An investigation of the temporal dynamics of *Staphylococcus aureus* nasal and oropharyngeal carriage amongst oral/dental healthcare workers

A thesis submitted to the University of Dublin in partial fulfilment of Doctorate in Dental Surgery D.Ch.Dent (Oral Surgery)

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May 2017
Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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_________________________
Keira M. Malone
‘Character cannot be developed in ease and quiet. Only through experience of trial and suffering can the soul be strengthened, ambition inspired, and success achieved’

Helen Keller
Summary

Background: Approximately 30% of humans are *Staphylococcus aureus* nasal carriers and trafficking of the organisms between the nose and oral cavity is frequent. Oro-nasal carriage of *S. aureus* can be persistent or transient. Healthcare workers (HCWs) have been implicated in transmission of methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) to patients. Oro-nasal colonisation prevalence data for dental HCWs is scarce.

Aims and Objectives: To investigate the prevalence of transient and persistent oro-nasal colonisation by *S. aureus* of two groups of dental HCWs including (A) Dublin Dental University Hospital HCWs working at outreach multicentre facilities and (B) dental HCWs in general practices in four different locations, and to characterise *S. aureus* isolates recovered. To achieve these objectives, volunteers from each group were screened twice for *S. aureus* carriage over three months.

Methods: Ethical permission for the study was granted by the Tallaght Hospital/St. James's Hospital Joint Research Ethics Committee. Nasal swabs and oral rinses were taken from 79 volunteer participants (n = 39 from group A, n = 40 from group B). Presumptive *S. aureus* were recovered on SaSelect™ (Bio-Rad) medium and confirmed by growth on mannitol salt agar and using the Pastorex™ Staph Plus kit (Bio-Rad). Twenty-four and 35 selected isolates from separate participants from the first and second collection phases, respectively, were screened for antimicrobial resistance and virulence-associated genes by DNA microarray profiling (Alere Technologies GmbH).

Results: MRSA was not detected from any of the participants tested. The MSSA nasal and oral carriage rate among participants during the first and second collection phases was 13.9% (11/79) and 16.5% (13/79), and 22.8% (18/79) and 21.5% (17/79), respectively. The first phase sampling identified 13 *S. aureus* nasal carriers, four of which were also oral *S. aureus* carriers. Following the second phase screening, 18 *S. aureus* nasal carriers were identified, seven of which were *S. aureus* nasal carriers during the first sampling phase. The second screening phase identified an additional 11 new nasal carriers and seven persistent nasal carriers, five of which retained the same *S. aureus* isolate CC from the first round.

Confluent growth of *S. aureus* from primary plating of oral swab and oral rinse samples was recorded for 21.5% (17/79) of *S. aureus* nasal and oral positive samples for the first round sampling with a reduced rate of 13.92% (11/79) for the second sampling round. The *S. aureus* cell density range recovered from *S. aureus* carriers yielding confluent growth from nasal swabs was determined between 2x10⁴ - 2.8 x10⁶ colony forming units (CFU)/swab.

A total of 24 MSSA isolates recovered from 21 participants during the first phase of sampling were investigated by DNA microarray profiling. DNA profiling detected 13 different multilocus sequence typing (MLST) clonal complexes (CCs), the most prevalent of which was CC30 (20.8%; 5/24 isolates). The most prevalent antimicrobial resistance gene detected among the 24 MSSA isolates was *sdrM* (91.7%; 22/24), encoding a non-specific efflux pump, and was identified in nine different CCs (CC1, CC5, CC7, CC8, CC20, CC22, CC30, CC45 and CC398). Immune
Evasion cluster genes were detected among 87.5% (21/24) of the isolates and included *scn* (87.5%; 21/24), *sak* (75%; 18/24), *chp* (62.5%; 15/24) and *sea* (29.2%; 7/24). The most common IEC type identified was IEC type B (41.7%; 10/24), followed by D (16.7%; 4/24), IEC type A (12.5%; 3/24), IEC types C and E both detected in 8.3% (2/24). The biofilm related gene, *icaA*, was identified in all 24 isolates. The enterotoxin gene cluster (*egc*) was detected in 50% (12/24) of isolates. The enterotoxin A gene *sea* was detected in 29.2% (7/24) of isolates belonging to four CCs (CC1, CC5, CC7 and CC30). The toxic shock toxin gene *tst* was detected in 12.5% (3/24) of isolates.

Thirty-five MSSA isolates recovered from 29 participants during the second phase of screening yielded 13 different MLST CC/sequence types (STs) following DNA profiling, of which CC30 (34.3%; 12/35) was the most common. Three additional CCs (CC15, CC59 and CC97) were identified, which were not detected during the first round. The beta-lactamase resistance gene *blaZ* was the most prevalent antimicrobial resistance gene identified (85.7%; 30/35), detected in eight different CCs (CC15, CC20, CC22, CC30, CC398, CC45, CC8 and CC97). IEC genes were detected among 85.7% (30/35) of the isolates. The most prevalent IEC type genes detected included *scn* (85.7%, 30/35), *sak* (71.4%, 25/35), *chp* (77.1%, 27/35) and *sea* (25.7%, 9/35). Similar to the first collection phase isolates, the most common IEC type for the second phase isolate collection was IEC type B (34.3%, 12/35), followed by IEC type C (20%, 7/35), IEC type A (22.9%, 8/35), IEC type D (8.6%, 3/35) and IEC type E (5.7%, 2/35). Clonal complexes CC30 and CC8 exhibited the most IEC types. The most prevalent virulence-associated gene detected was the biofilm related *icaA* gene, identified in all 35 isolates. The most prevalent toxin genes identified were the *egc* genes, detected in 19/35 (54.3%) isolates, most frequently belonging to CC30 with a total of 11/35 isolates. The enterotoxin A gene, *sea* was detected in 25.7% (9/35) isolates belonging to six CCs (CC7, CC8, CC15, CC30, CC45 and CC398). The toxic shock toxin gene *tst*, was detected in 31.4% (11/35).

**Conclusion:** These results indicate that there is a significant reservoir of *S. aureus* in dental HCWs including a subgroup that were heavily colonised. These latter individuals represent a significant health risk to both patients and fellow co-workers with the potential for onward transmission. There is no similar published data in the dental setting. Pre-employment screening may assist in identifying HCW carriers and reduce the risk of trans-contamination HCW-patient-HCW. Larger studies are warranted to investigate *S. aureus* carriage among dental HCWs.
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Acknowledgements

I would like to thank my research supervisor, Professor David Coleman for his advice, guidance, expertise and unwavering support both in the study design and completion of this thesis.

I am especially grateful to the staff of the microbiology laboratory at the Dublin Dental University Hospital, in particular Dr. Peter Kinnevey for his kindness and empirical facilitation given to me particularly throughout the laboratory-based period of this study; a gentleman and a scholar.

I extend thanks to Dr. Mary O Donnell who kept me laughing when times got tight.

I greatly appreciate and give thanks to each of the volunteer study participants for their time and repeated sample donations, without which, this study would not have been completed.

I would like to thank Professor Stassen for facilitating research sessions in my timetable to complete the sample collections and supporting me throughout my study.

I wish to acknowledge the stoic support coupled with personal and financial sacrifices bestowed upon me by my loving, beautiful best friend and husband Brian. His steadfast belief in me has guided me and steered us through good times-not least the recent birth of our baby daughter Claudia and challenging times.

Finally, a very special thank you to my parents for their continued invaluable support and belief in me throughout this thesis and all of my life adventures.
## Abbreviations

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<th>Definition</th>
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<td>ACME</td>
<td>Arginine-catabolic mobile element</td>
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<td>BSI</td>
<td>Blood stream infection</td>
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<td>CA</td>
<td>Community-associated</td>
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<tr>
<td>CBA</td>
<td>Columbia blood agar</td>
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<tr>
<td>CC</td>
<td>Clonal complex</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>cfu</td>
<td>Colony forming unit</td>
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<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>DDUH</td>
<td>Dublin Dental University Hospital</td>
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<td>DHCP</td>
<td>Dental Health Care Professional</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>e.g.</td>
<td>For example</td>
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<tr>
<td>et al.</td>
<td>And others</td>
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<tr>
<td>g</td>
<td>Acceleration due to gravity at the Earth’s surface</td>
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<td>h</td>
<td>Hour(s)</td>
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<tr>
<td>HA</td>
<td>Healthcare-associated</td>
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<td>HCW</td>
<td>Healthcare worker</td>
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<td>HRP</td>
<td>Horseradish-peroxidase</td>
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<tr>
<td>ICAS</td>
<td>Infection Control And Antibiotic Stewardship</td>
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<tr>
<td>i.e.</td>
<td>For example</td>
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<tr>
<td>IEC</td>
<td>Immune evasion cluster</td>
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<td>IgG</td>
<td>Immunoglobulin G</td>
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<td>IWG</td>
<td>International Working Group</td>
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<td>JADA</td>
<td>Journal of the American Dental Association</td>
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<tr>
<td>L</td>
<td>Litre</td>
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<tr>
<td>LA</td>
<td>Livestock-associated</td>
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<tr>
<td>MGE</td>
<td>Mobile genetic element</td>
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<td>min</td>
<td>Minute(s)</td>
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<td>ml</td>
<td>Mililitre</td>
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<td>MLST</td>
<td>Multilocus-sequence typing</td>
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<td>MRSA</td>
<td>Methicillin-resistant <em>S. aureus</em></td>
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<td>MSA</td>
<td>Mannitol salt agar</td>
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<td>MSSA</td>
<td>Methicillin-susceptible <em>S. aureus</em></td>
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<td>n</td>
<td>number</td>
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<td>NB</td>
<td>Nutrient broth</td>
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<td>NHANES</td>
<td>National Health And Nutrition Examination Survey</td>
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<td>NHS</td>
<td>National Health Service.</td>
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<td>NMRSAL</td>
<td>National Methicillin-resistant <em>S. aureus</em> Reference Laboratory</td>
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<td>NNIS</td>
<td>Nosocomial Infections Surveillance System</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
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<tr>
<td>PVL</td>
<td>Panton-Valentine leucocidin</td>
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Awards and Poster Presentations Associated with this Thesis

I presented and won an open paper research competition prize relating to this research at the British Association of Oral Surgeons (BAOS) annual scientific conference; held in the Assembly Rooms, Edinburgh on the 28th-30th September 2016.

I presented a poster relating to this research at the British Association of Oral Surgeons (BAOS) annual conference; held in the Assembly Rooms, Edinburgh on the 28th-30th September 2016.

I won the David Ryan Research Prize for a poster I presented relating to this research at the Irish Association of Oral Surgeons (IAOS) inaugural scientific meeting; held at the Radisson Blu Royal Hotel, Dublin on the 19th November 2016.
Chapter 1 Introduction
1.1 General overview of *Staphylococcus aureus*

*Staphylococcus aureus* is a Gram-positive coccus bacterium, which asymptotically colonises both animals and humans; and exhibits pathogenic capabilities. Approximately 30% of the human population are asymptomatic carriers of *S. aureus* (Mainous *et al.*, 2006). The anterior nares of the nose is the predominant site for human carriage (Harbarth *et al.*, 2001), however, other body sites can also be colonised namely the skin, perineum, pharynx (Armstrong-Esther, 1976; Pathak *et al.*, 2010; Ridley, 1959; Wertheim *et al.*, 2005a; Williams, 1963) and axillae (Dancer and Noble, 1991; Ridley, 1959; Williams, 1963). A feature of *S. aureus* is its peptidoglycan cell wall structure and its ability to produce coagulase which interacts with blood prothrombin resulting in the conversion of fibrinogen to fibrin. Blood coagulation is used to distinguish *S. aureus* from other members of the *Staphylococcus* genus. Protein A is a cell wall component of *S. aureus*. Laboratory testing for the confirmation of *S. aureus* utilises a triad of clumping factor, protein A and capsular polysaccharides which can be easily performed with the Pastorex™, Staph-Plus Kit latex agglutination kit (Bio-Rad) (Section 2.5.1).

Nasal colonisation of *S. aureus* has been identified as an important risk factor for the development of infection by the colonising isolate. Three main nasal carriage patterns of *S. aureus* have been described including persistent, intermittent and non-carriage. Persistent carriage accounts for 20% of the general human population, 60% remain intermittently colonised and 20% of people are never colonised by *S. aureus* (Harbarth *et al.*, 2001; von Eiff *et al.*, 2001). Individuals who are colonised by *S. aureus* are at increased risk for developing *S. aureus* infections; ranging from mild skin and soft tissue infections, to more severe diseases such as bacteraemia, osteomyelitis, sepsis, pneumonia and endocarditis. Nasally colonised individuals are at increased risk for developing infections with their colonising isolate; and these infections can be both hospital-acquired and community-acquired. The anterior nares provide the perfect niche for harbouring *S. aureus* with critical
interactions occurring between staphylococcal proteins and mucin carbohydrates (Shuter et al., 1996). A causal relationship exists between nasal carriage of *S. aureus* and infection, which is supported twofold. Firstly, the infecting strain often shares the same genotype as the nasal carriage strain (Valentine and Hall-Smith, 1952; von Eiff et al., 2001) and secondly, following nasal application of mupirocin, infections can be prevented during the period that the drug temporarily decolonises the nose and other colonised sites (Kluytmans et al., 1996; Wertheim et al., 2005b). Exhaustive studies have been carried out to determine the nasal carriage rate of *S. aureus* within the general healthy population, patients and amongst hospital staff (Armstrong-Esther, 1976; Noble et al., 1967; Williams, 1963). It has been established that most adult populations exhibit a nasal carriage rate of between 30-50% at any one time (Armstrong-Esther, 1976; Williams, 1963). Nasal colonisation of *S. aureus* is a multi-dependant process relying on a combination of four prerequisites, namely nasal contact with *S. aureus*, adherence of *S. aureus* to nasal receptors, overriding of the host defences to *S. aureus* and finally following adaption, *S. aureus* needs to be able to replicate in the nose (Wertheim et al., 2005a).

1.2 Clinical significance of *Staphylococcus aureus*

The clinical consequences of *S. aureus* are at best, most commonly superficial skin and soft tissue infections; but at worst deep-seated infections and life-threatening conditions such as toxic shock syndrome, septicaemia (Boyce et al., 1997; Diep et al., 2008; Ferry et al., 2005; Lowy, 1998; Murray, 2005; Todd, 2005) or death. Toxins and other virulence factors produced by *S. aureus* can mediate food poisoning, tissue destruction, immune evasion or toxic shock syndrome. It has been reported that *S. aureus* related bacteraemia accounts for mortality rates between 15-60% (Cluff et al., 1968; Julander, 1985). A meta-analysis by Cosgrove and colleagues demonstrated that the odds ratio (OR) of patient death caused by a MRSA bacteraemia compared to a MSSA bacteraemia was 1.93 (Cosgrove et al., 2003).
Main infections caused by *S. aureus* tend to be sinusitis or dermatitis with progress to chronic conditions including cellulitis, abscess, impetigo, carbuncles and furuncles and are associated with higher rates of persistent colonisation (Bertin et al., 2006; Boyce et al., 1993; Faibis et al., 2005; Gaynes et al., 1991; Kniehl et al., 2005; Locksley et al., 1982).

Specific to the oro-facial region, *S. aureus* is implicated in angular cheilitis (MacFarlane and Helinarska, 1976), and oral mucositis in elderly patients receiving parenteral nutrition (Bagg et al., 1995), parotitis and osteomyelitis. Oral cancer patients have been reported to be at increased risk of MRSA colonisation (Mitchell and Pickles, 1998; Parton et al., 1997; Supriya et al., 2009).

**1.3 Virulence of *S. aureus***

Staphylococcus aureus exhibits its virulence and subsequent pathogenicity through structural and excreted products. Microbial surface components recognising adhesive matrix molecules (MSCRAMMs) are surface proteins that mediate attachment of *S. aureus* to host tissue initiating colonisation leading to infection. Staphylococcus aureus carries numerous MSCRAMMs. Protein A, is a cell wall associated protein, that binds to immunoglobulin G (IgG) and is a hallmark of *S. aureus*. The specific binding orientation in which Protein A binds IgG on the surface of *S. aureus* is thought to disrupt phagocytosis and opsonisation. Another characteristic of *S. aureus* is the ability to secrete toxins that can either disrupt host cell membranes, or create pores in cytoplasmic membranes causing outpour of cellular contents and resultant lysis.

In the presence of fibronectin, clumping factor A and B mediate adherence of bacterial cells to fibrinogen. It is believed that clumping factors have a significant role to play in foreign body infections (Foster and Hook, 1998). Biofilm is a complex bacterial population enclosed in a polysaccharide matrix. The sticky matrix production is dependent on proteins encoded
by the intracellular adhesion (ica) operon. It has been reported that almost two thirds of S. aureus strains can produce biofilm (Aricola et al., 2001a).

Superantigen toxins produced by S. aureus are responsible for toxic shock syndrome, gastroenteritis and immune modulation. TSST-1 (tst) encodes toxic shock syndrome and enterotoxins A, B, C, D, E, G, Q are responsible for staphylococcal foodborne diseases and immune modulation. These toxins are resistant to heat denaturation and primarily weaken the host immunity system sufficiently thus allowing the pathogen to propagate and allow for disease to progress. These superantigen toxins are typically encoded by mobile genetic elements.

In addition to mobile genetic elements, S. aureus strains contain genomic islands and plasmids. Genomic islands carry genes that encode for approximately half of the S. aureus toxins and virulence factors which contributes greatly to the pathogenicity of the species (Gill et al., 2005) Plasmids are extrachromosomal genetic elements bearing only non-essential genes that encode factors relating to virulence factors and antimicrobial resistance factors.

1.4 Mechanism of methicillin resistance

The staphylococcal chromosome cassette (SCC) element is a large mobile genetic element (MGE) that harbours the mecA or the mecC genes, which encode a penicillin binding protein 2a (PBP2a) (Figure 1.3). SCCmec is a relatively stable DNA element incorporated into the S. aureus chromosome at the orfX locus. It is through the presence of this alternative PBP2a, that resistance to all beta lactam antibiotics is conferred (Chambers and Deleo, 2009; Malachowa and DeLeo, 2010). Isolates that acquire SCCmec are termed MRSA. SCCmec is passed to daughter cells during bacterial replication.
1.5 History of *S. aureus* in Ireland

Prior to the introduction of penicillin in the 1940s for the treatment of MSSA infections, mortality rates were approximately 80% (Cocchi et al., 2013). By 1950, the first reports of penicillin-resistant *S. aureus* strains began to appear (Levinson et al., 1950). A decade later, in 1959 semi-synthetic methicillin was introduced for the treatment of infections caused by beta-lactamase-producing staphylococci, and two years later MRSA was first reported in the U.K. (Barber, 1961; Jevons, 1961). In 1968, the first outbreak of MRSA was reported in a U.S. hospital (Barrett et al., 1968). Ten years after the initial first report in England, MRSA was first reported in 1971 in Irish hospitals (Hone and Keane, 1974). Following hospital-based infectious disease outbreaks, the CDC along with volunteer hospitals developed the National Nosocomial Infections Surveillance (NNIS) system in 1970. This group monitors the incidence of nosocomial (healthcare-associated) infections along with their associated pathogens and risk factors. Data from NNIS demonstrated an increase from 2% in *S. aureus* infections caused by MRSA in 1975 to 29% in 1991 (Panlilio et al., 1992). In line with global trends, MRSA prevalence increased in Ireland in the 1990’s with a fourfold increase in one hospital with MRSA affected patients between 1989-1998 (Rossney and Keane, 2002). MRSA have now been endemic in Irish hospitals for more than 40 years (Carroll et al., 1989; Coleman et al., 1985; Rossney and Keane, 2002; Rossney et al., 2006; Rossney et al., 2003; Shore et al., 2010) and during this time clonal replacement of prominent nosocomial MRSA strains in Irish hospitals has occurred several times (Shore et al., 2005). During the 1970’s and early 1980’s the ST250-MRSA lineage was the prominent nosocomial strain. This was replaced by ST239-MRSA during the mid-1980’s up to the early 1990’s. ST8-MRSA predominated during the 1990’s along with ST36-MRSA and ST22-MRSA-IV towards the latter end of the decade. Since 2002, ST22-MRSA-IV has been the dominant MRSA clone in Irish hospitals (Shore et al., 2005; Shore et al., 2010). Interestingly, prior to 1999, the ST22-MRSA-IV clone was only found sporadically in Ireland but four years later accounted
for 80% of all MRSA BSIs in Ireland and continues to account for an annual rate of between 70 to 80% of MRSA BSIs each year. Genetically diverse sporadic MRSA strains make up the final 20 to 30% of MRSA from BSIs in Ireland, which has approximately doubled from 12.1% in 2005 to 23.1% in 2011 (National MRSA Reference Laboratory annual report. 2009., National MRSA Reference Laboratory annual report. 2015., Kinnevey et al., 2014). Because it has been shown in Ireland that a previously sporadic strain such as ST22-MRSA-IV can emerge to predominate countrywide within a short period of time, it is essential to monitor new and emerging MSSA/MRSA strains, any one or more of which could emerge as prevalent strains in the future.

1.5.1 European blood stream infection (BSI) trends

Countries with the lowest incidence of MRSA BSIs include those of Scandinavia, Denmark and the Netherlands (Figure 1.1) (European Centre for Disease Prevention and Control, 2015, p. 74). MRSA prevalence increases geographically into the heart of Europe and reaches its highest levels in the Mediterranean region, most likely due to varying levels of infection control and antibiotic stewardship (ICAS) interventions within hospitals amongst different countries (Borg et al., 2012). Historically, Ireland has had a high incidence of MRSA related infections (Shore et al., 2010). Annual rates of MRSA among S. aureus strains causing BSIs in Ireland reached 41.9% in 2006, but have declined in recent years, with a rate of 19.5% for 2014 as reported by the European Antimicrobial Resistance Surveillance network (European Centre for Disease Prevention and Control, 2015). This is a European Union project that monitors and investigates antimicrobial resistance in member states. The most recent Irish data for MRSA BSI pertaining to 2016, published by the Health Protection Surveillance Centre, revealed that the number of S. aureus BSI that were methicillin resistant (i.e., MRSA) reduced from 18.4% (2015) to 14.7% (2016). Interestingly, the rate of MSSA BSI increased from 0.223 (2015) to 0.244 cases per 1,000 bed days used (Health Protection Surveillance Centre, 2017). The prevalence of MRSA in
the Netherlands is <5% probably best explained by the national policy that entails strict screening and isolation of all persons who are considered at high risk for MRSA when admitted to a hospital. Portugal has one of the highest levels of MRSA within Europe with more than 50% of *S. aureus* isolates which were reported as MRSA (European Centre for Disease Prevention and Control, 2015).

1.5.2 Global blood stream infection (BSI) trends

The largest study of reported *S. aureus* BSIs evaluating temporal and regional differences to date was carried out over a nine-year period from 2000-2008 that involved almost 20,000 patients from three continents. Countries involved included Finland, Australia, Western Sweden, Canada and Denmark. This study included cases of both MSSA and MRSA BSIs that occurred in both the community and hospitals and as such provided information on multiple populations around the world. Geographic incidence varied between the five global regions studied, along with regional variation observed within individual countries. This study revealed that 95.6% (17,618/18,430) of *S. aureus* BSI cases were attributed to MSSA and only 4.4% (812/18,430) attributed to MRSA. These results yielded an annual incidence rate of 24.2 per 100,000 MSSA BSI and 1.9 per 100,000 for MRSA BSIs. A significant limitation of this population-based design study included the lack of utilisation of a central laboratory (Laupland *et al.*, 2013).

1.6 MSSA and MRSA clones

A number of studies have concluded that each *S. aureus* lineage has evolved independently and *SCCmec* was introduced at different times in different lineages. These major clones are named CC5, CC8, C22, CC30, CC45 (McCarthy and Lindsay, 2010). The central European CC5-MSSA lineage is the only clone that has been shown to have multiple *SCCmec* elements that have integrated independently on several occasions into the local CC5-MSSA populations (Nubel *et al.*, 2008). Whole genome sequencing of *S. aureus* isolates has allowed useful studies to compare different isolates from geographically diverse locations belonging
Figure 1.1. Geographical distribution of methicillin-resistant *Staphylococcus aureus* bloodstream infections as a percentage of methicillin-susceptible *Staphylococcus aureus* infections detected within Europe in 2014 (European Centre for Disease Prevention and Control, 2015).
to the same clone. Studies with ST239, CC30, and ST225 (a variant of CC5) have revealed a stable HA-MRSA genome with frequent acquisition or loss of MGEs (Harris et al., 2010; McAdam et al., 2012; Nubel et al., 2010). The clones mainly associated with transmission in healthcare settings in the USA are USA100 (CC5-MRSA-II), USA500 (CC8-MRSA-IV) and USA800 (CC5-MRSA-IV), while in the U.K. they are CC22-MRSA-IV (EMRSA-15) and CC30-MRSA-II (EMRSA-16), in Germany CC5-MRSA and CC45-MRSA-IV, and in South America and Asia ST239-MRSA-III. In Irish hospitals ST22-MRSA harbouring SCCmec IV (ST22-MRSA-IV) has predominated since the late 1990s along with eight distinct clones of community associated MRSA (CA-MRSA) among which include CC1 and CC772 (Brennan et al., 2012; Earls et al., 2017).

1.7 Antibiotic resistance

Multidrug resistant bacteria have emerged worldwide for which there are reduced, or indeed no treatment options for infections caused by certain microorganisms. The CDC in 2013, reported an annual death toll of 23,000 individuals and more than two million infections attributable to antibiotic-resistant bacteria in the U.S. alone (Centers for Disease Control and Prevention, 2017). The single primary cause for the increase in antibiotic resistance is the excessive use of antibiotics (Carlet, 2015). A survey carried out in the U.S. in 2016 looking at physician’s offices, hospital clinics and emergency departments estimated that 30% of antibiotic prescriptions were inappropriate to treat a specific condition (Fleming-Dutra et al., 2016). This could very well be a conservative estimate as the study did not include prescriptions issued by physician assistants and nurse practitioners or correct dosage used.

In the U.K. it has been estimated from clinical audit, that around 50% of dental prescriptions for antibacterials are inappropriate (Chate et al., 2006; Palmer et al., 2001). It has been reported that dental antimicrobial prescribing in Scotland has been rising year on year with dental prescriptions accounting for almost 9% of all oral antibacterials dispensed in NHS primary care for 2013/2014 (Scottish Dental Clinical Effectiveness Programme, 2016).
similar rate of dental antimicrobial prescriptions has been reported in Norway. Analysis of 268,834 prescriptions issued by 4765 dentists from 2004 and 2005 showed that the dentists' prescriptions contributed 8% of the total national consumption antibiotics (Al-Haroni and Skaug, 2007). Recent guidelines for antibiotic use in dentistry have been published relating to appropriate antibiotic use (Fluent et al., 2016). Prescribing policy adjustments can mitigate selective pressures for antibiotic resistance and subsequently influence the incidence of MSSA or MRSA infection. Studies by Knight et al. have linked a decrease in the prescribing of ciprofloxacin to a decrease in MRSA incidence, which was not associated with infection control measures (Knight et al., 2012). Prudent appropriate use of antimicrobials will slow the emergence of bacterial resistance and preserve the usefulness of existing drugs for future generations.

Overuse of antimicrobials is not solely related to inappropriate antibiotic prescribing. Antibiotics are used by the agriculture and aquaculture industrial communities to enhance the growth of animals, vegetables, fruit and fish for human and animal consumption. A staggering 80% of all antibiotics, consumed in the U.S. annually is used within agriculture and aquaculture, not to combat disease however, but to promote growth (Glick, 2016). It is inevitable that these antibiotics will have a significant impact on the environment by increasing the selective pressures for the emergence of antibiotic resistance. Previous investigations have highlighted that the use of antibiotics has a detrimental effect on the human microbiome causing an imbalance in its overall function (Deshmukh et al., 2014; Jess, 2014). Antibiotics may affect the quantity, quality and diversity of the microbiota, which may ultimately affect host immunity; for example in the case of asthma, an immune-regulated disease that can occur later in life as a result of early-life antibiotic use affecting the microbiome (Russell et al., 2012).

MRSA exhibit resistance to the majority of beta-lactam antibiotics (Stefani and Goglio, 2010). An increasing prevalence of CA-MRSA infections limits the use of penicillin or
cephalosporins for complicated skin and soft-tissue infections (LaMar et al., 2003; Salgado et al., 2003; Spellberg et al., 2004). Vancomycin remains the treatment of choice for invasive MRSA infections. To compound the matter of antibiotic resistance, the emergence of vancomycin-resistant *S. aureus* (VRSA) is a direct result of increased use of vancomycin globally and an increase has occurred in colonisation and infection with VRSA strains (2002). Linezolid has become the last line of antimicrobial defence for infections resistant to all other agents. However, the emergence of linezolid-resistant *S. aureus* may limit treatment options for infections previously managed readily with conventional antimicrobials (Flamm et al., 2016; Rybak et al., 2014; Tsiodras et al., 2001).

**1.8 Healthcare associated MRSA (HA-MRSA)**

It is well established that patients in healthcare settings, in particular acute care hospitals, are more vulnerable to infections. This is mainly due to patient factors relating to age, underlying co-morbidities, treatment-related immunosuppression and procedures that perforate the body’s normal continuous protective skin and mucosal barriers. It has been established that only a small number of MRSA clones are responsible for the majority of infections in hospitals. Dominant clonal variation exists geographically. Epidemic HA-MRSA emerged in the 1980s and 1990s due to the evolution of new clones (Chambers and Deleo, 2009; Knight et al., 2012). It has been shown that replacement of predominant MRSA strains in Ireland occurs about once every ten years (Section 1.5). It has been postulated that the current endemic ST22-MRSA-IV clone in Ireland may be displaced in the near future by an emerging MRSA clone such as ST772-MRSA-V, CC1-MRSA-IV or another emerging strain. ST772-MRSA-V was the cause of an outbreak in a neonatal intensive care unit in a maternity hospital in Ireland in 2010 (Brennan et al., 2012) and CC1 is currently causing problems in several hospitals in Dublin (Earls et al., 2017).

Interestingly, it has been shown in Ireland that some predominant MRSA clones that have spread successfully over the years have not caused major problems in other countries.
and *vice versa*. For example, some ST239-MRSA-III strains caused major problems in Irish hospitals but other strains did not spread or were contained within a particular unit within a single hospital. Furthermore, ST247-MRSA-IA is the dominant MRSA clone within many European hospitals, especially in Spain and Portugal, but has never predominated in Irish hospitals (Shore *et al.*, 2005). Contrastingly the current predominant ST22-MRSA-IV within hospitals in Ireland is also the most prevalent epidemic strain in the U.K., known also as EMRSA-15.

Similarly, in a London hospital it was shown that HA-CC22-MRSA replaced CC30 and HA-ST239-MRSA with implied increased fitness and acquisition of multiple antibiotic resistances by the dominant clone CC22 also reported throughout England (Ellington *et al.*, 2010). A recent study in Italy reported HA-CC22-MRSA overtaking ST228 from the CC5 lineage, indicating CC22 to be a fitter strain, to grow faster and to be more virulent in a murine pneumonia model (Baldan *et al.*, 2012). CC22-MRSA has become more prevalent in Portugal in recent years, but is in strong competition with CC5 clones (Aires-de-Sousa *et al.*, 2008).

Results from a National U.S. survey from 2001 to 2003 revealed that there were 11.6 million *S. aureus*-related outpatient visits annually associated with skin and soft tissue infections. In 2005 in the U.S. an estimated 94,370 new invasive infections and 18,650 deaths were ascribed to MRSA. The increased frequency of MRSA was attributed to an increasing aging population along with an increased frequency of co-morbidities, such as diabetes. However, a decrease in relative *S. aureus* proportions has been shown in recent time blocks, possibly due to improved detection along with prompt isolation and control measures. Using the Nationwide Inpatient Sample from 1998 to 2003, Noskin and co-workers observed a significant increase in *S. aureus* rates per 1,000 patient-days (Noskin *et al.*, 2007).
A study by Avery et al. in 2006, investigated the acquisition of MRSA by two oral and maxillofacial patient groups in the U.K. following free flap surgery to reconstruct defects following the excision of lesions in the head and neck (such as oral carcinoma n = 58, osteoradionecrosis n = 2, dysplasia n = 1, postoperative deformity n= 1, or a benign tumour n= 2). The first group of 33 patients received a long-term 5-day post-operative course of antibiotics. The second group of 33 patients had a short-term 24-hour course of antibiotics. Results revealed that all infections occurred post-operatively. Delayed healing or discharge occurred in five MRSA patients. A total of 27% (17/64) of the patients developed an MRSA infection, seven of which occurred at the donor site. There were significantly fewer patients with MRSA infected sites in the short-term antibiotic group (4/33) compared to the group who had taken long-term antibiotic prophylaxis of five days duration (13/31). MRSA infected patients had a median longer hospital stay than those not infected with MRSA (Avery et al., 2006). In summary, this study reported a 27% MRSA rate in 64 patients who had free flap reconstruction, with a lower rate among those taking prophylactic antibiotics (24 h) than those patients taking a longer (5 day) course. In addition, patients involved in this study who had the shorter antibiotic course had significantly fewer infections post-operatively.

Another study carried out in a Regional Maxillofacial ward in Liverpool over a five year period identified that MRSA infection was also more prevalent among oncology patients who had undergone surgery for oral and oropharyngeal squamous cell carcinoma that required free tissue transfer (Rogers et al., 2008).

Outpatient and emergency department visits for these conditions rose by 59% from 1992 to 1994, and 31% from 2001 to 2003, likely reflecting the emergence of CA-MRSA. Visit rates were highest for children under two year of age and adults over 65 and higher geographically for people living in the South than in the Mid-west U.S. (McCaig et al., 2006). A study of adult patients with purulent skin and soft tissue infections presenting to emergency
departments in 11 U.S. cities revealed that MRSA was attributable for 78% of *S. aureus* skin and soft tissue infections (Moran et al., 2006). A study specific to the head and neck region carried out in one otolaryngology clinic revealed that the rate of CA-MRSA infections increased from 21% in 2000 to 64% in 2004 (Bothwell et al., 2007).

### 1.9 Community-associated MRSA (CA-MRSA)

The first reported CA-MRSA infections were among injecting drug users in Detroit, Michigan, in 1981 (Saravolatz et al., 1982). CA-MRSA has since emerged worldwide (Petti and Polimeni, 2011; Song et al., 2011). Some of the earliest reported cases of CA-MRSA infection occurred in Western Australia amongst the indigenous aboriginal people in the early 1990’s. Coupled with their susceptibility to most antibiotics other than beta-lactams and following PFGE, these MRSA strains were distinguishable from their hospital counterparts suggesting that these strains had either acquired *mecA* via horizontal gene transfer or were ‘rogue’ or ‘feral’ hospital-derived descendants (Chambers and Deleo, 2009). In Minnesota and North Dakota in the U.S. in 1997, four previously healthy children lost their lives as a direct result of MRSA systemic infections, namely sepsis and severe pneumonia associated with the USA400 (ST1-MRSA-IV) clone. These children had previously been healthy with no associated or established MRSA risk factors (Centers for Disease Control and Prevention (CDC), 1999). This commenced the worldwide recognition and emergence of CA-MRSA.

CA-MRSA in Ireland is largely under reported in contrast to the well characterised widespread HA-MRSA isolates found and reported in Irish hospitals (Kinnevey et al., 2014; Rossney et al., 2006; Shore et al., 2005; Shore et al., 2014). Since the Minnesota and North Dakota outbreak, CA-MRSA outbreaks have been reported in a younger healthier population without traditional risk factors in settings where people live in close contact, have frequent skin-to-skin contact, compromised skin (cuts and grazes), poor hygiene standards and practices that involve the sharing of personal equipment (Carleton et al., 2004; Saiman et
Populations affected include prison inmates, gym members, sports teams and the military. These groups typically have little or no previous contact with the healthcare system. Additional risk groups include intravenous drug users and men who have sex with other men.

1.10 Diverging movement of HA- and CA- *S. aureus*

There has been an increasing number of reports worldwide indicating that HA-MRSA strains in hospitals are being gradually replaced by CA-MRSA strains (Chatterjee and Otto, 2013; Earls *et al.*, 2017). A large prospective surveillance study conducted over two years involving seventeen hospitals from eight countries within Asia including Korea, Taiwan, Hong Kong, Thailand, the Philippines, Vietnam, India and Sri Lanka reported that not only are MRSA infections spreading between hospitals and the community; various MRSA clones have been displaying inter-country dispersion (Song *et al.*, 2011).

Reported prevalence of CA-MRSA rates vary from 1% (among adults in Chicago and children in New York) to 42% in some Western Australian rural communities (Rossney *et al.*, 2007). Epidemiologically, CA-MRSA isolates are distinct from their fellow HA-MRSA counterparts in many ways. These characteristics include differences in the populations affected as stated above and associated clinical symptoms. Specific to antibiotic resistance, CA-MRSA strains are distinguished primarily from HA-MRSA by several characteristics: firstly, CA-MRSA are associated with different types of SCCmeC elements. Traditionally SCCmeC elements found in HA-MRSA are most often the larger types I, II and III, in contrast to the smaller and less fitness burden causative CA-MRSA harbouring SCCmeC element types IV and V (Daum *et al.*, 2002; Hiramatsu *et al.*, 2001; Ito *et al.*, 2012; Lee *et al.*, 2007). Secondly, CA-MRSA isolates are usually susceptible to most antibiotics other than methicillin and beta-lactams. Some studies have suggested that the presence of SCCmeC IV coupled with the lack of a multi-antibiotic resistance phenotype may be used to identify CA-MRSA (Naimi *et al.*, 2003; Okuma *et al.*, 2002). This apparent simple
distinction was deemed unhelpful in Ireland however, in 2003 following reports that 80% of MRSA BSI isolates did not exhibit a multiantibiotic-resistant phenotype and carried SCCmec IV (Rossney et al., 2006).

What therefore has enabled CA-MRSA to spread sustainably and cause disease in an otherwise healthy population? CA-MRSA strains combine methicillin resistance with enhanced virulence (such as evasion of neutrophil killing (Voyich et al., 2005)) and fitness, and demonstrate acquisition of specific toxin genes, frequently affect a younger healthier population and cause skin and soft tissue infections that have not previously been seen in HA-MRSA strains. CA-MRSA often encode the cytotoxin Panton-Valentine Leukocidin (PVL) which consists of two proteins, LukS-PVL and LukF-PVL encoded by the lukS and lukF genes. The toxin targets both mononuclear and polymorphonuclear cells which damage host defence mechanisms causing leukocyte destruction and tissue death via cellular necrosis or apoptosis (Boyle-Vavra and Daum, 2007; Lina et al., 1999).

1.11 Livestock-associated MRSA (LA-MRSA)

Epidemiologically, LA-MRSA colonises livestock and their human contacts (Lindsay, 2010). The term livestock-associated (LA)-MRSA became established in 2005 following the emergence of the CC398 clone in the Netherlands among pigs and pig handlers (Voss et al., 2005). Since then, CC398 has emerged not only among pigs but horses and poultry along with humans who share close animal contact throughout other European countries and worldwide (Asai et al., 2012; Graveland et al., 2011; Lim et al., 2012; Witte et al., 2007). MRSA was first isolated from an animal source in 1971, when recovered from a milk sample from a dairy herd in Belgium (Devriese et al., 1972). Since then MRSA has been isolated from not only farm animals, but from companion animals including cats and dogs (Abbott et al., 2010; Catry et al., 2010; Cefai et al., 1994; Moodley et al., 2006; O’Mahony et al., 2005; Scott et al., 1988). Domestic animal MRSA strains are similar to strains isolated from humans (e.g. ST22-MRSA-IV), whereas those from livestock appear to have an animal
origin. Worryingly, several studies have reported MRSA from food intended for human consumption, namely pork and beef (Pu et al., 2009; Van Loo, 2007; Weese, 2010) either from colonised MRSA humans or MRSA clones that have emerged in animals.

1.12 Financial consequences of MSSA/MRSA

In addition to clinical consequences, MRSA places a huge financial burden and poses a real and significant economic challenge to hospitals. Currently in Ireland, latest published point prevalence reports indicate that MSSA are responsible for fifteen percent of hospital associated infections in Ireland (Health Protection Surveillance Centre, 2012). In the U.S., surgical site infections are the third most common nosocomial infection (Horan et al., 1993) and cost the U.S. healthcare system annually an additional $5-10 billion (Boyce et al., 1990; Emori and Gaynes, 1993; Green and Wenzel, 1977; Kirkland et al., 1999; Nelson and Dries, 1986). A cohort study of 348 patients with S. aureus bacteraemia of which 96 patients had MRSA carried out over a three-year period revealed that MRSA bacteraemia is associated with significant increases in hospital stay time periods and hospital charges. Patients with MRSA bacteraemia had an average nine-day hospital stay versus MSSA patients having a seven-day hospital stay. A 1.4-fold increase in hospital charges were attributed to MRSA bacteraemia patients compared to MSSA bacteraemia. This additional MRSA cost averaged at $3,836 per patient (Cosgrove et al., 2005). Studies have indicated that within Europe, the average additional costs per MRSA-positive patient range from between €5,700-10,000 (Chaberny et al., 2005; Kanerva et al., 2007; Wernitz et al., 2005). The European economic cost due to MRSA is estimated at €380 million per annum (Kock et al., 2010). The annual cost-of-illness due to antimicrobial resistance amounted to over 50 billion dollars for the U.S. (Smith and Coast, 2013).
1.13 Incidence of MSSA/MRSA nasal carriage

There are various reported instances of *S. aureus* nasal colonisation published in the literature. The *S. aureus* nasal carriage rate can vary with the population studied. Host characteristics co-determine *S. aureus* carrier rate as revealed by work published by Nouwen and colleagues. This study involved inoculating nasal carriers and non-nasal carriers of *S. aureus* with a mixture of *S. aureus* strains. The non-carriers eliminated the introduced strains whereas the carriers selectively eliminated the introduced strains thus retaining the original resident *S. aureus* strains (Nouwen et al., 2004). Nasal carriage rates as low as 10-15% amongst healthy adults in the general population have been reported. Other studies approximate 25 to 30% of the general population carry *S. aureus* in their noses (Gorwitz et al., 2008; Graham et al., 2006; Kluytmans et al., 1997; Peacock et al., 2001; Perl and Golub, 1998; Trochesset and Walker, 2012; Wenzel and Perl, 1995). A small percentage of this population (<2%) are colonised with MRSA (Gorwitz et al., 2008; Petti and Polimeni, 2011; Trochesset and Walker, 2012). Nasal carriage rates of *S. aureus* amongst hospital personnel are higher and rates of 20-35% have been published (Willems, 1990) with a MRSA colonisation rate of 4.1% amongst HCWs (Albrich and Harbarth, 2008). Specific to the dental field, limited reports are available. In Houston, Texas, Reddy and colleagues report a similar MRSA nasal carriage of 4.2% amongst dentists and 1.5% amongst hygienists (Reddy, 2010). The dental student population has been studied recently in Asia, South America and the U.S. Most recently, reports from Seoul National University School of Dentistry indicate that nasal samples from 159 dental students yielded an MRSA nasal carriage rate of 3.1% (Yoo Sang Baek, 2016). A study by Martinez-Ruiz and colleagues, in 2014 at the National University of Mexico, revealed that 20% of dental students with a five year clinical exposure accumulation carried MRSA (Martinez-Ruiz et al., 2014). Roberts and colleagues found similar high MRSA nasal carriage rates of 21% amongst dental students at the University of Washington (Roberts et al., 2011).
The first nationally representative sample used to estimate the prevalence of *S. aureus* nasal carriage in the U.S. was collected from 9,622 persons for The National Health and Nutrition Examination Survey (NHANES), 2001-2002. Results from this study indicated a *S. aureus* nasal colonisation prevalence of 32.4%, or 89.4 million individuals, and was highest in the 6-11 year age group. MRSA nasal colonisation prevalence for this group was 0.8% or 2.3 million persons. (Kuehnert et al., 2006). In a multicentre studied carried out by VonEiff and colleagues over 80% of *S. aureus* bacteraemia were found to be endogenous in origin, with the site of origin identified as the nasal mucosa as blood isolates were clonally identical to those recovered from the nose (von Eiff et al., 2001). By eliminating nasal carriage of *S. aureus* other authors have shown a reduction in the incidence of *S. aureus* infections (Boelaert et al., 1993; Chow and Yu, 1989; Holton et al., 1991; Kluytmans et al., 1996; Wenzel and Perl, 1995; Yu et al., 1986). Nasal MRSA colonisation can act as a reservoir for transmission and is a risk factor for MRSA infection development (Klevens et al., 2008). In a review of the literature Albrich and Harbarth found that the mean MRSA nasal carriage rate for HCWs was 4.1% from 104 studies (Albrich and Harbarth, 2008). In a recent study of two groups of dental students which included students with and without clinical experience, it was found that 5/159 (3.1%) of the dental students had nasal MRSA colonisation. Nasal MRSA carriage was associated significantly with previous clinical exposure (Baek et al., 2016b).

### 1.14 Incidence of MSSA/MRSA oral carriage

Depending on the population studied, it has been found that *S. aureus* is detected in the oral cavities of between 24%-84% of healthy dentate individuals (Jackson et al., 1999; Ohara-Nemoto et al., 2008) with an incidence of 48% amongst the denture wearing population (Tawara et al., 1996). A 10-year retrospective analysis of laboratory data reporting isolation of *S. aureus* from oral specimens revealed that *S. aureus* isolates accounted for 18% of the 11,312 specimens analysed. Of these, 90% (1,782) were MSSA and 10% (204) were MRSA.
The most common specimen type from which MSSA was isolated was an oral rinse (McCormack et al., 2015). Some older studies by several authors have identified that staphylococci reside transiently in the oral cavity (Marsh and Martin, 1999; Percival et al., 1991). A study by Ohara-Nemoto and colleagues looking at nasal and oral bacteria of pre-clinical and laboratory research staff of Iwate School of Dentistry demonstrated evidence of nasal-oral trafficking of *S. aureus* amongst other staphylococcal species including *S. epidermidis, S. hominis* and *S. lugdunensis* using PFGE genotyping. They postulated that the staphylococcal species found in the oral cavity may be provided continuously from the nasal cavity (Ohara-Nemoto et al., 2008). Oral cavity MRSA colonisation has been reported in a small number of studies. Small et al., investigated MRSA nasal and tongue colonisation of renal dialysis patients which yielded a 25% carriage rate (Small et al., 2007). Researchers investigating three years of records from a regional oral microbiology laboratory isolated *S. aureus* from 1,017 of 5,005 specimens of which 6% were MRSA. The same study reported that MRSA is frequently found in the oral cavity of people 70 years of age and older (Smith et al., 2003). It has been established that a risk factor for oral carriage of MRSA amongst the older population is related to antibiotic use, low serum albumin levels and poor nutrition (Tada et al., 2002).

### 1.15 MRSA HCW prevalence

Nasal carriage of MRSA has been reported to vary between 6.3% and 17% amongst the general population and between 18.2% and 43.8% amongst HCWs (Akhtar, 2010; Elie-Turenne et al., 2010; Kitti et al., 2011; Ozguven et al., 2008; Pathak et al., 2010; Treesirichod et al., 2013). A review of MRSA transmission in the dental healthcare setting revealed that MRSA carriage amongst dental HCWs was not above that of the normal healthy adult population (Petti and Polimeni, 2011). A study by Reddy revealed that in the greater Houston metropolitan area 4.2% of dentists and 1.5% of dental hygienists were carriers of MRSA (Reddy, 2010). Another study reported that 21% of dental students were
colonised with nasal MRSA (Roberts et al., 2011). These studies however did not use chromogenic media for MRSA detection and therefore the estimates are probably very conservative. An extensive literature review carried out by Albrich and Harbarth found that 23.7% (2508/10589) of HCWs were found to carry MSSA; 4.6% of the total screened which were colonised with MRSA of whom about 5.1% went on to develop clinical disease. Symptoms included most frequently skin and soft tissue infections (folliculitis, abscess, cellulitis, impetigo, lymphangitis, osteomyelitis) followed by upper respiratory tract infections (such as sinusitis, rhinitis, conjunctivitis), ear infections (otitis externa, mastoiditis), lower respiratory tract infections (pneumonia, chronic pneumonia, exacerbation of cystic fibrosis), toxic shock syndrome (Albrich and Harbarth, 2008). Specifically this study revealed that mean nasal MRSA carriage rate in HCWs was 4.1%, 6.4% for hands, 1.6% for perineum, 0.3% for pharynx. The main risk factors for acquisition and transmission of MRSA by HCWs were identified as being chronic skin conditions, poor hygiene and having previously worked in MRSA-endemic countries. MRSA eradication was achieved in 88% of HCWs. This study included 127 investigations involving 33,318 screened HCWs. The study revealed that forward transmission from personnel to patients was likely in 93% of cases. Limitations of this study revealed that no controlled intervention studies specifically addressed the role of HCW in MRSA transmission and included outbreak investigations, point prevalence studies, prospective surveillance studies, case reports or case series. The authors did not grade the evidence to a systematic review level or perform a meta-analysis. A review by Dulon et al. revealed that nurses are the group of HCWs with the highest prevalence of MRSA due to their high contact rate with patients (Dulon et al., 2014). Persistent MRSA carriage by HCWs has been rarely reported, and found to be more common in personnel with extranasal carriage (Cookson et al., 1989; Hill et al., 1988; Maeder et al., 1993; Reboli et al., 1990; Wagenvoort et al., 2005).
1.16 MSSA and MRSA in dental healthcare environments

There are relatively few MSSA or MRSA carriage investigations reported in the dental field compared with the medical field. Recent increased numbers of reports indicate that MRSA is present in the dental setting, including dental patients, on dental clinical surfaces, and in dental healthcare workers, including students (Apolonio-Alonso et al., 2011; Horiba et al., 1995; Kurita et al., 2006; Martinez-Ruiz et al., 2014; Roberts et al., 2011). An investigation conducted in a dental school clinic investigating *S. aureus* found MRSA isolates in the emergency treatment area from sites including the dental chair operating buttons, light handles, air-and-water syringes and a computer keyboard. This study did not evaluate the transmission from the environment to patients or fellow dental HCWs (Motta et al., 2007). These studies indicate that transmission can potentially occur via the hands of dental healthcare workers or through contaminated dental work surfaces. Specific to the dental environment, contamination of dental patients or dental healthcare workers (bidirectional) is possible via airborne droplets created from aerosolisation of dental hand pieces during tooth preparation, scaling or surgical procedures. A study by Bennett and colleagues identified that the microbial aerosol concentration in the dental treatment room was found to be \( \leq 10^3 \) colony forming units (CFUs)/m\(^3\) (Bennett et al., 2000). This research concludes heightened exposure of dental professionals to salivary aerosols containing pathogens including *S. aureus* which may lead to onward transmission to patients or *vice versa*.

Pathways of transmission can be bidirectional and MRSA can be transferred from the patient to HCWs but also *vice versa* as was seen when a confirmed documented transmission of MRSA occurred from a British dental practitioner to two patients. Two patients within three weeks of undergoing oral surgical procedures developed MRSA infections of the same type, phage type 29. Molecular typing identified the dentist as the source of transmission through samples isolated from his fingers and nares, which also identified phage type 29 with identical antibiotic susceptibility and resistance patterns to
those isolated from both of the infected patients. The dentist had a recent episode of hospitalisation for emergency surgery at a time when the surgical unit was managing an outbreak of MRSA. This is one possible source where the dentist became colonised. Following treatment and instigating infection control measures including the routine use of gloves, no further MRSA was detected after nine weeks (Martin and Hardy, 1991). Dental impression guns have been reported to be contaminated with MRSA (Westergard et al., 2011), along with heavy MRSA contamination of gypsum casts (Egusa et al., 2008).

In a study by Kurita et al. 8/140 special dental care and oral surgery patients had no evidence of MRSA when admitted to hospital. These individuals subsequently became MRSA carriers during their hospital stay (Kurita et al., 2006). It was suggested by the authors that the dental operatory work surfaces were the reservoirs for MRSA transmission to patients via the hands or gloves of medical staff and following infection prevention measures the investigators found no subsequent MRSA transmission.

There are very few published studies on the topic of nasal MRSA carriage amongst dental school students. Martinez-Ruiz et al. showed that dental students from the National University of Mexico had a 20% carriage rate of MRSA with five to six years of cumulative clinical exposure (Martinez-Ruiz et al., 2014). There are only three other published articles that report the MRSA nasal carriage rate amongst dental students with conflicting results indicating an association between clinical experience and nasal MRSA colonisation. Two studies have shown that dental students with clinical experience had a higher rate of MRSA nasal colonisation compared to those without clinical experience (Baek et al., 2016b; Roberts et al., 2011). Roberts and colleagues showed that dental students from the University of Washington had an overall MRSA carriage rate of 21% with a 26% MRSA carrier rate for fourth-year dental students compared with a rate of 6.6% for first year dental students. Dental students in Seoul National University School of Dentistry had a nasal colonisation of MRSA rate of 3.1% with clinical experience dental students having a higher rate of being a
nasal MRSA carrier (6.4%) than non-clinical dental students (0%). In contrast, Petti et al. demonstrated that MRSA nasal carriage was higher amongst pre-clinical dental students. The pooled risk difference of these three studies shows there is no significant association between clinical experience and nasal carriage rate of MRSA. In a letter to the JADA editor, Yoo Sang Baek suggested that due to a small study sample size of 477 students in 3 single-centre studies, a causal relationship between clinical experience and nasal MRSA carriage cannot be made (Baek et al., 2016a). Researchers have shown that the MRSA carriage rate amongst dental health care professionals (DHCPs) is relatively lower than the rate of MRSA carriage amongst HCWs and the DHCP MRSA carriage rate is close to the MRSA general population rate (Petti and Polimeni, 2011). It is possible that the relatively low rate of 3.1% for the Seoul research group may be attributed to the relatively short duration (that is 0-6 months) of clinical exposure for the cohort studied.

There is a clear lack of prospective longitudinal studies investigating the incidence of healthcare-associated infections caused by MSSA or MRSA in the dental setting and the likelihood of these infections being detected, reported, documented and/or published in the field of dentistry is small. This reservoir represents a potential risk for onward transmission between dental healthcare workers and patients.

1.17 Typing of *Staphylococcus aureus*

1.17.1 Molecular typing

Until recently, pulsed-field gel electrophoresis (PFGE) typing was the “gold-standard” typing method for *S. aureus* (Frenay et al., 1996; Melin et al., 2009; Murchan et al., 2003). This method involves the digestion of high molecular weight genomic DNA with restriction endonucleases that cleave DNA infrequently. The resulting fragments are then separated by agarose gel electrophoresis using special electrophoresis equipment that alternates the electric field during electrophoresis. The resulting fragment patterns are then used to define isolate relationships following computer-assisted analysis of fragment banding patterns. A
standardised nomenclature for PFGE isolate typing has been developed (Tenover et al., 1995). The technique suffers from significant drawbacks however, including poor intra-laboratory reproducibility, expense, and the technique is labour intensive. PFGE has proved to be a useful method for isolate typing in outbreak situations (Cookson et al., 2007).

Multilocus-sequence typing (MLST) is a well-established and powerful molecular technique for genotyping S. aureus isolates in both long-term and worldwide epidemiological studies (Enright et al., 2002; Oliveira et al., 2002). It identifies single nucleotide variations of seven S. aureus housekeeping genes (each variant is termed an allele), which provide an allele based profile known as a sequence type (ST) for each isolate. Presence of the same 5/7 or more identified housekeeping alleles identifies a clonal complex (CC) (Figure 1.2) MLST is a good typing method for long-term investigations of S. aureus and utilises a web-based standardised nomenclature to describe different methicillin-susceptible S. aureus (MSSA) and MRSA strains. However, MLST is time consuming to perform.

*Spa* typing, is an inexpensive robust method often used to investigate S. aureus outbreaks that analyses tandem repeats in the gene (*spa*) that encodes protein A. Specifically, the *spa* locus of S. aureus codes for a species-specific protein A. DNA sequencing of the *spa* gene can therefore provide unambiguous molecular typing data that can be utilised for epidemiological purposes such as transmission and outbreak investigations at various geographical levels (Harmsen et al., 2003; Mellmann et al., 2006). *Spa* typing provides relatively rapid typing of repeat regions of one gene with good correlation with MLST, in addition to being quick with good reproducibility. *Spa* typing is supported by a web-based curated database (Ridom GmbH, 2003) of *spa* types of global S. aureus isolates and utilises an algorithm developed by Mellman et al., (2007) which groups different *spa* types into CC groups (Mellmann et al., 2007).
Another typing method that is utilised in conjunction with MLST, is typing of the staphylococcal chromosomal cassette (SCC) element; which upon acquisition by *S. aureus*, confers methicillin resistance. Isolates that acquire SCC\textit{mec} are termed MRSA. The large mobile SCC\textit{mec} element harbours the \textit{mecA} or \textit{mecC} gene (encoding methicillin resistance), together with regulatory genes, and genes involved in the insertion and excision of SCC\textit{mec} from the *S. aureus* chromosome (Ito \textit{et al}., 2012; Katayama \textit{et al}., 2000; Shore \textit{et al}., 2011a (Figure 1.3). It has been revealed through whole genome sequencing (WGS), that MRSA clones are very similar to their respective MSSA counterparts of the same lineage apart from SCC\textit{mec} (Holden \textit{et al}., 2004; McCarthy and Lindsay, 2010). Each MRSA clone carries a specific SCC\textit{mec} element. Twelve distinct types of SCC\textit{mec} element (SCC\textit{mec} types I-XII) have been described to date in MRSA (International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009; Ito \textit{et al}., 2012; Shore \textit{et al}., 2011a; Wu \textit{et al}., 2015). Each type is structurally different and can harbour different antimicrobial resistance genes in addition to \textit{mecA} or \textit{mecC} (Shore \textit{et al}., 2011a; Wu \textit{et al}., 2015). SCC\textit{mec} typing involves typing of the cassette recombinase gene, \textit{ccr} and the methicillin resistance gene, \textit{mecA/mecC} and its regulatory genes, \textit{mecR} and \textit{mecI}. For example, the current endemic MRSA strain in Irish hospitals is referred to by its clonal type, ST22, and its SCC\textit{mec} type, SCC\textit{mec} IV (i.e. ST22-MRSA-IV). Originally, healthcare associated (HA)-MRSA infection were associated with SCC\textit{mec} types I, II and III, whereas community-associated (CA)-MRSA infections were associated with SCC\textit{mec} type IV (Cohen, 2007). However, some studies have shown that HA-MRSA infections were historically associated with SCC\textit{mec} type IV and CA-MRSA infections also associated with types II and III (Classification of staphylococcal cassette chromosome \textit{mec} (SCC\textit{mec}): guidelines for reporting novel SCC\textit{mec} elements, 2009). Therefore, SCC\textit{mec} typing alone cannot be used to classify a strain as CA-MRSA or HA-MRSA. The United States (U.S.) Centers for Disease Control and Prevention (CDC) defined healthcare-associated (HA)
Figure 1.2. Multilocus sequence typing (MLST) for *Staphylococcus aureus* incorporating seven loci or housekeeping genes (yellow dots), located around the *Staphylococcus aureus* genome. Each of the seven loci on the *Staphylococcus aureus* genome (blue circle), is amplified and sequenced. The resulting sequence of each locus equates to a unique seven series allelic number. The combined seven alleles for the *Staphylococcus aureus* is subsequently assigned a sequence type (ST). If five out of the seven alleles are identical in any two *Staphylococcus aureus* isolates, they are assigned to the same clonal complex (CC) (Figure adapted from Kinnevey *et al.*, 2011).
**Figure 1.3.** Fundamental structure of staphylococcal chromosomal cassette *mec* (SCCmec). Upon acquisition of SCCmec by *Staphylococcus aureus*, beta-lactam and penicillin resistance is conferred, and isolates are subsequently termed MRSA. SCCmec is a large mobile genetic element, which integrates directly at the *orfX* site, demonstrated in the above figure on the left hand side, upstream of the inverted repeats (IR). Integration of SCCmec at the *orfX* site is both site and orientation-specific. The SCCmec gene complex harbours either the *mecA* or *mecC* genes. SCCmec typing involves typing of the *mec* gene complex which encodes both penicillin binding protein 2a (PBP2a), which is encoded by *mecA* and resistance regulators (*mecI* and *mecR1*), and typing of the *ccr* gene complex, which encodes the integration and excision of entire SCC element. Both the *mec* gene complex and *ccr* gene complexes are flanked by characteristic nucleotide sequences, as demonstrated in the above figure on the far left *IS431* (blue box), inverted repeats (IR) and direct repeats (DR), at both ends. There are currently twelve different SCCmec types assigned by Roman numerals I-XII, according to the order in which they are reported to date (Figure adapted from Hiramatsu *et al.*, 2014).
infections as occurring in patients who underwent hospitalisation, surgery, dialysis, long-term care facility stay or use of indwelling catheters in the previous twelve months. CA-MRSA infection, as defined epidemiologically occurs either within the community or in patients less within 48 hours following hospital admission who do not have healthcare-associated (HA) risk factors (Song et al., 2011).

The more prominent S. aureus clones can be described by their PFGE type, ST, CC, spa type or lineage (Stefani et al., 2012). These clones are derived from distinct lineages with a different genetic backbone and have individual variations of combined genes that encode for surface proteins and immune evasion pathways. Mobile genetic elements account for up to 20% of the S. aureus genome and much variation can exist between isolates with evidence to show that MGEs display frequent horizontal transfer between isolates of the same lineage (Lindsay et al., 2006; McCarthy and Lindsay, 2010). With the recent advance in WGS technologies, a number of DNA microarray systems have been developed. One in particular, the S. aureus DNA microarray developed by Alere Technologies GmbH (Jena, Germany) targets 333 genes including antimicrobial resistance genes, virulence-associated genes, clone specific markers and control markers. The microarray patterns generated from isolates under investigation are compared to a large global database, which includes all of the S. aureus clones for assignment to CCs and STs (Monecke et al., 2008b).

1.18 Study Aims

This study had three overall aims. Firstly, to investigate the range of MRSA and MSSA strains carried by dental healthcare staff over two 3-month screening phases and including two groups. One group of dental HCWs was based at the Dublin Dental University Hospital (DDUH), but who also worked at multi-centre facilities including prisons and outreach city hospitals. The second group of dental HCWs worked in general dental practice. Both groups were exposed to aerosols generated by dental hand pieces from oral fluids potentially
containing *S. aureus* trafficked from the nares and therefore at risk of becoming colonised by *S. aureus*. The second aim was to establish the oral and nasal carriage pattern of persistent and intermittent carriage of MSSA and MRSA among oral/dental healthcare staff of both groups. The third aim was to undertake further in-depth analysis in order to identify if the MSSA/MRSA genotypes carried by HCWs are consistent with the main geographical genotypes found in Ireland and to investigate the prevalence of mobile genetic elements harbouring antimicrobial resistance and virulence genes. There is no other study of this kind published. The potential for onward transmission of MSSA or MRSA to fellow dental healthcare workers or patients is currently unknown, and this study will provide useful pilot data pertaining to carriage rates, virulence and pathogenicity amongst dental healthcare workers.
Chapter 2 Materials and Methods
2.1 Ethical approval

Ethical approval for this study was sought from the Tallaght Hospital / St. James's Hospital Joint Research Ethics Committee (REC), The Adelaide and Meath Hospital, Dublin (SJH/AMNCH). It was granted in September 2015 (Appendix I). This project did not involve any potential risk or harm to the safety of healthcare worker participants as the sampling methods, which included nasal swabs and oral rinse sampling, were non-invasive.

2.2 Study participants

The sample population consisted of dental HCW volunteers from two groups (group 1, \( n = 39 \), group 2, \( n = 40 \)), working out of five separate institutions. The first group of dental HCWs were staff and students based at the DDUH, Trinity College Dublin, but who also worked at multi-centre facilities including prisons and outreach city hospitals. The second group of dental HCWs worked in general dental practice. The sample group consisted of males and females, operators and assistants. Recruited volunteer dental operators consisted of hygienists, dentists, full time postgraduate dentists, oral surgeons and oral and maxillofacial surgeons. Dental assistants included exclusively registered nurses, dental nurses and student dental nurses. Daily work for all study participants involved active clinical exposure to dental handpiece aerosolisation. Through inhalation, aerosols and droplets generated by dental handpiece use have been recognised as an important source of microbial cross-infection and cross-contamination between patients and health personnel (Coleman et al., 2007).

The study involved a bi-phasic sampling collection regime performed at two single time points for each participant. The researcher (i.e. the author of this study) was analysed prior to the first and second sample collections for nasal carriage of MRSA or MSSA carriage and was deemed a non-carrier. Samples were collected on a designated day for individual participants taking care to anonymise participants. Data relating to gender and
subject category was recorded on the sample containers (oral rinse and nasal swab) at the time of sampling.

Initial contact was made with potential volunteers via an independent designated gatekeeper who issued an invitation to participate in the study, along with an information leaflet regarding the purpose of the study and investigations required (Appendix II). Participants were allowed a week following the first email contact from the gatekeeper to consider their participation in the study. The gatekeeper then issued individual volunteer participants designated sampling times that suited both the participants and the sampler. All samples were collected and processed by the author. Inclusion criteria for participation required that all participants were over eighteen years of age, were free of any local or systemic disease, were in general good health, had not received any antimicrobial agents within 28 days prior to sample collection, and were able to comply with the simple nasal sampling and oral rinse sampling techniques.

The initial sampling collection appointment was the first point of contact between the researcher and the volunteer participants. At this time, the researcher took the opportunity to introduce herself to the volunteers and explained in person the purpose of the study. Participants were afforded the opportunity to ask detailed questions regarding the study and its purpose. Personal instruction was provided to all participants prior to sample collection. Ethical approval for the research study had previously been obtained from (SJH/AMNCH) in September 2015 (Section 2.1 above). Written consent to participate in the study was obtained from each individual (Appendix III). Following the first sample collection, the researcher thereafter contacted the volunteers to arrange and co-ordinate a second round of sample collection after a three-month period. The second and final sampling procedure consisted of the same as the first, ensuring that volunteers were able to comply with the inclusion criteria and informed consent was signed by participants once again.
2.3 Nasal swab sampling

Nasal swab samples were collected from the anterior nares of each volunteer using individual sterile cotton swabs (Copan Venturi Transystem, Copan Innovation, Brescia, Italy), labelled carefully taking care to pseudo-anonymise the samples (Section 2.1 above). The anterior nares were sampled by rotating the swab tip in both nostrils, after which the swab was returned into the transport medium tube supplied with each swab and transportation to the DDUH Microbiology Laboratory at ambient temperature in an insulated trolley within 24 h after collection. Samples were either processed immediately or stored at 4-8°C no longer than seven days following collection.

2.4 Oral rinse sampling

Participants were instructed to rinse their mouths with 20 ml of sterile phosphate buffered saline (PBS) provided in a 62 mm diameter 100 ml polypropylene container with screw cap (Sarstedt Ltd., AG & Co., Numbrecht, Germany) for 60 s whilst keeping the container lid over the sampling pot to reduce the risk of introducing airborne contaminants. Following the timed oral rinse, volunteers were then requested to return as much of the rinse fluid as possible back into the container, after which the container lid was replaced and secured. Sample containers were labelled in a similar fashion to the nasal swab samples, pseudo-anonymised and returned at ambient temperature to the DDUH Microbiology Laboratory in an insulated trolley within 24 h after collection. Samples were either processed immediately or stored at 4-8°C no longer than seven days following collection.

2.5 Microbiological culture of nasal swab samples

Swab samples were inoculated onto SaSelect™ selective chromogenic agar medium for the isolation and direct identification of S. aureus (Bio-Rad, Marnes-la-Coquette, France) by rubbing the swab over the entire surface area of the agar plate while rotating the swab. SaSelect permits the detection of specific enzyme activities allowing direct identification of
S. aureus (pink to orange colonies) and its differentiation from other staphylococci (Figure 2.1).

Following inoculation, plates were incubated at 37°C in a static incubator (Gallenkamp, Leicester, U.K.) for 24 h. Following incubation, plates were examined and the number of colonies was recorded. Staphylococcus aureus colonies were presumptively identified as pink to orange coloured colonies in accordance with the manufacturer’s instructions as described below.

2.5.1 Phenotypic identification of S. aureus
SaSelect™ agar medium (Bio-Rad, Marnes-la-Coquette, France) is a chromogenic medium that enriches for growth of S. aureus. Pink to orange-coloured colonies after 18-24 h at 37°C are indicative of S. aureus due to phosphatase activity. Glycosidic activity allows differentiation of several other staphylococcal species including Staphylococcus saprophyticus, Staphylococcus simulans, Staphylococcus cohnii or Staphylococcus xylosus colonies of which appear blue. In the absence of phosphatase or glycosidic activity, the colonies appear white to yellow indicating Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus warneri and Staphylococcus epidermidis. The selective medium is composed of an optimised base for the growth of staphylococci, a mixture of antifungal agents, antibiotics and a limited salt concentration to inhibit the majority of yeasts, Gram-negative and Gram-positive bacteria other than staphylococci. Chromogenic substrates help to differentiate S. aureus from other staphylococci. A study conducted on the performance of SaSelect™ amongst two other chromogenic media on 86 known staphylococcus isolates revealed that SaSelect™ medium achieved a 100% sensitivity and 100% specificity to detect S aureus after 24 h of incubation (Hirvonen et al., 2014).
2.5.2 Additional microbiological culture of nasal swab samples

Following the initial incubation period on SaSelect™ agar medium, selected presumptive *S. aureus* isolates from each sample were chosen by the researcher with sample bias removed by always having a second opinion given by a consistent second party with comparison against a positive control. Chosen isolates were then sub cultured onto Mannitol Salt Agar (MSA) (Oxoid Ltd., Basingstoke, U.K.) followed by incubation at 37°C for 24 h. The high salt concentration present in MSA (7.5%) selects for staphylococcal species, since they can tolerate high salt levels. MSA also contains mannitol and the pH indicator phenol red. If an organism can ferment the sugar mannitol, such as *S. aureus*, acid is produced that causes the phenol red indicator to turn yellow (Figure 2.1). Most other staphylococci such as *S. epidermidis* will not ferment mannitol. Presumptive *S. aureus* isolates were then purified by subculture on Columbia Blood Agar (CBA) (Lip Diagnostic Services, Galway, Ireland).

Quantitative counts of *S. aureus* from selected nasal swab samples were determined by placing individual swab tips into 5 ml of nutrient broth (NB) (Oxoid Ltd., Hampshire, U.K.) in a 25 ml polypropylene tube (Starstedt AG & Co., Numbrecht, Germany) followed by vortexing at maximum speed for 30 s using a Heidolph Reax laboratory vortex (Heidolph UK, Essex, U.K.). Serial ten-fold dilutions were then prepared in NB and 0.1 ml of each dilution plated on SaSelect™ plates followed by incubation for 24 h at 37°C in a static incubator. Following incubation, plates were examined and the number of presumptive *S. aureus* colonies were recorded in CFUs per swab.

2.6 Microbiological culture of oral rinse samples

A one ml aliquot of each oral rinse sample was transferred aseptically into separate sterile 1.5 ml Eppendorf Safe-Lock (Eppendorf UK Ltd., Stevenage, U.K.) microcentrifuge tubes and centrifuged at 20,000 x g for three min. The supernatant from each sample was discarded and the pellet resuspended by vortexing in 0.1 ml of phosphate buffered saline and 0.1 ml aliquots were spread onto SaSelect™ plates followed by incubation for 24 h at 37°C in a
Figure 2.1. Presumptive identification of *S. aureus* on SaSelect™ selective medium (panels A and B) and mannitol salt agar (MSA) (panel C). SaSelect™ is a selective medium for the rapid isolation of *S. aureus* within 18-24 h growth. The detection of specific enzyme activities permits direct identification of *S. aureus* (pink to orange colonies) and its differentiation from other staphylococci (panels A and B). MSA is a selective and differential medium. The high salt concentration present in MSA (7.5%) selects for staphylococcal species, since they can tolerate high salt levels. MSA also contains mannitol and the pH indicator phenol red. If an organism can ferment the sugar mannitol, such as *S. aureus*, acid is produced that causes the phenol red indicator to turn yellow (see lower part of panel C). Most other staphylococci such as *S. epidermidis* will not ferment mannitol (see upper part of panel C).
with the Qiagen DNeasy Blood and Tissue kit Qiagen, Crawley, West Sussex, U.K.). Total genomic DNA was extracted from each isolate following the manufacturer’s instructions supplied with the Qiagen DNeasy Blood and Tissue kit. In brief, following the removal of the cellular protein components, the resultant free DNA was bound selectively to a silica-gel mini-column membrane provided with the Qiagen DNeasy Blood and tissue kit. A series of buffer washes containing ethanol and salt also provided with the kit were undertaken to remove any cellular debris from the DNA and bound to the membrane. Elution of the DNA followed by incubating for 10 min at room temperature with 50 µl of molecular biology grade water (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland) was followed by centrifugation at 6,000 x g for 1 min. Concentration of samples was achieved by heating at 70°C for 30 min with the microfuge lids open to allow for evaporation. The quality of the DNA and the absence of RNA was assessed by conventional agarose gel electrophoresis, which involved electrophoresing DNA samples diluted in Sigma water to a ratio of 1:50 in a 0.8% (w/v) agarose gel. DNA concentration (µg/µl) was determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc, Massachusetts, U.S.A.) and diluted with Sigma water to a concentration of between 0.5-1.5 µg/µl in 5 µl for linear polymerase chain reaction (PCR) amplification.

2.11 DNA microarray profiling of S. aureus isolates

DNA microarray profiling facilitated the comprehensive genomic profiling of S. aureus isolates investigated using genomic DNA extracted as described above in section 2.10. The DNA microarray method was performed as described previously in detail by Monecke et al. (2008) and in accordance with the manufacturer’s instructions using the S. aureus Genotyping Kit 2.0 (Alere Technologies GmbH). Comprehensive detail of the 336 targeted genes can be found at this publication (Monecke et al., 2008b). This kit consists of an eight-well microtiter strip within which an individual DNA microarray chip is mounted at the base (Figure 2.5). The microarray chip contains 336 oligonucleotide probes that detect gene
markers relating to antimicrobial resistance, virulence factors, exotoxin production, pathogenicity, enterotoxins, immune evasion cluster (IEC) genes and microbial surface components recognizing adhesive matrix molecules (MSCRAMMS). Isolates are also assigned to clonal complexes (CCs) or multilocus sequence types (STs) by comparison of the DNA microarray hybridisation pattern with a large international database.

2.11.1 Linear PCR amplification
A mastermix was prepared by adding 4.9 µl of labelling buffer (B1) and 0.1 µl of labelling enzyme B2), both of which were supplied by the microarray kit to 5 µl of the template DNA for each isolate. Thermocycling conditions were as follows: initial denaturation at 96°C for 5 min, followed by 45 cycles of 96°C for 20 s (denaturation), 50°C for 20 s (annealing) and 72°C for 30 s (extension). This thermally synchronised primer elongation reaction produced a linear PCR amplification and labelling reaction that allowed for the simultaneous amplification of all 336 gene targets if present.

2.11.2 Array prewashing and hybridisation
The wells of the microarray were prewashed using the hybridisation buffer C1 (supplied by the microarray kit) and following this 90 µl of hybridisation buffer C1 was added to each PCR reaction (total volume 100 µl). Each sample was then transferred to a prewashed array strip well. Caps were placed over the wells and incubated at 55°C with shaking at 550 rpm for 1 h in order for hybridisation of the PCR products to occur to the probes on the array chip.

2.11.3 Conjugation and precipitation staining
The hybridisation mixture was discarded from the array strip and the wells were washed carefully three times successively with 200 µl of washing buffer C2 (supplied with the microarray kit). Following the washing step, 100 µl of horseradish-peroxidase (HPR)-conjugate (reagent C3) and buffer C4 in a 1:100 ratio was added to each well (supplied by the microarray kit) and incubated at 30°C at 550 rpm for 10 min. A final wash step involved
**Figure 2.2.** Photograph showing an example of the identification of *S. aureus* using the Bio-Rad Pastorex™ Staph Plus (Bio-Rad, Marnes-la-Coquette, France) rapid latex agglutination test. This latex agglutination test identifies *S. aureus* isolates using latex particles sensitised by fibrinogen and IgG as well as specific monoclonal antibodies raised against *S. aureus* capsular polysaccharides. In the above example, samples of bacterial colonies were mixed with aliquots of control latex particles on the test card spots in the upper right-hand portion of the card. No latex agglutination is evident. Additional samples from the same bacterial colonies were mixed with sensitised test latex particles on the test card in the bottom right-hand portion of the card. The test latex particles in the right-most spot have agglutinated indicated the bacterial isolate tested was *S. aureus*. The other isolate tested in the adjacent spot did not agglutinate the latex particles, indicating it was not *S. aureus*. 
Figure 2.3. Photograph showing an example of a CBA agar plate used to culture *S. aureus* isolates from storage on cryogenic beads. Using a sterile loop, a single cryogenic bead harbouring *S. aureus* was streak inoculated onto a CBA plate to yield single colonies following incubation for 24 h at 37°C in a static incubator.
Figure 2.4. This figure shows a CBA plate with a lawned *S. aureus* colony on each half. Using a sterile inoculating loop, a single colony of each purified isolate was then further lawned onto one half of a new CBA plate, inverted and incubated for 24 hours at 37°C in a static incubator (Gallenkamp).
with the Qiagen DNeasy Blood and Tissue kit Qiagen, Crawley, West Sussex, U.K.). Total genomic DNA was extracted from each isolate following the manufacturer’s instructions supplied with the Qiagen DNeasy Blood and Tissue kit. In brief, following the removal of the cellular protein components, the resultant free DNA was bound selectively to a silica-gel mini-column membrane provided with the Qiagen DNeasy Blood and tissue kit. A series of buffer washes containing ethanol and salt also provided with the kit were undertaken to remove any cellular debris from the DNA and bound to the membrane. Elution of the DNA followed by incubating for 10 min at room temperature with 50 µl of molecular biology grade water (Sigma-Aldrich Ireland Ltd., Wicklow, Ireland) was followed by centrifugation at 6,000 x g for 1 min. Concentration of samples was achieved by heating at 70°C for 30 min with the microfuge lids open to allow for evaporation. The quality of the DNA and the absence of RNA was assessed by conventional agarose gel electrophoresis, which involved electrophoresing DNA samples diluted in Sigma water to a ratio of 1:50 in a 0.8% (w/v) agarose gel. DNA concentration (µg/µl) was determined using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc, Massachusetts, U.S.A.) and diluted with Sigma water to a concentration of between 0.5-1.5 µg/µl in 5 µl for linear polymerase chain reaction (PCR) amplification.

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markers relating to antimicrobial resistance, virulence factors, exotoxin production, pathogenicity, enterotoxins, immune evasion cluster (IEC) genes and microbial surface components recognizing adhesive matrix molecules (MSCRAMMS). Isolates are also assigned to clonal complexes (CCs) or multilocus sequence types (STs) by comparison of the DNA microarray hybridisation pattern with a large international database.

2.11.1 Linear PCR amplification
A mastermix was prepared by adding 4.9 µl of labelling buffer (B1) and 0.1 µl of labelling enzyme B2), both of which were supplied by the microarray kit to 5 µl of the template DNA for each isolate. Thermocycling conditions were as follows: initial denaturation at 96 °C for 5 min, followed by 45 cycles of 96°C for 20 s (denaturation), 50°C for 20 s (annealing) and 72°C for 30 s (extension). This thermally synchronised primer elongation reaction produced a linear PCR amplification and labelling reaction that allowed for the simultaneous amplification of all 336 gene targets if present.

2.11.2 Array prewashing and hybridisation
The wells of the microarray were prewashed using the hybridisation buffer C1 (supplied by the microarray kit) and following this 90 µl of hybridisation buffer C1 was added to each PCR reaction (total volume 100 µl). Each sample was then transferred to a prewashed array strip well. Caps were placed over the wells and incubated at 55°C with shaking at 550 rpm for 1 h in order for hybridisation of the PCR products to occur to the probes on the array chip.

2.11.3 Conjugation and precipitation staining
The hybridisation mixture was discarded from the array strip and the wells were washed carefully three times successively with 200 µl of washing buffer C2 (supplied with the microarray kit). Following the washing step, 100 µl of horseradish-peroxidase (HPR)-conjugate (reagent C3) and buffer C4 in a 1:100 ratio was added to each well (supplied by the microarray kit) and incubated at 30°C at 550 rpm for 10 min. A final wash step involved
Figure 2.5. A DNA microarray is a benchtop computer (C) that facilitates the genomic profiling of *Staphylococcus aureus* isolates. Extracted genomic DNA from *S. aureus* isolates is hybridised onto a microarray chip present at the base of an eight well microarray strip (A) following amplification of 334 probes. These probes detect gene markers relating to antimicrobial resistance, virulence factors, pathogenicity, exotoxins, enterotoxins, immune evasion cluster (IEC) genes and microbial surface components recognising adhesive matrix molecules (MSCRAMMS). Following application of horseradish-peroxidase dye, a resulting microarray chip pattern is formed at positions where dye binds to hybridised DNA (B). The benchtop microarray compares resultant patterns created, to a global database which are also assigned to clonal complexes (CC’s) or multilocus sequence types (ST’s) (Figure adapted from Monecke *et al.*, 2008).
removing the HRP-conjugate solution followed by one wash of the array with 200 µl of washing buffer C5 (supplied by the microarray kit). Finally, staining of the bound HRP-conjugate was performed by adding 100 µl of tetramethylbenzidine (D1) (supplied by the array kit) to each well and incubating at room temperature for 5 min without agitation resulting in a blue/green reaction and a subsequent hybridisation pattern at the base of the well. The D1 reagent was then discarded and each pattern was primed to be read and analysed using the Arraymate reader and Iconoclust software (Arraymate, Alere Technologies GmbH) (version 2.0) (Figure 2.5).

2.11.4 DNA microarray analysis

An image of each chip and hybridisation pattern was recorded by the Arraymate reader which was analysed for the presence or absence of genes by the Iconoclust software. The software interpreted the image and compared the individual pattern to an existing international database of *S. aureus* isolate microarray profiles, based on a previously described algorithm. The software also assigned each *S. aureus* isolate to a MLST CC and ST along with a SCCmec type (Monecke *et al.*, 2008b) (Figure 2.5).
Chapter 3 Results
3.1 Participant sample collection

Two groups of dental HCW volunteers (group one; \(n = 39\) and group two; \(n = 40\)) consisting of 79 individuals from five separate workplaces were screened for staphylococci by nasal swab and oral rinse sampling. An attempt was made to collect data from one region only but to improve sample numbers, the number of locations was expanded to five locations in total, from two regions. Each volunteer underwent two separate screenings at least three months apart. Samples were collected from January 2016 to August 2016. The first volunteer group consisted of 39 dental HCWs based at DDUH and who also work at outreach city facilities including general hospitals and prisons (location 1) (Table 3.1). The second volunteer group consisted of 40 dental HCWs from general dental practice working in four separate dental clinics (Location 2-4) (Table 3.1). The entire volunteer cohort consisted of males \((n = 20)\) and females \((n = 59)\), operators \((n = 38)\) and assistants \((n = 41)\). The dental HCW operators included hygienists, dentists, full time postgraduate dentists, oral surgeons, oral and maxillofacial surgeons. Dental HCW assistants included mainly student and dental nurses along with registered nurses who participate daily in clinical dentistry with active exposure to dental hand piece-generated aerosols. Volunteers were categorised according to place of work (Locations 1-5) and sub-categorised according to age range \((18-25, 26-35, 36-45, 46-55, 56-65, 65+ years)\) (Table 3.1).

3.2 Phases one and two sample recovery

Each volunteer from dental workplace Locations 1-5 underwent nasal swab sampling (as described in section 2.3) and oral rinse sampling (as described in section 2.4) on prearranged designated days for each of two sample recovery phases. Phase one sampling occurred between January 12\(^{th}\) 2016 and 26\(^{th}\) April 2016 (Table 3.1). Phase two sampling commenced on 25\(^{th}\) April and concluded on the 1\(^{st}\) of August 2016 (Table 3.2). Microbiological culture was performed on all nasal swab and oral rinse samples recovered using the methods described in sections 2.6 and 2.7.
3.3 Recovery of *S. aureus* from participants from Location 1

Location 1 participants included dental HCWs who worked in the DDUH in addition to prisons and general hospitals in the greater Dublin area. The total number of volunteers screened in this group was 39, which consisted of 11 males and 28 females varying in age from 18-66+ (Table 3.1). Following nasal swab sampling (as described in section 2.3) and oral rinse sampling (as described in section 2.4), microbiological culture identified five positive nasal swab samples for *S. aureus* and identified seven positive *S. aureus* oral rinse samples during the first phase of sampling (Table 3.1). First phase participants from Location 1 demonstrated a 12.8% (5/39) *S. aureus* nasal carriage rate and 17.9% (7/39) *S. aureus* oral carriage rate. The second sampling phase three months later identified six positive nasal swab samples for *S. aureus* and seven oral rinse samples positive for *S. aureus* (Table 3.2). Second phase participants from Location 1 demonstrated a 15.4% (6/39) *S. aureus* nasal carriage rate and the same *S. aureus* oral carriage rate of 17.9% (7/39).

3.4 Recovery of *S. aureus* from participants from Location 2

Location 2 participants included 17 dental HCWs who worked in a general dental practice in Northern Ireland. The total number of volunteers screened in this group included two males and 15 females varying in age from 18-55 (Table 3.1). Following nasal swab sampling (as described in section 2.3) and oral rinse sampling (as described in section 2.4), microbiological culture identified one positive nasal swab sample for *S. aureus* and identified four positive *S. aureus* oral rinse samples during the first phase of sampling (Table 3.1). First phase participants from Location 2 demonstrated a 5.9% (1/17) *S. aureus* nasal carriage rate and 23.5% (4/17) *S. aureus* oral carriage rate. The second sampling phase three months later identified six positive nasal swab samples for *S. aureus* and three oral rinse samples positive for *S. aureus* (Table 3.2). Second phase participants from Location 2 demonstrated a 35.3% (6/17) *S. aureus* nasal carriage rate and 17.6% (3/17) *S. aureus* oral carriage rate.
Table 3.1. Recovery of *S. aureus* from nasal and oral samples from 79 dental healthcare workers sampled between 12\textsuperscript{th} January-26\textsuperscript{th} April, 2016

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## First phase collection

### Breakdown of swab and oral rinse collection

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### Notes
- SaSelect™ is a preliminary test for detecting SARS-CoV-2.
- MSA refers to Microbiology System Agar.
- Latex agglutination is a method for detecting antibodies against SARS-CoV-2.
### First phase collection

<table>
<thead>
<tr>
<th>Location</th>
<th>Age-range</th>
<th>Nasal Location</th>
<th>Oral Location</th>
<th>SaSelect™</th>
<th>MSA</th>
<th>Latex agglutination</th>
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Under the breakdown of swab and oral rinse collection column, the number of swab and oral rinses recovered during the first collection phase from each dental clinic location [1-5] are shown. These are sub-categorised according to age-range and worker categories defined by operator (O), assistants (A) and their gender, male (M) or female (F). Under the phenotypic testing column, the number of *S. aureus*-positive samples recovered from each location/age-range is shown for nasal (N) and oral rinse (O) samples by culture on SaSelect™ agar (Bio-Rad, Marnes-la-Coquette, France) (pink to orange coloured colonies), with numbers of *S. aureus*-positive samples shown in parenthesis. Presumptive *S. aureus* colonies were confirmed by growth on mannitol salt agar (MSA) (Oxoid, Hampshire, United Kingdom) (yellow colonies surrounded by golden halo) and by latex agglutination testing using the Pastorex™ Staph Plus kit (Bio-Rad), with numbers shown in parenthesis. SaSelect™ culture plates were also assessed for *S. aureus* confluence (>500 colony forming units (CFU)/plate) and semi-confluence (250-500 CFU/plate) (shown in parenthesis).
Table 3.2. Recovery of *S. aureus* from nasal and oral samples from 79 dental healthcare workers sampled between 25th April-1st August, 2016

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### Second phase collection

#### Breakdown of swab and rinse collection

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</table>
Under the breakdown of swab and oral rinse collection column, the number of swab and oral rinses recovered during the second collection phase from each dental clinic location [1-5] are shown. These are sub-categorised according to age-range and worker categories defined by operator (O), assistants (A) and their gender, male (M) or female (F). Under the phenotypic testing column, the number of *S. aureus*-positive samples recovered from each location/age-range is shown for nasal (N) and oral rinse (O) samples by culture on SaSelect™ agar (Bio-Rad, Marnes-la-Coquette, France) (pink to orange coloured colonies), with numbers of *S. aureus*-positive samples shown in parenthesis. Presumptive *S. aureus* colonies were confirmed by growth on mannitol salt agar (MSA) (Oxoid, Hampshire, United Kingdom) (yellow colonies surrounded by golden halo) and by latex agglutination testing using the Pastorex™ Staph Plus kit (Bio-Rad), with numbers shown in parenthesis. SaSelect™ culture plates were also assessed for *S. aureus* confluence (>500 colony forming units (CFU)/plate) and semi-confluence (250-500 CFU/plate) (shown in parenthesis).

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Under the breakdown of swab and oral rinse collection column, the number of swab and oral rinses recovered during the second collection phase from each dental clinic location [1-5] are shown. These are sub-categorised according to age-range and worker categories defined by operator (O), assistants (A) and their gender, male (M) or female (F). Under the phenotypic testing column, the number of *S. aureus*-positive samples recovered from each location/age-range is shown for nasal (N) and oral rinse (O) samples by culture on SaSelect™ agar (Bio-Rad, Marnes-la-Coquette, France) (pink to orange coloured colonies), with numbers of *S. aureus*-positive samples shown in parenthesis. Presumptive *S. aureus* colonies were confirmed by growth on mannitol salt agar (MSA) (Oxoid, Hampshire, United Kingdom) (yellow colonies surrounded by golden halo) and by latex agglutination testing using the Pastorex™ Staph Plus kit (Bio-Rad), with numbers shown in parenthesis. SaSelect™ culture plates were also assessed for *S. aureus* confluence (>500 colony forming units (CFU)/plate) and semi-confluence (250-500 CFU/plate) (shown in parenthesis).
3.5 Recovery of *S. aureus* from participants from Location 3

Location 3 involved seven dental HCWs who worked in a general dental practice in Northern Ireland. The total number of volunteers screened in this group included two males and five females varying in age from 26-65 (Table 3.1). Following nasal swab sampling (as described in section 2.3) and oral rinse sampling (as described in section 2.4), microbiological culture identified two positive nasal swab samples for *S. aureus* and identified two positive *S. aureus* oral rinse samples during the first phase of sampling (Table 3.1). First phase participants from Location 3 demonstrated a 28.6% (2/7) *S. aureus* nasal and oral carriage rate. The second sampling phase three months later identified three positive nasal swab samples for *S. aureus* and two oral rinse samples positive for *S. aureus* (Table 3.2). Second phase participants from Location 3 demonstrated a 42.9% (3/7) *S. aureus* nasal carriage rate and 28.6% (2/7) *S. aureus* oral carriage rate.

3.6 Recovery of *S. aureus* from participants from Location 4

Location 4 involved 12 dental HCWs who worked in a general dental practice in Northern Ireland. The total number of volunteers screened in this group included three males and nine females varying in age from 18-55 (Table 3.1). Following nasal swab sampling (as described in section 2.3) and oral rinse sampling (as described in section 2.4), microbiological culture identified two positive nasal swab samples for *S. aureus* and identified one positive *S. aureus* oral rinse sample during the first phase of sampling (Table 3.1). First phase participants from Location 4 demonstrated a 16.7% (2/12) *S. aureus* nasal carriage rate and 8.3% (1/12) *S. aureus* oral carriage rate. The second sampling phase three months later identified two positive nasal swab samples for *S. aureus* and four oral rinse samples positive for *S. aureus* (Table 3.2). Second phase participants from Location 4 demonstrated the same *S. aureus* nasal carriage rate of 16.7% (2/12) with a *S. aureus* oral carriage rate of 33.3% (4/12).
3.7 Recovery of *S. aureus* from participants from Location 5

Location 5 involved four dental HCWs who worked in a general dental practice in Dublin. The total number of volunteers screened in this group included two males and two females varying in age from 26-55 (Table 3.1). Following nasal swab sampling (as described in section 2.3) and oral rinse sampling (as described in section 2.4), microbiological culture identified three positive nasal swab samples for *S. aureus* and identified zero positive *S. aureus* oral rinse sample during the first phase of sampling (Table 3.1). First phase participants from Location 5 demonstrated a 75% (3/4) *S. aureus* nasal carriage rate and 0% *S. aureus* oral carriage rate. The second sampling phase three months later identified one positive nasal swab samples for *S. aureus* and one oral rinse sample positive for *S. aureus* (Table 3.2). Second phase participants from Location 5 demonstrated equal *S. aureus* nasal and oral carriage rates of 25% (1/4).

3.8 Overall recovery of *S. aureus* nasal and oral carriage from first and second phase sampling

The *S. aureus* nasal and oral carriage rate among volunteer participants during the first collection phase was 16.5% (13/79) and 13.9% (11/79) respectively. Out of the thirteen nasal carriers amongst the first collection phase, confluence was demonstrated in 84.6% (11/13) of cases. The *S. aureus* nasal and oral carriage rate among volunteer participants during the second collection phase was 22.8% (18/79) and 21.5% (17/79) respectively. First collection phase carriage rates for operators and assistants was 75% (18/24) and 25% (6/24) respectively. Second collection phase carriage rates for operators and assistants was 62.9% (22/35) and 37.1% (13/35) respectively. Out of eighteen nasal carriers amongst the second collection, confluence was demonstrated in 44.4% (8/18) of cases. All of the *S. aureus* isolates recovered during the study were MSSA. MRSA was not detected in the present investigation.
The first phase sampling identified 13 nasally positive *S. aureus* isolates, four of which were also positive for *S. aureus* orally, which is suggestive of nasal-oral trafficking. Following the second phase screening, 18 positive nasal swabs were identified, seven of which that had remained positive from the first sampling phase. The second phase screening revealed therefore an additional 11 new nasal carriers and seven persistent nasal carriers over the two collection phases. From the 18 positive nasal carriers during the second phase collection, six were also positive for oral *S. aureus* carriage. Over both screening phases, only two participants harboured *S. aureus* orally and nasally during both screening phases. Results identified 7/13 positive *S. aureus* carriers from the first round that remained positive nasally during the second collection phase and 4/13 nasal positive carriers from the first collection phase that lost nasal carriage during the second collection phase.

Persistent nasal *S. aureus* carriers, defined as individuals harbouring MSSA nasally during both collection phases accounted for 7/79 (8.9%). Of the seven persistent nasal carriers, six were operators (which included five males and one female) and one was a hospital based assistant. Of the seven persistent nasal carriers, all demonstrated confluence during the first collection phase. Confluence was observed amongst three of those during the second collection phase.

Persistent oral *S. aureus* carriers, defined as individuals harbouring MSSA orally during both collection phases accounted for 3/79 (3.8%). Intermittent nasal *S. aureus* carriers, defined as individuals harbouring MSSA nasally on one occasion, either following loss of the first collection or gain following the second sampling phase accounted for 24/79 (30.4%). Intermittent oral *S. aureus* carriers, defined as individuals harbouring MSSA orally on one occasion, either following loss of the first collection or gain following the second sampling phase accounted for 23/79 (29.1%). As mentioned previously, two individuals (2/79) 2.5% harboured *S. aureus* both orally and nasally following both collection phases.
The total number of positive *S. aureus* colonisation among operators for both hospital and community-based dental HCWs was 18.99% (15/79) and assistants was 10.13% (8/79). The overall oral carriage rates for all dental HCWs (79 volunteers) from the first collection round was 16.46% (13/79) and from the second collection was 21.52% (17/79) resulting in similar oral carriage rates for both collection phases. The total number of *S. aureus* colonised operators for both hospital and community-based dental HCWs was 20.3% (16/79) and assistants was 13.92% (11/79) in the second round demonstrating similar numbers of operators and assistants being affected with a slightly higher rate of *S. aureus* carriage among operators for both collection phases. Similarly, community-based dental HCWs demonstrated an overall slightly higher carriage rate than those working in hospital based dental care provision.

3.8.1 Quantitative counts of *S. aureus* from selected nasal swab samples

Determination of quantitative counts of *S. aureus* CFUs from five selected nasal swab samples from separate participants was undertaken to obtain an estimate of the range of CFUs present in swab samples that yielded confluent or semi-confluent growth on SaSelect™ agar on primary plating (Section 2.6). This was achieved by vortexing the tips of individual swabs in broth, followed by plating serial dilutions on SaSelect™ agar. Following incubation at 37°C the bacterial counts recovered from each swab were calculated in CFU/swab sample. The *S. aureus* cell density range was determined between 2\times10^4 - 2.8\times10^6 CFU/swab.

Confluent growth of *S. aureus* from primary plating of oral swab and oral rinse samples was recorded for 21.5% (17/79) of *S. aureus* nasal and oral positive samples for the first round sampling with a reduced rate of 13.92% (11/79) for the second sampling round. Only 2.53% (2/79) of combined oral and nasal positive *S. aureus* samples yielded semi-confluent growth of *S. aureus* for the first sampling round, increasing to a rate of 7.6% (6/79) for the second round of sampling.
These results indicate that there is a significant reservoir of \textit{S. aureus} in dental HCWs including a subgroup that are heavily colonised. These latter individuals represent a significant health risk to both patients and fellow co-workers with the potential for onward transmission. There is no similar published data in the dental setting.

3.9 DNA microarray profiling

3.9.1 Microarray profiling of first round sampling \textit{S. aureus} isolates

3.9.1.1 Prevalence of \textit{S. aureus} CCs/STs

A total of 24 \textit{S. aureus} isolates recovered from 20 study participants during the first phase of sampling between 12\textsuperscript{th} January-26\textsuperscript{th} April, 2016 were investigated by DNA microarray profiling (Table 3.3). Isolates were selected as described in section 2.7. All isolates were identified as MSSA. Microarray profiling permitted the identification of MLST clonal types for all 24 isolates by comparing the resultant pattern created for individual \textit{S. aureus} isolates to a global database (Figure 2.5). Thirteen different MLST CC/STs were identified amongst the 24 isolates, two of which were represented by just one isolate (Table 3.3). DNA microarray analysis detected 10 different MLST CCs, the most prevalent of which was CC30-MSSA (20.8%; 5/24 isolates) including ST30/ST39-MSSA (n = 3/5), ST39-MSSA (n = 1/5) and ST34/42-MSSA (n = 1/5) followed by CC398-MSSA (16.7%; 4/24) including ST398-MSSA (n = 2/4) and ST291/813-MSSA (n = 2/4). The next most frequent was CC20/ST20-MSSA (12.5%; 3/24), CC45/ST45-MSSA (12.5%; 3/24) and CC7-MSSA (12.5%; 3/24) followed by CC8-MSSA (8.3%; 2/24 isolates) including ST8-MSSA (n = 1/2) and ST254/ST870-MSSA (n = 1/2), CC22/ST22-MSSA (8.3%; 2/24), CC5-MSSA (8.3%; 2/24) and CC1/ST1-MSSA was detected in one isolate (4.1%) (Table 3.3).
Overall, there were similar numbers of hospital-associated and community-associated dental HCWs harbouring MSSA isolates. MSSA isolates, despite being less antibiotic resistant than MRSA isolates; have the potential to be as destructive due to their virulence-associated genes. The total number of isolates recovered during the first screening phase from hospital based DDUH (Location 1) HCWs was 11/24 (45.8%) and community HCWs was 13/24 (54.2%). Of the community based dental HCWs, Location 2 harboured 2/24 (8.3%) of isolates, Location 3 harboured 4/24 (16.7%) of isolates, Location 4 harboured 4/24 (16.7%) of isolates and Location 5 harboured 3/24 (12.5%) of isolates. Other than Location 1 in which the largest total number of S. aureus-positive samples were recovered (11/24; 45.8%), Locations 3 and 4 followed with the next greatest total number of isolates from a location (4/24; 16.7%), followed by Location 5 (3/24; 12.5%), and Location 2 with the lease amount (2/24; 8.3%). Overall, the highest recovery rate, one third (33.3%) of S. aureus positive samples, were recovered from the age group 46-55, with the lease amount recovered from the youngest age group 18-25, at 2/24 (8.3%) of isolates. Overall, the total number of isolates recovered from operators from the first collection phase was 18/24 (75%), and among assistants was 6/24 (25%). Exact equal numbers of male and females were affected, each cohort accounting for 12/24 (50%). Similarly, oral and nasal carriage was found in exact equal proportions amongst positive carriers at 12/24 respectively (50%) (Table 3.3).

3.9.1.2 Detection of virulence-associated genes

The immune evasion cluster (IEC) genes and other virulence-associated genes detected among the majority of the 24 isolates investigated belonging to the different CCs are shown in Table 3.3 and Figure. 3.1. Immune evasion cluster genes were detected among 87.5% (21/24) of the 24 isolates and included *scn* (87.5%; 21/24), *sak* (75%; 18/24), *chp* (62.5%; 15/24) and *sea* (29.2%; 7/24) (Table 3.3). The most common IEC type identified among the 24 isolates investigated as defined by Van Wamel *et al.* (2006) (Table 3.3 for explanation) was IEC type B (41.7%; 10/24), followed by D (16.7%; 4/24), IEC type A (12.5%; 3/24),
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<td>MLST</td>
<td>Source</td>
<td>Location&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>agr type</td>
<td>Capsule type</td>
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<td>Virulence-associated genes</td>
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<td>sak, chp, scn, icaA</td>
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<sup>a</sup> Isolate name includes a number which was assigned to each HCW volunteer based on their order of attendance during screening phase one and subsequent screening phases. This number was followed by letters; O, oral rinse; A, oral rinse; S, nasal swab; B, nasal swab.

<sup>b</sup> The sample population consisted of dental healthcare worker (HCW) volunteers working out of five separate institutions (1-5). Location 1 refers to dental HCWs including staff and students who were based at DDUH, but who also worked at multi-centre facilities including prisons and outreach city hospitals. Locations 2-5 refers to dental HCWs who worked in general dental practices in Locations 2-5.

<sup>c</sup>ccrAB1 is a recombinase gene frequently associated with staphylococcal chromosome cassette (SCC) elements, including SCCmec as involved in integration and excision of such elements into the orfX locus on the S. aureus chromosome. The detection of ccrAB1 in this isolate in the absence of mecA or mecC suggest the presence of an SCC element.

agr type, capsule type, IEC type, antimicrobial-resistance genes and virulence-associated genes were detected using by DNA microarray profiling as previously described (Alere technologies, Jena, Germany). The antimicrobial-resistant genes encode resistance as follows: sdrM (tetracycline efflux pump resistance), blaZ (beta-lactamase resistance), fosB (fosfomicin resistance), fusC (fusidic acid resistance), sat (streptothricin resistance) and aphA3 (aminoglycoside-streptothricin resistance). The virulence-associated genes encode the following virulence factors: tst (toxic shock toxin), sea (enterotoxin A), egc (enterotoxin gene cluster), seh (enterotoxin H), sec & sel (enterotoxins C & L) and sed (enterotoxin D). The letters A-E refer to IEC types A (sea, sak, chp, scn), B (sak, chp, scn), C (chp, scn), D (sea, sak, scn) and E (sak, scn). Other genes present include sei (enterotoxin I), seg (enterotoxin G), selm (enterotoxin-like gene/Protein M), seln (enterotoxin-like gene/Protein N), icaA (intercellular protein A). N/A (non applicable, does not contain any genes belonging to the egc cluster) based on previously published classifications (Van Wamel et al. 2006).

Abbreviations: N, nasal; O, oral; HCW, health care worker; M, male; F, female; CA, community associated; HA, hospital associated; IEC, immune evasion cluster.
Figure 3.1. A DNA microarray profiling (Alere Technologies GmbH, Jena, Germany) was used to detect (A) virulence-associated genes and (B) antimicrobial agent resistance genes among 24 MSSA isolates recovered from 79 dental HCWs from five locations including a group of staff and students based at DDUH but who also worked at multi-centre facilities including prisons and outreach city hospitals. The second group of dental HCWs worked in general dental practice. The number of isolates is shown on the vertical axis and the number of genes associated with *S. aureus* isolates belonging to various CCs is shown on
the horizontal axis. The virulence-associated genes listed in panel A encode the following virulence factors: \textit{tst} (toxic shock toxin), \textit{sea} (enterotoxin A), \textit{ege} (enterotoxin gene cluster), \textit{seh} (enterotoxin H), \textit{sec\&sel} (enterotoxins C \& L) and \textit{sed} (enterotoxin D). The letters A-E refer to IEC types A (\textit{sea, sak, chp, scn}), B (\textit{sak, chp, scn}), C (\textit{chp, scn}), D (\textit{sea, sak, scn}) and E (\textit{sak, scn}). The antimicrobial-resistant genes listed in panel B encode resistance as follows: \textit{sdrM} (tetracycline efflux pump resistance), \textit{blaZ} (beta-lactamase resistance), \textit{fosB} (fosfomicin resistance), \textit{fusC} (fusidic acid resistance), \textit{sat} (streptothricin resistance) and \textit{aphA3} (aminoglycoside-streptothricin resistance).
Overall, there were similar numbers of hospital-associated and community-associated dental HCWs harbouring MSSA isolates. MSSA isolates, despite being less antibiotic resistant than MRSA isolates; have the potential to be as destructive due to their virulence-associated genes. The total number of isolates recovered during the first screening phase from hospital based DDUH (Location 1) HCWs was 11/24 (45.8%) and community HCWs was 13/24 (54.2%). Of the community based dental HCWs, Location 2 harboured 2/24 (8.3%) of isolates, Location 3 harboured 4/24 (16.7%) of isolates, Location 4 harboured 4/24 (16.7%) of isolates and Location 5 harboured 3/24 (12.5%) of isolates. Other than Location 1 in which the largest total number of S. aureus-positive samples were recovered (11/24; 45.8%), Locations 3 and 4 followed with the next greatest total number of isolates from a location (4/24; 16.7%), followed by Location 5 (3/24; 12.5%), and Location 2 with the least amount (2/24; 8.3%). Overall, the highest recovery rate, one third (33.3%) of S. aureus positive samples, were recovered from the age group 46-55, with the least amount recovered from the youngest age group 18-25, at 2/24 (8.3%) of isolates. Overall, the total number of isolates recovered from operators from the first collection phase was 18/24 (75%), and among assistants was 6/24 (25%). Exact equal numbers of male and females were affected, each cohort accounting for 12/24 (50%). Similarly, oral and nasal carriage was found in exact equal proportions amongst positive carriers at 12/24 respectively (50%) (Table 3.3).

3.9.1.2 Detection of virulence-associated genes

The immune evasion cluster (IEC) genes and other virulence-associated genes detected among the majority of the 24 isolates investigated belonging to the different CCs are shown in Table 3.3 and Figure. 3.1. Immune evasion cluster genes were detected among 87.5% (21/24) of the 24 isolates and included *scn* (87.5%; 21/24), *sak* (75%; 18/24), *chp* (62.5%; 15/24) and *sea* (29.2%; 7/24) (Table 3.3). The most common IEC type identified among the 24 isolates investigated as defined by Van Wamel *et al.* (2006) (Table 3.3 for explanation) was IEC type B (41.7%; 10/24), followed by D (16.7%; 4/24), IEC type A (12.5%; 3/24),
IEC types C and E both detected in 8.3% (2/24) (Table 3.3). Three isolates including two CC30-MSSA (isolates 45R and 33S1) and one CC8-MSSA (isolate 48R) had no IEC-associated genes detected by array profiling. All of these three isolates (45R, 33S1 and 48R) exhibited evidence of a disrupted hlb gene implying that there may have been an issue with microarray profiling data or some other MGE was inserted into the hlb gene undetectable by the DNA microarray. Clonal complex 30 isolates (n = 5) exhibited the most IEC types, including IEC types A, B and C. CC5-MSSA isolates (n =2) harboured IEC type A only, CC8-MSSA isolates (n =2) harboured IEC type E only, CC1-MSSA isolates (n=1) and CC7-MSSA isolates (n =3) harboured IEC type D only. CC20-MSSA isolates (n =3), CC22-MSSA isolates (n =2), CC45-MSSA (n =3) and ST291/813 isolates (n =4) harboured IEC type B only. CC398-MSSA isolates (n =4) harboured IEC type C only. The accessory gene regulator (agr) allele I was the most dominant agr type (66.7%; 6/24), followed by agr III (20.8%; 5/24) and agr II (8.3%; 2/24). The capsule gene types 5 and 8 were similarly distributed amongst the 24 MSSA isolates. Capsule type 5 predominated slightly and was detected in 54.2% (13/24) of isolates with capsule type 8 in 45.8% (11/24) isolates.

The biofilm related gene, icaA, was identified in all 24 isolates. The next most common virulence-associated genes detected were the enterotoxin gene cluster (egc), which was detected in 50% (12/24) of all isolates. The egc cluster was most common in CC30-MRSA and CC45-MRSA (12.5%; 3/24) of all isolates for each CC. The egc cluster was detected CC5/ST5-MRSA (8.3%; 2/24), ST73-MRSA (8.3%; 2/24), CC20/ST20-MRSA (8.3%; 2/24) and ST389-MSSA (8.3%; 2/24) and once in a ST22-MRSA isolate (4.2%) (Table 3.3 and Figure 3.1). The enterotoxin A gene sea was detected in 29.2% (7/24) belonging to four CCs (CC1, CC5, CC7 and CC30). In total, 8.3% (2/24) isolates harboured the enterotoxin gene seh, one each by CC1-MRSA and CC30/ST34-MRSA. The toxic shock toxin gene tst was detected in 12.5% (3/24) of isolates, one each belonged to CC30/ST30-
Table 3.4. Molecular characteristics identified by DNA microarray profiling of 35 methicillin-susceptible *Staphylococcus aureus* isolates recovered from second round screening of 29 individual dental healthcare workers from five locations during the time period 25<sup>th</sup> April –1<sup>st</sup> August, 2016

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<th>SCC associated/antimicrobial-resistance genes</th>
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Isolate name includes a number which was assigned to each HCW volunteer based on their order of attendance during screening phase one and subsequent screening phases. This number was followed by letters; O, oral rinse; A, oral rinse; S, nasal swab; B, nasal swab.

The sample population consisted of dental healthcare worker (HCW) volunteers working out of five separate institutions (1-5). Location 1 refers to dental HCWs including staff and students who were based at DDUH, but who also worked at multi-centre facilities including prisons and outreach city hospitals. Locations 2-5 refers to dental HCWs who worked in general dental practices in Locations 2-5.

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<th>Location&lt;sup&gt;b&lt;/sup&gt;</th>
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<th>agr type</th>
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<sup>a</sup> Isolate name includes a number which was assigned to each HCW volunteer based on their order of attendance during screening phase one and subsequent screening phases. This number was followed by letters; O, oral rinse; A, oral rinse; S, nasal swab; B, nasal swab.

<sup>b</sup> The sample population consisted of dental healthcare worker (HCW) volunteers working out of five separate institutions (1-5). Location 1 refers to dental HCWs including staff and students who were based at DDUH, but who also worked at multi-centre facilities including prisons and outreach city hospitals. Locations 2-5 refers to dental HCWs who worked in general dental practices in Locations 2-5.

agr type, capsule type, IEC type, antimicrobial-resistance genes and virulence-associated genes were detected using by DNA microarray profiling as previously described (Alere technologies, Jena, Germany). The antimicrobial-resistant genes encode resistance as follows: sdrM (tetracycline efflux pump resistance), blaZ (beta-lactamase resistance), fosB (fosfomycin resistance), fusC (fusidic acid resistance), sat (streptothricin resistance) and aphA3 (aminoglycoside-streptothricin resistance). The virulence-associated genes encode the following virulence factors: tst (toxic shock toxin), sea (enterotoxin A), egc (enterotoxin gene cluster), seh (enterotoxin H), sec & sel (enterotoxins C & L) and sed (enterotoxin D). The letters A-E refer to IEC types A (sea, sak, chp, scn), B (sak, chp, scn), C (chp, scn), D (sea, sak, scn) and E (sak, scn). Other genes present include sei (enterotoxin I), seg (enterotoxin G), selm (enterotoxin-like gene/Protein M), seln (enterotoxin-like gene/Protein N), icaA (intercellular protein A). N/A (non applicable, does not contain any genes belonging to the egc cluster) based on previously published classifications (Van Wamel et al. 2006).

Abbreviations: N, nasal; O, oral; HCW, health care worker; M, male; F, female; CA, community associated; HA, hospital associated; IEC, immune evasion cluster.
CC45-MSSA (11.4%; 4/35). The next most frequent was CC15-MSSA (8.6%; 3/35) followed by CC22-MSSA (8.6%; 3/35) and CC8/ST8-MSSA, (8.6%; 3/35), CC7-MSSA (5.7%; 2/35), and the following were identified in one isolate each CC2-MSSA (2.9%), CC59-MSSA (2.9%) and CC97-MSSA (2.9%).

3.9.2.2 Virulence-associated genes

The IEC type genes and other virulence-associated genes and their distribution among the different clonal complexes are in Table 3.4. Second round sampling revealed that IEC genes were detected among 85.7% (30/35) of sporadic MSSA isolates. During the 2nd sampling phase the most prevalent IEC type genes detected included scn (85.7%, 30/35), sak (71.4%, 25/35), chp (77.1%, 27/35) and sea (25.7%, 9/35) (Table 3.4.). Similar to the first collection phase, the most common IEC type for the second phase collection was IEC type B (34.3%, 12/35), followed by IEC type C (20%, 7/35), followed very closely by IEC type A (22.9%, 8/35), IEC type D (8.6%, 3/35) and IEC type E (5.7%, 2/35) (Table 3.4). Clonal complexes CC30 and CC8 exhibited the most IEC types (Figure 3.2).

The accessory gene regulator (agr) allele I was the most prevalent agr type for the second phase collection (65.7%, 23/35) similar to the first phase collection (66.7%) and agr III was detected in 34.3% (12/35) of all isolates (Table 3.4 and Figure 3.2). The capsule gene type 8, cap8 was detected in 62.9% (22/35) of all isolates and capsule type 5, cap5 was detected in 37.1% (13/35).

The most prevalent virulence-associated gene detected was the biofilm related icaA gene, which was recovered in all thirty-five isolates (100%) in the second round of sampling. The most prevalent toxin genes detected were the egc genes, which were detected in slightly more than half of all isolates 19/35 (54.3%). The egc cluster was detected most frequently among CC30-MSSA with a total of 11/35 isolates (Table 3.4 and Figure 3.2). The enterotoxin A gene, sea was detected in 25.7% (9/35) belonging to six CCs (CC7, CC8,
CC15, CC30, CC45 and CC398) and the enterotoxin gene seh, was detected in one CC30-MSSA isolate (2.9%; 1/35) of all isolates. The toxic shock toxin gene tst, was detected in 31.4% (11/35). The enterotoxin genes sec and sel were harboured by four isolates (11.4%), including two CC30/ST39-MSSA isolates, one CC398/398-MSSA isolate and one CC45/ST45-MSSA isolate.

3.9.2.3 Antimicrobial resistance genes.

The antimicrobial resistance genes detected among the isolates belonging to the different CCs are shown in Table 3.4. The most prevalent antimicrobial resistance genes detected among the 35 sporadic MSSA isolates was the beta-lactamase resistance gene blaZ (85.7%; 30/35), detected in eight different CCs (CC15, CC20, CC22, CC30, CC398, CC45, CC8 and CC97). The general efflux gene, sdrM was the next most prevalent antimicrobial resistance gene detected in 82.9% (29/35) isolates from ten different CCs (CC15, CC20, CC30, CC398, CC45, CC59, CC7, CC8 and CC97). The only other antimicrobial resistance gene detected in the second phase of sampling included the fosfomycin B resistance gene fosB, detected in 60% (21/35) of isolates from six different CCs (CC15, CC20, CC30, CC398, CC45 and CC8).

3.9.3 Comparing results of molecular characteristics for MSSA isolates recovered from nasal swab and oral rinse samples during first and second time-period screening phases

CC1, CC5 and ST22 were observed only in the first sampling phase. CC15, CC59 and CC97 were only observed in the second sampling phase (Table 3.3 and Table 3.4). These included three CC15-MSSA isolates, recovered in Locations 1 and 2 from two operators and one assistant, one CC59-MSSA isolate recovered from a female assistant in Location 2 and one CC97 recovered from a female hospital-based operator from Location 1. The second collection phase revealed that no isolates were recovered belonging to CC1-MSSA, CC5-MSSA or ST22-MSSA (Table 3.3 and Table 3.4). The second sampling phase also revealed
Virulence-associated genes (2nd screening phase)

No. of isolates
Figure 3.2. A DNA microarray (Alere technologies, Jena, Germany) was used to detect (A) virulence-associated genes and (B) antimicrobial resistance genes among 59 MSSA isolates recovered from 79 dental HCWs from five locations including a group of staff and students based at DDUH but who also worked at multi-centre facilities including prisons and outreach city hospitals. The second group of dental HCWs worked in general dental practice. The number of isolates is shown on the vertical axis and the number of genes associated with S. aureus isolates belonging to various CCs is shown on the horizontal axis. The virulence-associated genes listed in panel A encode the following virulence factors: *tst* (toxic shock toxin), *sea* (enterotoxin A), *ege* (enterotoxin gene cluster), *seh* (enterotoxin H), *sec&sel* (enterotoxins C & L) and *sed* (enterotoxin D). The letters A-E refer to IEC types A (*sea, sak, chp, scn*), B (*sak, chp, scn*), C (*chp, scn*), D (*sea, sak, scn*) and E (*sak, sen*). The antimicrobial-resistant genes listed in panel B...
encode resistance as follows: \textit{sdrM} (tetracycline efflux pump resistance), \textit{blaZ} (beta-lactamase resistance), \textit{fosB} (fosfomycin resistance), \textit{fusC} (fusidic acid resistance), \textit{sat} (streptothricin resistance) and \textit{aphA3} (aminoglycoside-streptothricin resistance).
additional isolates for CC30-MSSA (n = 6), CC398 isolates (n = 5), CC8 including one ST8-MSSA and one ST291/813-MSSA and one additional CC45-MSSA. There was one less isolate of CC7-MSSA (Table 3.3 and Table 3.4). Overall, roughly equal numbers of hospital-associated and community-associated dental HCWs harboured MSSA isolates. Five out of the seven persistent individuals harboured the same strains (CC22, CC30 (n = 2), CC398, CC8) nasally during both collection phases. Two individuals harboured strains nasally and orally during both collection phases (21, 42). One volunteer (42) harboured the same strains nasally (CC8) during both collection phases and demonstrated confluence during both collection phases. This individual harboured CC45 orally during the first collection phase but CC8 during the second collection phase. The second persistent carrier (21) during both collection phases, harboured ST291/813 with confluence during the first collection phase and ST29/813 with an affiliation with CC398 during the second screening phase. This individual (21) demonstrated the same strain orally during both collection phases (ST291/813).

Fifteen out of 35 isolates (42.9%) recovered from the five locations during the second screening phase were from hospital based DDUH HCWs (Location 1). Community HCWs (locations 2-5) were represented by 20/35 (57.1%). This finding is very similar to the results collected during the first collection phase. Location 2 yielded the majority of isolates 8/35 (22.9%) recovered from community based dental HCWs, followed by Location 3 & 4 (5/35; 14.3%) and Location 5 yielded the least number of isolates during the second collection phase with 2/35 (5.7%) of isolates. The 26-35 age-group yielded the highest number of isolates with 10/35 (28.6%) of all isolates collected during the second phase collection followed by the 36-45 age-group (25.7%, 9/35), the 46-55 age-group (22.9%, 8/35) and the 18-25 and 56-65 age-groups both yielded the lowest number of isolates (11.4%, 4/35) per age group (Tables 3.1 and Table 3.2). An overview of this data can be found in Table 3.5.
Compared to the first phase collection, hospital-based and community-based distribution remained roughly equal with a slightly higher carriage rate for the second phase collection among the community group with an additional 2.9%. Community-based Location 2 had a significant increase in carriage rate during the second collection phase (from 8.3% to 22.9%). Interestingly, Locations 3 and 4 demonstrated exact equal distribution during both collection phases with a slightly reduced overall carriage rate of 14.3% for both locations during the second collection phase compared to 16.7% for Locations 3 and 4 for the first collection phase. Location 5 had the lowest overall incidence during the second round of 5.7% compared with a rate of 12.5% during the first collection phase.

Overall, the total number of isolates recovered from operators during the second collection phase was 62.9% (22/35) and amongst assistants was 37.1% (13/35). This was an overall reduction of 12.1% in operators affected and increase in assistants harbouring *S. aureus* compared to the first collection phase. A higher carriage rate of 57.1% (20/35) was detected in females compared to 50% in the 1st sampling phase. A slightly higher nasal carriage rate of 54.3% (19/35) compared to oral carriage rate of 45.7% (16/35) was detected in the 2nd sampling phase. When compared to first sampling round results nasal carriage overall increased by 4.3% for the second sampling phase.
Table 3.5. Summary of carriage rates from collection phase one and collection phase two for oral and nasal S. aureus from 79 volunteers.

<table>
<thead>
<tr>
<th></th>
<th>First collection phase</th>
<th>Second collection phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of volunteers</td>
<td>79</td>
<td>79</td>
</tr>
<tr>
<td>Number of S. aureus isolates recovered</td>
<td>24</td>
<td>35</td>
</tr>
<tr>
<td>MRSA</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>MSSA nasal carriage rate</td>
<td>16.5% (13/79)</td>
<td>22.8% (18/79)</td>
</tr>
<tr>
<td>MSSA oral carriage rate</td>
<td>13.9% (11/79)</td>
<td>21.5% (17/79)</td>
</tr>
<tr>
<td>Number of S. aureus clonal types identified</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Most prevalent CCs</td>
<td>CC30</td>
<td>CC30</td>
</tr>
<tr>
<td>Number of operators colonised</td>
<td>75% (18/24)</td>
<td>62.9% (22/35)</td>
</tr>
<tr>
<td>Number of assistants colonised</td>
<td>25% (6/24)</td>
<td>37.1% (13/35)</td>
</tr>
<tr>
<td>Number of Hospital based MSSA isolates recovered (Location 1)</td>
<td>45.8% (11/24)</td>
<td>42.9% (15/35)</td>
</tr>
<tr>
<td>Number of Community based MSSA isolated recovered (Locations 2, 3, 4 and 5)</td>
<td>54.2% (13/24)</td>
<td>57.1% (20/35)</td>
</tr>
<tr>
<td>Age group most frequently colonised</td>
<td>46-55</td>
<td>26-35</td>
</tr>
</tbody>
</table>

Summary data showing recovery of S. aureus from nasal and oral samples from 79 dental healthcare workers sampled over two collection phases. The first phase included samples collected between 12<sup>th</sup> January-26<sup>th</sup> January 2016. The second phase included samples collected between 25<sup>th</sup> April-1<sup>st</sup> August 2016.
Chapter 4 Discussion
4.1 Discussion

There are relatively few MSSA or MRSA carriage studies in the dental field compared with the medical field and no published Irish data. This investigation, whilst the sample population is limited in number, studied a sample population of dental healthcare workers (n = 79) from both hospital and community settings which represented the diverse array of clinical operators and assistants in dental practice within Ireland. MRSA was undetected among these 79 participants, perhaps as MRSA select media was not used. Additionally, it is acknowledged that MRSA can be recovered from hospital air and from environmental surfaces as reported by Creamer and colleagues. This prospective observational study involved researchers sampling patients, air and surfaces in eight wards of a seven hundred bed tertiary care hospital that captured MRSA from 14.4% (19/132) of air samples (Creamer et al., 2014). Environmental air sampling was not carried out during sample collection for this dental study due to time and practical constraints.

It has been established that the *S. aureus* carriage rate varies with the population studied. Reports of nasal carriage rates have been as low as 10-15% among healthy adults rising to 20-35% of hospital personnel (Willems, 1990). Other studies approximate that 25-30% of the general population carry *S. aureus* in their noses (Gorwitz et al., 2008; Graham et al., 2006; Kluymans et al., 1997; Peacock et al., 2001; Perl and Golub, 1998; Trochesset and Walker, 2012; Wenzel and Perl, 1995). A low percentage of this population (less than 2%) are colonised with MRSA (Gorwitz et al., 2008; Petti and Polimeni, 2011; Trochesset and Walker, 2012). An extensive literature review carried out by Albrich and Harbarth found that 23.7% (2508/10589) of HCWs were found to carry MSSA, 4.6% of which were colonised with MRSA. A study by Reddy revealed that in the greater Houston metropolitan area, 4.2% of dentists and 1.5% of dental hygienists were carriers of MRSA (Reddy, 2010). Another study reported that 21% of dental students were colonised with nasal MRSA (Roberts et al., 2011). Nasal MRSA colonisation can act as a reservoir for transmission and...
is a risk factor for the development of MRSA infection (Klevens et al., 2008). Recent increased numbers of reports indicate that MRSA is present in the dental setting including dental patients, on dental clinical surfaces, and in dental HCWs including students (Apolonio-Alonso et al., 2011; Horiba et al., 1995; Kurita et al., 2006; Martinez-Ruiz et al., 2014; Roberts et al., 2011).

In the present study dental HCW volunteers (n = 79) underwent screening for MSSA and MRSA (79 saliva & 79 nasal swab samples) from five separate institutions on two occasions with a minimum of 12 weeks between samplings. The Dublin (Locations 1 and 5) and Belfast groups (Location 2, 3 and 4), were analysed separately with no differences found between the groups so the data were pooled for further analysis. MRSA was not detected among 79 volunteers during two sample periods which is, in itself, an interesting finding, as previous investigations have detected dental HCW MRSA carriage varies between 1.2-21% among dental students (Reddy, 2010; Roberts et al., 2011). This may be as a result of low abundance and MRSA may have gone undetected in this study as a result of not using MRSA select chromogenic media or broth enrichment. MSSA was detected in both oral and nasal samples amongst both groups from every institution during both collection phases. Previously, there was renewed interest in the use of cefoxitin disc susceptibility for the differentiation of MSSA from MRSA (Mougeot et al., 2001). MRSA select medium is the gold standard detection medium in the Dublin Dental University Hospital microbiology laboratory but was not used in the present study due to cost limitations. It was deemed however, sufficient to assess for MRSA utilising the methods previously described of which included the use of SaSelect™ selective chromogenic agar medium, MSA, CBA and latex agglutination followed by well established microarray analysis. Results of the first round of nasal and oral sampling for hospital based group 1 (DDUH dental HCWs) revealed a S. aureus nasal carriage rate of 12.83 % (5/39 volunteers) and S. aureus oral carriage rate of 17.95% (7/39), operators accounted for 12.82% (5/39) and assistants amounted to 7.7%
Community based group 2 (which included GDPs and assistants from Locations 2, 3, 4 and 5) yielded a nasal carriage rate of 20% (8/40) and an oral carriage rate of 17.5% (7/40) indicating slightly higher nasal carriage rates amongst the community group and almost identical oral carriage rates among both the hospital and community groups. It is important to recognise a limitation of this study includes the small sample number for both groups and as such, care must be given to the interpretation of the results.

The combined nasal carriage rate for all dental HCWs (79 volunteers) from the first collection round yielded a rate of 16.5% (13/79) and from the second collection round yielded a nasal carriage rate of 22.78% (18/79). The overall carriage rate of nasal and oral \( S.\ aureus \) detected among this specific cohort of dental HCW population is less than existing published data from other groups studied. In addition, this study revealed the unusual finding that no MRSA was found among any dental HCW working in either hospital or community-based practice. Of the 24 MSSA isolates recovered from the first collection phase, 18/24 (75%) were recovered from operators. During the second collection phase, 22/35 (62.9%) of MSSA isolates were recovered from operators. These results indicate that operators involved in open procedures can potentially cause forward transmission to patients and fellow dental healthcare workers.

Longitudinal studies distinguish at least three patterns of \( S.\ aureus \) nasal carriage in healthy individuals including persistent carriage (20%), intermittent (30%) and non-carriage (50%) (Eriksen et al., 1995; Hu et al., 1995; Kluytmans et al., 1997; Nouwen et al., 2004). No international consensus exists on how to identify different carrier states exactly (VandenBergh et al., 1999). Persistent nasal carriers harbour more \( S.\ aureus \) measured as CFUs compared to intermittent carriers, allowing for more environmental dispersal and a higher risk of infection (Nouwen et al., 2005; Nouwen et al., 2004; White, 1963). Persistent carriers would theoretically be identified by repeated screening over a prolonged time period. Persistent nasal \( S.\ aureus \) carriers as defined for this investigation harboured MSSA nasally
during both collection phases and accounted for 8.9% (7/79). This study revealed seven persistent nasal carriers of MSSA; of these seven persistent carriers, six were operators and one was a hospital-based assistant. All seven persistent nasal carriers demonstrated confluence during the first collection phase, which confirms a high potential risk for onward nasal transmission of MSSA/MRSA to patients and fellow dental healthcare workers. Intermittent carriers harbour staphylococci for a few weeks at a time followed by comparable periods of non-carriage, carrying different *S. aureus* strains during successive periods of carriage (Williams, 1963) whereas persistent carriers tend to carry a single strain of *S. aureus* over a long period of time (Eriksen et al., 1995; Hu et al., 1995; VandenBergh et al., 1999). This study revealed a persistent nasal *S. aureus* carriage rate of 7/79 (8.9%), that is nasal carriers harbouring *S. aureus* in both collection phases (Section 3.8). Intermittent nasal carriage as defined as either loss or gain of *S. aureus* during either collection phase accounted for 24/79 (30.4%). Due to time constraints, this study involved a biphasic collection period only. This is a drawback of the study, as more spaced collection periods would allow for more data to determine *S. aureus* carriage patterns and rates more precisely.

A study by Ohara-Nemoto and colleagues investigating nasal and oral bacteria of pre-clinical and laboratory research staff of Iwate School of Dentistry demonstrated evidence of nasal-oral trafficking of the *S. aureus* species using PFGE genotyping (Ohara-Nemoto et al., 2008). They postulated that *S. aureus* present in the oral cavity may be provided continuously from the nasal cavity. The present study detected evidence for persistent *S. aureus* nasal carriage (positive *S. aureus* carriage occurring during both collection phases) which as discussed above, accounted for seven individuals. This investigation revealed that two individuals harboured *S. aureus* both orally and nasally during both collection phases. One individual harboured the same strain (CC8) nasally during both collection phases. This volunteer however, harboured CC45 orally during the first collection phase but CC8 during the second collection phase. The second persistent oral and nasal carrier harboured the same
strain nasally and orally during both collection phases. This supports and demonstrates evidence of nasal-oral trafficking.

The results of the current investigation reflect *S. aureus* isolates recovered from a range of individuals working in the dental profession in Ireland. Recovered strains from this investigation span a variety of lineages seen at the global level. Trans-global migration patterns with an increase in cultural variety represented amongst the working population in Ireland may account for the diverse range of *S. aureus* strains identified from this study. This may be indicative of the importation of strains from other countries by dental HCWs. It is noteworthy that the majority of the MSSA clones detected in the present investigation shared ancestry with the major MRSA clones that have been successful on a global scale. It is possible some of these MSSA clones may have harboured SCCmec previously and lost it.

The majority of volunteers from both hospital and community settings appeared to harbour MSSA transiently in this investigation (Section 3.8). All *S. aureus* isolates recovered harboured antimicrobial resistant genes encoding resistance to combinations of streptomycin, beta-lactam, fusidic acid, fosfomycin, aminoglycoside and quinolones (Figure 3.1 and Figure 3.2). A wider array of virulence-associated genes were detected (Figure 3.1 and Figure 3.2). This investigation revealed a variety of global CCs circulating among dental HCWs working both in Irish hospitals and within the Irish community namely CC1, CC8, CC15, CC22, CC30, CC45, CC7, CC2, CC59, CC97 and the previously reported livestock associated CC398-MSSA was also detected. Chua et al. recently documented that the most common strains of hospital-associated MRSA belong to just five CCs (CC5, CC8, CC22, CC30, CC45) with a much larger and more diverse range of community-associated isolates (Chua et al., 2014). This may also indicate that MSSA isolates recovered from the participants in the present study may be more sporadic and diverse revealing an extensive diversity in genetic backgrounds on a molecular epidemiologic basis. Coupled with the fact that globally there is a far more diverse range of MSSA clones than MRSA clones, it is not
surprising therefore, that this study yielded such a broad variety of MSSA isolates. The diverse number of MSSA clones identified from the isolates investigated may also reflect the number of locations and different settings investigated. The wide range of CCs carrying different genes encoding antimicrobial resistance and virulence factors was evident amongst the 24 *S. aureus* isolates recovered from the first collection phase (Table 3.3) and the 35 *S. aureus* isolates recovered from the second collection phase (Table 3.4).

In the present investigation, CC30 predominated during both collection phases. Both HA-MSSA and CA-MSSA isolates originate from CC30, which has been reported in many countries with widespread global distribution; amongst countries including Malta (Gould *et al*., 2008; Seicluna *et al*., 2010), South Africa (Jansen van Rensburg *et al*., 2011), Canada (Christianson *et al*., 2007), Egypt (Enany *et al*., 2007) and was frequently isolated in Ireland and the U.K. in the 1990’s (Shore *et al*., 2005). An important CC30 strain variant, the PVL-positive ST30-MRSA-IV strain known as the Southwest Pacific clone, USA1100 or West Samoan Phage Pattern (WSPP) clone has been reported in Germany, Switzerland, Australia, the U.K. (Monecke *et al*., 2007; Monecke *et al*., 2008a), Hong Kong and Taiwan (Takano *et al*., 2008). Panton-Valentine leucocidin (PVL) is an exotoxin that is expressed by a significant proportion of CA-MRSA isolates which when produced causes enhanced virulence. The PVL-positive ST30-MRSA-IV strain is the predominant PVL-positive MRSA strain in Ireland, Scandinavia, Latvia and Kuwait (Monecke *et al*., 2011). No PVL-positive isolates were found amongst any of the CC30 isolates during the present study. Interestingly, the WSPP MSSA clone emerged in the 1950s causing severe infections worldwide but has since become quiescent globally, yet CC30 MSSA derivatives (CC30-ST30/ST39, CC30-ST24, ST42, CC30/ST39) remained the most prominent isolates recovered from the 79 participants in this study.

CC22-MSSA was detected as a nasal isolate in study participant 75 for both collection phases and this volunteer was positive for oral carriage of CC22 during the second
collection phase. CC22-MRSA, which is also known as UK-EMRSA-15 or Irish AR06, has the same genetic background as CC22-MSSA but lacks the SCCmec element. The ST22-MRSA-IV strain that has been endemic in Irish hospitals since 2002 may eventually be displaced by an emerging sporadic clone, which may exhibit enhanced virulence and be resistant to more antimicrobial agents. Shore et al. previously reported, a history of predominant MRSA strain replacement in Ireland, with strain replacement occurring approximately once per decade for the past forty years (Shore et al., 2005; Shore and Coleman, 2013). Another distinct ST22-MRSA-IV strain harbouring the arginine-catabolic mobile element (ACME), an element that has been associated with increased pathogenicity, in the predominant ST8-MRSA-IVa strain in the U.S.A., was reported in Dublin in 2011 (Monecke et al., 2011; Shore et al., 2011b).

MSSA are also harboured by animals including livestock, wild and domestic animals. This is another important S. aureus reservoir. Current global livestock populations now exceed populations of wild animals and serve as hosts for MSSA. Two sub-lineages of the CC398-MSSA clone were detected in five individuals during the present study indicating possible zoonotic spread of these strains within Ireland. CC398-MRSA is frequently found in association with pigs, poultry and livestock including horses and among humans with close animal contact (Asai et al., 2012; Graveland et al., 2011; Lim et al., 2012; Witte et al., 2007). In addition, CC398-MRSA has been found in turkey meat samples (Feßler et al., 2011; Monecke et al., 2013). CC398-MRSA was first discovered from members of a family pig farm in the Netherlands in 2006 and has since been observed in humans, cattle, horses, dogs, poultry, chickens and poultry, in addition to retail meat of domestic animals. Animals may act as reservoirs for MSSA/MRSA carriage and highlights the need to use active surveillance to minimise the spread of these clones both in hospital and amongst the community (Monecke et al., 2011).
CC45-MSSA is another well-known lineage globally. It has been recovered in Belgium, Germany, North America, Hong Kong, Australia, Saxony, the Netherlands, Switzerland, Croatia, Portugal and Portugal. Monecke et al. have observed sporadic isolates recovered in Ireland (Monecke et al., 2011). It was a prominent strain found during the present study. CC15-MSSA has been reported to be abundant amongst healthy carriers and the MRSA variant from this lineage is known to be very rare (Monecke et al., 2011). This study identified similar findings with CC15-MSSA isolates \((n = 3)\) recovered only in the second sampling phase, and no CC15-MRSA isolates detected from the 79 healthy volunteers. The CC8-MSSA clone, often referred to the Ancestral/Early MRSA or Irish AR02 is a pandemic strain and has both CA- and HA-MRSA associated strains. Several CC8-MSSA strains \((n = 6)\) were detected in the current investigation. ST8-MRSA predominated in the North of Ireland in 1999 (Monecke et al., 2011) where 2/3 locations in this study yielded CC8/ST8 during both collection phases (Locations 2, 3 and 4). One individual harboured CC8 nasally during the first collection phase and as a likely result of oro-nasal trafficking, also harboured CC8 both nasally and orally during the second collection phase.

CC5-MSSA is another common and widespread clonal complex comprising of both CA- and HA-MRSA associated strains. CC5-MSSA was only found during the first collection phase carried by two dental assistants working in different locations; one based in the community, the other a hospital based dental HCW. One HCW harboured the strain orally, the other nasally and both were non-carriers during the second collection phase.

CC7-MSSA was found exclusively in one location (Location 1) in this study predominantly among operators \((3/4)\). The two operators that carried the strain during the first sampling round all harboured CC7 orally and all were found to be non-oral \(S.\) *aureus* carriers for the second collection phase. During the second collection phase the operator harboured the strain nasally and the assistant, orally. CC7-MRSA are rare and have been reported in Saxony and Australia (CA and HA-MRSA associated strains).
CC20-MSSA has been found from both human and veterinary origins (Monecke et al., 2011) and only one single CC20-MRSA isolate has been found in Australia, identified in 2009. One hospital-based volunteer from this study harboured CC20-MSSA during both collection phases. The isolate was found both orally and nasally during the first collection phase and the volunteer lost nasal carriage during the second phase collection. CC59-MSSA was harboured orally as a single isolate during the second collection phase only in community based Location 2. The ST59-MRSA-IV variant is mainly restricted to the USA but other CC59 strains have been isolated from Western Australia and Taiwan mainly. CC97-MSSA is another clonal complex often isolated from cattle but has been identified among humans. MRSA strains are rare from this lineage. This study revealed a single isolate carried only during the second collection phase on a female operator from hospital-based Location 1. CC1-MSSA isolate was detected during the first collection phase only. The female operator volunteer was from community based Location 2. This isolate harboured \( fusC \) and \( ccrAB1 \) genes. These two genes when identified together are marker genes for a SCC\( fus \) cassette (Scicluna et al., 2010). This cassette is a MGE that harbours a gene encoding resistance to fusidic acid. Derivatives of the community associated CC1-MRSA clone was the cause of fatal infections of children from Minnesota and North Dakota in the late 1990s (1999), with four paediatric deaths from CA-MRSA. Recent studies from Ireland reported the emergence of community-associated multidrug resistant CC1-MRSA strains in many Irish hospitals and other healthcare facilities (Earls et al., 2017). One strain was responsible for a protracted outbreak in one large acute hospital, where the majority of isolates harboured a conjugative plasmid encoding high level mupirocin resistance (Earls et al., 2017).

*Staphylococcus aureus* is an important human pathogen and MRSA are a major cause of both hospital and community-acquired infections worldwide. It is well established that the carriage rate of *S. aureus* varies amongst the population studied and is estimated to be between 30-50% at any one point in time. It is unknown why some people may be carriers
and others remain unaffected. Recent research however has identified that some individuals have a specific nasal receptor for *S. aureus* (*Mulcahy et al.*, 2012). Staphylococci have evolved and adapted to combat detection by the immune system and survival via carriage of a wide arsenal of components allowing evasion of the host immune response. As such, it is postulated that the carrier-state is multifactorial and may result from nasal ecology and structure, nasal secretion, environmental factors, genetic predisposition (*Kinsman et al.*, 1983), local IgA or systemic IgG antibody production (*Eriksen et al.*, 1995). Repeated exposure to the pathogen via household contacts is considered an important determining factor for *S. aureus* nasal carriage (*Wertheim et al.*, 2005a). It is well accepted that nasal carriage of *S. aureus* is an important risk factor for nosocomial and surgical site infections and numerous studies have reported large reductions of surgical site infections following nasal decolonisation with mupirocin (*Cimochowski et al.*, 2001; *Gernaat-van der Sluis et al.*, 1998; *Kluytmans et al.*, 1996; *VandenBergh et al.*, 1996). These success rates however, failed to be reproduced through randomised controlled trials (*Kalmeijer et al.*, 2002; *Perl et al.*, 2002; *Wertheim et al.*, 2004).

A major difficulty for MRSA or MSSA control is the fact that patients or HCWs can act as reservoirs with unrecognised colonisation. Persistently colonised or infected HCWs have been found to be the main source of MRSA clusters among patients (*Ben-David et al.*, 2008; *Saravolatz et al.*, 1982). Successful control of MRSA transmission after identifying colonised HCWs and eradicating employee carriage with topical or systemic antimicrobial therapy has been reported several times (*Boyce et al.*, 1993; *Reboli et al.*, 1990; *Saravolatz et al.*, 1982). As such, HCWs may act not only as the source of horizontal transmission to patients, but also cross transmission to other HCWs. Educational programmes, proper hand hygiene, and strict adherence to universal precautions represent the fundamental cornerstone to control MRSA spread with credence associated to additional methods of HCW screening,
decolonisation and treatment of HCWs in MRSA outbreaks. Well-designed intervention studies are required to assess the effect of CA-MRSA on healthcare institutions.

The relative frequency of healthcare-associated infections may be due to a combination of factors relating to microorganism viability in the environment along with the frequency of antimicrobial use (Goldstein, 2011). Treatment of bacterial infections has become problematic with the advent of antibiotic resistance, resulting primarily from antibiotic overuse, which has ultimately caused the spread of resistant strains within the population. As a result, there are now many strains of highly pathogenic bacterial pathogens circulating globally that are resistant to some or multiple different antibiotics such as *S. aureus*.

Eliminating nasal carriage of *S. aureus* has previously been shown to reduce the incidence of *S. aureus* infections (Chow and Yu, 1989; Holton et al., 1991; Thodis et al., 1998; Yu et al., 1986). Further studies have shown that application of nasal mupirocin reduces overall the rate of surgical-wound infection (Kluytmans et al., 1996) along with a reduction in *S. aureus* bacteraemia in patients receiving haemodialysis (Boelaert et al., 1993). Topical nasal treatment using mupirocin has also successfully eliminated *S. aureus* from colonised body sites (Boelaert et al., 1996; Parras et al., 1995; Reagan et al., 1991) and importantly eliminates hand carriage (Reagan et al., 1991). CA-MRSA strains have been shown to have increased virulence and fitness properties compared to their traditional HA-MRSA counterparts due to a combination of genetic acquisitions such as SCC*me* and the genes encoding PVL and enhanced toxin expression.

Numerous reports have detailed the transmission of MRSA between HCWs and patients (Albrich and Harbarth, 2008; Ben-David et al., 2008; Bertin et al., 2006; Kassis et al., 2011; Stein et al., 2006; Wang et al., 2001). It has been suggested that random periodic screening of HCW staff would help identify asymptomatic carriers of MRSA, as colonisation
rates are higher in endemic settings (Boyle-Vavra and Daum, 2007; Edmundson et al., 2011). In a review paper, Albrich and Harbarth suggested that pre-employment HCW screening may assist in identifying *S. aureus* carriers (Albrich and Harbarth, 2008). Cost implications of routine HCW screening has been a contentious issue; however in countries and regions of low MRSA prevalence such as Scandinavia, the Netherlands and Western Australia, HCW surveillance is carried out routinely (Andersen et al., 1999; Andersen et al., 2002; Blok et al., 2003; Verhoef et al., 1999). Screening of HCWs is both expensive and contentious and there is no consensus regarding the best time to screen HCWs.

Current Irish guidelines recommend that staff be screened for MRSA carriage only as part of an outbreak investigation (Strategy for the Control of Antimicrobial Resistance in Ireland (SARI), 2005). Similarly, current U. K. guidelines do not recommend routine MRSA screening of staff either (Coia et al., 2006), however 63% of U.K. doctors attending two national conferences were in favour of routine staff screening for MRSA (Brady et al., 2009). As such there is little current evidence to support screening of HCWs in the NHS beyond the current national guideline recommendations (Hawkins et al., 2011).
Chapter 5 Conclusions
5.1 Conclusion

In addition to large cost implications, routine screening of HCWs is associated with potential stigmatisation of the HCW and has uncertain benefit. Importantly, HCWs are most often transiently colonised but may become persistent carriers particularly if they have skin lesions (Ben-David et al., 2008). Due consideration of implementing screening of all patients or staff at high risk for MRSA carriage (such as those hospitalised in foreign countries) ought to be considered, as has been policy in the Netherlands to help reduce and maintain low levels of MRSA carriage. In Western Australia, all HCWs, who will have clinical contact, are required to have their MRSA risk status assessed as part of a pre-employment / commencement process. In addition, MRSA surveillance screening is required for HCWs in Western Australia prior to commencement of work if the HCW has been hospitalised or worked in any healthcare facility outside of Western Australia in the previous 12 months. This study of a specific cohort of dental HCWs through nasal swab and oral rinse has revealed that amongst a population of 79 individuals, no MRSA was detected in either of two sampling phases over a six-month period. That said the nasal carriers, in particular the persistent nasal carriers (6/7) who were operators and conducting open procedures) were identified as high confluent carriers with high potential for onward transmission. MRSA selective chromogenic media was not used in this study, and as such, may be a valid reason as to why no MRSA was detected amongst the cohort studied. Based on the results from this study, it is reasonable to consider implementing dental HCW screening based on the high confluence rates alone. Despite the fact that no MRSA was identified, a wide variety of MSSA isolates were recovered which harboured multiple virulence factors with the potential to cause severe infections. It is reasonable therefore to suggest that HCWs involved in invasive procedures ought to be screened for MSSA/MRSA carriage. Further additional studies may be useful to expand both the numbers of volunteers screened, using multiple locations to capture a larger population and geographic spread facilitating collection from
dental HCWs in both Queens University Dental hospital Belfast and Cork University Dental hospitals. Additional work and further study on the existing cohort of dental HCWs may reveal further information relating to range of strains and carriage pattern differences. There is merit to consider environmental surveillance in future study. In order to detect low abundant carriers with further study, broth enrichment could be used followed by chromogenic MRSA media.
References


Appendices
Appendix I
30th September 2015

RE: ‘To investigate the temporal dynamics of Staphylococcus aureus nasal and oropharyngeal carriage amongst oral/dental healthcare staff’

REC Reference : REC Reference: 2015-0 Chairman’s Action (12)
(Please quote reference on all correspondence)

Dear Dr. Malone,

Thank you for your recent correspondence to SJH/AMNCH Research Ethics Committee in which you enquired about ethical approval for the above referenced study.

The Chairman, on behalf of the Research Ethics Committee, has reviewed your correspondence and has given ethical approval for this study.

Yours sincerely,

Claire Hartin
Administrator
SJH/AMNCH Research Ethics Committee
Appendix II
Appendix II

Participant information leaflet

Dear Staff Member,

You are being requested to participate in a research study. In order to make an informed judgement on whether you would like to be part of this research study or not, you should understand any associated potential risks and benefits. This is the principle of informed consent. This leaflet gives you information about the research study which will be discussed with you fully. Once you understand the nature of the study and you wish to participate, you will be asked to sign a consent form.

Title of the research study:

“To investigate the temporal dynamics of *Staphylococcus aureus* nasal and oropharyngeal carriage amongst oral/dental healthcare staff”.

Names of researchers:

Dr Keira Malone

Professor David Coleman

Professor Leo F.S Stassen

Description of the study:

This study that we have proposed you participate in is an attempt to further our understanding of the temporal patterns of carriage of the bacterium *Staphylococcus aureus* amongst dental healthcare workers. This bacterium can cause serious and sometimes fatal infections in vulnerable individuals. *Staphylococcus aureus* is carried harmlessly by a large portion of the general population. Amongst the human population it naturally resides on the skin and most commonly occurs in the nose and mouth. Evidence distinguishes at least three patterns of carriage of the bacteria in healthy individuals including persistent carriage, intermittent carriage and non-carriage. We aim to investigate the patterns of carriage of *S. aureus* strains amongst dental healthcare workers to determine whether the risk of transmission to dental patients warrants due consideration to recommend routine screening of *S. aureus* and decolonisation of dental healthcare workers.

If you decide to participate in this study, you will be asked to rinse your mouth briefly with a dilute salt solution and then return it into a sample container. This simple technique retrieves bacteria present on the surfaces of the oral tissues. A soft cotton swab will also be used to take samples from the nostril region. It is expected that samples will be taken on a pre-arranged day on three occasions for six months and sampling should take no longer than two minutes each time. You should not expect any pain or complications to you during these procedures.
Samples will be pseudo anonymised and identified with a numerical code. Microbiological testing and results will also be anonymised and not traceable back to you. There will be no personal or professional consequences for you because of your decision to participate or not in this study.

Procedure:

To be included in this study:

- You must not have received antibiotic treatment in the previous 28-day period.
- You must not have any active localised oral or nasal infection.
- You must be over 18 years of age.
- You must be free of systemic infection.
- You must be in good overall health and free of any diseases impairing on ability to co-operate with oral rinsing.
- Be willing to co-operate with the requirements of the study protocol and be able to attend all required sampling appointments.
- Be able to give consent to participate in this study and sign a consent form approved by The Research Committee.

You will not be able to participate if any of the above stated criteria is not fulfilled.

Other information of relevance to this study:

- Your identity will remain confidential and your name will not be disclosed or published.
- If you decide to participate in this study, you may withdraw at any stage. If you decide not to participate, or withdraw during the study, your decision will be respected.
- You understand that the investigators may withdraw your participation in the study at any time without your consent.
- If you are initially included in the study but fail to complete all stages, you will have to be excluded.

At this stage, I invite you to reflect on the previous information given and encourage you to ask any questions regarding the process.

Thank you for your kind assistance.

With Regards,

Ms. Keira Malone

BDS MFD RCSi DipConSed
Appendix III
Appendix III

Consent form for participation in study

Title of study: “To investigate the temporal dynamics of *Staphylococcus aureus* nasal and oropharyngeal carriage amongst oral/dental healthcare staff”

Names of researchers:

Ms. Keira Malone (Dublin Dental University Hospital)

Professor David Coleman (Dublin Dental University Hospital)

Professor Leo F.A Stassen (Dublin Dental University Hospital/St James’s Hospital)

I ……………………………… confirm that this study and the consent form have been explained to me. The investigator has answered all questions to my satisfaction. I believe that I understand what will happen if I agree to be part of this study. I have read, or had read to me this consent form and have had the opportunity to ask questions and all of my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study. I have received a copy of this agreement.

I have been assured that the microbiological samples taken from me will not be identified by any name associated to me, and that testing and results of treatment will be pseudo-anonymised and not traceable to me. I have also been assured that there will be no personal or professional consequences for me as a result of participating in this research study.

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                                        Name of participant      Date                           Signature

Statement of investigator’s responsibility:

I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent.

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                                        Name of Researcher        Date                           Signature

Contact details: 0861542355