to forward a copy of the protocol as required. Feel free to contact me if you have any queries on extension 01-809133 or on my mobile 087-1957264. A similar letter has been forwarded to the patient's Beaumont Hospital consultant.

Yours sincerely,

_____

Toney Thomas,
Health Research Board Research Fellow
Beaumont Hospital

Appendix - XV: List and source of MRSA isolates with the comparison of *spa* types

Natural Honey for Nasal MRSA Eradication A RCT - *spa* type comparison and analysis of historic clinical, baseline and end of study nasal MRSA isolates

No	Source of Historic (First) isolate	Study No	*spa* type - Historic	*spa* type - Baseline	*spa* type - Final	*spa* type comparison of historic and baseline isolates	*spa* type comparison of baseline and final isolates
1	NOSE	1111	t515	t6764	9999	D	NA
2	NOSE	1112	t032	t032	t032	ID	ID
3	SP CATH	1113	t032	t4559	9999	D	NA
4	NOSE	1114	t032	t7636	t7636	D	ID
5	GROIN	1115	t032	t020	t020	D	ID
6	NOSE	1116	t032	t032	9999	ID	NA
7	NOSE	1117	t032	t032	9999	ID	NA
8	GROIN	1118	t032	t4559	9999	D	NA
9	SupPdev site	1119	9999	Failed	9999	Napp	NA
10	NOSE	1120	t032	t4559	9999	D	NA
11	Line tip Jr	1121	9999	t4559	t032	Napp	D
12	BAL	1122	t127	t4559	t127	D	D
13	CELLULITISLL	1123	t022	t4559	9999	D	NA
14	MSU	1124	t515	t4559	9999	D	NA
15	ULCER	1125	t557	t4559	9999	D	NA
16	ULCER L	1126	t032	t4559	t4559	D	ID
17	NOSE	1127	t15373	t15373	t15373	ID	ID
18	Abd Cellulitis	1128	t190	t190	9999	ID	NA
19	NOSE	1129	t515	t515	t515	ID	ID
20	Wound	1130	9999	t515	9999	Napp	NA
21	NOSE	1131	9999	t515	t515	Napp	ID
22	GROIN	1132	9999	t020	t020	Napp	ID
23	NOSE	1133	t032	t032	9999	ID	NA
24	RESP	1134	t032	t032	9999	ID	NA
25	SKIN	1135	t032	t040	9999	D	NA
26	NOSE	1136	9999	t032	t032	Napp	ID
27	Wound	1137	t2436	t2436	t2436	ID	ID
28	CELLULITISLL	1138	t032	t032	t032	ID	ID
29	GROIN	1139	t020	t020	9999	ID	NA
30	DISCHARGE ns	1140	9999	t515	9999	Napp	NA
31	NOSE	1141	t032	t032	t032	ID	ID
32	NOSE	1142	t379	t379	9999	ID	NA

33	Wound TOE	1143	t2436	t2436	9999	ID	NA
34	NOSE	1144	t032	t032	9999	ID	NA
35	NOSE	1145	t032	t032	9999	ID	NA
36	NOSE	1146	t032	t032	9999	ID	NA
37	NOSE	1147	t1214	t032	t032	D	ID
38	BLOOD	1148	t032	not typeable	9999	D	NA
39	SPUTUM	1149	t032	t032	9999	ID	NA
40	NOSE	1150	t032	t032	9999	ID	NA
41	Ulcer L	1151	9999	t032	9999	Napp	NA
42	NOSE	1152	t032	t032	t032	ID	ID
43	SPUTUM	1153	9999	t515	t515	Napp	ID
44	Ulcer L	1154	unknown	t127	t127	D	ID
45	Pus Penis	1155	t515	t515	t515	ID	ID
46	CSU	1156	9999	t032	9999	Napp	NA
47	Nose	1157	t127	t127	9999	ID	NA
48	Nose	1158	t515	t515	Unknown type	ID	D
49	NOSE	1159	9999	t515	t515	Napp	ID
50	NOSE	1160	t8046	t8046	9999	ID	NA
51	NOSE	1161	t127	t127	t127	ID	ID
52	Wound	1162	t127	t127	t127	ID	ID
53	CELLULITISLL	1163	9999	t032	t032	Napp	ID
54	GROIN	1164	t032	t032	9999	ID	NA
55	DISCHARGE LL	1165	t032	t032	t032	ID	ID
56	NOSE	1166	t008	t127	t127	D	ID
57	GROIN	1167	t020	t020	9999	ID	NA
58	Blood CS PVC	1168	9999	t045	9999	Napp	NA
59	NOSE	1169	t515	t515	9999	ID	NA
60	NOSE	1170	9999	t032	t032	Napp	ID
61	WOUND FOOT	1171	9999	t032	9999	Napp	NA
62	WOUND FOOT	1172	t032	t032	9999	ID	NA
63	ULCER FOOT R	1173	9999	t032	9999	Napp	NA
64	ULCER LEG	1174	9999	t032	t032	Napp	ID
65	WOUND FOOT L	1175	9999	t515	9999	Napp	NA
66	NOSE	1176	9999	t022	9999	Napp	NA
67	NOSE	1177	9999	t032	9999	Napp	NA
68	WOUND TOE	1178	t032	t032	9999	ID	NA
69	ULCER LEG L	1179	t223	t127	9999	D	NA
70	CANNULA TIP FEM LINE	1180	9999	t032	t032	Napp	ID
71	Blood CS PVC	1181	9999	t022	t032	Napp	D

72	NOSE	1182	t032	t032	t032	ID	ID
73	NOSE	1183	9999	t127	9999	Napp	NA
74	NOSE	1184	t1612	t1612	t1612	ID	ID
75	ILEAL STOMA	1185	9999	t578	9999	Napp	NA
76	SPUTUM	1186	t1370	t1370	9999	ID	NA
77	NOSE	1187	t1612	t1612	t1612	ID	ID
78	MSU	1188	9999	t9570	9999	Napp	NA
79	NOSE	1189	t022	t022	9999	ID	NA
80	GROIN	1190	t022	t022	t032	ID	ID
81	NOSE	1191	t032	t032	t032	ID	ID
82	THROAT DISCHARGE	1192	9999	t032	9999	Napp	NA
83	WOUND SURGICAL RT UP LEG	1193	9999	t032	t032	Napp	ID
84	SPUTUM	1194	9999	t15959	9999	Napp	NA
85	BLOOD -PVC	1195	t127	t127	t127	ID	ID
86	NOSE	1196	9999	t032	t032	Napp	ID
87	NOSE	1197	t032	t515	t515	D	ID
88	ULCER LL	1198	9999	t032	9999	Napp	NA
89	GROIN	1199	t032	t127	9999	D	NA
90	URINE	1200	9999	t020	9999	Napp	NA
91	GROIN	1201	9999	t1499	9999	Napp	NA
92	NOSE	1202	t127	t032	9999	D	NA
93	NOSE	1203	9999	t515	t515	Napp	ID
94	WOUND ABDM	1204	9999	t032	9999	Napp	NA
95	ULCER LL	1205	t032	t032	9999	ID	NA
96	NOSE	1206	t032	t032	9999	ID	NA
97	PENIS DISCH	1207	t032	t032	9999	ID	NA
98	WOUND LL	1208	t1612	t1612	t1612	ID	ID
99	NOSE	1209	t084	t127	t127	D	ID
100	MSU	1210	t032	t032	t032	ID	ID

ID - *spa* type indistinguishable, D - *spa* type distinguishable, Napp - Not applicable (Historic isolate not available), NA - Not available (participant decolonised or final outcome not available), 9999 – isolate not available

Appendix - XVI: The Brief Illness perception questionnaire (BIPQ)

The Brief Illness Perception Questionnaire

For the following questions, please circle the number that best corresponds to your views:

How much does your illness affect your life?										
0 no affect at all	1	2	3	4	5	6	7	8	9	10 severely affects my life
How long do you think your illness will continue?										
0 a very short time	1	2	3	4	5	6	7	8	9	10 forever
How much control do you feel you have over your illness?										
0 absolutely no control	1	2	3	4	5	6	7	8	9	10 extreme amount of control
How much do you think your treatment can help your illness?										
0 not at all	1	2	3	4	5	6	7	8	9	10 extremely helpful
How much do you experience symptoms from your illness?										
0 no symptoms at all	1	2	3	4	5	6	7	8	9	10 many severe symptoms
How concerned are you about your illness?										
0 not at all concerned	1	2	3	4	5	6	7	8	9	10 extremely concerned
How well do you feel you understand your illness?										
0 don't understand at all	1	2	3	4	5	6	7	8	9	10 understand very clearly
How much does your illness affect you emotionally? (e.g. does it make you angry, scared, upset or depressed?										
0 not at all affected emotionally	1	2	3	4	5	6	7	8	9	10 extremely affected emotionally
Please list in rank-order the three most important factors that you believe caused your illness. *The most important causes for me:-*										
1. _____										
2. _____										
3. _____										

Appendix - XVII: NHNMRSA RCT: Abbreviations

No	Abbreviation	Full form
1	ADL	Activities of daily living
2	AE	Adverse Event
3	AIIR	Airborne infection isolation room
4	atl	Autolysin
5	*B. cereus*	*Bacillus cereus*
6	*B. subtilis*	*Bacillus subtilis*
7	BH	Beaumont Hospital
8	BIPQ	Brief illness perception questionnaire
9	BSI	Bloodstream infection
10	C. diff	*Clostridium difficile*
11	χ^2	Chi-square
12	CA-MRSA	Community-acquired MRSA
13	*ccr*	Cassette chromosome recombinase
14	CE	*Conformité Européene*
15	cfu	Colony forming units
16	CHD	Chronic Heart disease
17	CHG	Chlorhexidine gluconate
18	CI	Confidence interval
19	*ClyS*	Staphylococcal-specific phage lysin
20	CoNS	*Coagulase negative Staphylococci*
21	CONSORT	Consolidated Standards for Reporting of Clinical Trials
22	COPD	Chronic obstructive pulmonary disease
23	CP	Contact precautions
24	CRE	Carbapenem resistant *enterobacteriacae*
25	CRF	Case report form
26	CSTAR	Centre for Support and Training in Analysis and Research
27	CT	Clinical Trial
28	CVC	Central vascular catheter
29	DFU	Diabetic foot ulcers
30	DM	Diabetes mellitus
31	*E. coli*	*Escherichia coli*
32	EARS-Net	European Antimicrobial Resistance Surveillance Network
33	ESBL	Extended spectrum *beta lactamase* organisms
34	ESRD	End stage renal disease
35	EU	European Union
36	FDA	US Food and Drug Administration
37	GAS	group A Streptococcus

38	GCP	Good clinical practice
39	GDP	Gross domestic product
40	HA-MRSA	Healthcare-associated MRSA
41	HCF	Health care facility
42	HCW	Health care worker
43	HIV	Human immuno-deficiency virus
44	HLMR	High level mupirocin resistance
45	HPRA	Health Products Regulatory Authority
46	HPSC	Health Protection Surveillance Centre
47	HSE	Health Services Executive
48	IL	Interlukin
49	IMB	Irish Medicines Board
50	IMP	Investigational medicinal product
51	IPC	Infection prevention and control
52	IPQ	Illness perception questionnaire
53	IQR	Inter-quartile range
54	ITT	Intention to treat
55	ITU	Intensive care unit
56	LG	Logistic regression
57	LLMR	Low level mupirocin resistance
58	LOS	Length of stay
59	LP-MIC	Laboratory protocol - MIC
60	MBC	Minimum bactericidal concentration
61	MDRO	Multi drug resistant organisms
62	*mecA*	methicillin resistance gene
63	MGH	Medical grade honey
64	MGO	Methylglyoxal
65	MIC	Minimum inhibition concentration
66	MM6	Mono Mac 6
67	MMP-9	Matrix metalloproteinase -9
68	MR	Mupirocin resistance
69	MRMRSA	Mupirocin resistant MRSA
70	MRSA	Methicillin resistant *Staphylococcus aureus*
71	MS	Mupirocin susceptibility
72	MSSA	Methicillin susceptible *Staphylococcus aureus*
73	*mupA*	MR isoleucyl-tRNA synthetase gene
74	NDFU	Neuropathic diabetic foot ulcers
75	NHNMRSA	Natural honey for nasal MRSA
76	NIS	Nursing dependency information system
77	NR^2	Nagelkarke R^2
78	PAG	Project Advisory Group
79	PBP	Penicillin binding protein
80	PCR	Polymerase chain reaction

81	PEQ	Product experience questionnaire
82	PICC	Peripherally inserted central vascular catheter
83	PIPE	Patient Information Profile Explorer
84	PP	Per protocol
85	PVC	Peripheral vascular catheter
86	*qacA/B*	Chlorhexidine resistance genes
87	RCT	Randomised control trial
88	RJ	Royal jelly
89	RNA	Ribo nucleic acid
90	ROS	Reactive oxygen species
91	*S. aureus*	*Staphylococcus aureus*
92	*S. epidermidis*	*Staphylococcus epidermidis*
93	*S. pyogenes*	*Streptococcus pyogenes*
94	SAE	Serious adverse Event
95	SCC	Staphylococcal cassette chromosome
96	SCCmec	Staphylococcal cassette chromosome *mec*
97	SD	Standard deviation
98	SD	Standard deviation
99	SEM	Scanning electron microscopy
100	*smr*	Low level chlorhexidine resistance gene
101	SP	Standard precautions
102	SSTI	Skin and soft tissue infections
103	ST	Sequence Type
104	TBP	Transmission-based precautions
105	TEM	Transmission electron microscopy
106	TNF-α	tumour necrosis factor-α
107	UC	Urinary catheter
108	UCD	University College Dublin
109	UspA	Universal stress protein A
110	VRE	Vancomycin resistant *enterococci*