An Investigation of the Prevalence, Population Structure and Relatedness of Environmental, Healthcare Worker and Patient *Staphylococcus aureus* Isolates Recovered from Multi-bed Patient Rooms in an Irish Teaching hospital by Whole-Genome Sequencing

A thesis submitted to the University of Dublin, Trinity College in fulfilment of the requirements for the degree of M.Sc. by Research

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Declaration

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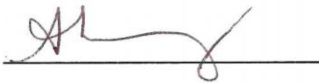

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Index of Tables

Table	Title	Page
1.1	Details of *S. aureus* bloodstream infection isolates reported to the Health Protection Surveillance Centre in Ireland from 2011-2018………...........................	15
1.2	Approaches used to ventilate hospital buildings………………………………...	31
2.1	Antimicrobial agents and breakpoints used for susceptibility testing in the present study………………………………………………………......................	61
3.1	Active air sampling results from the four study days included in the present study…………………………………………………………...........................	82
3.2	Antimicrobial resistance profiles of six discrete *S. aureus* isolates recovered from three active air sampling plates in the presentstudy………..	83
3.3	Details of *S. aureus* isolates (all MSSA) that were recovered using passive air sampling during the present study………………………………………………..	84
3.4	Results of surface sampling using Colorex™ Staph Aureus contact plates for the recovery of *S. aureus* in the present study………………………………………..	86
3.5	Antimicrobial resistance testing results of *S. aureus* isolates recovered during the present study……………………………………………………………….	90
3.6	Results of hygiene assessments, ATP measurements and *S. aureus* culture from 120 surfaces tested in Wards A and B over the four-day study period in the present study…………………………………………………………………...	93
3.7	Details of active air sampling culture results, airborne particle count values, room occupancy and movement of privacy curtains……………………………..	103
3.8	Demographic data provided by HCWs who participated in the study…...............	108
3.9	Demographic data provided by patients who participated in the study…………..	111
4.1	Summary of 62 *S. aureus* isolates subjected to whole-genome sequencing during the present study………………………………...................................	129
4.2	Quality assurance measures undertaken throughout whole-genome sequencing process…………………………………………………………………………	137
4.3	Gene targets included for *in-silico* genotyping by Ridom SeqSphere (version 7.0; Ridom GmbH)…………………………………………...........................	140

4.4 Whole-genome sequencing quality data for all 62 study isolates………………... 149

4.5 Population structure of the 62 *S. aureus* isolates recovered during the present study………………………………………………………………........................... 152

4.6 Virulence factors and resistance genes detected by *in-silico* genotyping of the 62 *S. aureus* isolates included in the present study………………………………….. 157

4.7 Isolates in the present study in which genes associated with SCC*mec* were detected……………………………………………………………………………... 166

4.8 Isolates assigned to related isolate groups in the present study………………….. 174

Index of Figures

Figure	Title	Page
1.1	The transmission cycle of *S. aureus*………………………………………	6
1.2	A stacked column chart representing *S. aureus* bloodstream infection isolates recovered in Ireland between 2004-2018, as reported to the European Antimicrobial Resistance Surveillance Network (EARS-Net)……………………………………………………………….….	28
2.1	Schematic diagram showing layout of Ward A. Sampling was completed in the 4- bedded bay indicated by the red arrow……………	47
2.2	Flow chart detailing the air handing unit servicing Ward A in the present study………………………………………………………………	48
2.3	Schematic diagram showing layout of Ward B………………………...	51
2.4	A photograph showing semi-confluent growth of *S. aureus* colonies on a Colorex™ Staph Aureus chromogenic agar plate from a sample obtained during the present study………………………………………	60
3.1	A flowchart describing hygiene assessments undertaken during the present study………………………………………………………………	71
3.2	Culture results of patient, HCW and environmental samples obtained from two multi-bed urology surgical wards in an Irish teaching hospital…………………………………………………………………	87
3.3	A clustered column chart detailing airborne particle counts recorded with respect to clinical activities that were being undertaken concurrently, and concurrently collected active air samples…………...	100
4.1	Minimum spanning tree (MST) based on whole-genome multilocus sequencing typing (wgMLST) analysis showing the relationships between the 62 *S. aureus* isolates recovered during the present study…	146
4.2	A schematic comparison of the SCCmec elements of an MRSA isolate, seven OS-MRSA isolates and a reference MRSA strain- all SCC*mec* V (5C2&5)……………………………………………………	167
4.3	The protein sequence alignment output of the *Fem*A and *Fem*X genes of eight ST8 OS-MRSA isolates and a reference strain (MRSA252) generated by Clustal Omega……………………………………………	170

Abbreviations

ACH	Air changes per hour
AHU	Air handling unit
ATP	Adenosine triphosphate
BLAST	Basic Local Assignment Search Tool
bp	Base pair
BSI	Bloodstream infection
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CBA	Columbia blood agar
CC	Clonal complex
cgMLST	Core-genome multilocus sequence typing
CHEF	Contour-clamped homogenous electric field electrophoresis
CLSI	Clinical Laboratory Standards Insititute
CoNS	Coagulase-negative staphylococci
CoPS	Coagulase-positive staphylococci
DDUH	Dublin Dental University Hospital
DNA	Deoxyribonucleic acid
e.g.	*Exemplia gratia*; for example
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Control
ED	Emergency department
et al.	*Et alia*; and others
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
g	Gravitational force

h	hour
HA-MRSA	Healthcare-associated methicillin-resistant *Staphylococcus aureus*
HCWs	Healthcare workers
HEPA	High-efficiency particulate air
HPSC	Health Protection Surveillance Centre
HSE	Health Service Executive
i.e.	*Id est*; that is
ICU	Intensive care unit
IEC	Immune evasion complex
IPC	Infection prevention and control
IS	Insertion sequence
L	Litre
LA-MRSA	Livestock-associated methicillin-resistant *Staphylococcus aureus*
LED	Light emitting diode
m	Metre
MDR	Multi-drug resistant/resistance
MGE	Mobile genetic element
MIC	Minimum inhibitory concentration
min	Minute
min	Minute
ml	Millilitre
MLS_b	Macrolides, lincosamides and streptogramin
MLST	Multilocus sequence typing
mm	Millimetre
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCRAMM	Microbial surface components recognising adhesive matrix

	molecules
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MST	Minimum spanning tree
ng	Nanogram
NGS	Next-generation sequencing
NICU	Neonatal intensive care unit
NICU	Neonatal intensive care unit
nm	Nanometre
NMRSARL	National methicillin-resistant *Staphylococcus aureus* reference laboratory
ºC	Degrees Celsius
OECD	Organisation for Economic Co-operation and Development
OS-MRSA	Oxacillin-susceptible methicillin-resistant *Staphylococcus aureus*
PBP	Penicillin-binding protein
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
pM	Picometre
PPE	Personal protective equipment
QAC	Quarternary ammonium compounds
RIG	Related isolate group
RLU	Relative light unit
s	seconds
SARI	Strategy for the Control of Antimicrobial Resistance in Ireland
SCC	Staphylococcal cassette chromosome
SD	Study day

SNP	Single nucleotide polymorphism
SNV	Single nucleotide variation
SSI	Surgical site infection
SSTIs	Skin and soft tissue infections
ST	Sequence type
TE	Transmission event
UK	United Kingdom
US	United States (of America)
USA	United States of America
v/v	Volume per volume
VRE	Vancomycin-resistant Enterococcus
wgMSLT	Whole-genome multilocus sequence typing
WGS	Whole-genome sequencing
µg	Microgram
µl	Microlitre
≤	Less than or equal to
≥	Greater than or equal to
%	Percentage
<	Less than
>	Greater than

Summary

Staphylococcus aureus (including methicillin-resistant *S. aureus*, or MRSA) are among the most significant nosocomial pathogens globally. *Staphylococcus aureus* can evade control measures such as hand hygiene and regular hospital cleaning by exploiting environmental transmission pathways. One such route is via hospital air. Studies that investigated the role of the air in *S. aureus* transmission were generally set in specialized areas, such as operating theatres or intensive care units. The putative role of the air in *S. aureus* transmission in multi-bed ward areas of hospitals, where the majority of in-patients are cared for, has not yet been elucidated. The work described in this thesis included extensive environmental sampling and concurrent in-depth observation of the clinical activities in two differently ventilated multi-bed wards (one supplied by a HEPA-filtered ventilation system, referred to as Ward A, and the other with ventilation provided by windows - Ward B). Patients and healthcare workers (HCWs) occupying the study area were invited to volunteer to be screened for *S. aureus* carriage. Isolates recovered were investigated by whole-genome sequencing (WGS). This study aimed (1) to investigate the prevalence of environmental *S. aureus* under active clinical conditions in the two multi-bed hospital wards, (2) to observe the impact of routine activities and surface hygiene on *S. aureus* recovery and (3) to identify the population structure, molecular characteristics and relatedness of isolates recovered. This study was set in two urology wards in an 820-bed tertiary referral hospital in Dublin, Ireland. The wards were chosen as they share similar patient caseloads and clinical activities, so are well-matched in most respects with the exception of how they are ventilated.

The first part of this study involved environmental and participant sampling and surface hygiene assessments alongside observation of ward activity. This was undertaken in five-hour periods spread over four days, with two days spent on each ward. Screening of patients and HCWs included sampling of the nares with a cotton-tipped swab (Copan, Italy) and an oral rinse using phosphate-buffered saline. Environmental sampling included the air and surfaces within the study area. Active air sampling was undertaken using an Oxoid/Thermo Scientific EM0100A air sampler (Oxoid, Ireland), which was programmed to collect 1000 L of air from a fixed point within the centre of the study room on a continuous basis for the duration of the study periods. Breaks in air sampling occurred only to clean the air sampler head and replace the culture plate. Passive air sampling involved the use of settle plates which were placed on the overbed tables of all patients occupying the study areas for a period of 60 min, and these were replaced hourly for the duration of the study periods. Surface sampling was undertaken by taking contact plate (Colorex™ Staph Aureus chromogenic medium (E&O Laboratories Ltd., Scotland)) samples of sites observed to be frequently touched by the hands of HCWs during the study. All participant and environmental samples were cultured using Colorex™ Staph Aureus chromogenic agar. Putative *S. aureus* colonies recovered on Colorex™ Staph Aureus agar were definitively identified using the tube coagulase test and by latex agglutination with the Pastorex Staph Plus latex agglutination kit (Bio-Rad, France). All *S. aureus* isolates were tested for growth on chromogenic agar selective for MRSA (Colorex™ MRSA, E&O Laboratories Ltd., Scotland). Surface contamination was assessed on sampled sites by visual assessment (currently advised in Irish national guidance for the audit of cleaning in hospitals) and surface adenosine triphosphate (ATP) was quantified (using a SystemSure II luminometer, (Hygiena Int. Ltd., UK)). Variables that have previously been associated with recovery of airborne *S. aureus* were recorded, including bed-making, movement of privacy curtains, occupant density and clinical activities. An airborne particle counter (ParticleScan Pro™ Airborne Particle Counter (IQ Air, Switzerland)) was used to enumerate suspended particles and investigate any potential correlation with environmental *S. aureus* contamination.

A total of 375 samples were taken including 117 active air samples, 100 settle plates, 120 contact plates and nasal and oral samples from 19 participants (five patients and 14 HCWs). It was not possible to sample all occupants of the study areas due to patients not meeting the study inclusion criteria (capacity to consent) or patients or HCWs declining participation. Six HCWs and one patient were found to be colonised with *S. aureus*. Active air sampling yielded 35 *S. aureus* isolates from 117 samples. Growth of colonies with different morphologies was observed with three samples, so 38 isolates from active air sampling were investigated. *Staphylococcus aureus* was recovered from six settle plates and 12 contact plates. Settle plate positivity reflected deposition of airborne *S. aureus* to the surface on which the contact plate was placed, whereas contact plate samples likely reflect such deposition in addition to hand-touch contamination. All isolates (n=65) were initially identified as methicillin-susceptible *S. aureus* (MSSA) as none grew on MRSA selective agar. Visual hygiene assessments were not significantly different on either ward, although ATP readings were higher on Ward A than Ward B. The majority of surfaces from which *S. aureus* was recovered were on Ward B, with nine surfaces yielding *S. aureus* compared with two surfaces on Ward A. Lower ATP values were observed on surfaces that appeared visually clean, but this difference was not significant. Seven surfaces that passed a visual hygiene assessment harboured *S. aureus*. Particle counts were significantly lower on the HEPA-filtered ward (Ward A). Airborne *S. aureus* was slightly (but not significantly) more prevalent on Ward A than Ward B, with *S. aureus* recovered from 37.7% and 29.0% of active air samples,

respectively. Airborne particle counts were observed to be higher when *S. aureus* was recovered from the concurrent air sample than when it was not, but not significantly. Privacy curtain movement was significantly associated with patients washing and dressing (p <000.1), and these actions were significantly associated with airborne *S. aureus* recovery (p <0.05). Antimicrobial susceptibility testing identified seven isolates that exhibited resistance to cefoxitin. These isolates failed to grow on MRSA selective agar but were confirmed as MRSA by the MRSA GeneXpert Assay (Cepheid, USA). All seven isolates were susceptible to oxacillin and were designated oxacillin-susceptible MRSA (OS-MRSA).

Overall, almost one third (32.2%) of air samples yielded *S. aureus*. *Staphylococcus aureus* was recovered from settle plates on six occasions (6%), representing contamination of surfaces via the air to a frequently touched site by patients- the overbed table. This likely under-estimates the frequency of such an occurrence, as the settle plate represents only a small portion of the surface area of the overbed table. Surfaces frequently touched by HCW hands were observed to be contaminated with *S. aureus* (11/120; 9.2%). Contamination of the near-patient environment did not appear to have resulted from shedding by the bed occupant, as on all occasions when *S. aureus* was recovered from a settle or contact plate, the individual occupying the bedspace was not known to be colonised with *S. aureus*. This highlights the permeability in the segregation of patients within multi-bed rooms, and the potential for transmission of *S. aureus* indirectly via the airborne route. The detection of OS-MRSA isolates among the study collection was unexpected. These were recovered from environmental samples (six active air samples and one contact plate used to sample a nightstand). Irish guidelines for the control of MRSA in hospitals recommend the use of chromogenic agar for the culture of screening samples, which would not have identified these isolates as MRSA.

In the second part of this study, recovered *S. aureus* isolates were investigated by short-read WGS using the Illumina MiSeq (Illumina, the Netherlands) platform. Three isolates recovered in the first part of the study were omitted as these represented HCW cases in which both nasal and oral samples yielded *S. aureus*. In all cases, identical antimicrobial resistance patterns were revealed by antimicrobial susceptibility testing and one representative isolate was subjected to WGS. Therefore, WGS was undertaken on 62 *S. aureus* isolates. Isolates were assigned to 13 multilocus sequence types (STs) within 11 clonal complexes (CCs). The most frequently identified CC was CC1 (22/62, 35.5%), comprising ST1 (n=19) and ST109 (n=3) isolates. The remaining 40 isolates were assigned to CC8 (9/62, 14.5%), CC15 (7/62, 11.3%), CC5 (6/62, 9.7%), CC45 (6/62, 9.7%), CC97 (5/62, 8.1%;), CC22 (2/62, 3.2%), CC30 (2/62, 3.2%) and one each (1/62, 1.6%) to CC12, CC398 and CC672. Isolates were considered related if they exhibited ≤ 24 allelic differences following whole genome (wg) MLST analysis. Using this criterion, 10 related isolate groups involving 46 isolates were identified. Each of these groups involved one or more isolates recovered from an active air sample. Four groups consisted exclusively of isolates recovered from air samples, two comprised isolates from active air samples, settle plates and contact plate samples, one involved isolates from an active air sample and contact plate and one involved isolates from active air samples, settle plates and a HCW. *In-silico* genotyping for virulence and resistance genes identified a wild type *mecA* gene in all seven OS-MRSA isolates identified in the first part of the study, and one additional OS-MRSA isolate originally identified as an MSSA, cultured from a settle plate and which was phenotypically susceptible to both oxacillin and cefoxitin and did not grow on MRSA chromogenic agar.

All eight OS-MRSA isolates were assigned to ST8 and staphylococcal chromosome cassette *mec* (SCC*mec*) type V (5C2&5) and all were closely related by wgMLST, exhibiting 0-2 allelic differences. OS-MRSA have previously been associated with mutations in the *femXAB* operon, which encodes proteins critical for peptidoglycan synthesis in MRSA when native penicillin binding proteins are inhibited by antibiotic agents. Mutations in *femA* and *femX* were identified in the eight OS-MRSA isolates. When compared to an MRSA reference strain, these mutations resulted in two amino acid substitutions within FemA (Y195F and E234D) and three within FemX (N18H, 151V and E261K). Similar mutations have been reported previously in investigations of OS-MRSA isolates and are thought to inhibit synthesis of a pentaglycine cross-linking structure which is integral to cell wall function in *S. aureus* and may contribute to oxacillin susceptibility.

This study investigated environmental contamination with *S. aureus* in two multi-bed surgical wards in a large Dublin teaching hospital under routine clinical conditions. Whole-genome sequencing of isolates recovered revealed that the majority of recovered isolates exhibited ≤ 24 allelic differences from one or more others, so were considered related. A number of isolates were recovered that were not linked to others, potential reflecting spurious shedding by room occupants and the dynamic nature of *S. aureus* in multi-bed rooms. A cluster of OS-MRSA was revealed which was not detected using chromogenic MRSA selective agar - the method of MRSA detection used routinely in the study hospital and is advised in current Irish guidance for the prevention of MRSA. The finding of OS-MRSA warrants further investigation as this has implications both clinically and for surveillance purposes. Prevalence of OS-MRSA in Ireland is currently not known. Finally, the results of this study indicate that the air may play an underappreciated role in contaminating near-patient sites in multi-bed hospital rooms with pathogenic bacteria.

Table of Contents

Acknowledgments…………………………………………………………….. i
Index of Tables……………………………………………………………... ii
Index of Figures…………………………………………………………….. iv
Abbreviations……………………………………………………………….. v
Summary……………………………………………………………………. ix

Chapter 1- General Introduction………………………………………………… 1
1.1 *Staphylococcus aureus*…………………………………………………… 2
1.1.1 Human carriage of *S. aureus*……………………………………….. 3
1.1.2 Transmission and acquisition of *S. aureus*…………………………. 5
1.1.3 *Staphylococcus aureus* infection…………………………………… 5
1.1.4 Antimicrobial resistance in *S. aureus*……………………………… 8
1.1.5 Virulence determinants in *S. aureus*……………………………….. 11
1.1.6 Molecular epidemiology of *S. aureus*……………………………… 11
1.1.6.1 Molecular epidemiology of MRSA in Ireland…………………….... 12
1.2 Impact and management of *S. aureus* in nosocomial settings……….. 13
1.2.1 Reservoirs of *S. aureus* in hospitals………………………………... 16
1.2.1.1 Patients……………………………………………………………... 16
12.1.2 Healthcare workers………………………………………………… 17
1.2.1.3 Contaminated Surfaces…………………………………………….... 17
1.2.1.4 Indoor Air…………………………………………………………... 18
1.2.2 Strategies for the control of *S. aureus* and MRSA in hospital settings……………………………………………………………... 23
1.2.2.1 Screening…………………………………………………………… 23
1.2.2.2 Antimicrobial stewardship…………………………………………. 24
1.2.2.3 Surveillance………………………………………………………… 25
1.2.2.4 Environmental control measures…………………………………… 26
1.2.2.5 Indoor hospital air and ventilation systems………………………… 30
1.3 *Staphylococcus aureus* typing…………………………………………… 34
1.3.1 Bacterial typing…………………………………………………….. 34
1.3.2 Strategies for typing *S. aureus*……………………………………... 35
1.3.2.1 Pulsed field gel electrophoresis…………………………………….. 35
1.3.2.2 Multilocus sequence typing………………………………………… 36
1.3.2.3 *Spa* typing…………………………………………………………... 38
1.3.3.4 Staphylococcal cassette chromosome *mec* typing………………….. 39
1.3.3.5 Whole-genome sequencing………………………………………… 40
1.4 Aims…………………………………………………………………….. 43
Chapter 2- Materials and Methods……………………………………………. 45
2.1 Ethical approval…………………………………………………………. 46
2.2 Study setting……………………………………………………………… 46
2.3 Sampling routine………………………………………………………… 50
2.4 Study participants……………………………………………………….. 52
2.4.1 Healthcare worker recruitment……………………………………... 52

2.4.2	Patient recruitment………………………………………………	53
2.4.3	Study participant enrolment and consent………………………...	53
2.4.4	Participant sampling……………………………………………...	54
2.5	**Environmental sampling………………………………………**	**54**
2.6	**Storage and transport of samples prior to laboratory processing…….**	**55**
2.7	**Microbiological processing of samples…………………………….**	**55**
2.7.1	Initial processing of samples……………………………………..	55
2.7.2	Identification of *S. aureus* and MRSA isolates……………………..	56
2.7.3	Antimicrobial susceptibility testing………………………………	57
2.7.4	Confirmation of isolates as MRSA………………………………	58
2.8	Statistical analysis………………………………………………..	58
Chapter 3- An investigation of environmental contamination by *Staphylococcus aureus* in two differently ventilated multi-bed hospital wards..		**62**
3.1	**Introduction……………………………………………………**	**63**
3.2	**Materials and methods………………………………………………..**	**66**
3.2.1	Ethical approval……………………………………………………..	66
3.2.2	Study setting…………………………………………………...	67
3.2.3	Sampling routine…………………………………………………	67
3.2.4	Study participants………………………………………………...	67
3.2.5	Study participant sampling………………………………………	67
3.2.5.1	Swab sampling of anterior nares…………………………………	68
3.2.5.2	Oral rinse sampling………………………………………………	68
3.2.6	Environmental sampling…………………………………………	68
3.2.6.1	Active air sampling………………………………………………	68
3.2.6.2	Passive air sampling……………………………………………...	69
3.2.6.3	Environmental surface sampling…………………………………	70
3.2.7	Storage and transport of samples prior to laboratory processing…...	74
3.2.8	Initial processing of samples……………………………………..	74
3.2.9	Identification of *S. aureus* and MRSA isolates……………………..	75
3.2.10	Antimicrobial susceptibility testing……………………………...	75
3.2.11	Observation of room activity……………………………………..	75
3.2.12	Airborne particle quantification…………………………………	76
3.2.13	Data Analysis……………………………………………………	77
3.3	**Results…………………………………………………………..**	**77**
3.3.1	Prevalence of *S. aureus* among sampled HCWs……………………	78
3.3.2	Prevalence of *S. aureus* among sampled patients…………………...	78
3.3.3	Prevalence of *S. aureus* in environmental samples…………………	79
3.3.3.1	Active air sampling………………………………………………	80
3.3.3.2	Passive air sampling……………………………………………...	81
3.3.3.3	Surface sampling…………………………………………………	85
3.3.4	Characterisation of *S. aureus* isolates recovered……………………	85
3.3.4.1	Antimicrobial agent resistance…………………………………...	85
3.3.4.2	Confirmation of methicillin-resistance…………………………...	89
3.3.5	Surface hygiene assessment, adenosine triphosphate and *S.aureus*	

	recovery……	89
3.3.6	Observational data from study area……	92
3.3.7	Demographic data relating to sampled healthcare workers……	107
3.3.8	Demographic data relating to sampled patients……	107
3.4	**Discussion……**	**112**
3.5	**Conclusion……**	**122**
Chapter 4- An investigation of 62 *Staphylococcus aureus* isolates recovered by sampling patient, healthcare worker and environmental sites in two surgical wards in an Irish tertiary referral hospital under active clinical conditions…..		**124**
4.1	**Introduction……**	**125**
4.2	**Materials and methods……**	**128**
4.2.1	Bacterial isolates……	128
4.2.2	Isolate storage……	128
4.2.3	Genomic DNA extraction from confirmed *S. aureus* isolates……	131
4.2.4	Preparation of extracted genomic DNA for WGS……	132
4.2.4.1	Tagmentation reaction……	132
4.2.4.2	PCR amplification and post-PCR cleaning……	133
4.2.4.3	Library pooling, denaturation and preparation of PhiX control……	135
4.2.5	MiSeq whole-genome sequencing……	135
4.2.5.1	WGS data processing and contig assembly……	136
4.2.5.2	Quality assessment of WGS data……	136
4.2.6	Genotyping……	139
4.2.7	Molecular typing of sequenced isolates……	139
4.2.7.1	Whole-genome multilocus sequence typing……	139
4.2.7.2	*Spa* typing……	139
4.2.8	Analysis of isolate relatedness……	144
4.2.9	Comparison of genes or sequences……	144
4.3	**Results……**	**144**
4.3.1	Population structure of *S. aureus* isolates investigated……	145
4.3.2	Detection of genes encoding virulence factors and antimicrobial agent resistance……	154
4.3.3	Investigation of detected staphylococcal cassette chromosome elements……	164
4.3.4	Investigation of OS-MRSA isolates……	164
4.3.5	Isolate relatedness……	169
4.4	**Discussion……**	**181**
4.5	**Conclusion……**	**191**
Chapter 5. General Discussion……		**192**
5.1	**The potential of whole-genome sequencing to enhance hospital infection prevention and control……**	**193**
5.2	**Detection of OS-MRSA……**	**197**
5.3	**The air as a route of transmission of *S. aureus* in hospitals……**	**201**
5.4	**Concluding statement……**	**203**
References……		**205**
Appendix……		**247**

Chapter 1

General Introduction

1.1 ***Staphylococcus aureus***

Staphylococci are Gram-positive, non-motile, non-spore forming, salt-tolerant aerobic and facultative anaerobic bacteria within the family *Staphylococcaceae*. Microscopically, they are spherical in shape and appear in clusters. Staphylococci form part of the normal commensal flora of mammals and birds, and are found environmentally. Although staphylococci frequently colonise human and animal hosts asymptomatically across a range of species, a number of species are pathogens or opportunistic pathogens, with significant attributable mortality and morbidity in community and healthcare settings globally.

Staphylococci comprise over 40 species and these are conventionally classified into one of two groups: coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS) based on their ability to produce the enzyme *coagulase*, an extracellular protein that induces blood clot formation. The presence of coagulase induces the conversion of fibrinogen to fibrin in mammalian blood by interacting with prothrombin.

Within the genus *Staphylococcus*, *Staphylococcus aureus* is the most pathogenic species and most frequently responsible for infections in humans. *Staphylococcus aureus* is abundant in human and animal populations globally. The organism is characterised by its ready ability to acquire or develop resistance to clinically relevant antibiotics, especially in the hospital environment where antibiotic selective pressures can be high (Guo *et al.*, 2020). The development of multidrug resistance (MDR) can significantly limit available treatment options for clinical infections. Clinical features of *S. aureus* infection range from minor and readily treatable skin and soft tissue infections (SSTIs) to life threatening invasive disease such as bloodstream infections and necrotizing pneumonia. Bloodstream

infection and sepsis are grave complications and pose serious risks to human life. *Staphylococcus aureus* are among the most common nosocomial pathogens worldwide.

1.1.1 Human carriage of *S. aureus*

Cross-sectional population studies have estimated that approximately 30% of humans are colonised by *S. aureus* at a given time (Graham *et al.*, 2006; Gorwitz *et al.*, 2008; Gamblin *et al.*, 2013). Repeated population sampling and investigation of colonisation trends have revealed two distinct patterns of colonisation in humans: transient colonisation and persistent colonisation. Persistent colonisation typically involves colonisation with a single strain over a prolonged period of time, whereas in the case of intermittent colonisation, an individual is colonised over an extended period but inconsistently and with distinguishably different strains (Kluytmans *et al.*, 1997; Von Eiff *et al.*, 2001). A subsection of the population appear to be 'non-carriers', and these individuals are never or rarely colonised by *S. aureus*, despite close contact with individuals that are colonised with *S. aureus* and via contact with *S. aureus* shed into the environment from human and/or animal sources (Nouwen *et al.*, 2004)

The mucous membranes, predominantly within the squamous epithelium of the *vestibulim nasi*, or anterior nares, are the primary site of *S. aureus* colonisation in humans, although extra-nasal colonisation is common including the axillae and groin (Wertheim *et al.*, 2005). Nasal colonisation is associated strongly with subsequent clinical infection (Von Eiff *et al.*, 2001; Wertheim *et al.*, 2005; Sakr *et al.*, 2018).

Mechanisms underpinning the acquisition and establishment of *S. aureus* within the nares of intermittent or persistent carriers are not yet fully understood, but appear to be multifactorial and dependent upon alignment of bacterial and host characteristics. Weirtheim *et al.* (2005) outlined four sequential steps associated with the establishment of

a nasal *S. aureus* reservoir: (1) inoculation of the nares with *S. aureus*, (2) adherence to receptor cells within the nares, (3) evasion of host immune response, and (4) population growth and establishment via replication.

Despite the ubiquity of *S. aureus* in human populations, nasal epithelial cells are primed to reject invading bacteria, and *S. aureus* must first overcome several host defences and competing nasal flora prior to establishing colonisation. Host defences include lysozyme, lactoferrin, IgA and various antimicrobial peptides (Weirtheim, 2005). Established residential flora, such as *Streptococcus pneumoniae* or *Staphylococcus epidermidis* that have secured adhesion to the target receptors of *S. aureus* may confound efforts by *S. aureus* to successfully colonise new hosts. Interaction between bacteria competing for the same niche may be multifaceted, so whilst *S. pneumoniae* may occupy target molecules for *S. aureus*, it can also secrete products (such as hydrogen peroxide), which stress and inhibit *S. aureus* growth (Regev-Yochay *et al.*, 2006).

Adherence to local cells and the overcoming of such defences must happen concurrently, and this process is facilitated by a group of proteins expressed by *S. aureus*, including microbial surface components recognizing adhesive matrix molecules (collectively referred to as MSCRAMMs). Nasal colonisation is a multifactorial process, and involves numerous bacterial molecules interacting with several host targets (Corrigan *et al.*, 2009). It is known that *S. aureus* molecules bind to epithelial cells within the nares via proteins encoding for adhesion, such clumping factor B (*clfB*) and iron-regulated surface determinant (*IsdA*) (Vitry *et al.*, 2017). A key host determinant for *S. aureus* colonisation involves an interaction between *clfB* and a major component of the squamous cell envelope, namely the protein loricrin (Mulcahy *et al.*, 2012). The molecular dynamics underpinning this binding interaction are not completely understood, but it has been shown by Vitry and colleagues (2017) that adhesion is the result of a high affinity "dock, lock and latch" mechanism, and bonds are strengthened under increased tensile stress.

1.1.2 Transmission and acquisition of *S. aureus*

The transmission cycle of *S. aureus* (Fig. 1.1) begins by initial exposure to *S. aureus*, which may be from a colonised individual or contaminated environmental site. Firstly, a colonised individual sheds *S. aureus* into their immediate environment. This may be by making contact between their fingertips and nares and subsequently contaminating a surface, or by shedding droplets containing *S. aureus* from their respiratory tract. Certain individuals shed *S. aureus* more readily and in greater quantities than others, such as in cases of persistent carriage or with colonisation of multiple sites. Even at an individual level, shedding of *S. aureus* is variable due to factors such as respiratory illness, which has been shown to increase dispersal (Sherertz *et al.*, 1996).

Illness resulting from direct inhalation of airborne *S. aureus* has been reported previously (Solberg, 2000; Gehanno *et al.*, 2009), and airborne droplets may also settle on surfaces, resulting in contamination. *Staphylococcus aureus* in the environment must remain viable until contact is made with a potential host, who may subsequently traffic *S. aureus* to their own nares via hand-touching following contact with a contaminated surface, or less commonly via inhalation of droplets. Infection may also result from the inadvertent inoculation of usually sterile anatomical sites (European Centre for Disease Control, 2018b; Schreiber *et al.*, 2018). At this point, the acquisition process begins and is ultimately determined, as outlined above, by a series of host/pathogen interactions, which may result in the establishment of a *S. aureus* reservoir within the new human host. Colonisation with *S. aureus* has been shown as a predisposing factor toward the development of subsequent infection (Von Eiff *et al.*, 2001).

1.1.3 *Staphylococcus aureus* infection

In community settings, endogenous *S. aureus* infection is strongly associated with

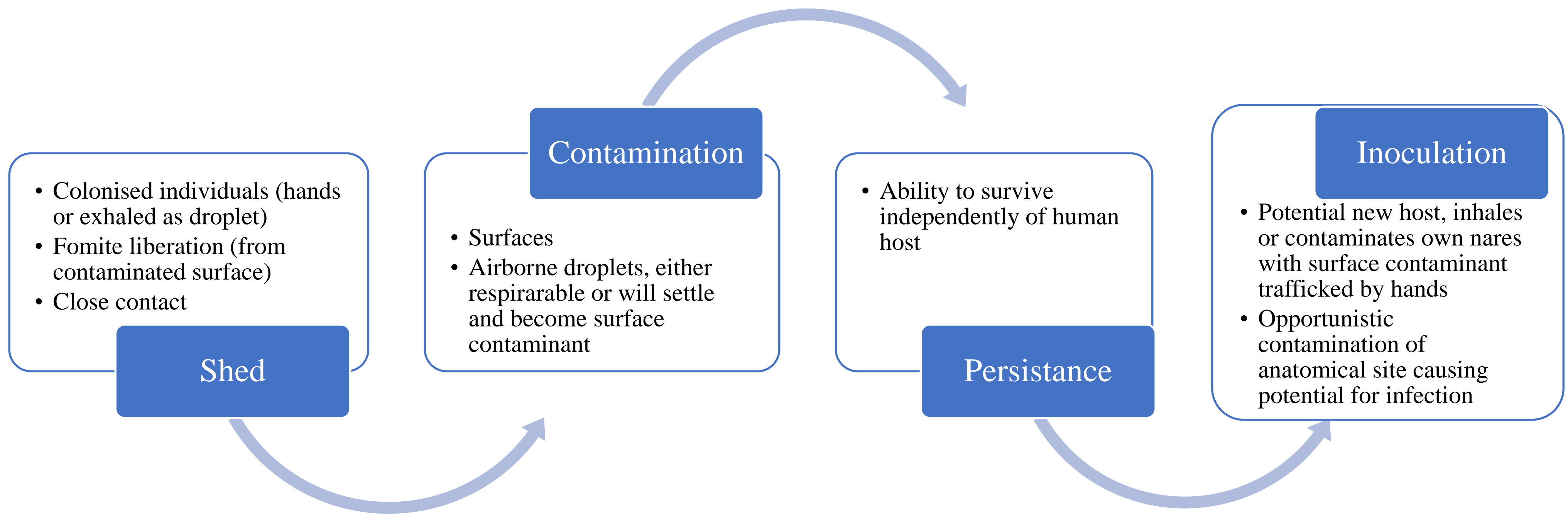


Figure 1.1: A process diagram outlining sequential elements of the cycle of *S. aureus* transmission. The cycle begins when *S. aureus* is shed by either a colonised individual or liberated from a contaminated fomite. Person-to-person transmission has been reported, such as between mothers and their infants. This generates bacterial contamination (either of surfaces or air). Provided these contaminants remain viable and evade threats, such as decontamination or dessication, contact may be made by a potential new host. This may be via inhation of dispersed droplets or by hand-touch contact. *Staphylococcus aureus* is trafficked to the potential host, where a complex and as yet incompletely understood set of processes will determine whether a *S. aureus* reservoir will be established within the host.

recurrent furunculosis (Demos *et al.*, 2012) and SSTIs in healthy populations (Chou *et al.*, 2015), and is the most frequent causative agent of diabetic foot ulcer infections (Dunyach-Remy *et al.*, 2016). In hospitalised patients, *S. aureus* is a predominant cause of infection, and was second only to enterobacterales as the most frequent causative microorganism of hospital-associated infection in a point prevalence study undertaken in European hospitals (European Centre for Disease Prevention and Control, 2013). In the same study, *S. aureus* was responsible for 12.3% of all infections, was the most prevalent cause of all surgical site infections (attributed to 17.9% of all reported cases) and among the most frequent cause of all reported pneumonia/lower respiratory tract infections (12.6% of all cases) and bloodstream infections (15.9% of cases).

In healthcare settings, both endogenous and exogenous *S. aureus* infection has been reported. Endogenous infection is thought to account for the majority of these illnesses, and can result as a consequence of medical care. An example would be the translocation of a patient's endogenous flora via the insertion of medical devices to a sterile site, such as a bloodstream infection arising from contamination of an intravascular device beneath the skin barrier (e.g. central venous catheter) (Dancer *et al.*, 2019). However, the hospital environment also facilitates *S. aureus* transmission, and has been implicated in *S. aureus* outbreaks in a range of clinical areas (Layton *et al.*, 1993; Shiomori *et al.*, 2001). Poor compliance with hygiene practices (including hand hygiene) has been shown to increase the risk of nosocomial infection (World Health Organization, 2009). It has been documented since the 1960s that poor hand hygiene by hospital staff is associated with *S. aureus* spread (Mortimer *et al.*, 1962), but hand hygiene practices remain substandard in many published studies, including in high risk areas such as critical care (Erasmus *et al.*, 2010; Musu *et al.*, 2017).

The success of *S. aureus* as a pathogen is partly due to its ubiquitous presence in human populations, but also in its ready ability to acquire and/or develop resistance to a wide

range of antibiotics classes used clinically (Chambers and Deleo, 2009). The emergence of multidrug resistance has limited available treatment options and in some cases has resulted in failure of widely used decolonisation therapies (e.g. plasmid encoded high-level mupirocin resistance) (Earls *et al.*, 2017).

1.1.4 Antimicrobial resistance in *S. aureus*

The ability of *S. aureus* to develop resistance to antibiotics, and the reality that this is not limited to one type or class of therapy, remains a global concern in the provision of healthcare (Guo *et al.*, 2020). Resistance to antimicrobial agents is mediated by a number of processes, both intrinsic and acquired (Hori and Hiramatsu, 1994; Khan *et al.*, 2014; Seifi and Khoshbakht, 2016).

Less than a century ago, in 1929, the incidental discovery by Alexander Fleming of penicillin revolutionised modern medicine. While working with staphylococci, Fleming observed lysis of bacterial colonies by a mould contaminant in Petri dishes that had been set aside on a windowsill while he was on leave. This mould was later identified as *Penicillin notatum,* and the active agent as benzylpenicillin (Penicillin G). The publication of this finding (Fleming, 1929) initiated work by other groups, and particularly at the University of Oxford, to isolate and further characterise this potential treatment agent. At the time, many bacterial infections were essentially untreatable, with case-fatality rates in *S. aureus* infections alone reportedly as high as 80% (Cocchi *et al.*, 2013).

Bacterial resistance to the action of penicillin was reported by Fleming alongside the penicillin discovery (1929). In 1940, prior to the introduction of penicillin clinically, *in-vitro* resistance to penicillin was reported (Abraham and Chain, 1940), alongside the observation that such resistance was likely mediated by an active substance, likely ‘enzymatic in nature’. Reports of clinically penicillin-resistant *S. aureus* infection emerged

in hospitalised patients soon after the introduction of penicillin to clinical practice in 1946 (Barber and Rozwadowska-Dowzenko, 1948). These penicillin-resistant *S. aureus* strains were embedded both in community and nosocomial settings by the early 1950s (Freeman *et al.*, 1955; Rountree and Freeman, 1955). The resistance to penicillin was mediated by penicillinase, which inhibited the antimicrobial effect of penicillin by compromising the agent's beta-lactam structure. The beta-lactam structure of penicillin had been confirmed by 1948, using X-ray crystallography (Hodgkin, 1949; Glusker, 1994). Mitigation efforts resulted in the production of semi-synthetic agents resistant to penicillinase, such as methicillin. Methicillin entered clinical practice in 1959 and methicillin-resistant *S. aureus* (MRSA) infections were reported just two years later (Jevons, 1961).

Antimicrobial resistance in *S. aureus* is now understood to occur in a number of ways: acquisition of resistance genes via horizontal transfer of mobile genetic elements (MGEs), from mutations which confound antimicrobial therapy ligands, and by upregulation of endogenous efflux pumps (Foster, 2017). Mobile genetic elements known to harbour *S. aureus* resistance genes include transposons and plasmids, in addition staphylococcal cassette chromosome *mec* (SCC*mec*) elements (Lindsay and Holden, 2006).

Methicillin-resistance in *S. aureus* results from the acquisition and integration of resistance genes, namely *mecA* (Matsuhashi *et al.*, 1986) and less often from *mecC*. Methicillin works to prevent cell wall synthesis in *S. aureus* through obstructing the action of penicillin-binding-proteins (PBP), which are innate to *S. aureus* (Ito *et al.*, 1999). In MRSA isolates that have acquired the *mecA* gene, production of a novel PBP (designated, PBP 2a) encoded by *mecA* means that cell wall synthesis is not inhibited (even if this action is dependent solely of PBP 2a, as other innate PBPs are susceptible) and therapeutic resistance is conferred (Matthews and Tomasz, 1990). In addition to *mecA*, a novel homologue, a *mecC* gene, has been detected in isolates that are phenotypically MRSA, but in which *mecA* was not detected (García-Álvarez *et al.*, 2011; Shore *et al.*, 2011a). The

resistance mechanisms encoded by both *mecA* and *mecC* are non-identical (Kim *et al.*, 2012).

Both *mecA* and *mecC* are encoded within SCC*mec* elements. These are MGEs that are inserted to the *S. aureus* chromosome at a specific site, which is designated *orfX* (Ito *et al.*, 1999). As per guidance provided by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC; 2009), SCC*mec* elements are designated to comprise: a cassette chromosome recombinase (*ccr*), a mec gene complex (*mec*) and joining regions (J), and may contain additional resistance or virulence determinants. The *mec* complex contains genes encoding methicillin resistance (*mecA* or *mecC*) and their regulators (such as *mecI*, *mecR*). The *ccr* complex is concerned with mobility and cassette dissemination (Rolo *et al.*, 2017). Rolo and colleagues (2017) sequenced the genomes of isolates including *Staphylococcus sciuri*, *Staphylococcus vitilinus* and *Staphylococcus fleuretti* to ascertain the ancestral heritage of SCC*mec*. They concluded that the probable origin of SCC*mec* was in *S. sciuri* by recombination, and that this was subsequently acquired by *S. aureus.*

Plasmids can harbour genetic sequences encoding resistance to a variety of antimicrobials, biocides and heavy metals (McCarthy and Lindsay, 2012), in addition to virulence traits (Malachowa and DeLeo, 2010). Transfer of staphylococcal plasmids between *S. aureus* cells is understood to occur predominantly by transduction or conjugation (Morikawa *et al.*, 2003). In terms of resistance within *S. aureus*, beta-lactam resistance (mediated by beta-lactamase) can be coded on plasmids (in the form of *bla* genes), in addition to within chromosomal DNA (Malachowa and DeLeo, 2010). The emergence of vancomycin-resistance in *S. aureus* isolates obtained from a cluster of human cases in Michigan, US (Zhu *et al.*, 2008) is understood to have resulted from the transfer of a conjugative plasmid containing *vanA*, from a vancomycin-resistant enterococcus (VRE) (Weigel *et al.*, 2003) during concurrent infections in the index patient.

This issue of antimicrobial resistance increased dramatically year on year since the 1950s, and MRSA particularly has remained a concern for both clinicians and the general public globally. A point prevalence study undertaken by the European Centre for Disease Control identified methicillin-resistance in 41% of all infective *S. aureus* isolates included, reflecting the scale of the issue of MRSA in contemporary clinical practice (European Centre for Disease Prevention and Control, 2013). The World Health Organization stated in 2020 that antimicrobial resistance represents a significant threat to human health and security, and serves as a major barrier to global developmental goals (World Health Organization, 2020).

1.1.5 Virulence determinants in *Staphylococcus aureus*

Staphylococcus aureus can express many virulence factors, including haemolysins, leukocidins, enterotoxins, exfoliative toxins, proteases and immune-modulatory factors (Oogai *et al.*, 2011) and these are typically encoded by MGEs (Malachowa and DeLeo, 2010). There is high-level regulation of the production of these factors during bacterial synthesis, and this is regulated by a number of processes, of which the *agr* system is considered the most integral (Cheung *et al.*, 2004). Isolates that exhibit high-level methicillin resistance often harbour large and therefore burdensome SCC*mec* elements, which incur a relative fitness cost in the form of reduced virulence (Rudkin *et al.*, 2012). Virulence factors in *S. aureus* concern both initial host colonisation (such as MSCRAMMS) in addition to those inducing pathologies.

1.1.6 Molecular epidemiology of *S. aureus*

Typing methods applied to *S. aureus* are discussed in detail in Section 1.3 below. Typing of MRSA isolates has been facilitated by the application of standardised nomenclature, for

example typing of the SCC*mec* element (IWG-SCC, 2009) in combination with multilocus sequencing typing (MLST) (Enright *et al.*, 2002). This has allowed comparison of isolates on an international and inter-laboratory level (Stefani *et al.*, 2012). Investigations of MRSA isolates has revealed a clonal population structure, reflecting a well conserved core genome, and has outlined that in *S. aureus*, intra-clonal variety arises predominantly from point mutation and not recombination (Feil *et al.*, 2003). Typing of MRSA specifically has revealed that MRSA have evolved several times in distinct ancestral lineages (Lakhundi and Zhang, 2018).

Certain countries and geographical regions tend to have dominant MRSA clones, whereas studies investigating methicillin-susceptible *S. aureus* (MSSA) have revealed a more diverse population structure, with less regional dominance by particular clones over an extended period (Grundmann *et al.*, 2014; Deasy *et al.*, 2019). Deasy *et al.* (2019) investigated Irish MSSA bloodstream infection (BSI) isolates recovered during the period 2006-2017, and found that although 24 MLST clonal complexes (CCs) were identified (which included 124 *spa* types), three of these clonal complexes represented almost 50% of the 252 isolates investigated.

1.1.6.1. Molecular epidemiology of MRSA in Ireland

The epidemiology of MRSA has been described as healthcare-associated (HA-MRSA), community-associated (CA-MRSA) or as associated with livestock, farming or animal husbandry (LA-MRSA), and this generally confers to classification of the lineage to which a specific isolate will have been assigned (Lakhundi and Zhang, 2018). However, in recent years, CA-MRSA have been associated with nosocomial outbreaks (Otter *et al.*, 2011). In some instances, CA-MRSA have become endemic in nosocomial settings, such as in the USA, where the CA-MRSA clone USA300 (MRSA-ST8-IV) has migrated from

community settings and has become endemic in nosocomial settings, displacing previously predominant HA-MRSA clones (Tenover and Goering, 2009; Otter and French, 2011). This has also been seen in India where a CA-MRSA ST772-MRSA-V clone (dubbed the 'Bengal Bay clone' due to its emergence and spread within countries in the region) displaced the previously predominant Indian HA-MRSA, ST239-MRSA-III clone (D'Souza *et al.*, 2010; Steinig *et al.*, 2019). Zoonotic transmission of LA-MRSA has been well described previously (Pirolo *et al.*, 2019). The traditional lineages of HA-MRSA, CA-MRSA and LA-MRSA can no longer be relied up to accurately infer epidemiology or to describe the population structure and temporal dynamics of MRSA.

The first report of an MRSA isolate recovered from an Irish hospital was in the early 1970s (Hone and Keane, 1974). Further reports in the following years reflected an increased MRSA prevalence, and MRSA has now been endemic in Irish hospitals for decades (Rossney *et al.*, 2006). The ST250-MRSA-I clone became predominant in the 1970s and was replaced in the 1980s by ST239-MRSA-III, which was in turn replaced by ST8-MRSA-II in the 1990s (Shore *et al.*, 2010). In the late 1990s ST22-MRSA-IV became predominant and replaced ST8-MRSA-II and has remained predominant since. ST22-MRSA-IV was responsible for 73.7% of MRSA BSIs in Ireland in 2018 (National MRSA Reference Laboratory, 2018).

1.2 Impact and management of *S. aureus* in nosocomial settings

As a pathogen, *S. aureus* impacts healthcare facilities globally, with significant effects both for individual patients who become infected and on a wider organisational scale, both in terms of efficiency and economics (Zhen *et al.*, 2020). In developed countries, it is estimated that there are between 80-190 cases of *S. aureus* BSI per 100,000 inhabitants per year (Laupland, 2013; Le Moing *et al.*, 2015) with a case fatality rate of up to 30% (van

Hal *et al.*, 2012). A point prevalence study of surgical site infections (SSI) undertaken by European Centres for Disease Control (ECDC) in 2016 identified *S. aureus* as the most commonly isolated pathogen in surgical site infections (attributed 17.9% of 7,431 SSIs) (European Centre for Disease Control, 2018b). In a point prevalence study of infections associated with intensive care unit (ICU), *S. aureus* were among the most frequently isolated microorganisms responsible for ICU-acquired pneumonia (17.8% of all infections and second only to *Pseudomonas aeruginosa*) and ICU-acquired BSI (8.7% of all isolates), and of the 2,025 isolates reported, 29.9% were methicillin-resistant (European Centre for Disease Control, 2018a). *Staphylococcus aureus* isolates reported in Irish hospitals are collated and the data published by the Health Protection Surveillance Centre (HPSC). Data relating to Irish *S. aureus* isolates reported between 2011-2018 obtained from the HPSC website are detailed in Table 1.1.

Infections caused by *S. aureus*, including MRSA have been endemic in many countries for decades. Policies to prevent nosocomial *S. aureus* transmission focus mainly on conventional infection prevention and control practices, including promotion of hand hygiene, environmental cleaning and oversight of infection rates or outbreaks by infection control teams. However, infection control and prevention remains a relatively young science, and ensuring adherence to best practice remains challenging. As mentioned previously, hand hygiene compliance remains sub-optimal despite evidence of its importance being demonstrated over 50 years ago (Mortimer *et al.*, 1962).

The inanimate hospital environment, for example, until relatively recently was thought to play a negligible role in pathogen transmission (Rhame, 1998), but has since been accepted as a driver or pathogen transfer between patients and HCWs (Boyce, 2007; Cohen *et al.*, 2017). Steady rates of *S. aureus* infection suggest that current control strategies remain imperfect, and further and ongoing work is required to minimise infection.

Table 1.1 Details of *S. aureus* bloodstream infection isolates reported to the Health Protection Surveillance Centre in Ireland from 2011-2018

Year	*S. aureus* isolates (n)	MRSA (n)	MSSA (n)
2011	1055	251	804
2012	1030	232	798
2013	1070	213	857
2014	1076	208	868
2015	1056	191	865
2016	1142	164	978
2017	1155	186	969
2018	1188	147	1041

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphlyococcus aureus*

1.2.1 Reservoirs of *S. aureus* in hospitals

Reservoirs of *S. aureus* in nosocomial settings are understood to comprise predominantly the human occupants of the hospital, i.e. patients and hospital workers, alongside environmental contamination (Dancer, 2008). The important role of the environment in facilitating *S. aureus* transmission has been recognized and accepted in recent decades, especially following reports of outbreaks or transmission events facilitated either by substandard environmental hygiene or environmental sources (Layton *et al.*, 1993; Cotterill *et al.*, 1996; Hardy *et al.*, 2006). Reservoirs (either environmental or human) likely reflect overall colonisation pressure. In hospital settings where MRSA is endemic, for example, this is reflected in higher HCW colonisation rates (Albrich and Harbarth, 2008). Additional *S. aureus* reservoirs may contribute to nosocomial infection. Based on cross-sectional population studies, hospital visitors (such as relatives of patients) could be colonised with *S. aureus* with prevalence rates of up to 30% (Graham *et al.*, 2006; Gamblin *et al.*, 2013). Hand hygiene practices to mitigate transmission to surfaces and patients among visitors are known to be poor, with a recently published study observing baseline compliance with recommended hand hygiene practices as low as 9.7% (El Marjiya Villarreal *et al.*, 2020). Personal equipment used by healthcare professionals may also harbour *S. aureus*. Mobile phones and computer keyboards, for example, may serve as insidious reservoirs of such bacteria. Indistinguishable *S. aureus* strains have been recovered from the hands and mobile phones of nursing staff (Katsuse Kanayama *et al.*, 2017).

1.2.1.1. Patients

In Ireland, current guidelines do not stipulate a requirement for routine screening for MSSA. so overall prevalence of MSSA carriage in hospitalised individuals is not known

(Department of Health- An Roinn Sláinte, 2013). However, consistent rates of infection and evidence from the published literature identify patients as comprising a significant nosocomial reservoir of *S. aureus*.

A study from this laboratory in 2020 undertaken in a large Dublin teaching hospital found that 30.4% of patients were colonised with MSSA and 6.4% with MRSA (margin of error ± 5%) (Kearney *et al.*, 2020), a lower carriage rate than has been observed in general populations (Graham *et al.*, 2006; Gamblin *et al.*, 2013). A review of rates of MRSA colonisation of patient in non-outbreak settings (including 31 observational studies) reported MRSA colonisation rates of 0.1-24% (Dulon *et al.*, 2011). A 2012 study undertaken in the hospital where the present work is set, and where the study by Kearney *et al.* (2020) was undertaken, reported MRSA carriage rates of 10% (Creamer *et al.*, 2012).

1.2.1.2 Healthcare workers

Albrich and Harbath (2008) undertook a review of MSSA carriage among HCWs that included 41 studies representing a total of 10,589 HCWs, and found an overall prevalence of *S. aureus* carriage of 23.7%. This figure was slightly lower than expected in healthy populations based on previous studies, which estimated a prevalence closer to 30% (Gamblin *et al*., 2013; Graham *et al*., 2006). In the hospital where the present study was undertaken, sampling of HCWs was undertaken on a voluntary basis between 2017-2019 and rates of nasal carriage of MSSA and MRSA of 31.5% and 3.7%, respectively, were observed (Kearney *et al.*, 2020).

1.2.1.3 Contaminated surfaces

Hospital wards and clinics provide many surfaces for microorganisms to reside on, and the risk of pathogen transmission is managed by regular cleaning and decontamination (Health Service Executive, 2006). Surfaces in hospitals are generally described as existing either within or external to the 'near-patient environment', and this is generally understood to comprise the hospital bed components and associated fabrics (such as bed linens, pillowcases), nightstand, over-bed table and privacy curtains. Other elements within the near-patient environment may include patient notes, which may be stored at the end of each bed, or medical equipment that must be adjacent to patients, such as infusion pumps or monitors. There are many reports of *S. aureus* (including MRSA) recovery from such sites (Cimolai, 2008; Dancer, 2008; Moore *et al.*, 2013; Tan *et al.*, 2013). However, MRSA has also been recovered from areas inaccessible to patients, such as staff-only bathrooms (Moore *et al.*, 2013).

A primary method of contamination of environmental sites is generally accepted to be by trafficking on hands, as these sites tend to correspond with sites frequently touched by the hands of HCWs and patients (Moore *et al.*, 2013). This has been supported by findings of *S. aureus* and MRSA contamination on the hands of HCWs when sampled (Tan *et al.*, 2013; Moore *et al.*, 2015; Shi *et al.*, 2015; Dancer *et al.*, 2019). Surface contamination also reflects particles deposited from the air, which may have been shed by a colonised or infected individual or have been liberated from a contaminated fomite(s) (Adams and Dancer, 2020), e.g. during bed-making (Shiomori *et al.*, 2002; Hansen *et al.*, 2010)

1.2.1.4 Indoor air

Air sampling in the hospital environment has yielded recovery of *S. aureus* (including MRSA) both in the presence and absence of colonised or infected patients (Dansby *et al.*, 2008), and from a variety of clinical settings. *Staphylococcus aureus* is not primarily

transmitted via the airborne route, such as occurs with other microbial pathogens including *Mycobacterium tuberculosis* and measles virus. *Staphylococcus aureus* liberated or shed into the air by colonised/infected individuals or from fomites has traditionally been shown to quickly settle on surfaces, remaining suspended in the air for only short periods. It is known that colonised individuals shed *S. aureus* to their surrounding environments at variable rates (Sherertz *et al.*, 1996) and that these bacteria will go on to settle on nearby surfaces, where they can survive for extended periods (Neely and Maley, 2000). This provides opportunity for hand contamination and trafficking to anatomical sites susceptible to colonisation or infection.

Many factors are thought to influence air contamination with *S. aureus*, and variables include the number of individuals present within a defined area, clinical activities being undertaken (Shiomori *et al.*, 2002; Roberts *et al.*, 2006), the clinical setting, infrastructure, cleaning standards and frequency (Dancer, 2008; Gizaw *et al.*, 2016) and the colonisation pressure, i.e. the number of colonised individuals occupying the space (Khojasteh *et al.*, 2007).

MRSA outbreaks linked to hospital air

MRSA outbreaks linked to hospital air have been reported in a neonatal intensive care unit (NICUs) (Hara *et al.*, 2016), in a burns unit (Dansby *et al.*, 2008) and in an orthopaedic ward (Kumari *et al.*, 1998). In these reports, air sampling was undertaken when usual infection prevention and control (IPC) measures failed.

Kumari and colleagues (1998) describe an outbreak of MRSA in a UK orthopaedic unit. The unit shared nursing staff at night with an adjacent rheumatology ward, so the outbreak management plan included screening HCWs employed on both wards. The outbreak control team identified acquisition of the outbreak strain (confirmed by pulsed field gel

electrophoresis typing (PFGE)) in patients that was not supported by epidemiological data, and this prompted environmental screening. A ventilation grille was subsequently found to be contaminated with *S. aureus*, and further investigations revealed that the ventilation system was switched off for a number of hours each day. This inadvertently led to air (contaminated with MRSA) entering the system, and being re-circulated when the system was switched back on. A similar event led to an outbreak when an electrical fault resulting in system downtime in a hospital ventilation system, and when the system was functional again contaminated dust was expelled to the patient area, resulting in an MRSA outbreak (Wagenvoort *et al.*, 1993).

Sub-optimal ventilation system design was implicated in an outbreak of MRSA in two single-bed rooms in a UK ICU (Cotterill *et al.*, 1996). Contaminated air from the ventilation exhaust removing air from one isolation room where a patient with MRSA was being cared for inadvertently entered the adjacent room. A number of patients admitted to the isolation room that the contaminated air was entering had previously acquired MRSA, and this was later found to be linked to the contaminated air. An investigation revealed an incomplete seal on the external window of the room, which was in close proximity to the ventilation exhaust outlet, and the outbreak ceased when this was repaired.

Dansby *et al.* (2008) reported on 'epidemic' transmission of MRSA in their US burns unit. Air sampling revealed dissemination of MRSA into the air when the dressings of MRSA colonised patients were being changed (Dansby *et al.*, 2008). Air samples yielded MRSA both within the patient room, and outside the door of the colonised patient room in the corridor. This was compounded by sub-optimal unit design, which was revealed by smoke plume investigation. This revealed that most rooms in the unit were subject to positive pressure, apart from two that were under negative pressure- potentially allowing contaminated air to enter rooms of non-colonised or infected patients. Rooms fitted with laminar airflow mechanisms were not functioning adequately, and it was found that the

ventilation system did not meet the minimum standard specification required for such rooms. The burns unit was renovated, and changes to the door closing mechanism prevented the migration of MRSA from the air of patient rooms to the corridor. The authors reported decreased MRSA infection rates since these changes were implemented. Two MRSA strains were identified by PFGE typing of isolates recovered from the burns unit and which had resulted in patient infections. Such isolates ceased to be recovered since the unit was renovated.

Hara *et al*. (2016) reported an MRSA outbreak in a NICU in Japan. Air sampling was undertaken adjacent to HCWs who were donning personal protective equipment (PPE) to observe contact precautions prior to caring for a neonate with an exfoliative skin condition (Netherton syndrome). Air samples were repeated when PPE was removed. Increased recovery of MRSA was observed in samples obtained when HCWs were removing their PPE, leading the investigators to suspect that airborne MRSA shedding from this neonate was facilitating the outbreak. This infant was being cared for in an open-plan NICU, and was moved to an isolation room when the air sampling results became known, at which point the outbreak ceased.

Transmission of MRSA between air and contaminated environmental surfaces

Transmission between environmental sites, HCWs and patients has been demonstrated by genotypic analysis of isolates recovered from settings including ICUs under non-outbreak conditions (Mirzaii *et al.*, 2015; Dancer *et al.*, 2019) and an otolaryngology/head and neck surgical ward (Shiomori *et al.*, 2001).

Dancer and colleagues (2019) used whole-genome sequencing (WGS) to investigate the relatedness of 140 *S. aureus* isolates recovered during 10 sampling days over a 10-month period in a Scottish ICU. Four such isolates were MSSA recovered by active air sampling

and three of these were implicated in transmission events; on two occasions MSSA recovered from air was linked to isolates recovered from the hands of HCWs, and on one occasion an MSSA isolate from the air was linked to an isolate obtained from a bedrail. The authors commented that the relatedness of these isolates likely reflects the role the air plays in influencing surface deposition, which has been suggested previously (Creamer *et al.*, 2014; Adams *et al.*, 2017).

A molecular investigation of isolates recovered from patients, HCWs and environmental sites (including air) in an ICU in Tehran used *spa* typing to analyze 37 *S. aureus* isolates, three of which had been recovered by active air sampling (Mirzaii *et al.*, 2015). The air was implicated in two transmission events (TE) when *spa* typing and antimicrobial resistance data was applied. The first TE (*spa* type t7689) involved five isolates of which one was recovered from the air, one from a refrigerator, one from a blanket and two from the surfaces of tables within the ICU.

Transmission of S. aureus *between patients and air*

Shiomori *et al.* (2001) investigated the role of air as a transmission pathway in an otolaryngology surgical unit. The unit housed a particularly vulnerable population at increased risk of nosocomial infection either due to the loss of host defences in the respiratory tract (such as by surgical laryngectomy) and/or the use of long-term packing of surgical sites with gauzes that could become contaminated with pathogens such as MRSA (Suh *et al.*, 1998). Sampling (including of the air) was undertaken of the rooms of three patients known to be colonised with MRSA, and transmission between patients, the air and inanimate surfaces was revealed. Clinical activities (such as bed-making) and increased occupant density were associated with MRSA recovery from the air.

Mirzaii and colleagues (2015) identified potential transmission events between environmental sites and a patient in an ICU. An air isolate had the same *spa* type (t7789) as an isolate recovered from the patients and an isolate recovered from the surface of a ventilator.

1.2.2 Strategies for control of *S. aureus* and MRSA in hospital settings

In Ireland, current guidance for the control of nosocomial *S. aureus* infections covers a number of areas, including: screening and decolonisation, infection prevention and control measures in acute hospitals, antimicrobial stewardship, prophylaxis and treatment of MRSA infections, surveillance and governance (Department of Health- An Roinn Sláinte, 2013). This guidance provided by the Irish Department of Health, states that despite the focus of the document being on MRSA, there is overlap with MSSA due to their 'similar characteristics and mechanisms of spread'. Thus, despite no standalone guidance provided for MSSA, MRSA prevention guidance provides overlapping strategies for both MRSA and MSSA in many instances (Department of Health- An Roinn Sláinte, 2013).

1.2.2.1 Screening

Active screening and decolonisation of certain groups of patients for MRSA is advised both in Ireland and the UK (Department of Health- An Roinn Sláinte, 2013; Department of Health Expert Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection (ARHAI), 2014). In Ireland, current guidelines recommend targeted MRSA screening of patients who have a known history of MRSA, have had recent inpatient hospital stays or are being transferred from another healthcare setting, patients with non-intact skin, patients admitted for or who are undergoing high-risk procedures (orthopaedic implant surgery, insertion of indwelling cardiovascular device, renal dialysis)

or are admitted to high-risk clinical areas (such as critical care units) (Department of Health- An Roinn Sláinte, 2013). Additional screening is recommended during outbreaks, following decolonisation (to determine whether this has been successful) or based on local risk assessment. Routine occupational screening of HCWs for MRSA carriage is not currently advised in Irish hospitals, except where an epidemiological link has been established between a HCW and a cluster of infections, in institutions without endemic MRSA, or in high-risk units based on risk-assessment by local infection prevention and control teams (Department of Health- An Roinn Sláinte, 2013).

Screening and decolonisation for MSSA is not currently recommended for patients or HCWs in Irish hospitals (Department of Health- An Roinn Sláinte, 2013). In recent years, screening and decolonisation of MSSA-positive patients undergoing elective orthopaedic surgeries has been investigated in research studies, with outcomes demonstrating a reduction in post-operative infection and associated healthcare costs (Dancer *et al.*, 2016; Higgins *et al.*, 2018; Tsang *et al.*, 2018) outlining potential benefits, although this is not yet reflected in national guidance or widespread clinical practice.

1.2.2.2. Antimicrobial stewardship

Exposure to antibiotics, although often absolutely necessary in cases of bacterial infection, poses risks to patients. Systematic antibiotic treatment results in disruptions to mammalian gut microbiota, predisposing hospitalised patients to antibiotic-related diarrhoea and potential *Clostridium difficile* infection (Francino, 2016). The principles of antimicrobial stewardship work to maximise the antibiotic treatment where clinically indicated, whilst avoiding inappropriate prescriptions of treatments.

Antibiotic stewardship is a horizontal control intervention, designed to prevent infections caused by many bacterial species, not solely *S. aureus*. The role that antibiotic

consumption plays in the acquisition of nosocomial infection is intersectional, i.e. treatment of an infection caused by a non-staphylococcal bacterium could paradoxically result in a *S. aureus* infection due to the exposure to antibiotic agents. A systematic review and meta-analysis that included 76 studies demonstrated a significant association in the development of an MRSA infection in patients who had received an antimicrobial agent (Tacconelli *et al.*, 2007). Patients who had received treatment with an antimicrobial agent were 1.8 times more likely to develop a laboratory-confirmed MRSA infection than patients who had not received an antibiotic, and of antibiotic classes, quinolones were most influential.

1.2.2.3 Surveillance

Surveillance of invasive *S. aureus* infection in Ireland is undertaken and reported as an element of the European Antimicrobial Resistance Surveillance Network (EARS-Net). This surveillance system was initially established in 1998, and is now co-ordinated by the ECDC, an independent agency of the European Union that was established in 2004 with headquarters located in Solna, Sweden. EARS-Net collates information on the antibiotic susceptibility of pathogens causing illness in humans from isolates recovered exclusively from blood or cerebrospinal fluid. In addition to *S. aureus*, EARS-Net undertakes surveillance on *Escherichia coli, Klebsiella pneumoniae, P. aeruginosa, Acinetobacter species, Streptococcus pneumoniae, Enterococcus faecalis* and *Enterococcus faecium*. Based on the numbers of Irish laboratories reporting results to EARS-Net, the sample representativeness for the population of Ireland is currently deemed to be 100%, i.e. all geographic areas are represented and data is reflective of epidemiology at a national level (European Centre for Disease Prevention and Control, 2019). Surveillance data from Ireland is collected via the Health Protection Surveillance Centre (HPSC), an agency of the

Health Service Executive, based in Dublin. MRSA-specific laboratory support is provided by the National MRSA Reference Laboratory (NMRSARL), based at St. James's Hospital, Dublin.

Annual reports of Irish surveillance data are published by the HPSC, with the latest report published in November 2019 detailing data up to the end of quarter 4, 2018 (HSE Health Protection Surveillance Centre, 2019). In 2018, 1,188 *S. aureus* isolates were reported, and 12.4% of these were MRSA. When EARS-Net was established in 1994, Ireland reported 1,323 *S. aureus* infections, of which 41.8% were MRSA. Numbers of reported MRSA BSIs have reduced steadily in intervening years, but rates of *S. aureus* invasive infection remain above 1,000 annually (Fig. 1.2). As per EARS-Net data, patients from whom MRSA was recovered were more commonly male (76%), and older than those from whom MSSA was recovered (median age for MRSA was 72.4 years compared to 62.6 years for MSSA). The majority of *S. aureus* infections were recovered > 48 hours after admission to hospital (59% for MRSA and 66% for MSSA). Fewer isolates were recovered from patients who had been in hospital for more than 5 days (30% for MRSA and 21% for MSSA). This suggests that a significant number of these infections were nosocomial in nature.

EARS-Net report a populated weighed mean of 16.4% of isolates exhibiting methicillin-resistance in 2018, with Ireland below this at 12.4%. Differences in MRSA prevalence rates between countries reporting to EARS-Net remain, with weighted mean of methicillin-resistance ranging 0-43%.

1.2.2.4 Environmental control measures

Environmental control measures for *S. aureus* involve both guidance for the built environment itself, and for the maintenance of effective cleaning of surfaces and

equipment in clinical areas. Each of these elements is said to be integral to the control of nosocomial *S. aureus* (Coia *et al.*, 2006; Department of Health- An Roinn Sláinte, 2013).

In Ireland, guidelines stipulate that hospitals should be built such that overcrowding can be avoided (Department of Health- An Roinn Sláinte, 2013). In reality, Irish hospitals consistently operate near to full capacity: Ireland reported the highest acute bed occupancy rates (94.7%) of all 37 member states of the Organisation for Economic Co-operation and Development in 2015 (Organisation for Economic Co-operation and Development, 2019). Stochastic modelling suggests that for optimal acute hospital functioning, occupancy rates should be maintained around 85% (Bagust *et al.*, 1999). Elevated occupancy rates on inpatient wards creates a bottleneck which hinders admissions from the Emergency Department (ED), resulting in overcrowding and acts as a barrier for patients who need access to emergency care. This issue is well documented in the Irish healthcare system, and an Emergency Department Taskforce was convened in 2014 to tackle ED overcrowding and the downstream impacts for patients (Health Service Executive, 2015). Overcrowding in emergency departments (as a result of high occupancy) can result in patients being admitted to 'additional capacity beds' on wards to facilitate patient flow through the system (McGowan *et al.*, 2018). This involves an additional bed being added to a multi-bed room, for example, a room designed to contain six bedspaces will have a seventh bed added. It has previously been shown that the addition of one bed to a hospital ward can increase the risk of MRSA transmission between patients (Kibbler *et al.*, 1998).

Hospital building infrastructure in Irish hospitals poses an additional challenge. Guidelines recommend that each hospital bed should be surrounded by a minimum floor space of 19 m^2, and that no more than three beds should be admitted to one multi-bed room (Strategy for the control of Antimicrobial Resistance in Ireland (SARI), 2008). An audit of Irish hospitals in 2007 revealed that of 49 acute hospitals included, 83% had 2.9 or more metres

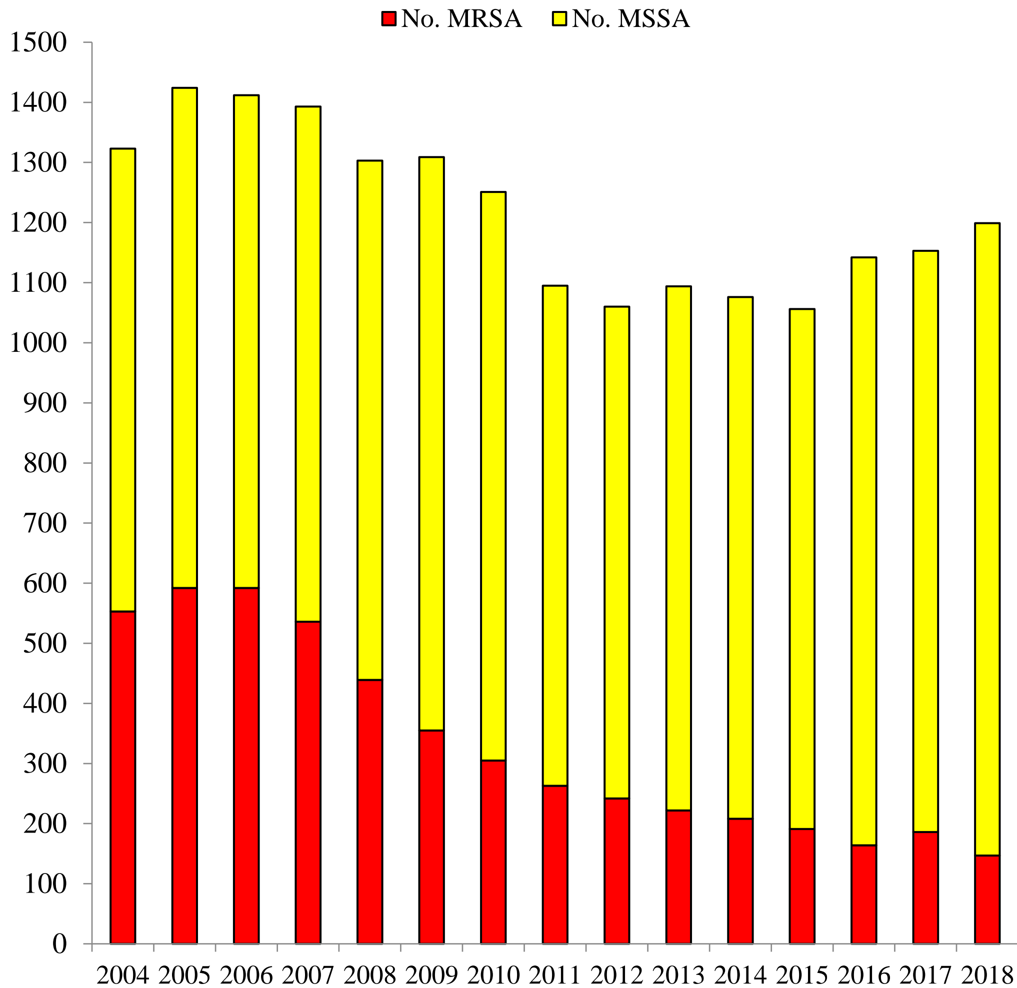


Figure 1.2 A stacked column chart representing *S. aureus* bloodstream infection isolates recovered in Ireland between 2004-2018, as reported to the European Antimicrobial Resistance Surveillance Network (EARS-Net). In Ireland, sufficient hospital laboratories submit data to to EARS-Net via the Health Protection Surveillance Centre to capture epidemioligical data for 100% of the population of the Republic of Ireland. Since the establishment of the EARS-Net reporting network in 2004, numbers of invasive *S. aureus* isolates recovered from Irish patients has remained relatively steady, ranging from 1,056-1424 per year. The prevelance of MRSA in Ireland has decreased during this time; rates of methicillin resistance among these isolates has decreased from a the highest prevalence of 41.9% in 2006 to the lowest yet reported levels of 12.3% in 2018)

between adjacent hospital beds (Strategy for the Control of Antimicrobial Resistance in Ireland Committee, 2007), which does not comply with the spatial guidelines outlined by SARI (2008). Guidance states that new builds or renovations of existing acute hospitals should provide no less than $19m^2$/bedpspace. Current adherence to this guideline is not known. Over a decade has passed since the audit by SARI, in which the overall number of acute hospital beds has reduced (An Roinn Slainte- Irish Department of Health, 2019). However, this reduction is attributed to a reduction in public expenditure in the healthcare system due to a financial crisis in the late 2000s (Keegan *et al.*, 2019), rather than as a result of allocating more space to each patient bed.

Maintenance of building components by technical services engineers is vital. Environmental reservoirs of MRSA that are beyond the scope of regular hospital cleaning operatives have been implicated in MRSA outbreaks, such as contaminated ventilation pipework (Cotterill *et al.*, 1996; Kumari *et al.*, 1998). Facilities management professionals, such as plumbers or technical services professionals, are tasked with such maintenance. Despite their vital role in preventing infections in hospitals, these groups self-report inadequate knowledge of IPC and a lack of training with regard to their role in preventing nosocomial infection (Liyanage and Egbu, 2005).

Hospital cleaning of surfaces in clinical and non-clinical areas is a fundamental control measure to mitigate *S. aureus* transmission. It is recommended that hospitals should be clean, uncluttered and free of dust, with all surfaces intact (National Hospitals Office, 2006; Department of Health- An Roinn Sláinte, 2013). Cleaning of patient areas is recommended at least daily. Use of detergent and warm water is recommended, with the addition of a disinfectant agent when performing a terminal clean on a room that previously housed an MRSA-positive patient or during an outbreak (Department of Health- An Roinn Sláinte, 2013), with particular attention paid to sites that are frequently touched (Health Service Executive, 2006).

1.2.2.5 Indoor hospital air and ventilation systems

Control of indoor air is undertaken both to maximise comfort and control temperature and humidity, in addition to infection prevention. Certain groups of HCWs, such as those working with biological hazards in clinical laboratories, are exposed to occupational hazards through air, so certain control measures, such as local exhaust ventilation may be advised to ensure occupational safety (Health and Safety Authority, 2014). There is a wide range of approaches for the control of indoor air quality, ranging from natural ventilation to technologically advanced systems that provide close to sterile air to specialised areas (Table 1.2).

Transmission of *S. aureus* is generally considered to occur via contact or droplet transmission, so the widespread use of mechanical ventilation systems is not currently advised in Ireland (Department of Health- An Roinn Sláinte, 2013). Specific guidance for ventilation and airflow management requirements pertaining to *S. aureus* is provided only in relation to critical areas, such as operating theatres, where air and ventilation-related interventions are undertaken to minimise the risk of surgical site infection caused by a patients own flora or from contamination by skin squames shed by operating theatre HCWs (Allegranzi *et al.*, 2016).

In addition to targeting intrinsic patient factors, the environment of operating theatres is tightly controlled, and ventilation involves at least a predetermined number of air changes per hour. Current Irish guidance recommends that where a patient known to be colonised or infected with MRSA is undergoing a surgical procedure, the ventilation system should allow for at least 20 air changes per hour (Department of Health- An Roinn Sláinte, 2013). Air filtering and temperature control should be maintained. Ventilation systems for operating theatres may also include laminar airflow or 'ultraclean' air, which aims to provide highly filtered air only to the theatre area, and is usually indicated only for prosthetic joint surgery. Evidence has emerged in recent years that the use of laminar flow

Table 1.2 Approaches used to ventilate hospital buildings[1]

Type of ventilation	Examples of use in hospitals	Principle
Natural ventilation	Widespread, used on most general patient wards	• Governed by thermo-convective effect of temperature differential between air within buildings and outside, and/or by outdoor wind speeds • Air enters building either through open windows or doors, or through ventilation openings in the building, such as vents • Air quality and temperature is difficult to control and highly variable, dependent on external conditions (e.g. weather, pollution, time of year)
Extract ventilation system	Used in bathrooms or dirty utility/sluice rooms	• Extraction of air which may be 'unpleasant' (i.e. odourous) but not toxic or dangerous to be expelled from the building • Generally consists of a single electric fan • System is generally intermittent and determined by user need, e.g. fan is activated by switching on light in bathroom
Supply-only ventilation	Units housing immunocompromised patients, instrument preparation rooms in operating theatres	• Supplied to areas where uncontrolled air entry may be detrimental either to patients within the room, or may result in contamination of high-risk objects (such as in instrument preparation areas for operating theatres) • Air is not considered harmful to others having entered the room it is intended for, so removal of air within the room may not be managed • Air can leave room via natural pressure differential (i.e. is pushed out of room by the addition of more controlled air by the ventilation system)
Supply and extract ventilation	Certain isolation units, operating theatres	• Air is controlled both in its supply and extraction • Controlled and minimally contaminated air is required for this area, and once the air has entered the area it is now considered contaminated, so cannot enter other parts of the building

Continued overleaf

Type of ventilation	Examples of use in hospitals	• Principle
Air conditioning	Areas with equipment or medication sensitive to climate conditions, critical care units, some operating theatres	• Air conditioning allows control of the climate within the provided space • Used either for comfort, or if a certain temperature range must be maintained (e.g. in pharmacy departments where medications must be stored within specific temperate parameters) • Encompasses control of temperature (air can be heated or cooled), humidity (can be increased or decreased) and some filtration • Indoor climate controlled independently of outdoor conditions • Climate can be adjusted either centrally (such as by hospital technical services department) or locally by user (if control panel is installed)
Local exhaust ventilation	Laboratory, areas where toxic gases may be released	• Where potentially harmful occupational exposure to chemicals, fumes, biological materials that require containment or gases may occur, local area ventilation must be provided. • Usually HCWs at risk of this, and certain occupational activities require local extract ventilation (e.g. Health and Safety Authority, 2014)
Ultra-clean ventilation systems	Certain operating theatres, i.e. orthopedic, asceptic pharmacy preparation areas	• Air is continuously and significantly diluted by the addition of highly filtered 'ultra-clean' air • Air enters operating theatre or pharmacy above critical site (i.e. incision or sterile pharmaceutical preparation area), passing over the this area when least contaminated, flowing down and outwards before extraction near floor level • Ultra-clean ventilation classified as air which typically harbours less than 10 CFU/m^3

Continued overleaf

Type of ventilation	Examples of use in hospitals	• Principle
HEPA-filtration	Either as part of exhaust ventilation in rooms housing infectious patients (e.g. tuberculosis patients) or as a protective measure for immunocompromised patients (e.g. bone marrow transplant units)	• A type of filter which can be part of a HVAC system (such as in Ward A in the present study) that is 99.97% efficient to ensure particles with a diameter ≤ 0.3 μm do not pass through system* • Used to filter out particles that may cause infections in immunocompromised patients, and to prevent egress of contaminated vented air from the rooms of patients with airborne diseases • Aerosolised *S. aureus* generated in a laboratory setting has been shown to have aerodynamic particle sizes of 0.723-0.777 μm, so should not pass through such a filter

Abbreviations: CFU, colony forming unit; HEPA-filter, high-efficiency particulate air filter; HVAC, heating ventilation and air conditioning.

[1]Adapted from NHS guidance for the ventilation of hospitals: Health Technical Memorandum (HTM) 03-01.

may increase rates of surgical site infection (Tayton *et al.*, 2016), with the latest guidelines for the prevention of surgical site infection advising against the use of lamniar flow in joint arthroplasty (World Health Organization, 2018). Conventionally ventilated theatres are recommended for the care of patients colonised with *S. aureus* under current Irish guidance, and sufficient air exchange reduces risk of transmission between patients who are operated on subsequently (Department of Health- An Roinn Sláinte, 2013). References to air in current Irish guidance for the control of *S. aureus* infection relate mainly to colonised HCWs (Department of Health- An Roinn Sláinte, 2013). Colonised HCWs may unknowingly shed *S. aureus* into the air, especially if they have an upper respiratory tract infection, which has been shown to increase dispersal (Sherertz *et al.*, 1996). The guidelines acknowledge evidence that wearing a surgical mask may decrease risk of dispersal, citing Sherertz *et al.* (1996a), but do not recommend this practice. There are currently no guidelines advising a specific type of ventilation system or metric of air quality for multi-bed patient rooms in Irish hospitals.

1.3 *Staphylococcus aureus* typing

1.3.1 Bacterial typing

Bacterial typing methods may be broadly characterised into approaches for investigating isolates either phenotypically or genotypically (Li *et al.*, 2009). Phenotypic typing is based on expressed traits elicited under laboratory conditions, such as resistance to antimicrobial agents, bacteriophage typing or the ability to ferment glucose. Genotypic typing involves the extraction and interrogation of genomic DNA, and may be band-based (i.e. pulsed field electrophoresis, or PFGE) or sequence-based (i.e. multi-locus sequence typing). Bacterial typing is undertaken for a number of reasons, including to provide clinical insights, to track pathogenic strains for surveillance purposes, to monitor environmental

contamination, to assess population structure, for forensic analysis and to monitor evolutionary traits in species or strains of interest (van Belkum *et al.*, 2007b).

The typing method undertaken is generally dependent on the reason for which a microorganism is being investigated, and each method has features supporting their choice of use. In general, the 'ideal' typing method has been described as one which: generates objective data that is relatively straightforward to process and store, is reproducible and portable at user and institutional level, adheres to a globally recognised and standardised nomenclature for output data, is subject to rigorous quality control assessment and is applicable to all isolates- encompassing many types or microorganisms where possible (van Belkum *et al.*, 2007a; Stefani *et al.*, 2012). Additional considerations include turnaround time, cost and demand of user (i.e. how much training and expertise is required) (Li *et al.*, 2009). Li *et al.* (2009) identify discriminatory power as among the most important criteria in selection of typing methods.

1.3.2 Strategies for typing *S. aureus*

1.3.2.1. Pulsed field gel electrophoresis

Typing of *S. aureus* once relied upon and was limited to the analysis of expressed phenotypic traits, but this has been superseded in recent decades by molecular or genotypic typing. These DNA-based techniques provide greater epidemiological insights than conventional phenotyping, as well as informing transmission dynamics and in-depth population structure with greater clarity (D. A. Williamson *et al.*, 2015). In the 1970s, the science of molecular epidemiology was established, and a number of techniques to analyse, compare and interrogate the genetic composition of *S. aureus* began to emerge.

The use of agarose gel electrophoresis to identify and visually display intra-cellular DNA was an important milestone in the shift from phenotypic to genotypic typing (Meyers *et al.*, 1976), and was the foundation for the method that would become the 'gold standard' method for *S. aureus* typing for decades, namely pulsed-field gel electrophoresis (PFGE). A number of methodological approaches to PFGE have been described, but the most common approach in widespread use is contour-clamped homogenous electric field electrophoresis, or CHEF (Chu *et al.*, 1986; Goering, 2010). This approach involves the cleaving of chromosomal DNA using restriction endonucleases that cleave DNA infrequently and separation of resulting fragments by agarose gel electrophoresis aided by a hexagonal arrangement of 24 discrete electrodes, which facilitates the development of a uniform electrophoretic gradient (Goering, 2010). The fragment patterns of separate isolates formed by this PFGE gradient can be compared using bespoke software packages, providing high-level discrimination between isolates (Tenover *et al.*, 1995) Despite the advantages associated with this method, PFGE never achieved full portability of results, remained laborious with a relatively long turnaround time and could not reliably classify all *S. aureus* lineages (Cookson *et al.*, 1996; Stefani *et al.*, 2012). These challenges underpinned the need for a standardised, inter-institutional approach to reporting typing results, such as that offered by sequence-based, rather than band-based approaches.

1.3.2.2 Multilocus sequence typing

A multilocus sequence typing (MLST) scheme for characterising *S. aureus* isolates was first published in 2000 (Enright *et al.*, 2000), but MLST has been used to characterise other bacteria, alongside certain yeasts and fungi prior to its application to the typing of *S. aureus*. Internal fragments (with lengths ranging from 400-500 bp) of seven highly conserved and unlinked housekeeping genes are amplified using polymerase chain reaction

technology and the amplimers sequenced. These housekeeping genes are: carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glp*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase (*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*). The sequencing of these genes generates a profile for each isolate based on the locus sequence of the housekeeping gene contained within the fragment. Isolates are then characterized based on this profile, and organised by sequence type (ST). A database of *S. aureus* sequence types, or MLSTs, is curated at https://pubmlst.org/saureus/ by the University of Oxford, and currently holds 619,325 sequences and 6,185 MLST profiles.

MLST provided a number of novel insights into *S. aureus* following widespread adaption by surveillance laboratories in the 2000s. The global population structure of *S. aureus* was characterised and revealed as being highly clonal. Lineages could be classified by geographic origin and by type of acquisition (i.e. community-associated, hospital-associated) (David and Daum, 2010). Evolutionary characteristics were informed using MLST output data (Feil *et al.*, 2004), by grouping isolates based on similarity of sequence types into groups, referred to as clonal complexes (CC). Within these complexes, algorithms identified the progenitor of the CC, and other isolates assigned to the CC were shown to have descended from this ST, differing at one locus (a single-locus variant), two loci (double-loci variant) or three loci (triple loci variant) (D. A. Williamson *et al.*, 2015), with these differences occurring either by recombination and/or point mutation.

Despite these crucial advances in understanding of global epidemiology of *S. aureus*, a number of limitations remained with MLST technology, particularly its lack of usefulness in investigating outbreaks of isolates of the same ST, which could not be further classified at a more granular level, and emerging clones could not be reliably identified due to subtle differences within STs. MLST represents only a small portion of the highly-conserved genes with the core genome (Maiden *et al.*, 2013), and was considered costly with a

relatively low throughput in comparison to other emerging *S. aureus* typing methods (Stefani *et al.*, 2012).

1.3.2.3 Spa typing

In the 1990s, sequencing of a single locus, namely the polymorphic X (or short sequence repeat) region of the *S. aureus* protein A gene (*spa*), emerged as a potential adjunct to other typing methods. It was postulated that *spa* typing would offer greater discrimination between local isolates, which was limited based on typing schemes and methods available at that time (such as PFGE or MLST), especially for applications such as outbreak investigations (Frénay *et al.*, 1996). The polymorphic X region was known to comprise 21-27 bp of variable number tandem repeats, and these varied between isolates based on point mutations, duplication or deletions (Shopsin *et al.*, 1999). The regions surrounding this target were well conserved, so amenable to primer amplification and sequence-based analysis. The genetic sequence identified is assigned to a *spa* type, and this is defined by the order of specific repeats (Strommenger *et al.*, 2006). Nomenclature is in the form of an alpha-numerical code (e.g. t127). Software is used to assign *spa* types, i.e. Ridom Spa server, available at http://spa.ridom.de. (Ridom GmbH, Würzburg, Germany). This database contains sequence data curated by SeqNet.org, and at the time of writing contains 19,496 *spa* types obtained from 432,337 strains from 143 countries.

Relatively low costs and short turnaround time positioned *spa* typing as a key typing strategy for *S. aureus*. From a processing perspective, *spa* typing was less technically challenging than PFGE, but was not more discriminatory (Tang *et al.*, 2000). The development of a standardised nomenclature meant that output data was portable and analysis transcended single institutions or countries (Stefani *et al.*, 2012). Advances in *spa* typing technology ameliorated its usefulness outside of outbreak studies. Application of

the algorithms that predicted the CC and ST based on *spa* typing meant that *spa* typing could be applied for epidemiological purposes, including longitudinal studies (Harmsen *et al.*, 2003). Limitations of this technology included misclassifications of certain lineages due to the focusing only on one genetic target (Sabat *et al.*, 2013) and limited insights into the ancestry of isolates with MLST. Poor discrimination between isolates meant that putative outbreaks suggested by *spa* typing results required further confirmatory typing, such as by PFGE- a more discriminatory method, negating the usefulness of *spa* typing as a standalone measure for *S. aureus* typing.

1.3.3.4 Staphylococcal cassette chromosome mec typing

Resistance to methicillin was first identified as a genotypic feature of MRSA in the 1970s (Sjöström *et al.*, 1975), and this was shown to be driven by a specific gene, *mec*, which is generally not found in MSSA isolates (Ito *et al.*, 1999). It is known that *mec* is contained within a MGE, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) element, which is 21-67 kb in size. Specific genes are contained with SCC*mec*, and typing schemes have been developed based on the combination of two fundamental parts: a cassette chromosome recombinase gene complex (*ccr*) which is concerned with mobility and integration of SCC*mec* to the *S. aureus* chromosome at the site of the *orfX* gene (Katayama *et al.*, 2000; Deurenberg and Stobberingh, 2008), and *mec* (encoding methicillin resistance via facilitating the production of PBP 2a). These are variable between isolates and are thus used for assigning types and facilitate inter-isolate discrimination. The combination of *mec* gene and *ccr* allotype determines the SCC*mec* type of an isolate, and further clarity and sub-typing is achieved by reporting the variation observed in the joining regions (Yoon *et al.*, 2019). This nomenclature was provided in 2009 by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) based

on multiplex PCR protocols (available at http://www.sccmeg/org). A database for identifying SCC*mec* types *in-silico* based on WGS data has been proposed (Kaya *et al.*, 2018). This tool, known as SCC*mec*Finder, is curated by a group at the Technical University of Denmark and is available at http://genomicepidemiology.org.

Despite the high-level discrimination provided by SCC*mec*, certain limitations have restricted its usefulness as a standalone typing strategy. SCC*mec*-type determination is laborious from a technical perspective. Nomenclature is continuously evolving (Baig *et al.*, 2018), which requires updating of protocols to ensure data integrity (Stefani *et al.*, 2012). However, SCC*mec* nomenclature remains in use today, and infers important information regarding the epidemiology and lineage of MRSA isolates. However, this is now commonly assigned using WGS data, which provides additional information outlining relatedness and phylogeny.

1.3.3.5 Whole-genome sequencing

The potential of WGS technology has emerged in recent years as the ultimate tool for identification and interrogation of DNA within any organism (Lakhundi and Zhang, 2018), and is expected to become mainstream for surveillance and research purposes, but also in clinical microbiology laboratories in the near future. WGS involves the extraction of DNA, preparing the DNA for sequencing (often referred to as library preparation), sequencing and the re-assembly of the genome thereafter. The first complete *S. aureus* genome was published in 2001 (Kuroda *et al.*, 2001). Early reports of *S. aureus* strains that had been subjected to WGS revealed that the *S. aureus* genome was circular, containing approximately 2,800,000 bp, which facilitated the coding of more than 2,600 proteins (Kuroda *et al.*, 2001; Baba *et al.*, 2002).

The initial sequencing methods detailed by Sanger *et al.* (1977) are now referred to as 'first generation sequencing'. 'Second generation sequencing', or 'next-generation sequencing' (NGS), which emerged in the mid-2000s, is now more commonly used due to the decreased costs, higher throughput and widespread access provided through the provision of commercial bench-top sequencers (Schadt *et al.*, 2010). Commercially available next generation sequencing (NGS) sequencing platforms include those provided by Roche 454 Life Sciences (e.g. GS FLX Titanium), Life Technologies (e.g. Ion Torrent and Ion Proton) and Illumina (e.g. MiSeq).

The process involved in NGS does not vary greatly based on the platform used. Bacterial DNA is first extracted and fragmented. Fragments are then ligated with adaptors containing primers for amplification and sequencing, and a barcode/identifier to enable downstream isolate de-multiplexing. Adaptors and DNA fragments undergo amplification by PCR and are then bound to a cartridge where complementary strand synthesis is used to determine the DNA sequence. The DNA sequence is expressed as 'reads' and stored alongside quality metrics in FASTQ file format. If isolates had been pooled for sequencing, identification primers will have been used (most commonly by the sequencing platform) to separate into discrete FASTQ files. Read quality will be ascertained, and low quality regions of sequences 'trimmed'. The volume and complexity of data generated by sequencing runs on NGS platforms requires the use of bioinformatics software for quality control and data analysis (Carriço *et al.*, 2018).

Whole-genome sequencing superseded other typing methods in terms of portability, reproducibility and un-paralleled discrimination between isolates, meaning the technology could be applied as a standalone typing measure in both clinical and epidemiological contexts.

Whole-genome sequencing for S. aureus typing

The data obtained by WGS of *S. aureus* isolates reveals markers that have been historically used for *S. aureus* typing (such as *spa* types, SCC-*mec* types and MLST), so facilitates retrospective as well as prospective analysis (Bartels *et al.*, 2014; Kaya *et al.*, 2018). However, WGS allows comparison of almost the entire *S. aureus* genome, in addition to these targets. To facilitate such comparisons a number of approaches have been developed, the most common of which are core-genome MLST (cgMLST) and whole-genome MLST (wgMLST). For the investigation of the relatedness of isolates, differences are generally enumerated by identifying either single nucleotide variations (SNVs) or allelic differences (Humphreys and Coleman, 2019). A variation that occurs once within a genomic sequence may be referred to as either a SNV or single nucleotide polymorphism (SNP), but the term SNP infers the variation has become established within a population, whereas the term SNV does not address this (Humphreys and Coleman, 2019).

Both cgMLST and wgMLST are extensions of conventional MLST schemes, but incorporate the genetic sequence of the core *S. aureus* genome (cgMLST), and in the case of wgMLST, both the core and accessory genomes. A publicly available curated scheme for *S. aureus* incorporating 3,897 loci (1861 core and 2036 accessory) has been developed using publicly available reference sequences (Leopold *et al.*, 2014; Roisin *et al.*, 2016). The extension of the MLST scheme facilitates increased discrimination between isolates that is not possible using conventional MLST (Stefani *et al.*, 2012).

Data derived by *S. aureus* WGS can be used to investigate isolate relatedness in outbreak investigations. A guideline threshold of relatedness have been proposed such that isolates that differ by ≤ 15 SNPs or ≤ 24 cgMLST/wgMLST allelic differences may be considered related (Schürch *et al.*, 2018). It is noted that these guidelines should be used in

conjunction with available epidemiological data (Schürch *et al.*, 2018; Humphreys and Coleman, 2019).

1.4 Aims

1. It is known that routine clinical activities in the hospital setting can result in dispersal of MSSA/MRSA into the air, which will eventually settle and result in surface contamination. In isolation rooms, this results in patients contaminating their own immediate environment, and the risk of onward transmission is mitigated by healthcare workers doffing single-use personal protective equipment and performing hand hygiene prior to leaving the patient area and providing care to others. However, in multi-bed rooms, shedding of MSSA/MRSA would inadvertently contaminate the near-patient environment of neighbouring patients. The aim of the early part of this study (detailed in Chapter 3) was to investigate surface contamination patterns within multi-bed rooms by intensive microbial sampling and to investigate systematically how routine care can result in contamination of multi-bed ward environments. Two surgical wards in Beaumont Hospital were chosen, and sampling was undertaken over four separate days (with two consecutive days on each ward).

2. The results of the investigations detailed in the component of the study detailed under 'Aim 1.' demonstrated widespread environmental contamination with *S. aureus*, with active air samples revealing an apparently diverse *S. aureus* population (based on phenotypic traits) in the air of two surgical wards, and an apparent link between clinical activities and *S. aureus* recovery from the air. Isolates of *S. aureus* from the air of multi-bed ward environments outside of critical

care areas haves not yet been investigated by whole-genome sequencing, and the role of the air in potential transmission pathways in such settings remains poorly understood. Whole-genome sequencing was undertaken on recovered *S. aureus* isolates with the aim of investigating (1) the population structure of *S. aureus* isolates recovered, (2) identifying virulence and resistance determinants carried by the isolates and (3) determining the relatedness of isolates recovered. Assessments of relatedness aimed to examine potential transmission pathways of *S. aureus* under non-outbreak conditions in the wards concerned and aimed to demonstrate to what extent hospital air may facilitate such transmission.

Chapter 2

Materials and Methods

2.1 Ethical approval

This work comprised part of a *S. aureus* transmission study spanning three years that commenced in May 2017. Ethical approval was initially applied for in November 2016 and granted by the Beaumont Hospital Medical Research Ethics Committee in February 2017 (application reference number: 17/01; Appendix A). An amendment was approved in February 2018 to allow access to patient notes to collect additional clinical data .

Participation in this study was solely on a voluntary basis for both patients and HCWs. There were no risks involved in the sampling protocol, and no changes or alterations to patient care were made as a result of inclusion in the study. The sampling undertaken in this study was in addition to routine hospital screening for multidrug resistant organisms. Results were not shared with either patients or HCW participants, and individuals who were found to be colonised with *S. aureus* were not decolonised or subject to altered clinical treatment based on the research findings.

2.2 Study setting

This study was undertaken in two separate wards (Wards A and B) located in Beaumont Hospital, a 820-bed referral hospital, located in Dublin, Ireland.

Ward A is a 21-bed area designated for patients who are under the care of a transplant surgery team. The ward consists of 12 single-occupancy patient rooms and two multi-bed patient areas, the first of which has five beds and the second has four beds. The patient area with four beds was selected for investigation in the present study (Fig. 2.1). This area is serviced by a modern air handling system, including high efficiency particulate air (HEPA) filtration and a heat recovery system (Fig. 2.2). The ward area contains two air inlet and outlet vents, with an air exchange rate of fifteen air changes per hour (ACH). At

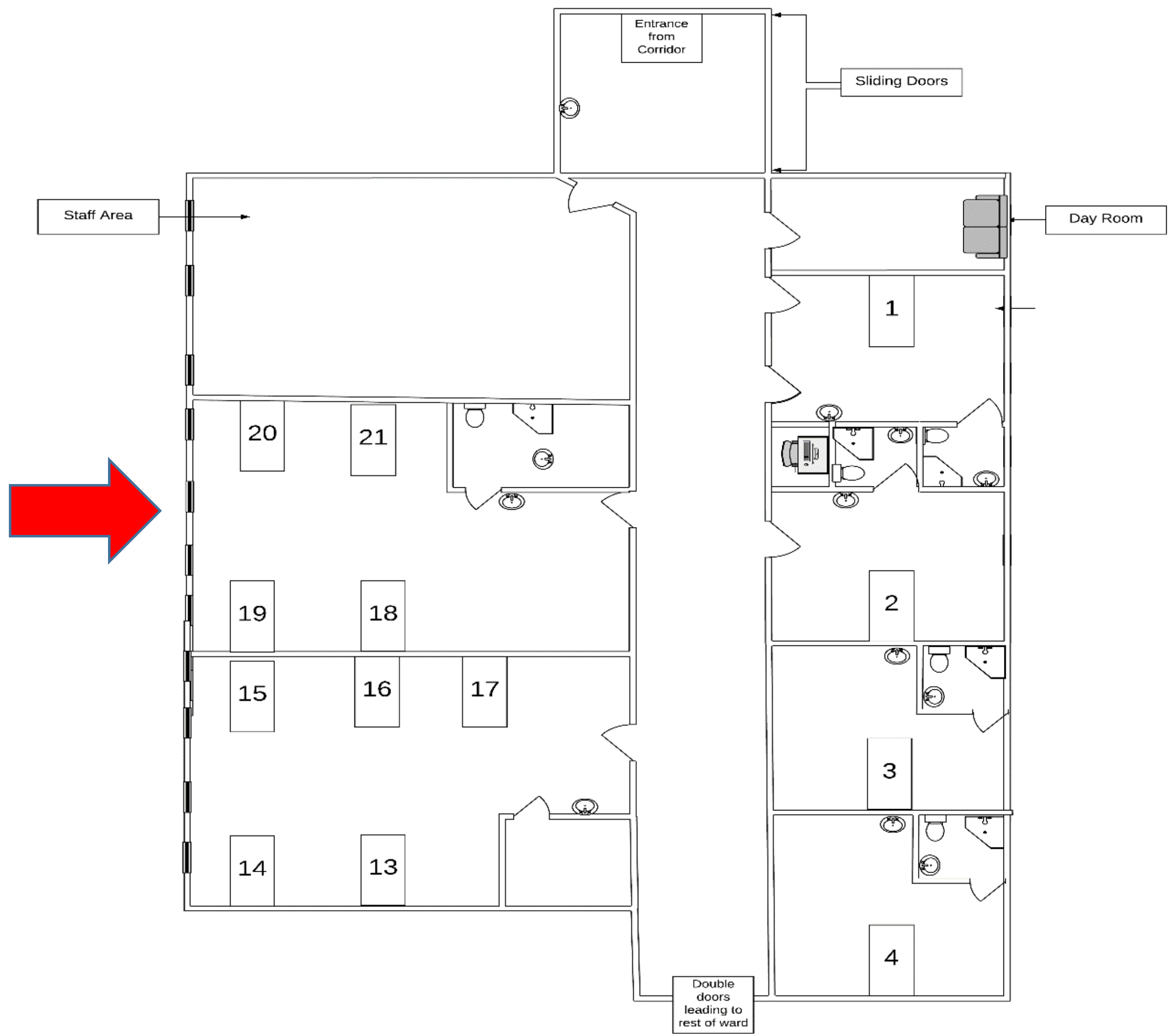


Figure 2.1 Schematic diagram showing layout of Ward A. Sampling was completed in the 4- bedded bay indicated by the red arrow.

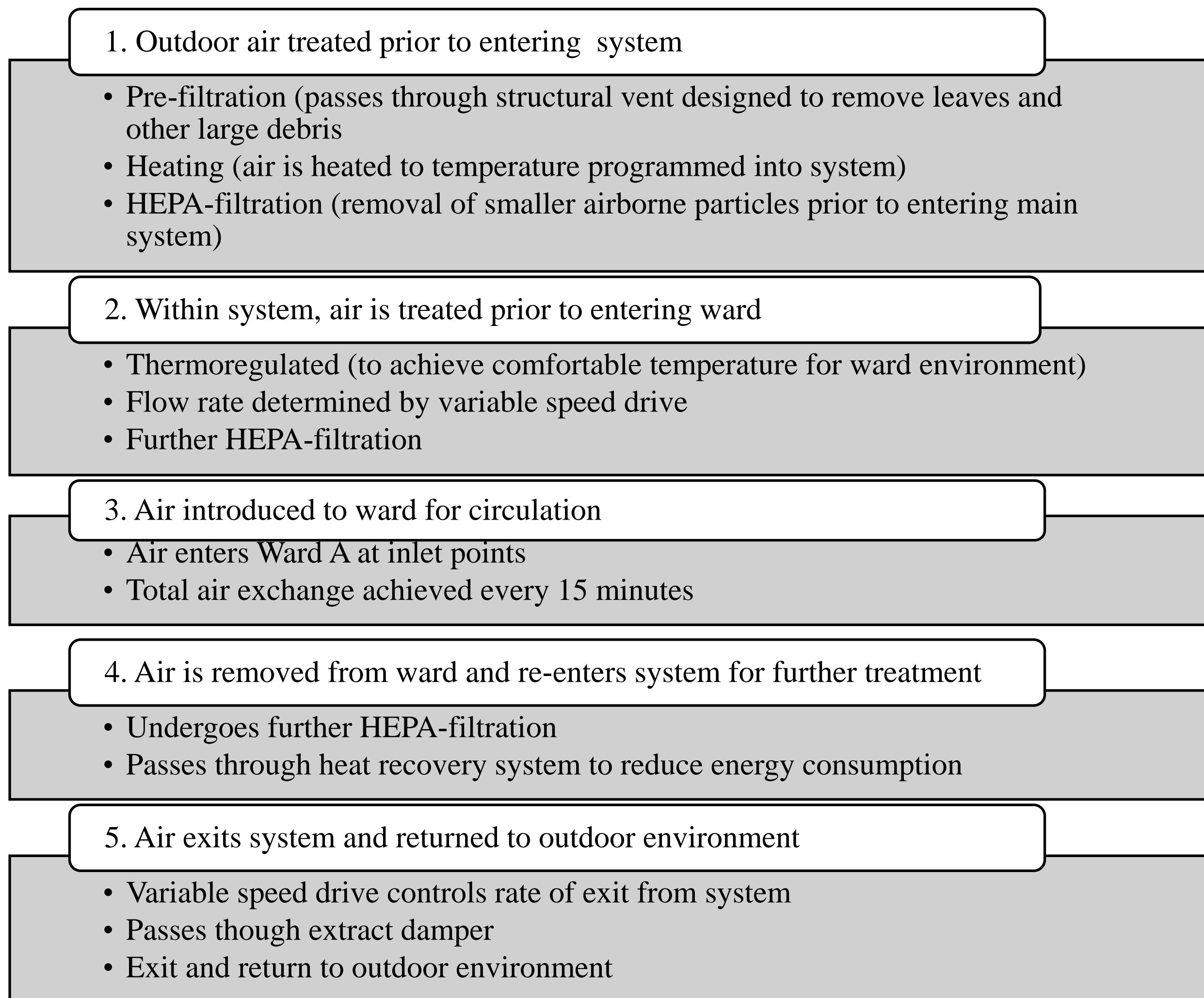


Figure 2.2 Flow chart detailing the air handing unit process servicing Ward A. Air enters the air handling unit (AHU) through mechanical vents that filter out large debris (e.g. leaves). Air is then heated and passed through a HEPA-filter. This air then passes through the system nexus, and undergoes thermoregulation and flow regulation. Flow regulation is controlled centrally by the hospital Technical Services Department. Thermoregulation is determined by the chosen temperature setting on the ward area and can be controlled both centrally and by ward occupants through wall-mounted thermostats. Following further HEPA-filtration, the air is supplied to the ward area. There is a heat recovery system in place, so that heated air that leaves the ward passes through a further HEPA-filter and is returned to the nexus. Extracted air is released to the atmosphere through the extract fan,

the flow rate of which is controlled centrally. Released air has been passed through a HEPA-filter prior to leaving the system.

the time of sampling the technical services manager at Beaumont Hospital confirmed that the ventilation system and HEPA-filtration system were operating within parameters detailed in the manufacturer’s instructions, as detailed by the hospital in-house real-time system surveillance software program.

Ward B comprises sixteen beds and predominantly houses patients who are under the care of a urological surgical team. It consists of two six-bed patient areas, a two-bed room and two single-occupancy isolation rooms (Fig. 2.3). One of the six-bed areas was selected for investigation in the present study. This room is naturally ventilated, with two windows that can be opened manually, and when opened there is a gap of eight inches to facilitate air exchange. Within the 6-bed room, there is an en-suite bathroom for patient use, and this has an extraction fan to remove foul air. The fan activates when the light switch in the bathroom is switched on, and operates for five minutes after the light is turned off.

Due to both the specialist knowledge required to provide patient care across both ward areas, there is frequent overlap of HCWs across the two areas, both among nursing and medical staff, but also allied HCWs and specialist nurses. Both wards are located on the same aspect of the hospital building, but separated by three floors. During the sampling period of this study, there was no transfer of patients between Ward A and Ward B.

2.3 Sampling routine

Sampling and data collection was undertaken over four separate sampling days in January 2019. The first two days of sampling, hereafter referred to as 'sampling day one' (SD1) and 'sampling day two' (SD2) were consecutive and spent on Ward A, followed by a break of seven days before 'sampling day 3' (SD3) and 'sampling day 4' (SD4) were undertaken over two consecutive days on Ward B. Active air sampling, passive air sampling and surface sampling were undertaken, and patients and HCW volunteers were recruited and screened for carriage of *S. aureus*. Only patients and HCWs from the designated study area were included, i.e. individuals that occupied or entered the multi-bed patient room being sampled on the sampling day(s) concerned.

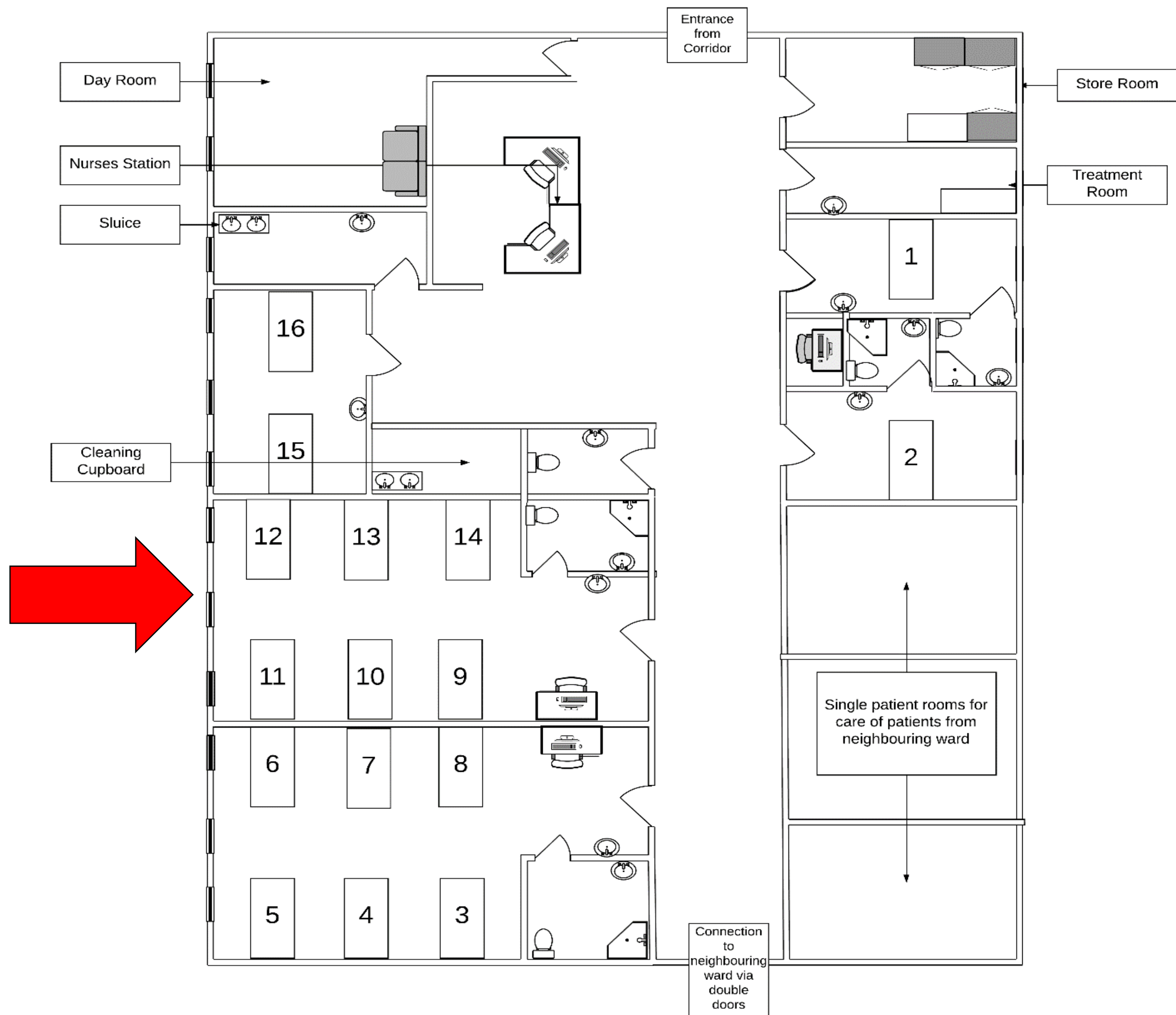


Figure 2.3 Schematic diagram showing layout of Ward B. Sampling was completed in the 6-bed bay indicated with the red arrow

2.4 Study participants

Patients and HCWs occupying the study area on all study days were eligible for inclusion in the study. Therefore, inclusion criteria for patients included being admitted to a bedspace within the study area, and for staff member involved working within the study area. Patient exclusion criteria included inability to provide informed consent. As per the Beaumont Hospital Ethics Committee, patients must be allowed a 24 h period to consider their participation in research studies. Therefore, patients could not be included if this time period was not feasible, i.e. if they were admitted to the study area without prior knowledge of the study. As the sampling protocol included an oral rinse, patients with dysphagia were not included, to avoid potential aspiration of the phosphate-buffered saline. There was no exclusion criteria applied to HCWs. For both patients and HCWs, recruitment was not mandatory and inclusion in the study was voluntary.

2.4.1 Healthcare worker recruitment

Initial contact was made in advance of sample collection to inform potential participants of the study and provide information for those who consented to be included as volunteers. Healthcare workers from all disciplines who entered the study area were invited to participate in the research study by undergoing screening for carriage of *S. aureus*. There were no exclusion criteria for HCWs for participation, but inclusion was non-mandatory and relied on HCWs to volunteer. Study information was provided both verbally and with a written project information leaflet (Appendix B).

2.4.2 Patient recruitment

Patients who were admitted to a bed within the sampling area were approached and informed about the study on the day prior to sampling. As with HCWs, information was provided via an information leaflet (Appendix C) and verbally by the researcher. The nurse manager on each ward acted as a gatekeeper and informed the researcher of patients who could not be included based on the exclusion criteria.

2.4.3 Study participant enrolment and consent

All volunteer participants were sampled by the researcher. Both patients and healthcare workers were given a period of at least 24 h to consider participation in the study from the point of initial contact. The sampling time was arranged with individual participants and kept informal and flexible to allow for the demands of active service provision, which this study aimed to observe in a realistic and authentic manner.

At the time of sampling, potential participants were reminded of the purpose and general aims of the study that had been discussed at the initial point of contact and was included in the information leaflet. At this point, participants were encouraged to explore and clarify any queries they had with the researcher, with time given by the researcher to answer and discuss any such queries prior to participation.

Consent was obtained from all participating patients and HCWs as outlined in the ethical submission granted by the Beaumont Hospital Medical Research Committee. Informed consent was documented by each participating HCW and patient, and also signed by the researcher (Appendices D and E, respectively). A copy of the consent form signed by both the participant and the researcher was returned to each participant. For participating patients, the researcher confirmed with the patient's designated nurse that there had been no changes in the preceding 24-h period (since initial contact) that would now exclude

them from participation, e.g. any changes to clinical condition that would compromise an ability to consent.

Participants were advised at the time of sampling that they would not be informed of the outcome of screening (i.e. whether they were colonised with *S. aureus* at the time of sampling).

From the point of participation onwards, all data was stored in a pseudonymised manner. Participants were allocated a unique identifier and all samples and results were processed under that identification. The researcher stored the consent forms (which held the participants names) and the link to the study identifier separately, and all data was stored either in a locked filing cabinet in an office requiring swipe access or as an encrypted password-protected electronic file. This file was held on the computer desktop of the researcher. A password is required to access this computer desktop, and access was available only to the researcher.

2.4.4 Participant sampling

All patients and HCWs who were enrolled in the study were sampled for *S. aureus* carriage by the researcher. This involved sampling at two anatomical sites- the anterior nares (sampled using a dry sterile cotton-tipped swab), and the oropharynx (sampled using an oral rinse of phosphate buffered saline). A more detailed description of participant sampling is provided in Chapter 3.

2.5 Environmental sampling

Extensive environmental sampling for *S. aureus* was undertaken on each sampling day. This sampling consisted of continuous active air sampling, passive air sampling for the

evaluation of *S. aureus* deposition on high-touch, near patient areas and surface sampling for the presence of *S. aureus* using contact plates. A more detailed description of environmental sampling is provided in Chapter 3.

2.6 Storage and transport of samples prior to laboratory processing

Samples were stored at ambient temperature during sample collection. Samples were transferred to the Dublin Dental University Hospital (DDUH) Microbiology Laboratory within four hours of collection. All samples were transported in UN3373-compliant packaging containers for Category B biological substances (Health and Safety Authority, 2017).

2.7 Microbiological processing of samples

2.7.1 Initial processing of samples

Upon arrival at the DDUH Microbiology Laboratory, environmental sampling plates (contact plates, air sample plates and settle plates) were incubated in a Sanyo Gallenkamp (Leicestershire, UK) static incubator at 37°C. Contact plates were incubated aerobically for 24 h, whereas air sample plates and settle plates were incubated for 48 h.

Participant samples (patient and HCW nasal swabs and oral rinses) underwent further processing prior to incubation. If processing was not undertaken immediately on arrival at the laboratory, samples were refrigerated at 4°C for no longer than 48 h prior to processing. Nasal swab samples were inoculated directly onto Colorex^{TM} Staph Aureus chromogenic agar plates (Colorex, E&O Laboratories, Bonnybridge, UK) by rotating the swab over the entire surface of the agar plate prior to incubation in a static incubator for 18-24 h at 37°C. To ensure maximum bacterial recovery, plates were lawned in three

different directions, while rotating the swab. Oral rinses were vortexed in their collection cups for 30 s at maximum speed, using a Heidolph Reax benchtop vortex (Heidolph Instruments GmbH & Co., Schwabach, Germany). After vortexing, a 1 ml aliquot of each oral rinse sample was transferred into a sterile 1.5 ml Safe-Lock microfuge tube (Eppendorf Ltd., Hamburg, Germany) and centrifuged in an Eppendorf model 5417C bench top centrifuge at 20,000 × *g* for 1 min. The supernatant from each sample was discarded and the pellet was resuspended in 300 µl of phosphate-buffered saline (PBS). A 100 µl volume of each resuspended sample was spread onto Colorex™ Staph Aureus chromogenic agar using sterile plastic L spreaders (Greiner Bio-One GmbH, Frickenhausen, Germany) and incubated at 37°C statically for a period of 18-24 h. In-house isolates that had previously been confirmed as MSSA and MRSA served as positive controls.

2.7.2 Identification of S. aureus and MRSA isolates

Following incubation, all plates were examined visually for the presence of presumptive *S. aureus* colonies based on colony colour. Colonies suggestive of *S. aureus* were deemed to be those mauve or pink in colour on Colorex™ Staph Aureus agar, as per the manufacturer's instructions (Fig. 2.4). Putative *S. aureus* colonies from sampling plates were subcultured onto Colorex™ Staph Aureus and Colorex™ MRSA chromogenic agar (Colorex) under asceptic conditions. MRSA colonies also exhibit a mauve/pink colour when cultured on Colorex™ MRSA agar plates. If there was more than one putative *S. aureus* colony morphology type evident per plate, each colony was labelled with a unique code that identified the sample and agar plate it was recovered from. For example, if a sample obtained from a patient yielded more than one putative *S. aureus* colony, the sampling naming system reflected this by referring to the sample with the unique patient

identifier, followed by the assignment of ‘a’, ‘b’, ‘c’, etc., to reflect multiple non-identical putative colonies recovered on the same sampling plate. Up to five putative *S. aureus* colonies (when present) were selected from each plate and purified by subculture on fresh plates of the original isolation agar, and incubated statically at 37℃ for 18-24 h. Presumptive *S. aureus* isolates were further tested using the Pastorex Staph Plus latex agglutination kit according to the manufacturer’s instructions (Bio-Rad, Marnes la Coquette, France). Detection of the *S. aureus* clumping factor, protein A and capsular polysaccharide is indicated by agglutination of latex particles sensitised with human fibrinogen and monoclonal antibodies.

Isolates that fit the phenotypic profile of *S. aureus*/MRSA based on colony colour on chromogenic media and a positive latex agglutination test underwent further confirmatory testing at the Irish National MRSA Reference Laboratory (NMRSARL, St. James’s Hospital, Dublin). Isolates were confirmed as *S. aureus* using the tube coagulase test, which detects the presence of the staphylocoagulase protein (Rossney *et al.*, 1990). Resistance to methicillin was evaluated using 30-µg cefoxitin discs (Oxoid, Basingstoke, England) using the methodology and interpretive criteria provided by the European Committee of Antimicrobial Susceptibility testing (EUCAST, 2019).

2.7.3 Antimicrobial susceptibility testing

All isolates confirmed at the NMRSARL as *S. aureus* underwent susceptibility testing using disk diffusion to a panel of twenty five heavy metals and antimicrobial agents using EUCAST methodology, previously described reference strains (ATCC29213 and ATCC25923) and interpretative criteria (McManus *et al.*, 2015; Clinical Laboratory Standards Institute, 2018; EUCAST, 2019) (Table 2.1).

2.7.4 Confirmation of isolates as MRSA

Isolates that were identified as resistant to either cefoxitin or oxacillin (as described in section 2.7.3) were deemed to be MRSA. This was further confirmed by testing such isolates using the GeneXpert® IV real-time PCR platform (Cepheid, Sunnyvale, USA) using the GeneXpert MRSA assay (Cepheid) as per the manufacturers instructions and as described previously (Rossney *et al.*, 2008). This PCR assay detects segments of MRSA-specific DNA within the SCC*mec* element, and has been validated for the detection of MRSA from clinical samples.

Isolates that exhibited resistance to cefoxitin, i.e. with a breakpoint value in excess of 22 mm were tested for oxacillin resistance, in addition to the antimicrobial agents outlined in Table 2.1. Briefly, isolates were recovered from stored conditions (-70°C) on CBA plates (Lip Diagnostic Services) incubated at 37°C for 20 h. An inoculum equivalent to 0.5 McFarland standard was prepared and, using a cotton swab applicator, lawned on to a Mueller-Hinton agar plate (E&O Laboratories, Bonnybridge). A oxacillin strip E-test strip (Biomérieux, Marcy l'Etoile, France) was applied to the agar plate. The strip was allowed to dry by leaving untouched at room temperature for no longer than 30 min, followed by static incubation at 35°C for a period of 24 h. Isolates were considered susceptible to oxacillin if the minimum inhibitory concentration (MIC) observed was ≤ 4 μg.

2.8 Statistical analysis

All patient and HCW data were first pseudonymised and then entered into a Microsoft Excel database (version 14.7.7, Microsoft Corporation, Washington, USA). The text-baseddataset was then translated into numerical code, using a data dictionary developed by the researcher. Once this was complete and had been interrogated for potential errors, the numerically-coded data were imported to SPSS data analysis software, version 25

(International Business Machines Corporation, Armonk, New York, USA). For comparison of independent continuous variables where the assumption of normality was violated, the Mann Whitney U was used. The Chi-square Test was used to analyse two or more categorical variables, and many such variables were dichotomous in nature. Analyses which generated *P* values of < 0.05 were considered signficant.

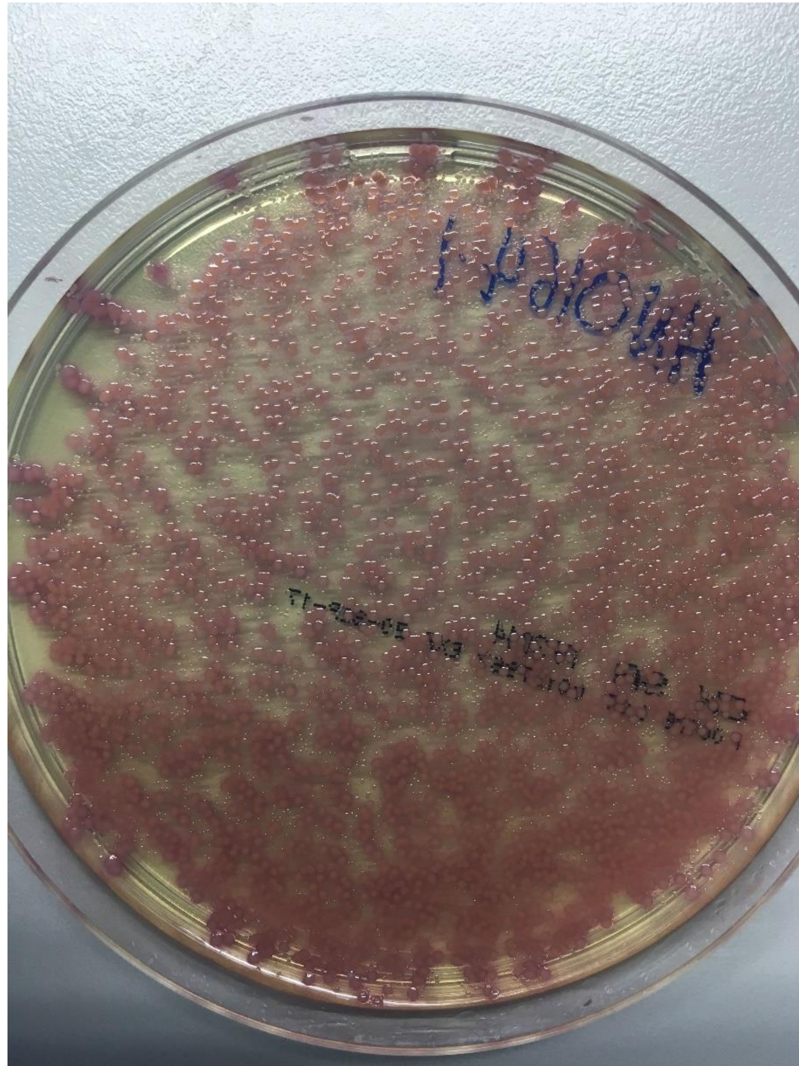


Figure 2.4 A photograph showing semi-confluent growth of *S. aureus* colonies on a Colorex^{TM} Staph Aureus chromogenic agar plate, which was used to culture a nasal specimen from a colonised patient. This agar permits the presumptive identification of *S. aureus* colonies based on colony colour (with *S. aureus* colonies appearing in mauve or pink) after 18-24 h. Putative *S. aureus* isolates underwent confimatory testing at the NMRSARL.

Table 2.1 Antimicrobial agents and breakpoints used for susceptibility testing in the present study

Antimicrobial agent[a]	Disk concentration (µg/disk)	Zone breakpoints (mm)[b]			Reference
		S ≥	I	R <	
Amikacin	30	18	None[c]	16	(EUCAST, 2019)
Ampicillin	10	29	None	28	(CLSI, 2018)
Cefoxitin	30	22	None	22	(EUCAST, 2019)
Chloramphenicol	30	18	None	18	(EUCAST, 2019)
Ciprofloxacin	5	21	None	21	(EUCAST, 2019)
Clindamycin	2	22	None	19	(EUCAST, 2019)
Erythromycin	15	21	None	18	(EUCAST, 2019)
Fusidic acid	10	24	None	24	(EUCAST, 2019)
Gentamicin	10	18	None	18	(EUCAST, 2019)
Kanamycin	30	18	14-17	13	(CLSI, 2018)
Linezolid	10	21	None	21	(EUCAST, 2019)
Mupirocin	200	30	None	18	(EUCAST, 2019)
Neomycin	30	18	16-17	15	(Rossney *et al.*, 2007)
Rifampicin	5	26	None	23	(EUCAST, 2019)
Spectinomycin	500	13	14-19	20	(Rossney *et al.*, 2007)
Streptomycin	25	16	14-15	13	(Rossney *et al.*, 2007)
Sulphonamide	300	17	13-16	12	(CLSI, 2018)
Tetracycline	30	22	None	19	(EUCAST, 2019)
Tobramycin	10	18	None	18	(EUCAST, 2019)
Trimethoprim	5	17	None	14	(EUCAST, 2019)
Vancomycin	30	15	None	14	(CLSI, 2018)

[a]Resistance to oxacillin was investigated using oxacillin E-test strips (Biomérieux) as described in Section 2.7.3, so is not shown in this table.

[b]Zones of growth inhibition were recorded in mm and interpreted as resistant (R), intermediate (I), or susceptible (S), according to the guidelines referenced.

[c]None, no intermediate breakpoint indicated in guidelines.

Chapter 3

An investigation of environmental contamination by *Staphylococcus aureus* in two differently ventilated multi-bed surgical wards

3.1 Introduction

The detection and physical isolation of MRSA-colonised or MRSA-infected patients, the administration of decolonisation agents and the use of transmission-based contact precautions are the primary strategies for the control of MRSA in Irish hospital settings. Despite the endemic nature of MRSA in Irish hospitals, MRSA decolonisation is not always feasible, and risk assessments for limited isolation rooms are undertaken by clinical staff based on patient and organism factors (Department of Health- An Roinn Sláinte, 2013). Methicillin-susceptible *S. aureus* colonisation or infection does not warrant isolation, regardless of the virulence of the strain concerned or the antimicrobial resistance profile exhibited. As such, the majority of patients colonised or infected with MRSA or MSSA in Irish hospitals are cared for in multi-bed patient rooms.

Given the known limitations of hospital cleaning (Guh and Carling, 2010), it is imperative to determine how and by what mechanisms rooms housing MRSA/MSSA-colonised patients become contaminated with MRSA/MSSA, so that dissemination can be minimised and effective evidence-based infection prevention and control measures implemented. Patterns and determinants of *S. aureus* surface contamination are not completely understood, but it is known that the presence of bacteria in the air has been associated with increased surface contamination (Carvalho *et al.*, 2007), which may contribute to environmental persistence and transmission pathways of *S. aureus* in nosocomial settings.

Staphylococcus aureus (including MRSA) has been recovered from multi-bed ward environments from various specialties in hospitals throughout the world. These include general medical and surgical wards (Shiomori *et al.*, 2002; McLarnon *et al.*, 2006; Roberts *et al.*, 2006; Carvalho *et al.*, 2007; Bernard *et al.*, 2012; Creamer *et al.*, 2014), and specialised areas housing especially vulnerable patients, including burns units (Rutala *et al.*, 1983; Khojasteh *et al.*, 2007; Dansby *et al.*, 2008), haematology and stem cell

transplantation units (He *et al.*, 2014), and critical care settings (Hathway *et al.*, 2013; Dancer *et al.*, 2019).

The presence of *S. aureus* in hospital air in reported studies is influenced by a number of factors including characteristics of the source (which may be a colonised individual or contaminated fomite), sampling technique, the ventilation system in use, the number of individuals present and by routine clinical activities that may liberate *S. aureus* to the air, which will eventually settle on surfaces. Colonised individuals shed *S. aureus* to their immediate environments at variable rates (Sherertz *et al.*, 1996) . Increased dissemination has been shown during viral illness or when experiencing respiratory symptoms (Thompson *et al.*, 2014). Furthermore, the site of colonisation has been observed to influence the degree of MRSA shedding and resulting environmental contamination. For example, colonisation of the groin of a patient associated with highest levels of contamination at environmental sites within their close proximity (Rohr *et al.*, 2009).

Airborne *S. aureus* may occur through shedding from a colonised individual, or as a result of liberation from a contaminated fomite, such as bedsheets contaminated with skin squames (Beggs *et al.*, 2008). Bacterial particles will then settle on surfaces or equipment, many of which are frequently touched by the hands of patients, visitors and HCWs (Smith *et al.*, 2012; Cheng *et al.*, 2015; Jinadatha *et al.*, 2017). An additional transmission opportunity is afforded when patients are nursed in multi-bed rooms. In such situations, no clear or built boundary exists between patient environments, which are often separated only by curtains which are pulled to provide privacy for personal tasks or medical examination. In such situations, colonised or infected patients will inadvertently shed *S. aureus* into their own environment (both air and surfaces), as well as the environment of the patients in adjacent beds, in addition to contributions from colonised HCWs and visitors.

Although MRSA is not considered ‘airborne’, or transmitted through droplets or aerosols in the same way that smaller particles may be, such as influenza virus or measles virus, aerial dissemination of MRSA has been shown. The role this may play in disease transmission is not yet quanitifiable or well understood. MRSA has been recovered from air both within isolation rooms (Sexton *et al.*, 2006) and in multi-bed areas (Creamer *et al.*, 2014), both in the presence and absence of colonised patients or HCWs, in both developed and less developed countries (Fekadu and Getachewu, 2015; Getachew *et al.*, 2018) and in adult and paediatric care settings (Khojasteh *et al.*, 2007; Creamer *et al.*, 2014). The air environment in certain units, such as operating theatres and critical care settings, is considered to pose a high risk for the dispersal of nosocomial pathogens (Gastmeier *et al.*, 2012; Birgand *et al.*, 2015; Stockwell *et al.*, 2019). As such, ventilation within these settings is subject to strict controls. Multi-bed hospital wards, such as those included in the present study and where the majority of patients colonised with *S. aureus* are housed, do not fall within the remit of such guidance (Beggs *et al.*, 2008).

Naturally ventilated areas tend to have less consistent microbial contamination patterns than areas where mechanical ventilation systems are in place, and are more likely to be influenced by outside environmental and weather factors, and tend to harbour greater numbers of bacteria (McLarnon *et al.*, 2006; Snitkin, 2019). Routine activities such as changing bedsheets (Shiomori *et al.*, 2002; Roberts *et al.*, 2006; Hathway *et al.*, 2013), changing of wound dressings (Dansby *et al.*, 2008), respiratory treatments such as the administration of nebulised medication and non-invasive airway ventilation (Roberts *et al.*, 2006) and personal hygiene activities (Hathway *et al.*, 2013) have previously been shown to increase airborne *S. aureus* in hospitals. Inappropriate cleaning techniques (such as dry sweeping) and poor waste management and infrastructure, as observed by Gizaw *et al.*, (2016) are also influential, and can result in greater levels of contamination. Gizaw and colleagues, for example, observed “indiscriminate” waste disposal practices, visibly

contaminated toilet facilities in close proximity to the patient area and lack of adherence to cleaning protocols during their study of microbial composition of indoor air at a teaching hospital in Ethiopia. Increased levels or airborne bacteria are likely a reflection of such practices, and may reflect overall hygiene in a healthcare setting.

Comparing the burden of *S. aureus* in the air of different settings is complicated by the use of different sampling tools, with a variety of available air samplers (i.e. cascade samplers, slit samplers, impact samplers), and variability in microbial processing techniques in published literature. For, example, the use of chromogenic agar for the detection of MSSA or MRSA has been shown to have greater sensitivity than conventional culture methods (Hirvonen *et al.*, 2014). Many studies do not mention the type of ventilation in use, the colonisation pressure of *S. aureus* during the investigative period or the location within the room where the air sampler was located, further complicating comparison between different studies (Gaudart *et al.*, 2013).

The primary aim of this part of the present study was to investigate the prevalence of MSSA and MRSA in near-patient environmental sites and the air in two differently ventilated hospital wards in order to investigate the transmission dynamics of these organisms in the hospital setting. Furthermore, due to the limited body of previous work that investigated the impact of routine care activities and hospital infrastructure on microbial contamination of the environment and the air, this study aimed to systematically investigate activities undertaken in the two differently ventilated hospital wards in relation to environmental MSSA and MRSA.

3.2 Materials and Methods

3.2.1 Ethics Approval

Ethics approval was granted by the Beaumont Hospital Medical Ethics Committee, as detailed in Chapter 2, section 2.1.

3.2.2 Study Setting

This study was undertaken in two separate wards located (Wards A and B) in Beaumont Hospital, as described in detail in chapter 2, section 2.2.

3.2.3 Sampling routine

Sampling and data collection was undertaken over four separate sampling days in January 2019 (SD1, SD2, SD3 and SD4) as described in Chapter 2, Section 2.3.

3.2.4 Study participants

The study involved screening voluntary consenting participants (who were either inpatients or HCWs) for carriage of *S. aureus* at a single time point that coincided with extensive environmental screening of the clinical area. Sampling of HCWs and patients was undertaken in the same manner and consisted of swab sampling of the anterior nares and sampling of the oropharnyx using an oral rinse. Details of recruitment of HCWs and patients to the study are described in Chapter 2, Sections 2.4.1, 2.4.2 and 2.4.3, respectively.

3.2.5 Study participant sampling

All volunteer participants were sampled by the researcher. The process of study obtaining informed consent from participants was as described in Chapter 2, Section 2.4.3.

3.2.5.1 Swab sampling of anterior nares

The anterior nares of each participant was sampled using a dry sterile cotton-tipped swab (Copan Italia S.p.A, Brescia, Italy). The swab was pre-moistened using the supplied aqueous transport medium. Swabs were rotated in both nostrils and then returned to the transport medium. The sample tube was then labelled with the identifying participant code by the researcher. Patient and HCW participants completed a questionnaire detailing demographic data at the time of sampling (Appendices F and G, respectively).

3.2.5.2 Oral rinse sampling

Participants were instructed to rinse their oral cavity with 20 ml of sterile PBS for 20 s, provided in a 100 ml disposable container (Sarstedt, Wexford, Ireland). While this oral rinse was being undertaken, the cap of the container was replaced to avoid contamination of the sample. Participants were advised to return the liquid to the same container, and the lid was securely replaced. The sample container was then labelled with the related identifying code to the nasal samples. The researcher ensured there were no patients with dysphagia who were eligible to participate in the study to negate the clinical risk of aspiration.

3.2.6 Environmental sampling

The study environment was sampled on each study day, and this consisted of continuous active air sampling, passive air sampling for the evaluation of *S. aureus* deposition on high-touch near patient areas and surface sampling for the presence of *S. aureus*.

3.2.6.1 Active air sampling

Active air sampling was undertaken using an Oxoid/Thermo Scientific model EM0100A air sampler (Oxoid Ireland, Fannin Healthcare, Dublin, Ireland). The air sampler was programmed to collect 1000 L (one cubic metre) of air onto Colorex^{TM} Staph Aureus chromogenic agar plates (Colorex). Collection of the required volume of air by the sampler took approximately 10 min (the sampler has an impact speed of < 20 m/s and a nominal air flow rate of 100 L air per min).

Sampling commenced at 07:00 h on each sampling day, and was undertaken continuously until 12:00 midday. Once 1000 L of air had been collected by the sampler, an alert was sounded by the equipment. The head and plate holder areas of the air sampler were decontaminated with 70% (v/v) ethanol, and the agar plate replaced before the sampler was reset to collect another 1000 L of air. This process was repeated from 07:00-12:00 h resulting in 30 individual cubic metres samples of air being collected on each sampling day.

Prior to taking air samplings, the air sampler was placed on a stainless steel trolley that had been de-contaminated using 70% (v/v) ethanol. The trolley and air sampler was placed in a location point near the centre of the room and this remained consistent throughout the sampling period. The head of the air sampler reached a height of 1.2 m above floor level. Agar plates used to take air samples were labelled with a code devised by the researcher, which allowed each sample to be traced to a defined time period and related to additional observation data collected concurrently.

3.2.6.2 Passive air sampling

Passive air sampling was undertaken using a previously described settle plate method (Pasquarella *et al*., 2000). Colorex^{TM} Staph Aureus chromogenic agar plates (Colorex) were placed on each of the six patient over bed tables within the study room, so that plates

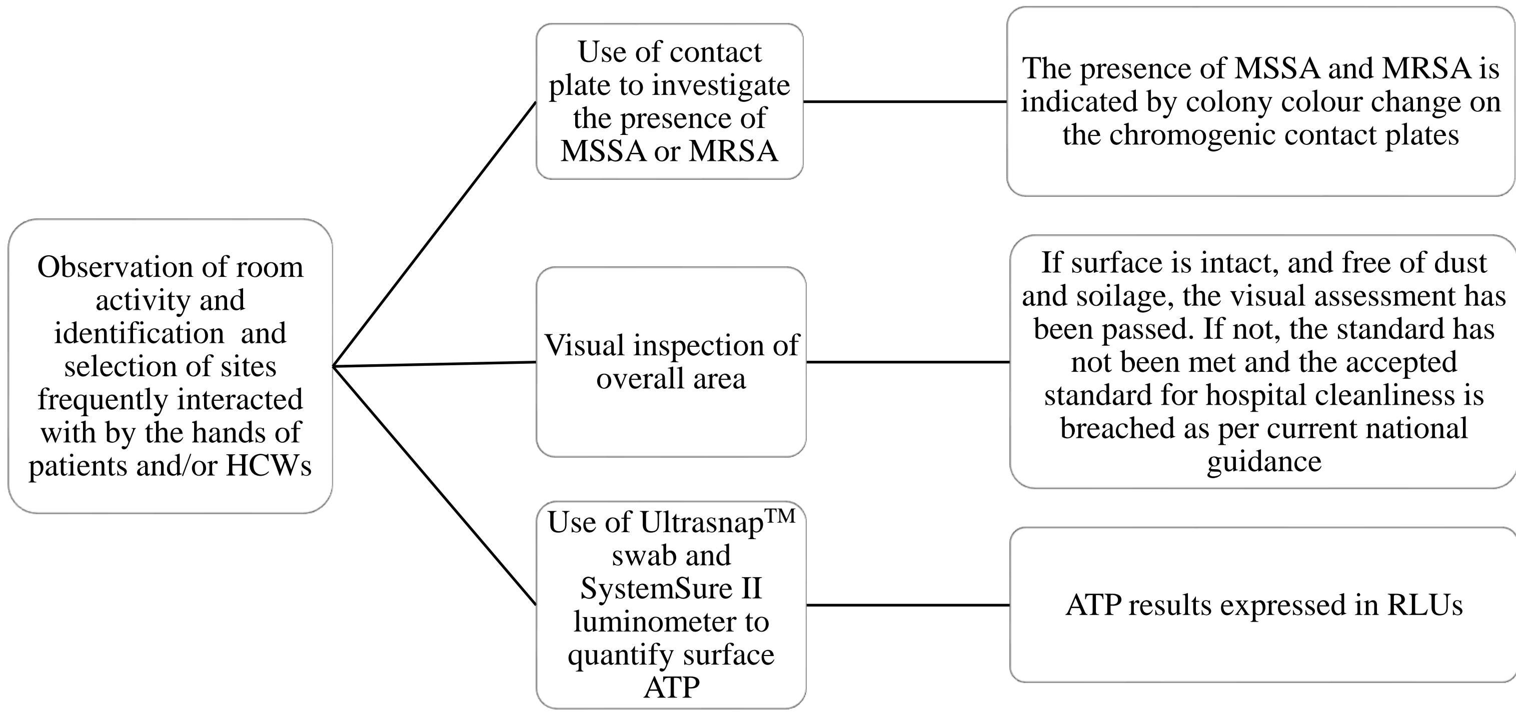
were left at a height of 1 m above floor level. The first plate was placed at 07:00 h and these were replaced with a new Colorex™ Staph Aureus agar plate every 60 min in the same location until 12:00 midday. This procedure resulted in one settle plate per hour from each bed and yielded five settle plates from each bed space over the five-hour study sampling period. As with the active air samples, each plate was labelled with a code relating to the location, date and time of collection.

3.2.6.3 Environmental surface sampling

Surface sampling was undertaken on a number of horizontal surfaces within the study area, and included contact plate sampling for the detection of *S. aureus*, adenosine-triphosphate (ATP) quantification measurements using a luminometer device, and the undertaking of visual assessments for hygiene and cleanliness. For each surface sampled, concurrent observations (visual assessment, contact plates and ATP measurements) were recorded such that it could be determined whether a surface appeared 'clean', ATP levels quantified and whether *S. aureus* was detected or not (Fig. 3.1)

Sampling using contact plates

Contact plates were 60 mm in diameter. The surface of the agar is curved in a deliberate convex line across the surface of the contact plate, resulting in a slight protrusion of the agar surface above the depth of the plate edge, permitting maximum surface contact. The surfaces sampled were not decontaminated during the observation period either by the either as part of the research study or by hospital staff. Surfaces selected for sampling were chosen as sites observed to be touched frequently by the hands of HCWs or patients during the sampling period.



Abbreviations: MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; ATP, adenosine triphosphate; RLUs, relative light units measured as bioluminesence.

Figure 3.1 A flowchart describing hygiene assessments undertaken in tandem with surface sampling. No quantitative or definitive standard exists currently for assessing cleanliness of hospital surfaces. Visual inspection is used currently as an audit tool. The measurement of ATP levels to quantify organic soil on surfaces, which is well established in other industries, has been suggested as an objective metric of hospital hygiene. Currently, there are no definitive thresholds or pass/fail results available for ATP measurements, but a guideline has been suggested by researchers that a reading >100 RLUs constitutes a breach in hygiene standards. Contact plates were used to assess whether MSSA or MRSA was present on each surface assessed. Following collection, these were incubated for 18 h at 37°C on Colorex™ Staph Aureus agar medium. This agar permits the presumptive identification of *S. aureus* colonies based on colony colour (with *S. aureus* colonies appearing in mauve or pink) after 18-24 h incubation at 37°C.

Surface sampling was undertaken in tandem with hygiene assessments of surfaces. For each surface included in sampling, initially the area was inspected for any issue that would warrant a 'hygiene fail' using the current audit tool used in Irish hospitals (Health Service Executive, 2006). This audit tool describes a hygiene failure if the surface is visibly damaged in any way, or if there is any visible dust or soiling present. The contact plate was applied to the surface after which the plate was removed and the lid replaced. Contact plates were incubated as described in Chapter 2, Section 2.7.1.

Measurement of ATP levels

A System Sure II luminometer (Hygiena Int. Ltd., Watford, UK) was used to sample a 10 cm^2 area immediately adjacent to a surface sampled using a contact plate to determine the presence or absence and relative quantity of contamination by determining levels of adenosine triphosphate (ATP) measured by bioluminescence. For ATP measurements, an UltraSnap^{TM} (Hygiena Int. Ltd, Watford, UK) swab was used to sample the area.

This process involved the application of the sterile UltraSnap swab to the surface to be sampled. The swab was first removed from its supply tube, and was not moistened prior to application in line with the manufacturer's instructions. Contact was made across the surface in ten discrete movements in a number of directions to ensure maximum coverage and contact between the swab and the surface. Following contact with the surface, the swab was returned to its tube. Once an adequate seal was made between the tube and the swab, a compartment at the top of the swab (i.e. at the most distal point from the tip of the swab used to sample) is 'snapped', triggering the release of liquid stable luciferase reagents throughout the tube containing the swab. The swab and reagents were then mixed by inverting the tube for 15 s. The luminometer (Hygiena Int. Ltd.) was turned on and allowed to undergo a routine self-calibration. Once this was complete, the tube (still

containing the swab and reagents) was placed in the testing compartment of the luminometer. Reading of bioluminescence was then commenced, which took approximately, 15 s before results are displayed on the luminometer display panel.

Bioluminescence is reported as relative light units (RLUs). The number of RLUs is directly proportional to the quantity of ATP present in the sample. Thus, ATP measurements are expressed in RLUs. The surface sampled with the ATP swab did not overlap with the area sampled using the contact plate, to avoid confounding results due to residual sampling material left from either the contact plates or ATP swabs. Surfaces that yielded > 100 RLUs and failed a visual hygiene assessment were considered contaminated in accordance with the recommendations of a previous study (Mulvey *et al.*, 2011).

Visual hygiene assessment

Each surface was examined to determine whether it appeared visibly clean. A surface passed if it was intact and free of dust or soilage. If these conditions were not met, the surface was deemed to have failed the hygiene assessment. This assessment process reflects current national guidance for the audit of cleanliness standards in hospitals (Health Service Executive, 2006).

3.2.7 Storage and transport of samples prior to laboratory processing

Samples were stored at ambient temperature during sample collection. Samples were transferred to the DDUH Microbiology Laboratory within 4 h of collection, as described in Chapter 2, Section 2.6.

3.2.8 Initial processing of samples

Upon arrival at the DDUH Microbiology Research Laboratory, initial processing was undertaken as described in Chapter 2, Section 2.7.1.

3.2.9 Identification of *S. aureus* and MRSA isolates

The identification of *S. aureus* and MRSA from processed samples was undertaken as described in Chapter 2, Section 2.7.2. and Section 2.7.4.

3.2.10 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was undertaken as described in Chapter 2, Section 2.7.3.

3.2.11 Observation of room activity

Information relating to the activity in each of the two ward rooms (Ward A and Ward B) included in the study was observed, and documented in such a way that each activity could be linked back to each environmental sample. The air sampler takes approximately 10 min to collect a cubic metre of air, and for each of the air samples taken in each ward data was collected relating to occupant density, activity, and whether an individual present in the room was screened for *S. aureus*. Information relating to activities that have been shown previously to increase airborne particles and total viable counts, such as bed making, washing and dressing, the administration of nebulised medications and dressing changes, was recorded. This data was linked directly to active air samples, passive air samples from the same period and surface sampling.

3.2.13 Airborne particle quantification

Airborne particles were measured throughout the sampling period using a ParticleScan Pro™ Airborne Particle Counter (IQ Air, INCEN AG, Goldach, Switzerland). For each active air sample that was collected, a corresponding measurement was taken using the ParticleScan device to reflect airborne particles at that time. The sample was collected adjacent to the air sampler, in an effort to represent the air sample being collected. The particle counter measurement was obtained halfway through the collection of each air sample, so where 1000L of air was being collected per sample, the particle counter was used when 500L was collected. A display panel on the air sampler provides this information in real-time.

The particle counter operates by using a light emitting diode (LED) light source when the test function is engaged. When engaged, a defined quantity of air is drawn into the device through an isokinetic probe, and particles within this air sample scatter the LED light output toward a series of collection optics. The light that is received by the collection optics (some of which will be inhibited or obscured due to the presence of particles within the sample) is concentrated toward a semiconductor, which converts the light signal input into a series of electrical impulses. Each electrical impulse generated corresponds to a single particle detected within the sample. The device then enumerates the impulses generated and the results are displayed on a digital panel, expressed as particles per cubic litre of air.

For the purpose of this study, the particle counter was used for each active air sample collected, and the sampler was set to measure all particles five microns or larger from each litre of air collected. There is no standard or benchmark available to provide comparison with findings from this study and other multi-bed hospital wards, and no standard exists for airborne particle counts in multi-bed or non-specialist ward areas under current guidance

(Department of Health- Estates and Facilities, 2013). However, airborne particle counts have been significantly correlated with bacterial counts previously, as shown in a study which undertook airborne particle count sampling alongside active air sampling in an operating theatre environment (Dai *et al.*, 2015). In the present study, the use of the particle counter aimed to explore differences in the quantity of airborne particles between two differently ventilated wards, and as an aid to investigate what impact, if any, routine clinical activities have on the number of airborne particles in a multi-bed hospital ward.

3.2.13 Data Analysis

The main outcomes of interest were the prevalence of MSSA and MRSA among individuals occupying a multi-bed hospital room, contamination of sites within the room, and specifically how routine care activites may influence such contamination. Details of statistical methods are provided in Chapter 2, Section 2.8.

3.3 Results

A total of 375 samples were taken over the four sampling days in both ward areas. This included environmental samples (117 active air samples, 100 passive air samples, 120 contact plates) and participant samples (5 patients and 14 HCWs). The nares and oropharynx of HCWs and patients were each sampled for *S. aureus*. Each participant therefore generated two samples that were tested independently for the presence of *S. aureus* (one from the anterior nares and one other from the oropharynx) resulting in a total of 10 samples from patients and 28 samples from HCWs, respectively.

A total of 117 active air samples were obtained over the four sampling days (31/117 and 25/117 during SD1 and SD2 on Ward A, and 30/117 and 31/117 on SD3 and SD4 on Ward B). Each sample equated to one cubic metre of air, and all were obtained in an identical

manner. A total of 100 passive air samples were obtained over the four sampling days using the settle plate method, with 27/100 and 28/100 obtained during SD1 and SD2 on Ward A and 35/100 and 30/100 collected during SD3 and SD4 on Ward B. Settle plates were placed on patient over bed tables for a period of 1 h. These were placed on bed tables of all patients, regardless of inclusion or exclusion in the study. Contact plates were used to sample high-touch horizontal surfaces. A total of 120 samples were taken over the four sampling days, with 27/120 and 28/120 from Ward A during SD1 and SD2, and 34/120 and 31/120 from Ward B on SD3 and SD4.

3.3.1 Prevalence of *S. aureus* among sampled HCWs

MSSA colonisation was observed in 6/14 (42.85%) sampled HCWs. Three of these HCWs were colonised both nasally and orally (Hx0570.1, Hx0536.1, and Hx0538.1) and three were colonised only nasally (Hx0572.2, Hx0574.1 and Hx0534.1). Differing antibiograms were exhibited by nasal and oral isolates from two HCWs colonised at both sites, and two isolates with identical antibiograms were obtained from the remaining HCW colonised at both sites. Of the six colonised HCWs, three were recruited from Ward A and three from Ward B. Two of these were staff nurses who worked the night shift (20:00-08:00) prior to both SD3 and SD4, and one was a student nurse who occupied the study area on SD3. None of the HCWs sampled were found to be colonised with MRSA.

3.3.2 Prevalence of *S. aureus* among sampled patients

Five patients were recruited and sampled as part of this study. *Staphylococcus aureus* was recovered from the nasal sample of only one patient, who was admitted to Ward B during SD4. This patient was 61.6 years of age at the time of sampling, was from Ireland, female,

and had been admitted to hospital in the 24-h period prior to sampling. This patient had not been admitted to hospital in the 12-months prior to the study, nor did she report use of antibiotics or steroids or any previous history of abcesses, boils, osteomyelitis or cellulitis. This patient reported contact with farm animals in the 12-months prior to sampling and reported travel within Europe during that time. No other patients included in the study were colonised at the time of sampling.

Sample rooms were occupied by patients who were not eligible for inclusion in this study as discussed previously. None of these patients were known to be colonised with MRSA at the time of sampling according to ward staff. It is not possible to say whether any of these patients were colonised with MSSA, as MSSA screening is not routinely undertaken in Irish hospitals, and would be identified only by the clinical microbiology laboratory upon culture of a clinical specimen. However, ward staff advised that none of the patients occupying these areas had been treated for a MSSA infection in the 72 h period prior to sample collection. This does not exclude the possibility that un-sampled patients who were not being treated for an MSSA infection could have been colonised with *S. aureus* and could have shed MSSA into the study environment.

3.3.3 Prevalence of *S. aureus* in environmental samples

In total, 117 active air samples, 100 passive air samples and 120 contact plate samples were obtained during the four study days. Putative *S. aureus* colonies cultured using Colorex™ Staph Aureus agar were sub-cultured to MRSA chromogenic media (Colorex) to detect for methicillin-resistance. No growth was observed on MRSA chromogenic media, therefore all recovered isolates were classified as MSSA. The majority were obtained from active air samples (n=38). Passive air samples (settle plates) and contact plates yielded 6 and 11 MSSA isolates, respectively- based on the results of chromogenic media culture.

3.3.3.1 Active air sampling

A total of 117 active air samples were collected, with each air sample representing one cubic metre of air. Slightly more air samples were obtained from Ward B than Ward A. In total, 56 active air samples (corresponding to 56 cubic metres of air) were collected on Ward A (31/56 on SD1 and 25/56 on SD2), whereas 61 active air samples were collected on Ward B (30/61 on SD3 and 31/61 on SD4). This was due to sampling finishing slightly earlier during SD2 during Ward A. A multi-disciplinary ward round commenced at 11:20, and sampling had been due to continue until 12:00 midday. To maintain patient privacy and confidentiality the charge nurse requested that sampling not continue to minimise persons present in the room when clinical information was being discussed. This resulted in six less samples being recovered on SD2.

Following incubation, putative *S. aureus* colonies were observed on 35/117 sample plates. Three air sample plates (A-0801-18, A1501-13 and A1601-10) harboured more than one putative *S. aureus* colony type, i.e. the colony morphology differed. Selected representatives of each colony type were subcultured and further categorised as 'colony a' or 'colony b' etc., from a specific sample and assigned a new isolate identifier (e.g. the two colonies cultured from air sample A0801-18 were sub-cultured and labelled A1801-18a and A1801-18b). Confirmatory identification testing was undertaken on each isolate and all but one (one putative colony of three recovered from air sample A-1601-10) were identified as *S. aureus* (all MSSA). Isolates underwent antimicrobial susceptibility testing, with no two isolates from one air sample exhibiting identical antimicrobial resistance profiles (Table 3.2). It was therefore presumed that these pairs of isolates, from the same air sample in each case (i.e. A-0801-18, A1501-13 and A1601-10) were not identical and were treated as separate isolates.. Therefore, 38 *S. aureus* isolates were recovered from 117 cubic metres of air, revealing an overall prevalence of 32.5%. An investigation of the

relatedness of *S. aureus* isolates recovered from active air sampling is described in Chapter 4.

The prevalence of *S. aureus* recovered from Ward A was higher than Ward B, with 20 *S. aureus colonies* recovered from 56 (20/56; 35.7%) cubic metres of air collected from Ward A samples. This was compared to 18 *S. aureus* colonies recovered from 61 cubic metres of air obtained on Ward B (18/61; 29.6%). Details of air sampling results are outlined in Table 3.1.

3.3.3.2 Passive air sampling

Passive air sampling yielded six *S. aureus* isolates from 100 samples, all of which were MSSA. On SD1 and SD2 on ward A, 40 settle plates samples were collected. *Staphylococcus aureus* was cultured from 3/20 samples collected on SD1, and 2/20 samples collected on SD2. On SD3 and SD4 on Ward B, *S. aureus* was cultured from 0/30 and 2/30 samples from SD3 and SD4, respectively. The sampling area on Ward A had four bedspaces, whereas the sampling area on Ward B had six bedspaces. Settle plates were placed within each near-patient area, on the overbed table. Due to the difference in patient bedspaces between Ward A and Ward B, more settle plates were collected from Ward B (n=60) than Ward A (n=40). No settle plates (used for passive air sampling) yielded more than one *S. aureus* colony per plate, although growth of other bacterial species on these plates was evident. The remit of this study extended only to *S. aureus* isolates, so these other species were not investigated further here. Details of settle plate results are shown in Table 3.3.

Table 3.1 Active air sampling results from the four study days included in the present study

Sample details	Air[1] samples collected (n)	*S. aureus* colonies[2]/cubic metres of air (n, % total)
Ward A (total)[3]	56	20, 35.7%
SD1	31	15, 48.4%
SD2	25	5, 25%
Ward B (total)	61	18, 29.6%
SD3	30	8, 26.6%
SD4	31	10, 32.2%

Abbreviations: SD, study day;

[1]Air samples were taken using an Oxoid/Thermo Scientific model EM0100A air sampler (Oxoid). Each air sample plate represents airborne *S. aureus* colonies recovered from one cubic metre of air.

[2] All samples were cultured using Colorex™ Staph Aureus chromogenic media for the identification of *S. aureus*. Putative *S. aureus* colonies were then subcultured using Colorex™ MRSA to determine whether the isolate was methicillin-susceptible *S. aureus* (MSSA) or methicillin-resistant. No samples exhibited growth using chromogenic media selective for methicillin-resistant *S. aureus* colonies, therefore all were considered MSSA.

[3]Sampling was undertaken on two wards (Ward A and Ward B). Two sampling days were undertaken on Ward A (SD1 and SD2), and two on Ward B (SD3 and SD4).

Table 3.2 Antimicrobial resistance profiles of six discrete *S. aureus* isolates recovered from three active air sampling plates in the present study

Sample	Isolate ID	SD	AR profile
A-0801-18	A-0801-18a	SD1	Ap, Cd,
A-0801-18	A-0801-18b	SD1	Ap, Fd, Cp
A-1501-13	A-1501-13a	SD3	Ap, Er, Sp, Cd
A-1501-13	A-1501-13b	SD3	Ap, Er, Fd, Cp, Sp, Cd
A-1601-10	A-1601-10b	SD4	Ap, Er, Su, Cd
A-1601-10	A-1601-10c	SD4	Ap, Cd

Abbreviations: SD, study day; AR, antimicrobial resistance; Ap, ampicillin; Cd, cadmium acetate; Fd, fusidic acid; Er, erythromycin; Sp, spectinomycin; Su, sulphonamides

Table 3.3: Details of *S. aureus* isolates (all MSSA) that were recovered using passive air sampling during the present study

Isolate ID	Ward	SD	Time sample taken	Details of patient occupying bedspace
S0801-0718	A	SD1	07:00-08:00	Patient was not sampled as part of the study, but was not known to be colonised with *S. aureus* as per ward staff
S0801-0821	A	SD1	08:00-09:00	Patient was enrolled in study, and was found not to be colonised with *S. aureus* at the time of sampling
S0801-1018	A	SD1	10:00-11:00	Patient was not sampled as part of the study, but was not known to be colonised with *S. aureus* as per ward staff
S0901-0918	A	SD2	09:00-10:00	Patient was not sampled as part of the study, but was not known to be colonised with *S. aureus* as per ward staff
S1601-0912	B	SD4	09:00-10:00	Patient was not sampled as part of the study, but was not known to be colonised with *S. aureus* as per ward staff
S1601-1010	B	SD4	10:00-11:00	Patient was enrolled in study, and was found now to be colonised with *S. aureus* at the time of sampling

Abbreviations: SD, study day.

3.3.3.3 Surface sampling

Eleven *S. aureus* isolates were recovered from 120 contact plates. Chromogenic media (Colorex™ Staph Aureus and Colorex™ MRSA, Colorex) determined all 11 isolates to be MSSA. Sampled sites within the near-patient environment included bedframes (n=20), pillows (soiled, following removal from beds and prior to going to laundry; n=6), blankets (n=7), nightstands (n=12), patient notes holders (n=21), tray tables (n=4), dripstands (n=1) and privacy curtains (n=20). Sites sampled at handwashing sinks within patient wards included a tap (n=1), paper towel dispensers (n=1), a hand soap dispenser (n=1), sink surfaces (n=1) and a hand gel dispenser (n=1). Other items included patient notes folders (n=15), vital signs monitors (n=1), window cills (n=7) and the lid of a wastepaper bin (n=1). *Staphylococcus aureus* was recovered from a variety of sites, and these are detailed in Table 3.4.

3.3.4 Characterisation of *S. aureus* isolates recovered

Samples from which *S. aureus* was cultured, and their putative identification based on these results are outlined in Fig 3.2. An in-depth molecular analysis of 62 of these *S. aureus* isolates recovered is described in Chapter 4. Three HCW isolates were omitted from further analysis, and these were obtained from HCWs who were colonised both orally and nasally. In these cases, the nasal and oral samples exhibited similar phenotypic morphologies and antimicrobial susceptibility patterns, i.e. it was felt that the nasal or oral isolates were related. In order to optimise resources for this study, in these instances a representative isolate was chosen to undergo further investigation.

3.3.4.1 Antimicrobial agent resistance

Table 3.4 Results of surface sampling using Colorex™ Staph Aureus contact plates[1] for the recovery of *S. aureus* in the present study

Surface sampled	No. Sampled	Surface finish	MSSA detected (n)	MRSA detected (n)
Bedframes	20	Rough plastic	0	0
Pillows	6	Cotton fabric	0	0
Blankets	7	Rough fabric	0	0
Nightstands	12	Polished vinyl	1	0
Patient notes holders	21	Rough plastic	1	0
Tray tables	4	Rough plastic	0	0
Dripstands	1	Stainless steel	0	0
Privacy curtains	20	Cotton fabric	6	0
Tap	1	Stainless steel	0	0
Paper towel dispenser	1	Smooth plastic	0	0
Hand soap dispenser	1	Smooth plastic	0	0
Sink surface	1	Porcelain	0	0
Hand gel dispenser	1	Smooth plastic	0	0
Notes folders	15	Rough plastic	3	0
Vital signs monitors	1	Smooth plastic	0	0
Window sills	7	Smooth paint	0	0
Wastepaper bin lid	1	Smooth plastic	0	0

Abbreviations: No., number; MSSA, methicillin-susceptible *S. aureus.*

[1] The contact plates used were Colorex™ Staph Aureus chromogenic medium (Colorex). Putative *S. aureus* colonies were then subcultured using Colorex™ MRSA (Colorex) to determine whether the isolate was MSSA or MRSA. The results of these culture results are expressed in this table.

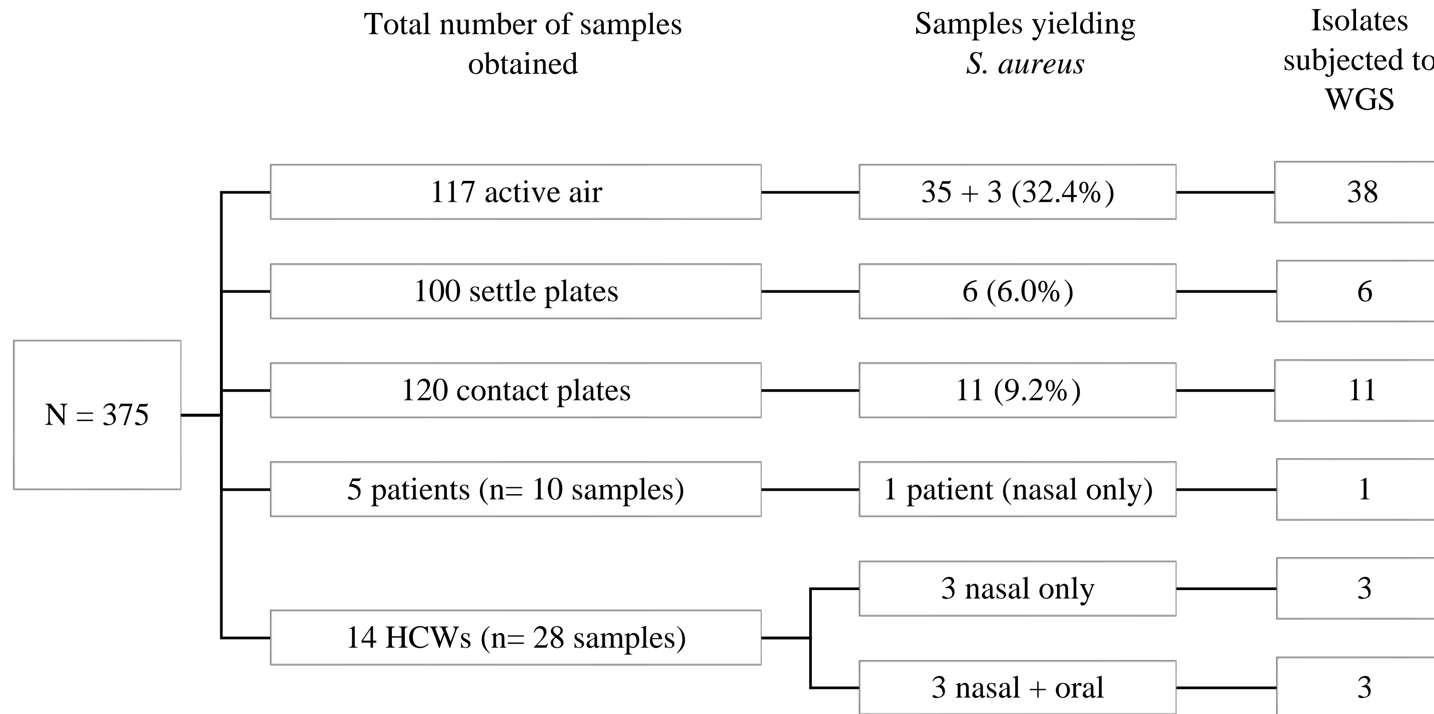


Figure 3.2 Culture results of patient, HCW and environmental samples obtained from two multi-bed urology surgical wards in an Irish teaching hospital. Patients and HCWs were invited to partake in the study on a voluntary basis and sampling included a nasal swab and oral rinse which aimed to detect *S. aureus* colonisation. Active air samples were collected using an Oxoid/Thermo Scientific EM0100A surface air sampler (Oxoid, Ireland) which was programmed to obtain 1000L of air per sample. Settle plates were used to passively sample the air, and each was placed an overbed table for a period of one hour. Contact plates were used to sample high touch surfaces. Chromogenic media were used for the culture and detection of *S. aureus* from all samples. Putative *S. aureus* colonies were then subcultured using Colorex™ MRSA to determine whether the isolate was MSSA or MRSA. None of the above isolates exhibited growth on Colorex ™ MRSA, therefore all were considered MSSA based on culture results.

The susceptibility of the 62 *S. aureus* isolates investigated to a range of antimicrobial agent tested is shown in Table 3.5. Resistance to erythromycin, sulphonamides, trimethoprim, tetracycline, and aminoglycosides was more common among isolates recovered on Ward B. Fusidic acid resistance was observed almost exclusively in isolates recovered on Ward A (of 15 fusidic acid resistant isolates recovered, 13/15 were recovered from Ward A and 2/15 from Ward B). All ciprofloxacin resistant isolates were recovered from Ward B.

All fusidic acid resistant isolates were recovered from environmental samples, which included active air samples (n=11), contact plates (n=1) and settle plates (n=3). Fusidic acid resistance was not observed in the patient or HCW isolates. Resistance to macrolide and tetracycline agents, erythromycin, sulphonamides, spectinomycin and trimethoprim was observed more commonly in environmental isolates. Of the three isolates exhibiting resistance to ciprofloxacin, one was obtained from an active air sample collected during SD3 and two were obtained from participant samples (both from HCWs, and specifically staff nurses who were both sampled on Ward B on SD3).

Seven isolates exhibited resistance to cefoxitin (A1501-05, A1601-08, A1501-14, A1601-09, A1501-11, A1601-01 and C87). These isolates had failed to grow on Colorex™ MRSA culture plates (Colorex), so were previously classified as MSSA. The diameter of the zone of inhibition for these isolates ranged from 17-19 mm (Table 3.5), with the breakpoint for methicillin-resistance considered less than 22 mm (EUCAST, 2019). These isolates were tested for oxacillin susceptibility by E-test strip as outlined in Section 2.7.3. All seven cefoxitin-resistant isolates were found to be susceptible to oxacillin based on E-test results, with results of 0.25-1.0 μg/ml, which is within the susceptible range outlined by CLSI criteria (2018), which classifies resistance to oxacillin as a result of $\geq$ 4 μg/ml. Therefore, these isolates were deemed resistant to cefoxitin and susceptible to oxacillin.

3.3.4.2 Confirmation of methicillin resistance

The seven isolates exhibiting resistance to cefoxitin and susceptibility to oxacillin as described in Section 3.3.4.1 (Table 3.5) were identified as MRSA by the GeneXpert IV MRSA assay (Cepheid). Therefore, these isolates were considered oxacillin-susceptible MRSA (OS-MRSA), as described previously (Hososaka *et al.*, 2007), and are examined in greater detail in Chapter 4.

3.3.5 Surface hygiene assessment, ATP, and surface *S. aureus* recovery

An assessment of surface hygiene and cleanliness was undertaken on each horizontal surface sampled using contact plates. For each contact plate sample obtained (n=120), a visual inspection of the surface was performed and an ATP hygiene assessment test was also undertaken. The hygiene assessment results and whether *S. aureus* was cultured from the corresponding contact plate samples are shown in Table 3.6.

Overall 93/120 surfaces appeared clean, i.e. the surface was intact and not visibly soiled or contaminated, while the remaining 27/120 surfaces failing to meet this standard. This level of surface hygiene appeared consistent over the four sampling days and did not differ significantly (p = 0.72), with 5/27 (19%) and 7/28 (25%) surfaces visibly contaminated on Ward A on SD1 and SD2, and 9/34 (26%) and 6/31 (19%) surfaces visibly contaminated on SD3 and SD4 on Ward B, respectively.

ATP measurements, expressed in relative light units (RLUs), were variable and those observed ranged from 0-581 RLUs (median 15.5 RLUs, interquartile range (IQR) 5-41 RLUs). Highest average levels of ATP were recovered on SD2 from Ward A (median 53 RLUs, IQR 20-115 RLUs), followed by SD1 (median 16 RLUs, IQR 3-24 RLUs), SD3 (median 15 RLUs, IQR 7-36 RLUs), and SD4 (median 6 RLUs, IQR 3-16 RLUs). Highest average levels of ATP were recovered (in the following order) from: blankets

Table 3.5 Antimicrobial resistance testing results of *S. aureus* isolates recovered during the present study

Isolate ID	Type	Result[1]	Resistance Profile	Oxacillin MIC[2]
A-0801-01	Air	MSSA	Ap, Fd, Cd	NT
A-0801-04	Air	MSSA	Ap, Fd, Cd	NT
A-0801-05	Air	MSSA	Ap, Cd	NT
A-0801-06	Air	MSSA	Ap, Fd, Cd	NT
A-0801-07	Air	MSSA	Ap, Er, Cd	NT
A-0801-08	Air	MSSA	Ap, Fd, Cd	NT
A-0801-09	Air	MSSA	Ap, Fd, Cd	NT
A-0801-10	Air	MSSA	Ap, Er, Cd	NT
A-0801-13	Air	MSSA	Ap, Fd, Cd	NT
A-0801-14	Air	MSSA	Ap, Fd, Cd	NT
A-0801-18a	Air	MSSA	Ap, Cd	NT
A-0801-18b	Air	MSSA	Ap, Fd, Cd	NT
A-0801-19	Air	MSSA	Ap, Er	NT
A-0801-22	Air	MSSA	Ap, Fd, Cd	NT
A-0901-08:36	Air	MSSA	Ap, Fd, Cd	NT
A-0901-08:50	Air	MSSA	Susceptible	NT
A-0901-11:03	Air	MSSA	Ap, Cd	NT
A-0901-11:51	Air	MSSA	Ap, Cd	NT
A-1501-05	Air	MRSA	Ap, Te, Su, TMP, Cd, Fox	0.5
A-1501-08b	Air	MSSA	Ap, Er, Cd	NT
A-1501-09	Air	MSSA	Ap, Er, Cd	NT
A-1501-11	Air	MRSA	Ap, Te,Su, TMP, Cd, Fox	0.5
A-1501-13a	Air	MSSA	Ap, Er, Sp, Cd	NT
A-1501-13b	Air	MSSA	Ap, Er, Fd, Cp, Sp, Cd	NT
A-1501-14	Air	MRSA	Te, TMP, Cd, Fox	1.0
A-1601-01	Air	MRSA	Ap, Te, Su, TMP, Cd, Fox	0.5
A-1601-03	Air	MSSA	Ap, Cd	NT
A-1601-05	Air	MSSA	Ap, Te, Su, TMP, Cd	NT
A-1601-08	Air	MRSA	Ap, Te, Su, TMP, Cd, Fox	0.5
A-1601-09	Air	MRSA	Ap, Te, Su, TMP, Cd, Fox	0.5
A-1601-10b	Air	MSSA	Ap, Er, Cd	NT
A-1601-10c	Air	MSSA	Ap, Cd	NT
A-1601-13	Air	MSSA	Ap, Er	NT
A-1601-16	Air	MSSA	Ap, Er, Cd	NT
A-1601-18	Air	MSSA	Ap	NT
A0801-17	Air	MSSA	Ap, Cd	NT
A0901-0721	Air	MSSA	Ap, Cd	NT
A1501-08a	Air	MSSA	Ap, Er,	NT

Continued overleaf

Isolate ID	Type	Result[1]	Resistance Profile	Oxacillin MIC
C104	Contact	MSSA	Ap, Er, Cd	NT
C122	Contact	MSSA	Ap, Er, Su, Cd	NT
C123	Contact	MSSA	Ap, Er, Cd	NT
C37	Contact	MSSA	Ap, Cd	NT
C52	Contact	MSSA	Ap, Cd	NT
C75	Contact	MSSA	Susceptible	NT
C78	Contact	MSSA	Ap, Er, Su	NT
C87	Contact	MRSA	Ap, Te,Su, TMP, Cd, Fox	0.25
C89	Contact	MSSA	Ap, Fd, Cd	NT
C93	Contact	MSSA	Ap, Er, Su	NT
C94	Contact	MSSA	Ap, Er	NT
Hx0534.1	HCW	MSSA	Ap, Cd	NT
Hx0536.1	HCW	MSSA	Ap, Er, Cd	NT
Hx0538.1	HCW	MSSA	Ap, Cd	NT
Hx0570.1	HCW	MSSA	Ap, Cp, Tmp, Cd	NT
Hx0572.1	HCW	MSSA	Ap, Cp, Tmp, Cd	NT
Hx0574.1	HCW	MSSA	Ap, Cd	NT
Px0621	Patient	MSSA	Ap, Cd	NT
S-0801-07-18	Settle	MSSA	Ap, Fd, Cd	NT
S-0801-08-21	Settle	MSSA	Ap, Fd, Cd	NT
S-0801-10-18	Settle	MSSA	Ap, Fd, Cd	NT
S-0901-09-18	Settle	MSSA	Ap, Fd, Cd	NT
S-1601-09-12	Settle	MSSA	Ap, Er, Cd	NT
S-1601-10-10	Settle	MSSA	Ap, Er, Su, Cd	NT

Abbreviations: ID, identifier; MSSA, methicillin-susceptible *S. aureus*; Ap, ampicillin; Cd, cadmium acetate; Cp, ciprofloxacin; Er, erythromycin; Fox, cefoxitin, Fd, fusidic acid; Fox, cefoxitin; Sp, spectinomycin; Su, sulphonamides; Te, tetracycline; TMP, trimethoprim, NT, not tested.

[1]Isolates were considered MRSA where a cefoxitin disc diffusion test result was in the resistant range (CLSI, 2018) and they were identified as MRSA by GeneXpert (Cepheid).

[2]The seven isolates that exhibited resistance to cefoxitin were further tested against oxacillin using oxacillin E-test strips (Biomérieux) and were found to be susceptible.

(n=7, median 22 RLUs, IQR 5-75 RLUs), over-bed tray tables (n=4, median 21 RLUs, IQR 9.5-52 RLUs), pillow cases (immediately prior to being sent to laundry) (n=6, median 17 RLUs, IQR 10-33 RLUs), window sills (n=7, median 17 RLUs, IQR 4-25 RLUs), bedframes (n=20, median 16 RLUs, IQR 6-49 RLUs), curtains (n=20, median 16 RLUs, IQR 5-46 RLUs), end-of-bed holders for patient notes (n=21, median 15 RLUs, IQR 5-48 RLUs), patient notes folders (n=15, median 13.5 RLUs, IQR 6-47 RLUs) and nightstands (n=12, median 11 RLUs, IQR 5-25 RLUs). A number of surface types were sampled on only one occasion, with variable ATP RLUs observed. These included an intravenous fluid stand (383 RLUs), a vital signs monitor (28 RLUs), a wastebin (26 RLUs), an alcohol hand gel rub dispenser (20 RLUs), a sink surface (3 RLUs), a paper towel dispenser (1 RLU), a soap dispenser (1 RLU), a handwashing sink tap handle (1 RLU). Lower values of ATP were recorded from visibly clean surfaces (median 15 RLUs, IQR 5-41 RLUs) compared to those that appeared visibly soiled or dusty (median 16 RLUs, IQR 5-46 RLUs), but this difference was not significant ($p = 0.40$).

MSSA was recovered from 10/120 surfaces sampled, and MRSA from 1/120. Of these 11 surfaces, seven appeared visibly clean. The ten surfaces from which MSSA was recovered included six curtains (of which 3/6 were not visibly soiled), three notes folders (with 3/3 not visibly soiled) and one end-of-bed container that holds patient notes which was visibly soiled. A nightstand, from which MRSA (C87) was recovered appeared visibly clean.

The ATP levels recorded were observed to be higher from surfaces where *S. aureus* was recovered (median 17 RLUs, IQR 6-47 RLUs) compared with surfaces where no *S. aureus* was recovered (median 15 RLUs, IQR 5-41 RLUs), although this difference was not significant ($p = 0.53$).

3.3.6 Observational data from study area

Table 3.6 Results of hygiene assessments, ATP measurements and *S. aureus* culture from 120 surfaces tested in Wards A and B over the four-day study period in the present study

Sample	Ward	Site	ATP (RLUs)[1]	Visual assessment[2]	MSSA/MRSA[3]
C1	A	Tray table	1	Pass	None
C2	A	Window sill	6	Pass	None
C3	A	Vital signs monitor	28	Pass	None
C4	A	Bedframe	0	Fail	None
C5	A	Curtain	21	Pass	None
C6	A	Curtain	47	Pass	None
C7	A	Bedframe	18	Pass	None
C8	A	Curtain	21	Pass	None
C9	A	Bedframe	64	Fail	None
C10	A	Curtain	17	Pass	None
C11	A	Bedframe	52	Fail	None
C12	A	Blanket	14	Pass	None
C13	A	Blanket	10	Pass	None
C14	A	Blanket	8	Pass	None
C15	A	Bin	26	Fail	None
C16	A	Notes holder	4	Pass	None
C17	A	Notes holder	16	Pass	None
C18	A	Notes holder	17	Pass	None
C19	A	Notes holder	36	Pass	None
C20	A	Tap	4	Pass	None
C21	A	Paper towel dispenser	1	Pass	None
C22	A	Sink	3	Pass	None
C23	A	Window sill	24	Pass	None
C24	A	Window sill	3	Pass	None
C25	A	Window sill	1	Pass	None
C26	A	Soap dispenser	1	Pass	None
C27	A	Hand gel	20	Fail	None
C30	A	Window sill	85	Fail	None
C31	A	Window sill	6	Pass	None
C32	A	Window sill	19	Pass	None
C33	A	Drip stand	383	Pass	None
C34	A	Blanket	401	Pass	None
C35	A	Bedframe	30	Pass	None
C36	A	Notes holder	247	Fail	None
C37	A	Notes folder	92	Pass	MSSA recovered
C38	A	Blanket	30	Pass	None
C39	A	Bedframe	325	Pass	None
C40	A	Notes holder	64	Fail	None
C41	A	Notes folder	55	Pass	None
C42	A	Blanket	47	Pass	None
C43	A	Bedframe	261	Fail	None

Continued overleaf

Sample	Ward	Site	ATP[1] (RLUs)	Visual assessment	MSSA/MRSA
C44	A	Notes holder	88	Fail	None
C45	A	Notes folder	4	Pass	None
C46	A	Blanket	22	Pass	None
C47	A	Bedframe	10	Pass	None
C48	A	Notes holder	122	Fail	None
C49	A	Notes folder	10	Fail	None
C50	A	Curtain	90	Pass	None
C51	A	Curtain	10	Pass	None
C52	A	Curtain	80	Pass	MSSA recovered
C53	A	Curtain	47	Pass	None
C54	A	Pillow	17	Pass	None
C55	A	Pillow	51	Pass	None
C56	A	Pillow	40	Pass	None
C57	A	Pillow	247	Pass	None
C60	B	Bedframe	24	Pass	None
C61	B	Tray table	3	Pass	None
C62	B	Pillow	1	Pass	None
C63	B	Nightstand	9	Pass	None
C64	B	Notes holder	15	Fail	None
C65	B	Folder	25	Fail	None
C66	B	Bedframe	22	Fail	None
C67	B	Tray table	13	Fail	None
C68	B	Pillow	11	Pass	None
C69	B	Nightstand	267	Fail	None
C70	B	Notes folder	36	Pass	None
C71	B	Notes holder	15	Pass	None
C72	B	Bedframe	24	Pass	None
C73	B	Tray table	2	Pass	None
C74	B	Nightstand	0	Pass	None
C75	B	Notes folder	1	Pass	MSSA recovered
C76	B	Notes holder	4	Pass	None
C77	B	Bedframe	5	Pass	None
C78	B	Notes holder	90	Fail	MSSA recovered
C79	B	Nightstand	7	Pass	None
C80	B	Folder	9	Pass	None
C81	B	Bedframe	16	Pass	None
C82	B	Notes holder	367	Fail	None
C83	B	Nightstand	33	Pass	None
C84	B	Folder	581	Fail	None
C85	B	Bedframe	55	Pass	None
C86	B	Notes holder	63	Pass	None

Continued overleaf

Sample	Ward	Site	ATP (RLUs)	Visual Assessment	MSSA/MRSA
C87	B	Nightstand	4	Pass	MRSA recovered
C88	B	Folder	50	Pass	None
C89	B	Curtain	7	Pass	MSSA recovered
C90	B	Curtain	33	Pass	None
C91	B	Curtain	11	Pass	None
C92	B	Curtain	8	Pass	None
C93	B	Curtain	57	Fail	MSSA recovered
C94	B	Curtain	12	Pass	MSSA recovered
C95	B	Bedframe	4	Pass	None
C96	B	Folder	4	Pass	None
C97	B	Notes holder	3	Pass	None
C98	B	Nightstand	6	Pass	None
C99	B	Bedframe	1	Pass	None
C100	B	Folder	6	Pass	None
C101	B	Notes holder	12	Pass	None
C102	B	Nightstand	5	Pass	None
C103	B	Bedframe	14	Pass	None
C104	B	Folder	13	Pass	MSSA recovered
C105	B	Notes holder	3	Pass	None
C106	B	Nightstand	23	Pass	None
C107	B	Bedframe	9	Pass	None
C108	B	Folder	17	Pass	None
C109	B	Notes holder	15	Pass	None
C110	B	Nightstand	1	Pass	None
C111	B	Bedframe	50	Fail	None
C112	B	Folder	41	Fail	None
C113	B	Notes holder	9	Pass	None
C114	B	Nightstand	6	Pass	None
C115	B	Bedframe	3	Pass	None
C116	B	Folder	2	Pass	None
C117	B	Notes holder	5	Fail	None
C118	B	Nightstand	5	Pass	None
C119	B	Curtain	25	Fail	None
C120	B	Curtain	10	Pass	None
C121	B	Curtain	3	Pass	None
C122	B	Curtain	17	Fail	MSSA recovered
C123	B	Curtain	20	Fail	MSSA recovered
C124	B	Curtain	2	Pass	None

[1]ATP values are expressed in relative light units (RLUs). There are no definitive thresholds or pass/fail results available for ATP measurements, as this is an emerging technology and is not yet embedded in guidelines for hospital cleaning. However, a proposed guideline in

the literature of a reading in excess of 100 RLUs constituting a breach in hygiene standards has been proposed by Mulvey *et al*. (2011) and further evaluated in subsequent studies in similar active acute hospital settings.

[2]Visual inspection is the current practice of hygiene assessment in nationally and internationally (Health Service Executive, 2006). Hygiene standards have not been met under these guidelines if the surface is damaged in any way, or if there is soil or dust present. For the purpose of this study, a 'hygiene pass' constitutes a smooth, intact and visibly clean surface.

[3] Colorex^{TM} Staph Aureus (chromogenic medium) contact plates were used to sample each assessed surface for the presence of *S. aureus*. Isolates underwent susceptibility testing and were deemed MRSA if resistance was exhibited to cefoxitin and MRSA was identified by GeneXpert (Cepheid). Results are expressed as whether MSSA or MRSA was cultured from a surface, i.e. 'MSSA recovered', 'MRSA recovered' or 'None'.

Throughout the sampling period, activities and conditions (occupant density, the presence of HCWs and patients who had not been screened for *S. aureus* carriage but were within the study areas) were recorded. Variables previously reported in the literature to increase levels of airborne *S. aureus* were also included, such as the use of nebulised medications, coughing or sneezing, washing and dressing, bedmaking and drawing or closing of privacy curtains. Observations were recorded at 10-min intervals and corresponded with an active air sample collected during the same 10-min period. Details of observed activities are provided in Table 3.7.

All periods of observation and sampling began in the early morning (07:00 h), towards the end of the night shift and prior to the beginning of daily clinical routines (e.g. clinical rounds, washing and dressing, bed-making). On all sampling days, the activity in each of the ward rooms at the commencement of the observation period reflected patient rest time, with the majority of patients having spent the preceding hours asleep or resting in bed. The only exception to this was on SD3, where a patient had been transferred out of the ward overnight, and in the 30-min period prior to sampling her bed had been stripped of linens and bedsheets. A clean of the area around this patient's specific bed area and of the hospital bed itself was undertaken by two housekeeping staff, and this was completed by 06:50 h, just prior to commencement of the sampling routine. This cleaning included the application of detergent/disinfectant to all surfaces of the locker, overbed table, bedframe, mattress using a microfiber cloth, and to the floor surrounding this patient's bed using a mop. The privacy curtains surrounding the bed were not changed, as the hospital policy advises changing of privacy curtains only if the patient is known or suspected to be colonised with a multidrug resistant organism, or has diarrhoeal symptoms prior to transfer, and this patient did not meet these criteria. This patient had not been deemed eligible to participate in the study, as it was determined during the previous day that she did not have capacity to consent. Both housekeeping staff declined inclusion in the study.

Ward area occupants

Occupant density per 10-min period was observed throughout the study period (Table 3.7). Highest numbers of people occupying the study areas were observed on the two sampling days undertaken on Ward B (median 9, IQR 9-11) compared to the days spent on Ward A (median 7, IQR 6-8), with these differences highly significant (p <0.001). The layout of these wards is different, with the room included in the study on Ward A having four beds, whereas there was six beds on Ward B. Also, on Ward B, there was one supernumerary nursing student assigned to assist the staff nurse in the study area on both days, whereas there were no students assigned to Ward A. No significant association was found with increased room occupants and airborne *S. aureus* recovery within the same period, and no pattern was observed with recovery of *S. aureus* in the samples following peaks in occupant density. The presence of a HCW who was known to be colonised with *S. aureus* was not significantly associated with recovery of *S. aureus* (p = 0.34). No HCWs or patients were known to be colonised with MRSA.

Airborne particle counts

Airborne particle counts were obtained, with significant differences observed between the HEPA-filtered Ward A (mean 64.0, standard deviation (SD) 59.1) and Ward B, which is naturally ventilated (mean 239.1, SD 114.7; p <0.001) (Table 3.7). Particle counts were higher, although not significantly so, in samples that yielded *S. aureus*, with average particle counts measured at 167.9 (SD 150.0) in *S. aureus*-positive episodes compared with 154 (SD 119.1) when there was no recovery of *S. aureus*. Increased particle counts were associated with bed-making and housekeeping, although this was not significantly different to periods where these activities did not occur. Use of nebulised medications resulted in significantly elevated airborne particle counts (p <0.05). Both movement of privacy

curtains and washing and dressing were also significantly associated with increased particle counts ($p < 0.05$), although these activities were highly associated ($p < 0.001$) with one another, as patients dressing at their bedside, or being assisted by HCWs, all first pulled the curtains before doing so. Details of activities observed and related airborne particle counts are provided in Fig. 3.3.

Although a greater number of ward occupants was not found to be significantly associated with airborne *S. aureus* recovery, a bivariate correlation analysis confirmed a significant positive linear relationship ($p < 0.001$) between increasing occupant density and increasing airborne particle counts.

Cleaning of study area

Cleaning of the study areas was observed on just four occasions during the observation period on each ward. This process included damp dusting of surfaces and mopping of floor areas, and was undertaken by housekeeping staff. On one of the four occasions where cleaning was undertaken, *S. aureus* was recovered from the active air sample. This may potentially reflect agitation of *S. aureus* contamination on floors or surfaces and dissemination into ward air by the mechanism of cleaning. The housekeeping staff undertaking the cleaning declined participation in the study, and a different member of staff completed cleaning on each sampling day.

Movement of privacy curtains

The movement of privacy curtains was observed on 52/117 sampling occasions (Table 3.7). *Staphylococcus aureus* was recovered during 17/52 (33.1%). On 26/52 occasions

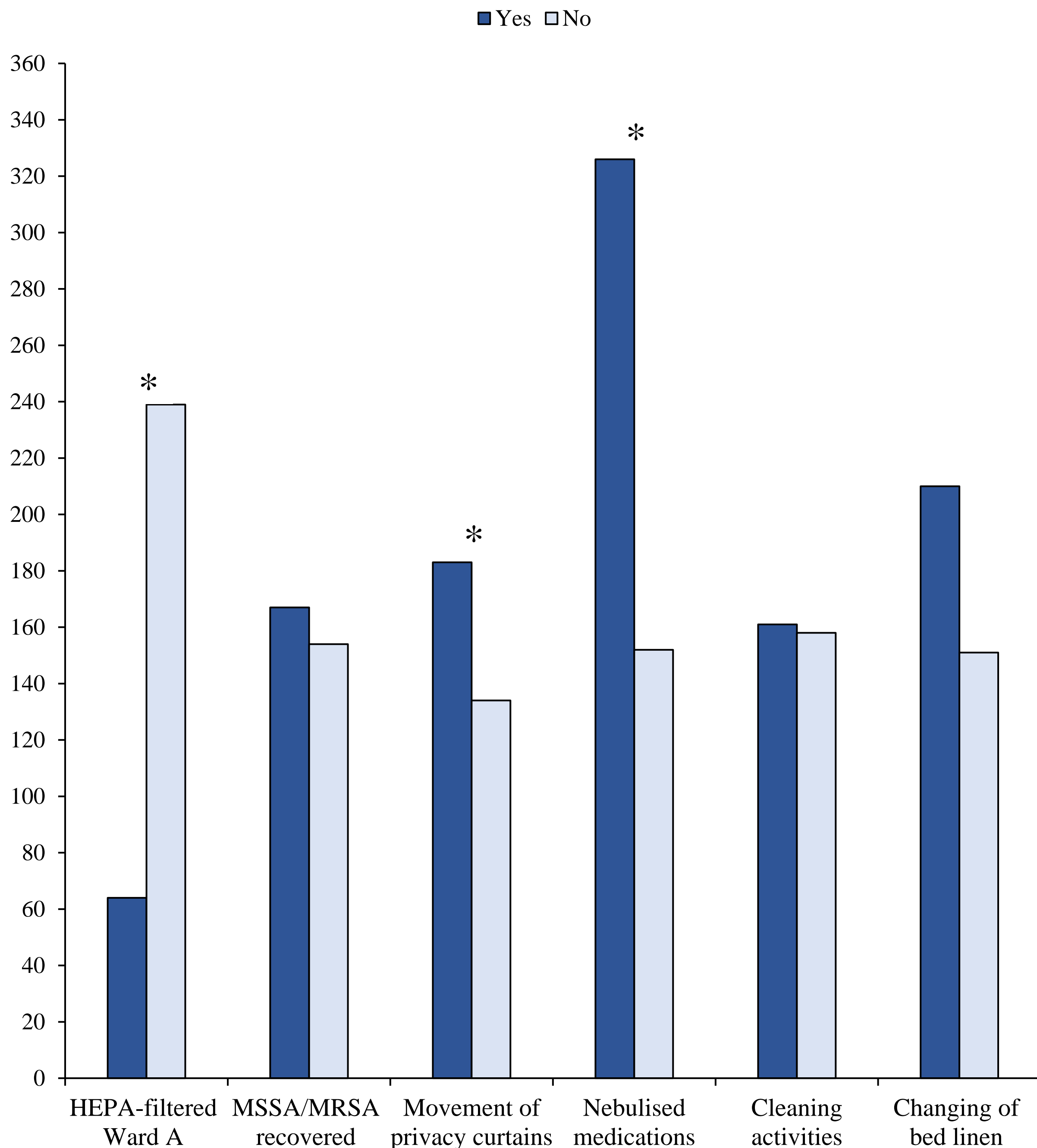


Figure 3.3 A clustered column chart detailing airborne particle counts recorded with respect to clinical activities that were being undertaken concurrently, and concurrently collected active air samples. Sampling was undertaken on two urology surgical wards in an Irish tertiary referral hospital. An airborne particle counter (ParticleScan Pro^{TM} Airborne Particle Counter (IQ Air, Switzerland)) was used to enumerate suspended particles. Clinical activities were observed. Active air samples were collected using an Oxoid/Thermo Scientific EM0100A surface air sampler (Oxoid, Ireland) and were cultured

using Colorex^{TM} Staph Aureus chromogenic media for the identification of *S. aureus*. Putative S. aureus colonies were then subcultured using Colorex^{TM} MRSA to determine whether the isolate was MSSA or MRSA. Differences in observed particle counts were investigated by the Mann Whitney U test, with P values < 0.05 considered statistically significant. Variables in which statistically significant differences were observed are marked in the chart by an asterisk symbol.

when privacy curtains were used, this was to facilitate patient washing and dressing at their bedside, with *S. aureus* recovered from 9/26 such occasions, and a non-significant association observed. On the remaining 26 occasions when curtains were agitated for other reasons, this was done by clinical staff to facilitate privacy either for communication, minor bedside procedures or clinical review. *Staphylococcus aureus* was recovered on only two occasions from active air samples when a curtain had not been agitated in the 20-min period prior to sample collection, showing that movement of privacy curtains, which are known fomites for environmental *S. aureus* is significantly associated with recovery of airborne *S. aureus* in the following twenty minutes ($p < 0.05$).

Changing bed linen

Healthcare workers were observed removing and changing bed linen on patient beds on 13/117 occasions during the study period. On seven occasions, *S. aureus* was recovered from the air sample collected during bed linen changing. In five of these seven occasions, *S. aureus* was recovered both from the concurrently collected sample, and in the subsequent sample obtained also.

Use of nebulisers

Nebulised medication was observed to be administered by clinical staff during 4/117 observation periods, with *S. aureus* recovered on one such occasion. Although nebulised medication was strongly associated with significantly elevated airborne particle counts ($p < 0.05$), an effect on airborne *S. aureus* was not observed. However, the patient who received these nebulised respiratory treatments was screened for *S. aureus* carriage as part of this investigation, and was found not to be colonised.

Table 3.7 Details of active air sampling culture results, airborne particle count values, room occupancy and movement of privacy curtains

Sample ID	Ward	SD	*S. aureus* culture results[1]	APC[2]	Occupant density[3]	Curtain movement[4]
A0801-01	A	1	MSSA	15	6	No
A0801-02	A	1	Not recovered	10	6	Yes
A0801-03	A	1	Not recovered	49	11	Yes
A0801-04	A	1	MSSA	14	8	Yes
A0801-05	A	1	MSSA	122	11	Yes
A0801-06	A	1	MSSA	175	9	Yes
A0801-07	A	1	MSSA	240	8	No
A0801-08	A	1	MSSA	166	8	Yes
A0801-09	A	1	MSSA	140	7	Yes
A0801-10	A	1	MSSA	120	5	Yes
A0801-11	A	1	Not recovered	44	6	No
A0801-12	A	1	Not recovered	35	12	Yes
A0801-13	A	1	MSSA	21	6	No
A0801-14	A	1	MSSA	52	6	No
A0801-15	A	1	Not recovered	95	9	No
A0801-16	A	1	Not recovered	67	11	Yes
A0801-17	A	1	MSSA	63	5	Yes
A0801-18	A	1	MSSA	27	3	No
A0801-19	A	1	MSSA	10	2	No
A0801-20	A	1	Not recovered	46	6	Yes
A0801-21	A	1	Not recovered	40	6	Yes
A0801-22	A	1	MSSA	114	7	Yes
A0801-23	A	1	Not recovered	39	6	Yes
A0801-24	A	1	Not recovered	64	8	Yes
A0801-25	A	1	Not recovered	27	8	No
A0801-26	A	1	Not recovered	44	8	No
A0801-27	A	1	Not recovered	32	8	No
A0801-28	A	1	Not recovered	30	6	Yes
A0801-29	A	1	Not recovered	41	8	No
A0801-30	A	1	Not recovered	38	8	No
A0801-31	A	1	Not recovered	30	7	No
A0901-0702	A	2	Not recovered	14	6	No
A0901-0712	A	2	Not recovered	17	6	No
A0901-0721	A	2	MSSA	189	8	No
A0901-0736	A	2	Not recovered	27	5	Yes
A0901-0750	A	2	Not recovered	38	5	Yes
A0901-0802	A	2	Not recovered	20	12	No
A0901-0813	A	2	Not recovered	20	7	Yes
A0901-0826	A	2	Not recovered	120	6	Yes

Continued overleaf

Sample ID	Ward	SD	*S. aureus* culture results	APC	Occupant density	Curtain movement
A0901-0836	A	2	MSSA	125	6	No
A0901-0850	A	2	MSSA	114	6	Yes
A0901-0902	A	2	Not recovered	96	6	Yes
A0901-0912	A	2	Not recovered	74	9	No
A0901-0925	A	2	Not recovered	81	4	Yes
A0901-0935	A	2	Not recovered	70	6	Yes
A0901-0949	A	2	Not recovered	150	6	Yes
A0901-1000	A	2	Not recovered	105	6	No
A0901-1013	A	2	Not recovered	80	12	No
A0901-1027	A	2	Not recovered	42	9	Yes
A0901-1038	A	2	Not recovered	28	5	Yes
A0901-1050	A	2	Not recovered	45	5	Yes
A0901-1103	A	2	MSSA	50	4	No
A0901-1120	A	2	Not recovered	24	5	No
A0901-1132	A	2	Not recovered	113	10	Yes
A0901-1145	A	2	Not recovered	100	10	Yes
A0901-1151	A	2	MSSA	195	10	No
A1501-01	B	3	Not recovered	360	6	No
A1501-02	B	3	Not recovered	275	7	No
A1501-03	B	3	Not recovered	218	7	No
A1501-04	B	3	MRSA	182	6	No
A1501-05	B	3	MRSA	191	8	No
A1501-06	B	3	Not recovered	244	9	No
A1501-07	B	3	Not recovered	361	9	Yes
A1501-08	B	3	MSSA	310	10	Yes
A1501-09	B	3	MSSA	390	8	No
A1501-10	B	3	Not recovered	270	8	No
A1501-11	B	3	MRSA	323	9	No
A1501-12	B	3	Not recovered	351	11	No
A1501-13	B	3	MSSA	295	9	No
A1501-14	B	3	MSSA	390	14	No
A1501-15	B	3	Not recovered	440	11	No
A1501-16	B	3	Not recovered	520	6	No
A1501-17	B	3	Not recovered	540	14	Yes
A1501-18	B	3	Not recovered	310	9	No
A1501-19	B	3	Not recovered	224	7	Yes
A1501-20	B	3	Not recovered	180	8	No
A1501-21	B	3	Not recovered	227	9	No
A1501-22	B	3	Not recovered	180	9	No
A1501-23	B	3	Not recovered	182	9	Yes
A1501-24	B	3	Not recovered	108	8	No
A1501-25	B	3	Not recovered	126	7	No
A1501-26	B	3	Not recovered	120	8	No

Continued overleaf

Sample ID	Ward	SD	*S. aureus* culture results	APC	Occupant density	Curtain movement
A1501-27	B	3	Not recovered	118	6	No
A1501-28	B	3	Not recovered	132	6	No
A1501-29	B	3	Not recovered	140	8	No
A1501-30	B	3	Not recovered	127	9	No
A1601-01	B	4	MRSA	354	7	No
A1601-02	B	4	Not recovered	184	6	Yes
A1601-03	B	4	MSSA	142	7	No
A1601-04	B	4	Not recovered	164	5	Yes
A1601-05	B	4	MSSA	290	7	Yes
A1601-06	B	4	Not recovered	142	12	Yes
A1601-07	B	4	Not recovered	157	12	Yes
A1601-08	B	4	MRSA	395	13	No
A1601-09	B	4	MRSA	380	12	No
A1601-10	B	4	MSSA	399	16	Yes
A1601-11	B	4	Not recovered	297	9	Yes
A1601-12	B	4	Not recovered	249	9	Yes
A1601-13	B	4	MSSA	295	9	No
A1601-14	B	4	Not recovered	172	9	Yes
A1601-15	B	4	Not recovered	181	9	No
A1601-16	B	4	MSSA	390	11	Yes
A1601-17	B	4	Not recovered	172	11	No
A1601-18	B	4	MSSA	340	12	Yes
A1601-19	B	4	Not recovered	185	8	No
A1601-20	B	4	Not recovered	190	10	Yes
A1601-21	B	4	Not recovered	195	10	Yes
A1601-22	B	4	Not recovered	100	10	Yes
A1601-23	B	4	Not recovered	190	10	Yes
A1601-24	B	4	Not recovered	271	9	Yes
A1601-25	B	4	Not recovered	277	11	Yes
A1601-26	B	4	Not recovered	189	7	No
A1601-27	B	4	Not recovered	185	7	No
A1601-28	B	4	Not recovered	220	7	No
A1601-29	B	4	Not recovered	210	8	No
A1601-30	B	4	Not recovered	229	7	No
A1601-31	B	4	Not recovered	230	8	No

Abbreviations: Sample ID, sample identifier; SD, study day; APC, airborne particle counts; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

[1]Active air samples were collected using an Oxoid/Thermo Scientific EM0100A surface air sampler (Oxoid, Ireland) and were cultured using Colorex^{TM} Staph Aureus chromogenic media for the identification of *S. aureus*. Putative S. aureus colonies were

then subcultured using Colorex™ MRSA to determine whether the isolate was MSSA or MRSA.

[2]An airborne particle counter (ParticleScan Pro™ Airborne Particle Counter (IQ Air, Switzerland)) was used to enumerate suspended particles.

[3]The number of individuals occupying the study area was recorded.

[4]Movement of privacy curtains was recorded.

3.3.7 Demographic data relating to sampled HCWs

Of the HCWs sampled, the majority were nurses (including students, specialists or managers) or healthcare assistants. In detail, the sample included staff nurses (n=8), nursing students (n=2), healthcare assistants (n=3) and one ward attendant (n=1). Demographic data relating to these HCWs is provided in Table 3.9.

Of the fourteen HCWs sampled, gender was equally distributed with 50% male and 50% female. The age profile of HCWs varied across the group, with 4/14 (28.6%) aged 18-24, 3/14 (21.3%) aged 25-34, 2/14 (14.3%) aged 35-44, 4/15 (28.6%) aged 45-54 and 1/14 (7.1%) aged 55-64. Participating HCWs were asked to quantify their average number of hours spent engaged in direct patient contact during a typical work shift. All HCWs sampled with the exception of the ward attendant reported between 8-12 h direct patient contact per working day, whereas the ward attendant reported between 1-2 h only. The majority of HCWs had been employed at this hospital for greater than 6 years (6/14), with 5/14 reporting an employment length of 1-5 years and 3/14 employed for less than one year. Previous employment in a healthcare facility was reported by 5/9 HCWs. In the 12 months prior to sampling, one HCW was admitted to hospital as an in-patient, 3/10 had contact with farm animals, 4/14 had consumed antibiotics, none used steroids, and half (n=7) had travelled abroad. Of those that travelled outside Ireland, five remained within Europe, one visited the USA and one other visited India. The majority of HCWs were born in Ireland (n=11), but also originated from Poland (n=1; less than 5 years in Ireland), India (n=1; less than 5 years in Ireland) and England (n= 1; greater than 10 years in Ireland). No HCWs reported any history of boils, abscesses, osteomyelitis or cellulitis.

3.3.8 Demographic data relating to sampled patients

Table 3.8 Demographic data provided by HCWs who participated in the study

HCW ID	Ward	*S. aureus* colonisation status[2]	Age range	Sex	Country of birth	Hours of patient contact[3]	Length of employment in years[4]	Previous HCF employment[5]	Previous HCF admission[6]	Antibiotic use[7]	Steroid use[8]
Hx0566.1	B	NC	25-34	Female	Ireland	>12	6-10	Yes	No	Yes	No
Hx0568.1	B	NC	18-24	Male	Ireland	8-12	<1	No	No	No	No
Hx0570.1	B	MSSA	25-34	Male	Poland	8-12	1-5	Yes	No	No	No
Hx0572.1	B	MSSA	18-24	Male	Ireland	8-12	1-5	No	No	No	No
Hx0574.1	B	MSSA	25-34	Male	Ireland	1-2	6-10	No	Yes	No	No
Hx0576.1	B	NC	45-54	Female	Ireland	8-12	6-10	Yes	No	No	No
Hx0534.1	A	MSSA	45-54	Male	Ireland	8-12	1-5	Yes	No	Yes	No
Hx0540.1	B	NC	18-24	Female	Ireland	8-12	<1	No	No	Yes	No
Hx0536.1	A	MSSA	45-54	Female	Ireland	8-12	>10	No	No	No	No
Hx0538.1	A	MSSA	35-44	Male	India	8-12	1-5	No	No	No	No
Hx0542.1	B	NC	45-54	Female	Ireland	>12	<1	No	No	No	No
Hx0022.3	B	NC	55-64	Female	England	8-12	>10	No	No	Yes	No
Hx0060.2	B	NC	18-24	Female	Ireland	>12	1-5	No	No	No	No
Hx0074.3	A	NC	35-44	Male	Ireland	8-12	>10	Yes	No	No	No

Abbreviations: NC, not colonised

[1]All HCWs were frontline nurses or healthcare assistants, with the exception of Hx0574.1, who was a ward attendant, so did not undertake clinical duties but worked closely with the ward team managing equipment and medication stocks. Direct contact may have included conversing with patients and assisting with escorting to different areas of the hospital, but would not have included clinical duties.

[2] Colonisation status reflect whether MSSA or MRSA was cultured from either a nasal or an oral sample from each participant.

[2]Participants were asked to estimate the average No. of hours they spend engaged in direct patient contact in a typical working day.

[3]Participants were asked how long they were employed in their current job role.

[4]Participants were asked whether they had previously worked in a different healthcare facility.

[5]Participants were asked whether they had been admitted (as a patient) to a healthcare facility in the 12 months prior to sampling.

[6]Participants were asked whether they had used antibiotics in the 12 months prior to sampling.

[7]Participants were asked whether they had used steroids in the 12 months prior to sampling.

Eleven patients occupied the study area. Four patients in Ward A occupied the study ward, and there was no changes to the patient cohort over the two study days. Two of these patients were included in the study, and two were not included as they were unable to provide consent. Seven patients occupied the study area on Ward B. One patient was unable to consent, and was moved to a different ward between the first and second study day. A patient was admitted to the same bed space during the course of the second study day, and this patient consented to be sampled. Two other patients who occupied the study room on both days were sampled, and three were not recruited, as they could not consent or declined. Therefore, of a population of eleven patients, five were included (Table 3.10)

The median age of patients sampled was 60.6 years (range 43.6-61.6 years). Both patients sampled on Ward A were male and all from Ward B were female, reflecting the same-sex room sharing policy in this hospital. Four patients were born in Ireland, with one female patient born in England but living in Ireland for longer than 10 years. The median length of inpatient stay was fifteen days (range 1-35). Within 12 months of sampling, 2/5 patients reported contact with farm animals, 3/5 patients had been admitted to hospital on a separate occasion (and all to this hospital), 4/5 reported using antibiotics and 3/5 had used steroids. All five patients had travelled abroad in the 12-month period prior to sampling, and this travel was limited to Europe. No patients had a history of abscesses, boils, osteomyelitis or cellulitis.

Of the five patients who consented to partake in the study, two were patients of Ward A and three of Ward B. The average length of stay of the patients sampled on Ward A was slightly longer (mean: 20 days) than patients sampled on Ward B where the mean length of stay was 13.7 days. However, the study was not powered to reflect differences in length of stays to either ward, so the duration of the participating patients hospital admissions is unlikely to reflect the typical length of stay of either ward, including during the sampling period.

Table 3.9 Demographic data provided by all patients who participated in the study

ID	Ward	LOS	Sampling Result[1]	Age (years)	Sex	Country of birth	Previous admission to HCF[2]	Antibiotic use[3]	Steroid use[4]	Travel abroad[5]
Px0617	B	5	NC	61.5	Female	UK	Yes	Yes	Yes	Yes
Px0619	B	35	NC	60.6	Female	Ireland	Yes	Yes	Yes	Yes
Px0621	B	1	MSSA	61.6	Female	Ireland	No	No	No	Yes
Px0613	A	18	NC	43.6	Male	Ireland	Yes	Yes	Yes	Yes
Px0615	A	15	NC	56.7	Male	Ireland	No	Yes	No	Yes

Abbreviations: LOS, length of stay; MSSA, methicillin-susceptible *S. aureus*; HCF, healthcare facility.

[1]Sampling results reflect whether MSSA or MRSA was cultured from either a nasal or an oral sample from each participant.

[2]Participants were asked whether they had been admitted to a healthcare facility in the 12 months prior to sampling.

[3]Participants were asked whether they had used antibiotics in the 12 months prior to sampling.

[4]Participants were asked whether they had used steroids in the 12 months prior to sampling.

[5]Participants were asked whether they had travelled outside the island of Ireland in the 12 months prior to sampling.

3.4 Discussion

This study investigated the prevalence of MSSA and MRSA in the environment and among patient and HCW occupants of two differently ventilated hospital wards in a historically MRSA-endemic Irish hospital (Fig 3.2). Clinical and housekeeping activities were observed with respect to their effect on environmental contamination with MSSA and MRSA, and on airborne particle counts. Sampling of 19 participants for carriage of *S. aureus* was undertaken, yielding 10 MSSA isolates. Air sampling was undertaken both actively (resulting in 117 samples) using an impact air sampler and passively (resulting in 100 samples) using the settle plate method as previously described (Pasquarella *et al.*, 2000). Air sampling yielded a total of 44 *S. aureus* isolates, with 38 of these recovered by active air sampling (32 MSSA and 6 MRSA) and the remaining six by passive air sampling (5 MSSA and 1 MRSA). Surfaces were assessed for *S. aureus* contamination on 120 occasions using contact plates (yielding 11 MSSA isolates and one MRSA isolate), and surface hygiene assessments were undertaken concurrently on each sampled surface both visually and using an ATP luminometer. Routine clinical activities were found to increase particle counts, and increased particle counts were associated with the presence of *S. aureus* in the air, although not significantly.

This study indicated that the air of general hospital wards (regardless of ventilation) comprises a seemingly dynamic population of *S. aureus*. The agitation of fomites contaminated with skin squames is likely to be a significant contributor to this. By identifying routine clinical activities that increase airborne and resultant surface contamination, cleaning routines can be targeted toward high-risk periods where these activities are concentrated, to mitigate onward dissemination to adjacent patients.

Participant sampling

MSSA colonisation of participants in this study was 35% (7/20), a slightly higher figure than anticipated based on cross-sectional population studies (Graham *et al.*, 2006; Gamblin *et al.*, 2013). However, this study did not aim to estimate prevalence of *S. aureus* within this population, so was not powered to do so. As such, this figure of 35% cannot be interpreted as a reflection of *S. aureus* prevalence within this setting, but rather a reflection of the contribution of room occupants to the environmental burden of *S. aureus* observed. No participants sampled were positive for MRSA.

Environmental sampling

Active air sampling yielded 38 *S. aureus* isolates, and six of these were MRSA. Ward A, the HEPA-filtered setting, yielded 20 *S. aureus* isolates from 56 separate cubic metre air samples taken during the study period (35.7%). This was a slightly higher rate than that observed in Ward B, which is naturally ventilated, where 61 separate one cubic metre air samples yielded 18 MSSA isolates (29.5%). The microbial composition of air in naturally ventilated spaces is less consistent than spaces equipped with mechanical ventilation systems and naturally ventilated hospital wards have been shown to harbour higher levels of airborne bacteria, the majority of which tend to be Gram-positive (Crimi *et al.*, 2006). In this study, the increased prevalence of airborne MSSA in the HEPA-filtered ward suggests a source within the space was contaminating the air consistently. As no patients were colonised with *S. aureus*, a colonised HCW may have been shedding *S. aureus* to the environment. However, sampling for the purpose of this study included only the nares and oropharynx, so colonisation limited to other anatomical sites not included in the sampling regimen could potentially account for this. All MRSA isolates were recovered from Ward on SD3 and SD4.

MRSA was recovered from 6/117 (5.1%) of active air samples within the present study, and no patients or HCWs known to be colonised with MRSA were present at this time- although not all those present were included in the study. Therefore, an unaccounted for source of MRSA within the study area may have influenced the recovery of MRSA from air samples. In a previous study undertaken in an open bay area of a naturally ventilated ward within this hospital, although six years ago, MRSA was isolated from the air in the absence of colonised patients on 10/132 (7.6%) sampling occasions (Creamer *et al.*, 2014). Studies undertaken in different settings have failed to recover MRSA from the air of rooms occupied with patients known not to be colonised with MRSA (Rohr *et al.*, 2009), or have noted MRSA is no longer recovered from ward air when a colonised patient has left the ward area (Khojasteh *et al.*, 2007). Factors such as ventilation and HCW colonisation tend not to be factored into such studies, which may explain this.

Passive air sampling using settle plates recovered *S. aureus* on 6/100 (6.0%) occasions, all of which were MSSA. Settle plates were placed on patients over-bed tables for a period of an 60 min as described by (Pasquarella *et al.*, 2000). Overbed tables are among the most frequently touched sites by the hands of patients in general ward settings (Adams *et al.*, 2017); one study observed that tables were touched on average 12 times per hour (Cheng *et al.*, 2015). In active clinical settings, the area immediately adjacent to patients has been shown to be most frequently contaminated with multiple bacterial species, including staphylococci (Moore *et al.*, 2013). Passive sampling has been well correlated with surface deposition and contamination, and it has been suggested that this can be used as a useful metric of hospital cleanliness (Smith *et al.*, 2018). Recovery rates of *S. aureus* from passive sampling is variable (Sexton *et al.*, 2006; Carvalho *et al.*, 2007; Khojasteh *et al.*, 2007; Gizaw *et al.*, 2016; Getachew *et al.*, 2018), and influenced by factors including lack of capacity or poor infrastructure (Gizaw *et al.*, 2016), overcrowding (Fekadu and Getachewu, 2015), agitation of contaminated surfaces such as during cleaning activities

(Gizaw *et al.*, 2016), and the presence of colonised individuals, all of which may contribute to the contamination surfaces at faster rates (Sexton *et al.*, 2006). The approach used to recover *S. aureus* can also be influential and the culture media used tends to vary between studies, as there is as yet no standardized approach. Chromogenic medium was used in the present study, which has been shown to be superior than conventional culture methods for the detection of *S. aureus* (Hirvonen *et al.*, 2014).

Assessment of surface hygiene

Surface hygiene assessment and sampling was undertaken on 120 high-touch sites in the study wards, yielding 10 MSSA isolates and one MRSA. For all surfaces sampled, documentation included whether the surface was visually clean or not, the ATP result and coded the contact plates such that these samples were all clearly identifiable as related for further analysis.

All *S. aureus* isolates were obtained from sites within the near patient environment. Contamination of hospital areas in close proximity to patients is a recognised phenomenon and identified as an extension of the patients own flora for the purposes of infection prevention and control. This is reflected in hand hygiene guidance provided by the World Health Organization, which advises hand hygiene should be performed by HCWs following contact with the near-patient environment, even if no contact with the patient has been made (World Health Organization, 2009). No *S. aureus* isolates were recovered from the surfaces sampled within the near-patient environment of the patient known to be colonised with *S. aureus*.

The use of ATP for the evaluation of cleaning efficacy and surface hygiene was explored in the present study. Currently, no objective assessment tool exists to determine the cleanliness of a hospital surface, and current guidance recommends that visual assessment

be used to assess hospital hygiene (Health Service Executive, 2006). ATP measurements have historically been used in food and drink industries to provide feedback on surface cleanliness during processing and as a quality assurance tool. However, this technology is increasingly being applied in healthcare settings, particularly during decontamination processes, to add an objective element to current hygiene assessments. There are known limitations with the application of ATP measurements in healthcare settings. Firstly, there is no direct correlation with microbial surface contamination and ATP RLUs recorded, as ATP levels reflect the presence of all organic material (Guh and Carling, 2010). The presence of a residual disinfectant and/or cleaning agents on a surface, which is common in healthcare facilities, can enhance or slightly inhibit the luciferin reaction, which can impact the ATP values recorded (Omidbakhsh *et al.*, 2014). Furthermore, the impact of such chemical activity varies between different luminometer devices (Omidbakhsh *et al.*, 2014). In the present study, surfaces that appeared visibly clean yielded lower ATP readings than those that were visibly soiled, and levels or recovered ATP were higher on surfaces from which *S. aureus* was recovered- although not significantly. This suggests that, overall, for this small-scale study the use of ATP performed well, despite the known limitations. However, the difference observed did not reach statistical significance.

Room activity, elevated particle counts and S. aureus recovery

The use of particle counters to correlate relationships between airborne bacterial density and particles of varying sizes has been previously undertaken in hospital environments (Roberts *et al.*, 2006; Hathway *et al.*, 2013). In the present study, airborne particle counts were found to be higher during periods where active air sampling recovered airborne *S. aureus*, although not significantly. In one study that investigated the effect of nursing activities on airborne particle concentrations in a respiratory high-dependency unit, a

significant relationship was observed both between increasing particle counts measured using a laser particle counter, and the recovery of total viable bacterial counts from the air (Hathway *et al.*, 2013). In both studies undertaken by Robert *et al*. (2006) and Hathway and colleagues (2013), a relationship was observed between the undertaking of routine care and increased particle counts.

Agitation of fomites within the sampling area was also observed to result in airborne dispersal of *S. aureus*. Movement of privacy curtains was significantly associated with recovery of *S. aureus* from active air samples. On 33/35 occasions where *S. aureus* was recovered from an active air sample, at least one privacy curtain within the room had been moved by a patient or HCW in the preceding 20-minute period. A relationship was also observed between changing of bed linen and airborne dispersal of *S. aureus*. Bed-linens were changed during 13/115 periods of observation, and *S. aureus* was recovered actively from the air on 7/13 occasions. On 5/7 such occasions, *S. aureus* was also recovered from the subsequent active air sample, inferring that liberated *S. aureus* may linger in the air for a short period prior to setting on a surface.

A patient in the present study received nebulised medications during the study period, which significantly elevated observed airborne particle counts. However, *S. aureus* recovery was not associated with use of nebulised medications by this patient. The patient concerned was not colonised with MSSA, which may explain this finding. Hathway *et al*. (2013) found that nebulised medications altered the composition of particle size in the room throughout their use. This study did not demonstrate a significant difference in *S. aureus* recovery when nebulisers were in use. However, the present study was undertaken on surgical wards where only one patient was receiving infrequent nebulised medication, whereas undertaking a similar study on a respiratory ward may yield more data, facilitating greater analysis and providing clarity.

Clinical significance of OS-MRSA

Chromogenic medium selectitive for methicillin-resistance did not identify MRSA in samples obtained in the course of this study. Isolates were identified as *S. aureus* by Colorex^{TM} Staph Aureus (Colorex), but no growth was observed on Colorex^{TM} MRSA for any isolates, despite growth being observed with a positive control MRSA isolate. Cefoxitin resistance in 7/62 isolates was identified following disc diffusion antimicrobial susceptibility testing, and none of these isolates exhibited oxacillin resistance. These isolates were confirmed as MRSA by the GeneXpert MRSA assay due to the presence of *mecA* gene and elements of SCC*mec*. Published reports of oxacillin/cefoxitin susceptible isolates harbouring a *mec*A gene generally use the term OS-MRSA (oxacillin-susceptible MRSA), a term which was introduced as "a new type of MRSA" in a report which identified 480 OS-MRSA isolates collected over two years from nine hospitals in Japan (Hososaka *et al.*, 2007). However, "OS-MRSA" had been reported prior to this, such as in a USA case study which recovered clinically oxacillin-susceptible isolates (later confirmed to harbour *mecA*) in August 1999. These isolates were obtained from a series of blood cultures and a shoulder aspirate, all obtained from the same patient in Beth Israel Hospital, Boston (Sakoulas *et al.*, 2001). The term 'dormant MRSA' has been used to describe *mec*A-positive isolates that were initially oxacillin-susceptible but in which high-level resistance could be induced *in-vivo* (Kampf *et al.*, 2003), and OS-MRSA have also been described as 'cryptic MRSA' (Saeed *et al.*, 2014) or 'stealth MRSA' (Goering *et al.*, 2019). There are published reports of genetically diverse OS-MRSA in many hospital settings (Saeed *et al.*, 2014; Andrade-Figueiredo and Leal-Balbino, 2016; Song *et al.*, 2017), associated with CA-MRSA lineages (Hososaka *et al.*, 2007), and with dairy livestock (Pu *et al.*, 2014; Mistry *et al.*, 2016; Guimarães *et al.*, 2017).

In 2016, Proulx and colleagues reviewed all available reports of OS-MRSA to date and concluded the prevalence of *S. aureus* strains among clinical isolates that harbour *mec*A

but that are not detectable as MRSA by conventional phenotypic methods to be 3% (Proulx *et al.*, 2015). Irish guidelines for the control of MRSA rely on conventional culture methods (Department of Health- An Roinn Sláinte, 2013), and state that these must adhere to internationally recognised standards and breakpoints for susceptibility testing (Clinical Laboratory Standards Institute, 2018; EUCAST, 2019). Although the advantages of molecular methods, namely PCR, are discussed in the guidance document (such as improved turnaround times in comparison to conventional culture methods), it is noted that these techniques are not widespread due to cost implications and staffing pressures in Irish laboratories (Department of Health- An Roinn Sláinte, 2013). As such, the prevalence of OS-MRSA in Ireland is not known, and the clinical significance cannot be reliably interpreted. Reports have emerged in other countries detailing the complicated nature of such infections, and clarifying the importance of clarity for treating physicians of the nature of *S. aureus* isolate they are treating (Proulx *et al.*, 2015; Jones *et al.*, 2018; Duarte *et al.*, 2019). In the present study, culture using chromogenic media failed to detect OS-MRSA. In the hospital in which this study is set, chromogenic culture media is used to screen all MRSA screening samples, so carriage of OS-MRSA would not have been detected.

A report from a tertiary care hospital in Brazil describes a case of fatal sepsis caused by OS-MRSA (SCC*mec*IVa/ST1) in a critically unwell patient who presented with septic shock to the Emergency Department (Duarte *et al.*, 2019). Blood cultures were processed using an automated blood culture processing system, as described previously (Minassian *et al.*, 2014). The identification of Gram-positive cocci, likely *Staphylococcus spp.*, prompted intravenous vancomycin therapy in addition to piperacillin-tazobactam. Culture results that became available on the third day of the hospital admission revealed an MSSA bacteraemia which was resistant only to penicillin when tested using the automated antimicrobial susceptibility system. Thus, vancomycin was discontinued and intravenous

oxacillin was commenced. The patient deteriorated further, and died on the tenth day of her hospital admission, despite the later addition of daptomycin to her treatment regimen. A molecular investigation of *S. aureus* isolates recovered from this patient after her death revealed the presence of the *mec*A gene. The authors state that her fatal sepsis was misidentified as MSSA by phenotypic methods, and report that inadequate antimicrobial therapy was administered as a result. The authors reference CLSI guidance (2010) which states that this infection should have been treated as MRSA (as *mec*A was present, although this was not known to the treating physicians at the time of antibiotic prescription).

Failure to detect OS-MRSA in a clinical specimen has also been reported a USA case report, with the authors postulating that this resulted in a serious MRSA infection (due to inadvertent induction of oxacillin-resistance by treatment of OS-MRSA with naficillin) (Proulx *et al.*, 2015). The authors describe the clinical course of a male patient who presented to a tertiary referral hospital due to sudden onset of pain and oedema of the left knee five years post left total knee arthroplasty. Clinical signs and biochemical markers indicated infection and blood cultures and synovial fluid aspirate grew oxacillin and cefoxitin-susceptible MSSA. Treatment with vancomycin and daptomycin failed, and surgical removal of the prosthesis and replacement with a tobramycin-impregnated spacer were not curative; blood cultures remained positive for MSSA. Extensive radiological imaging did not identify other sources of infection. Escalation of treatment to naficillin resulted in sterile blood cultures. Thereafter, osteomyelitis was identified in the lumbar spine, so the patient completed a further 10 weeks of naficillin therapy. Within three weeks of ceasing naficillin treatment the patient was re-admitted to hospital and MRSA (oxacillin-resistant *S. aureus*) was obtained from a biopsy of fluid in the interspace region of the lumbar spine. A further 10 weeks of antibiotic therapy (nafcillin, vancomycin and rifampicin) were curative. Retrospective analysis of all isolates recovered from this patient

revealed the presence of *mec*A, which was not recognised at the time of antibiotic prescription due to reliance on phenotypic methods.

Further analysis of these isolates by the authors determined *IS*1181 had inserted into the *mec*A gene, resulting in a sequence interruption and inhibiting the function of *mecA*, rendering the isolates susceptible to oxacillin. The authors demonstrated an *in-vitro* reversion to MRSA by induction with oxacillin, and hypothesised that this reversion also occurred in this patient's clinical infection due to the inability to distinguish the MSSA and MRSA isolates recovered from this patient by PFGE. The precise excision of *IS*1181 from the MSSA isolates was observed *in-vitro*, and a significant increase in the rate of precise excision of this insertion sequence from *mec*A was observed in the presence of oxacillin. The authors suggest that this reversion occurred *in-vivo* due to the prolonged course of naficillin received by the patient. As per CLSI guidance (2010), had this been reported as MRSA from the initial isolate, naficillin would not have been administered.

An economic impact assessment was undertaken as part of the development of guidance for the control of MRSA in Ireland (Department of Health- An Roinn Sláinte, 2013). This report considered the economic advantages of implementing PCR for the detection of MRSA in hospital laboratories. In high and medium prevalence settings, the use of chromogenic agar was financially optimal, when considering turnaround times, time to isolation and exposure of patients to a colonised individual, occupational exposure of staff and number of unavailable room hours per patient. However, OS-MRSA, the prevalence of which may be as high as 3% (Proulx *et al.*, 2015), was not part of this consideration. Inappropriate antimicrobial therapy due to mis-identifying OS-MRSA as MSSA, such as described by Duarte (2019), will likely add days to the patients' length of stay, and may result in additional costs, such as radiological and laboratory investigations. Additional and prolonged therapies, as described by Proulx and colleagues (2015) do not appear to be accounted for. A case-matched retrospective analysis of OS-MRSA and conventional

MRSA BSIs revealed that, even with molecular tests available at the time of clinical decision-making and the knowledge that isolates are OS-MRSA at that time, patients with OS-MRSA were significantly more likely to require changes to antibiotic therapies and less likely to experience infection clearance on vancomycin monotherapy (Jones *et al.*, 2018). Therefore, the authors identify these infections as requiring additional attention from treating physicians to ensure optimal outcomes for patients.

OS-MRSA have been reported in Ireland previously (Brennan *et al.*, 2016). All seven OS-MRSA isolates in the present study were recovered from environmental sites (air samples one nightstand) in a urology surgical ward in an Irish hospital. The source, or colonised individual, who likely shed this OS-MRSA to the environment is not known. The method used for MRSA screening in this hospital is reliant upon conventional culture methods, so if the OS-MRSA was theoretically carried by a patient, this would not be detected as harbouring a *mec* gene due to reliance upon culture using chromogenic agar. The clinical impact of this could be significant, especially in cases where, for example, an opportunity to decolonise a patient harbouring MRSA prior to surgery could be missed. Furthermore, local surveillance of MRSA may prove falsely reassuring, due to failure to detect MRSA from screening samples. The prevalence of OS-MRSA in Ireland should be established.

3.5 Conclusion

Despite increasing acceptance of the role of the environment in the transmission of *S. aureus* in healthcare settings, the role of the air remains poorly understood. An integral element of infection control is the separation of patients from one another. This may involve isolation in a single room in the case of infection or immunosuppression. However, the majority of patients in Irish hospitals are cared for in multi-bed rooms. Cohorting patients in such spaces relies on adequate cleaning and adherence to optimal hand hygiene

to prevent pathogen transmission. The role that the air may play in circumventing such strategies in multibed rooms remains poorly understood. Data from this study identified a relatively abundant *S. aureus* population in the air and settling on surfaces in multibed rooms, likely reflecting a dynamic model of contamination which is occurring on a constant basis. Clinical activities were linked with increased *S. aureus* dispersal, which will result in surface contamination, and in multibed rooms the surface deposition patterns of such contamination are likely variable and extend outside the bedspace of any one patient. This small-scale and single-centre study has shown that the role of the air, and the impact on resultant surface contamination, should be examined in further detail. Potential strategies to control this putative transmission route could include targeting cleaning routines to periods after activities which cause dispersal of bacteria to the air, and high-touch surfaces within the near-patient environment should be assumed to become contaminated on an ongoing basis and as a result of routine care. By including room occupants, activities and ventilation mechanisms, studies can be more easily contextualized and more robust evidence can be generated.

The finding of methicillin-resistance in isolates that failed to grow on media selective for MRSA is concerning, as chromogenic media are routinely used to culture MRSA screening samples in Irish hospitals. Further studies should establish the prevalence of OS-MRSA in Ireland. The inclusion of a molecular-based diagnostic step (such as GeneXpert) would provide reassurance that phenotypically MSSA isolates harbouring *mecA* genes are not misclassified. A disadvantage of such testing is that it is expensive. The economic impact of such a measure should be considered, with attention paid to the avoidable costs, suffering and potential litigation that may arise from failing to detect MRSA in a clinical laboratory.

Chapter 4

An investigation of 62 *S. aureus* isolates recovered by sampling patient, healthcare workers and environmental sites in two surgical wards in an Irish tertiary referral hospital under active clinical conditions

4.1 Introduction

Whole-genome sequencing has emerged as the 'gold standard' typing method for *S. aureus* in recent years, and allows the ability to interrogate isolates in a manner that previously could not be facilitated, providing unrivalled discriminatory power (Moore *et al.*, 2015; Quainoo *et al.*, 2017). The availability of next-generation sequencing (NGS) platforms has made this technology available to a greater number of laboratories (Humphreys and Coleman, 2019). Currently, the use of WGS is still largely restricted to research or surveillance laboratories, but future iterations of the technology and streamlining of data analyses are being developed such that clinical laboratories may also benefit from the routine use of WGS both for diagnostics and to support local outbreak investigations (Quainoo *et al.*, 2017).

Whole-genome sequencing technology has been applied retrospectively to isolates retained from historical *S. aureus* outbreaks, and has assisted investigators in identifying transmission between patients, HCWs or environmental sites that was previously either supported by epidemiological evidence only or were not apparent to investigators (Köser *et al.*, 2012; Kong *et al.*, 2016; Roe *et al.*, 2016; Weterings *et al.*, 2017; Donkor *et al.*, 2018; Earls *et al.*, 2018; Madigan *et al.*, 2018; Ugolotti *et al.*, 2018; Cremers *et al.*, 2020). The majority of these studies investigated outbreak isolates recovered from neonatal intensive care units (NICUs) (Köser *et al.*, 2012; Earls *et al.*, 2018; Madigan *et al.*, 2018; Cremers *et al.*, 2020). Outbreaks in paediatric settings (Donkor *et al.*, 2018; Ugolotti *et al.*, 2018), on an oncology unit (Weterings *et al.*, 2017) and from wards in 280-bed rural hospital (Roe *et al.*, 2016) have also been undertaken. The application of WGS to aid in the investigation of ongoing outbreaks has been undertaken in obstetric (Gideskog and Melhus, 2019; Kossow *et al.*, 2019) and neonatal nosocomial populations (Cheng *et al.*, 2019; Kossow *et al.*, 2019; Kristinsdottir *et al.*, 2019).

Previously, the majority consensus in the literature concerning the main mode of *S. aureus* transmission was by the patient-to-patient route (via HCW hands), with some reports of outbreaks from colonised HCWs (Haill *et al.*, 2013; Harris *et al.*, 2013; Otter *et al.*, 2013). The role of the environment in the transmission of nosocomial pathogens, including *S. aureus*, has now been established and has gained acceptance in the literature (Boyce, 2007; Otter *et al.*, 2011; Mitchell *et al.*, 2015), and is being increasingly investigated using WGS technology. A number of studies have undertaken WGS on environmental isolates that were collected as part of prospective sampling in settings such as NICU (Christoff *et al.*, 2020), ICU (Moore *et al.*, 2015; Tong *et al.*, 2015; Price *et al.*, 2017; Smibert *et al.*, 2018; Dancer *et al.*, 2019; Christoff *et al.*, 2020; McKew *et al.*, 2020), a surgical ward (Kinnevey *et al.*, 2016), a wound treatment centre (Kpeli *et al.*, 2016), long term care facilities/nursing homes (Harrison *et al.*, 2016) and on a burns unit (Tong *et al.*, 2015) in order to investigate the role of the environment in the transmission of *S. aureus*. A number of these studies have linked isolates obtained from HCWs and patients to environmental sites (predominantly high-touch surfaces), outlining the role of the environment in *S. aureus* transmission in these settings.

Nosocomial surface contamination is managed by regular cleaning and decontamination (Health Service Executive, 2006). It is known that individuals colonised with *S. aureus* will contaminate their surroundings at variable rates, and upper respiratory symptoms can increase the rate of shedding (Sherertz *et al.*, 1996). This is mediated by air, whereby *S. aureus*, expelled in droplet form will remain airborne for variable periods before landing on a surface. Contaminated fomites can also liberate *S. aureus* into air when agitated (Kumari *et al.*, 1998). Indoor air in hospitals is a recognised transmission pathway of a number of pathogens, and has been implicated in the transmission of *S. aureus* and MRSA previously (as discussed in Chapter 1, Section 1.2.2.5). A systematic review which included 36 articles and investigated the impact of hospital ventilation on airborne

bioaerosols identified inpatient areas as those with the highest levels of bioaerosol counts, and concluded that, in agreement with a previous systematic review over a decade previously (Beggs *et al.*, 2008), multi-bed patient rooms could promote opportunistic transmission of pathogens through the air (Stockwell *et al.*, 2019). The role of the air on the transmission of *S. aureus*, or in influencing *S. aureus* contamination has been investigated by WGS in ICUs (Moore *et al.*, 2015; Price *et al.*, 2017; Dancer *et al.*, 2019; Adams and Dancer, 2020), but not in multi-bed hospital rooms on general wards, where the majority of patients are admitted. In two of these ICU-based studies, sampling of HCWs was limited to their hands, and not nares or anatomical sites amenable to *S. aureus* colonisation (Moore *et al.*, 2015; Dancer *et al.*, 2019). This likely reflects contact with surface contamination and lapsed hand hygiene rather than colonisation, and may omit transmission between HCWs and air that occurs from shedding of *S. aureus* by a colonised HCW.

Patterns of contamination (Moore *et al.*, 2013), patient populations, HCW work behaviours and treatments differ between general wards and ICUs (Schenk *et al.*, 2017), so the role of the air in the transmission of *S. aureus* in ICUs cannot be directly extrapolated to general wards from the current evidence available. Furthermore, these studies collected isolates over extended time periods. Moore *et al*. (2015) collected environmental samples three times per week for the duration of their study period, Dancer *et al*. (2019) sampled on one day per month over a ten-month period and Price *et al*. (2017) sampled ten sites per month.

In this study, we applied WGS technology to determine *S. aureus* transmission events by ascertaining relatedness of isolates, but also to describe the diversity of *S. aureus* circulating in a multi-bed ward environment over a short period, and did this by using isolates that were collected over four study days (each comprising five hours) and with all study days occurring within a period of 8 days.

4.2 Materials and methods

4.2.1 Bacterial isolates

A total of 62 recovered isolates were investigated by WGS. These were recovered from environmental sites (n = 52), HCWs (n = 9) and from the nasal sample of the single *S. aureus* colonised patient included in the study.

All isolates investigated in this chapter were obtained by sampling patients, healthcare workers and environmental sites in two wards of a large Dublin teaching hospital, as described in Chapter 2, Sections 2.2 - 2.4.4 (Table 4.1). Samples were initially processed with the aim of identifying the presence of *S. aureus* using routine culture methods (as described in Chapter 2, Section 2.7.1), and with results detailing prevalence provided in Chapter 3. Putative *S. aureus* isolates underwent confirmatory testing, as described in Chapter 2, Section 2.7.2. A total of 65 *S. aureus* isolates were recovered. Of the 62 isolates, culture-based susceptibility testing and use of the GeneXpert MRSA assay (as described in Chapter 2, Sections 2.7.3 and 2.7.4) identified seven OS-MRSA isolates, with the remainder designated MSSA.

As described in Chapter 3, Section 3.3.4, *S. aureus* was recovered from three HCWs, from both their anterior nares and oropharynx. Antimicrobial susceptibility testing revealed that in each case, isolates resistance phenotypes exhibited by both nasal and oral samples were identical, suggesting they were related. To optimise resources for this study, one representative isolate was chosen from each of these three HCWs for molecular analysis and whole-genome sequencing. Thus, three isolates obtained from HCWs were not included in WGS.

4.2.2 Isolate storage

Table 4.1 Summary of 62 *S. aureus* isolates subjected to whole-genome sequencing during the present study.

Isolate ID	Isolate Type	MSSA/MRSA[1]	Ward	Study day (SD)
A0801-01	Active air	MSSA	A	SD1
A0801-04	Active air	MSSA	A	SD1
A0801-05	Active air	MSSA	A	SD1
A0801-06	Active air	MSSA	A	SD1
A0801-07	Active air	MSSA	A	SD1
A0801-09	Active air	MSSA	A	SD1
A0801-10	Active air	MSSA	A	SD1
A0801-13	Active air	MSSA	A	SD1
A0801-14	Active air	MSSA	A	SD1
A0801-17	Active air	MSSA	A	SD1
A0801-18a	Active air	MSSA	A	SD1
A0801-18b	Active air	MSSA	A	SD1
A0801-19	Active air	MSSA	A	SD1
A0801-22	Active air	MSSA	A	SD1
A0801-8C	Active air	MSSA	A	SD1
A0901-0721	Active air	MSSA	A	SD2
A0901-0836	Active air	MSSA	A	SD2
A0901-0850	Active air	MSSA	A	SD2
A0901-1103a	Active air	MSSA	A	SD2
A0901-1151	Active air	MSSA	A	SD2
A1501-05	Active air	MRSA	B	SD3
A1501-8A	Active air	MSSA	B	SD3
A1501-08b	Active air	MSSA	B	SD3
A1501-09	Active air	MSSA	B	SD3
A1501-11	Active air	MRSA	B	SD3
A1501-13a	Active air	MSSA	B	SD3
A1501-13b	Active air	MSSA	B	SD3
A1501-14	Active air	MRSA	B	SD3
A1601-01	Active air	MRSA	B	SD4
A1601-03	Active air	MSSA	B	SD4
A1601-05	Active air	MSSA	B	SD4
A1601-08	Active air	MRSA	B	SD4
A1601-09	Active air	MRSA	B	SD4
A1601-10b	Active air	MSSA	B	SD4
A1601-10c	Active air	MSSA	B	SD4
A1601-13	Active air	MSSA	B	SD4
A1601-16	Active air	MSSA	B	SD4
A1601-18	Active air	MSSA	B	SD4
C104	Contact plate	MSSA	B	SD4

Continued overleaf

Isolate ID	Isolate Type	MSSA/MRSA	Ward	Study day (SD)
C122	Contact plate	MSSA	B	SD4
C123	Contact plate	MSSA	B	SD4
C37a	Contact plate	MSSA	A	SD2
C52	Contact plate	MSSA	A	SD2
C75	Contact plate	MSSA	B	SD3
C78	Contact plate	MSSA	B	SD3
C87	Contact plate	MRSA	B	SD3
C89	Contact plate	MSSA	B	SD3
C93	Contact plate	MSSA	B	SD3
C94	Contact plate	MSSA	B	SD3
HN0534.1	HCW	MSSA	A	SD1
HN0538.1	HCW	MSSA	A	SD2
HN0570.1	HCW	MSSA	B	SD3
HN0572.1	HCW	MSSA	B	SD3
HN0574.1	HCW	MSSA	B	SD4
HO0536.1	HCW	MSSA	A B	SD1
PN0621	Patient	MSSA	B	SD4
S0801-0718	Settle plate	MSSA	A	SD1
S0801-0821	Settle plate	MSSA	A	SD1
S0801-1018	Settle plate	MSSA	A	SD1
S0901-0918	Settle plate	MSSA	A	SD2
S1601-0912	Settle plate	MSSA	B	SD4
S1601-1010	Settle plate	MSSA	B	SD4

Abbreviations: HCW, healthcare worker; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*; SD, study day.

[1]Determination of whether isolates were MSSA or MRSA was based on resistance to cefoxitin and classification as MRSA by the GeneXpert MRSA assay (Cepheid).

The 62 confirmed *S. aureus* isolates investigated by WGS were stored on cryoprotective beads (Protect, Thermo Fischer Scientific, Ireland) at -70°C in the Microbiology Research Laboratory at the DDUH. Immediately prior to processing, isolates were reactivated by inoculating a single bead on Columbia Blood Agar (CBA) containing 7% (v/v) defibrinated horse blood (Lip Diagnostic Services, Galway, Ireland), followed by incubation for 18 h at 37°C in a Sanyo Gallenkamp static incubator. Following incubation, a sterile plastic loop was used to transfer roughly one square inch of culture growth to a new CBA plate, and plates were lawned in three directions to ensure confluent growth. These plates were then incubated as previously, for a further period of 20 h prior to further processing.

4.2.3 Genomic DNA extraction from confirmed *S. aureus* isolates

Genomic DNA for WGS analysis was extracted by enzymatic lysis using the buffers and reagents supplied with the *S. aureus* Genotyping Kit 2.0 (Abbott [Alere Technologies GmbH, Jena, Germany]) and the Qiagen DNeasy blood and tissue kit (Qiagen, Crawley, West Sussex, UK), as follows: following overnight incubation of isolates on CBA, a 1 µl inoculation loop of *S. aureus* culture growth was added to 200 µl of lysis buffer (supplied with Alere kit) in a 1.5 ml Eppendorf Safelock microfuge tube, which was then vortexed using a Heidolph Reax laboratory vortex set to maximum speed for 20 s, and incubated at 37°C for 3 h in a waterbath with shaking. Following incubation, a 25 µl aliquot of Proteinase K (supplied with Qiagen kit) was added to each tube, along with 200 µl buffer A1 (supplied with Alere kit). Samples were vortexed using a Heidolph Reax laboratory vortex on maximum speed for 5 s and incubated for 40 min at 70°C, after which time 200 µl of 100% (v/v) ethanol at -20°C (Merck [Sigma-Aldrich Ireland Ltd., Wicklow, Ireland]) was added. Samples were carefully inverted to mix the contents. The entire contents of the

samples were then transferred to DNeasy mini spin columns in 2 ml collection tubes (supplied with the Qiagen kit). The mini spin columns contain a silica resin that binds to DNA. This acts as a filter, retaining DNA in the column and allowing debris and other cellular elements to pass through the membrane to the collection tube, which is then discarded. DNA is then eluted using the supplied buffers, and the inclusion of the spin column step ensures that impurities are removed, improving end-point DNA quality. The spin column was then placed in a sterile 1.5 ml Eppendorf tube and 100 µl of molecular biology grade water (Sigma-Aldrich) (which had been pre-warmed at 37°C in a static incubator) was added to induce release of DNA from the silica resin. Samples were incubated at room temperature for 10 min to encourage optimal elution, and were then centrifuged at 5,000 × *g* for 1 min.

The quality and concentrations (ng/µl) of the extracted genomic DNA was evaluated using a Nanodrop 2000c thermal spectrophometer (Thermo Fischer Scientific, Massachusetts, USA). To ensure appropriate quality of the diluted samples, absorbance ratios were recorded. Samples did not meet the quality standard if 260/280 nm absorbance ratio was not within the range 1.8-2.0. Extracted genomic DNA was stored at 4-8°C.

4.2.4 Preparation of extracted genomic DNA for WGS

Extracted genomic DNA was prepared for WGS using the Illumina DNA Prep Tagmentation Library Prep Kit (Illumina, Eindhoven, the Netherlands).

4.2.4.1 Tagmentation reaction

For each sample of genomic DNA, a tagmentation reaction was induced by adding 5.5 µl bead- linked transposomes (Illumina*),* 5.5 µl tagmentation buffer (Illumina) and 10 µl of

genomic DNA per sample together in a 0.2 ml PCR tube and incubating at 55°C for 15 min. To inhibit the tagmentation reaction after the incubation step, 5 µl of the supplied tagment stop buffer (Illumina) was added to each sample. Each mixture was then incubated with the tagment stop buffer for 15 min at 37°C. Samples were then cleaned in preparation for amplification. Samples were transferred from PCR tubes to a 96-well plate (Sigma-Aldrich). The plate was placed on a magnetic stand (Thermo Fischer Scientific, Dublin) until the solution became clear and a dark precipitate could be seen adhering to the side of the tube under magnetic force. This process is induced by the attraction and subsequent movement of the bead linked transposomes (added as part of the tagmentation reaction) toward the magnet, resulting in bead accumulation at the edge of each well within the 96-well plate. The supernatant was discarded and washing of the DNA was undertaken using tagmentation wash buffer (a solution containing salts and ethanol; supplied with the Illumina kit) with the magnet used to facilitate removal of the supernatant and avoid disruption of the DNA. This wash step was repeated three times.

4.2.4.2. PCR amplification and post-PCR cleaning

The tagmented and cleaned DNA was then amplified. The 96-well plate was removed from the magnet and 20 µl of mastermix containing 11 µl Enhanced PCR mix (Illumina) and 11 µl molecular biology grade water (Sigma-Aldrich) per sample was added to the wells, the solution was transferred to 0.2 ml Thermo Scientific PCR tubes (Thermo Scientific, Dublin) from wells wihin the 96-well plates. Using the supplied primer mix, 2.5 µl of index adaptors i5 and i7 (Illumina) were added and the well from which the sample was obtained was noted. Each primer index represents one sample, so this step serves as an identifier for each sample processed. The solution was thoroughly mixed using a pipette. The samples then underwent PCR amplification by thermal cycling under the following

conditions: 68℃ for 3 min, 98℃ for 3 min, six cycles at 98℃ for 45 s, 62℃ for 30 s, 68℃ for 2 min, and finally 60 s at 68℃. The amplified library was then purified using a bead purification procedure. Samples were then centrifuged at 280 × *g* for 60 s.

Samples were then transferred from PCR tubes to a new 96-well plate. This plate was then placed on a magnetic stand until the supernatant was clear, at which point 22.5 µl of the supernatant was transferred to a fresh 96-well plate. The use of the magnet at this point facilitates cleaning of samples by removing unwanted high-molecular weight fragments, which are within the solution following PCR. Removal of lower weight fragments is undertaken by using paramagnetic sample purification beads (Illumina). These beads bind to DNA fragments of the size necessary for WGS, facilitating removal of unwanted fragments that can compromise the quality of the final elution.

A mastermix was then prepared containing 22.5 µl of sample purification beads and 20 µl biological grade nuclease free water (Sigma-Aldrich) per sample. Following the mixing of 42.5 µl of each PCR product with the mastermix, solutions were thoroughly mixed in the 96-well plate using a pipette, and then incubated at room temperature for five min. A further 7.5 µl of sample purification beads were added to each sample, and these were not diluted as previously. Samples were again completely mixed using a pipette, incubated at room temperature for 5 min and then returned to the magnetic stand until the supernatant was clear. The supernatant was then removed and discarded, and 100 µl of 80% (v/v) ethanol was added to each sample and left for 30 s, before removal and discarding of ethanol without disrupting the pellet. The ethanol rinse was repeated twice more. The beads were then allowed to dry by leaving untouched, on the magnetic stand and at room temperature for a 5 min. The samples were removed from the magnetic stand and 17 µl of resuspension buffer was then added, and thoroughly mixed using a pipette. A final incubation step at room temperature for 2 min was undertaken, followed again by placing

the samples on the magnetic stand. When the solution was clear, 15 µl was transferred to a fresh well on the 96-well plate.

4.2.4.3 Library pooling and denaturation and preparation of PhiX control

Prepared libraries were measured and quantified using a Qubit 4 Fluorometer (Thermo-Fisher Scientific, Massachusetts, USA). To ensure adequate and relatively equally distributed quantities of each sample were present in the pool, all samples were normalised to a concentration of 4 nm/µl. The pool now contained the extracted DNA and subsequent prepared library for each sample. The pooled library was contained in a single volume within a 0.2 ml PCR tube (Thermo Scientific, Dublin).

Using a pipette, 5 µl of the sample pool was combined with 5 µl of sodium hydroxide in a 1.5 ml microcentrifuge tube, and this solution was agitated to ensure complete mixing. Following a 5 min incubation at room temperature, 990 µl of hybridization buffer (Illumina) was added to the solution, which was then diluted to a concentration of 12 pM. This solution was stored on ice whilst the PhiX library was prepared. This was done by combining 2 µl of 10 nM PhiX library, 2 µl of Tris-Cl and 5 µl of freshly diluted sodium hydroxide in a 1.5 ml microcentrifuge tube, vortexing, and incubating the solution for five min at room temperature. Hybridisation buffer (Illumina) was then added (990 µl). The denatured PhiX library was then diluted to 12.5 pM.

The denatured library and PhiX control were combined immediately prior to MiSeq loading and incubated at 96°C for 2 min, ensuring complete denaturation prior to sequencing.

4.2.5 MiSeq whole-genome sequencing

Paired end whole-genome sequencing was undertaken using the 500-cycle MiSeq reagent kit v2 (Illumina).

4.2.5.1 WGS data processing and contig assembly

Upon completion of the 500-cycle MiSeq sequencing process, sequencing data were stored as FASTQ files. Each FASTQ file contains results of sequencing reads, and each sample processed as part of the pooled library generates a discrete FASTQ file. These were first trimmed and then exported from the MiSeq platform and uploaded to BaseSpace (Illumina) for temporary storage. FASTQ files were then imported to BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium) for data analysis. Initial quality assurance of isolates was undertaken at the point of importation to BioNumerics in the FASTQ format, using the default BioNumerics trimming settings. Isolate genomes were assembled within BioNumerics, using the BioNumerics SPAdes *de novo* contig assembly algorithm.

4.2.5.2 Quality assessment of WGS data

Quality assessment of all sequenced isolates was undertaken using the BioNumerics quality experiment function. If the core genome of any isolate sequenced was not present at a level of above 97%, the sequencing procedure was repeated from the point of library preparation onwards. Assessments also included: average trimmed isolate quality, number of contigs per isolate and N50 value.

Quality assurance of the WGS process was undertaken at a number of points between DNA extraction and final data analysis (Table 4.2) with a number of standardised quality metrics recorded.

Table 4.2 Quality assurance measures undertaken throughout whole-genome sequencing process

Metric(s)	Description of metric	Measurement tool	Accepted values
DNA quantification	Quantity of extracted genomic DNA required for further processing	Nanodrop 2000c thermal spectrophometer (Thermo Fischer Scientific)	100-500 ng of genomic DNA
Extracted DNA quality	Extracted DNA was assessed for evidence of impurity or contamination	Nanodrop 2000c thermal spectrophometer (Thermo Fischer Scientific)	Nextera Library preparation optimised to process absorbance ratio values 1.8-2.0
Amplified DNA quantification	DNA which has been tagmented, amplified and undergone cleaning is quantified. This facilitates normalisation of each sample added to the library pool, and insures a relatively equal distribution of DNA from each sample	Qubit Fluorometer 1.0 (ThermoFisher Scientific)	DNA concentrations are normalised to 4 nM, so this quantity must be present
Average read coverage	The number of bases present in the reads and the expected sequence length (based on known genome size) is used to calculate the degree of coverage of each base, and how much of the genome has been sequenced.	BioNumerics v7.6 (Applied Maths)	>30x Samples with values <10x coverage would be removed and re-sequenced due to insufficient quality.
			Continued overleaf

Metric(s)	Description of metric	Measurement tool	Accepted values
N50	Length of the median contiguous sequence (‘contig’). The genome sequence will comprise contigs of different length, and the N50 value represents the shortest sequence in the group of larger sequences which together cover half the genome.	BioNumerics v7.6 (Applied Maths)	>100,000 bp
Number of contigs	Number of contigs, or sets of overlapping DNA sequence that can be concatenated to represent a larger genomic region than if the overlapping sequences were viewed separately.	BioNumerics v7.6 (Applied Maths)	<400[1]
Percentage of core genome present	Number of loci detected in core genome which comprise total number of core loci known. BioNumerics curate a *S. aureus* genomic scheme that currently comprises 1,861 core loci and 2,036 accessory loci, as defined by Leopold *et al*. (2014)	wgMLST plug-in, BioNumerics v7.6 (Applied Maths)	Scheme contains 1,861 core genome loci, no threshold given but as many of these as possible should be present. As such, for the purpose of this study, isolates with <97% of cgMLST alleles detected were repeated.

Abbrevations: wgMLST, whole-genome multi-locus sequence typing; bp, base pair.

[1]According to the BioNumerics wgMLST user manual, less than 400 contigs are acceptable as a read quality measure. However, this varies based on the bacterial species being subjected to WGS. As such, and for the purpose of this study, if the number of contigs exceeded 400, the isolate would have been repeated.

4.2.6 Genotyping

In silico detection of virulence and resistance genes was undertaken for all isolates. Assembled contigs were first exported from BioNumerics in GenBank flat file format, and then uploaded to Ridom SeqSphere (version 7.0; Ridom GmbH, Würzburg, Germany). The coding sequences of 160 genes were searched within the complete assembled genomes to determine the presence of virulence genes, resistance genes and SCC*mec* type (Table 4.3). Data were then exported to Microsoft Excel (2016) for further analysis.

4.2.7 Molecular typing of sequenced isolates

4.2.7.1 Whole-genome multilocus sequence typing

Whole-genome multilocus sequence-typing (wgMLST) was undertaken *in-silico* using the BioNumerics wgMLST plug-in application (BioNumerics v7.6). Completion of the wgMLST function facilitates assignment of conventional MLST STs (using seven housekeeping genes *arcC, aroE, glpF, gmk, pta, tpi,* and *yqiL*) for all schemes adhering to PubMLST allele numbering. In addition, WGS is undertaken to interrogate the presence of target loci within the core genome (n=1861 loci; Leopold *et al*, 2014) and accessory genomes (n=2036 loci).

4.2.7.2 *spa* typing

In-silico spa typing was performed. WGS data was first exported from BioNumerics in GenBank flat file format and imported to SeqSphere for *spa* typing. Ridom curate a database of 19,463 discrete *S. aureus spa* types from 143 countries (https://spaserver.ridom.de/), and this was used to assign *spa* types to all sequenced isolates.

Table 4.3 Gene targets included for *in-silico* genotyping by Ridom SeqSphere (version 7.0; Ridom GmbH)

Gene[1]	Gene product or function	Reference
aac-aphD	Aminoglycaside resistance	(Strauß *et al.*, 2016)
aadD	Kanamycin resistance	(Strauß *et al.*, 2016)
aphA3	Aminoglycaside resistance	(Strauß *et al.*, 2016)
blaI	Penicillinase repressor	(Strauß *et al.*, 2016)
blaR	Beta-lacatamase regulatory protein	(Strauß *et al.*, 2016)
blaZ	Beta-lactamase resistance	(Strauß *et al.*, 2016)
cat	Chloramphenicol resistance	(Strauß *et al.*, 2016)
cfr	Chloramphenicol resistance	(Strauß *et al.*, 2016)
dfrA	Trimethoprim resistance	(Vickers *et al.*, 2009)
erm(A)	MLS_B agent resistance	(Strauß *et al.*, 2016)
erm(B)	MLS_B agent resistance	(Strauß *et al.*, 2016)
erm(C)	MLS_B agent resistance	(Strauß *et al.*, 2016)
fexA	Chloramphenicol resistance	(Strauß *et al.*, 2016)
fosB	Fosfomycin resistance	(Strauß *et al.*, 2016)
fusB	Fusidic acid resistance	(Strauß *et al.*, 2016)
fusC	Fusidic acid resistance	(Strauß *et al.*, 2016)
kdpA-E	Potassium uptake system	(Strauß *et al.*, 2016)
lmrP	Transporter protein	(Strauß *et al.*, 2016)
lnuA	Lincosamide resistance	(Strauß *et al.*, 2016)
mefA	Macrolide resistance	(Strauß *et al.*, 2016)
merA	Mercuric reducatase	(Strauß *et al.*, 2016)
merB	Mercuric reducatase	(Strauß *et al.*, 2016)
mph(C)	Macrolide resistance	(Strauß *et al.*, 2016)
mprF	Daptomycin resistance	(Strauß *et al.*, 2016)
msr(A)	Macrolide resistance	(Strauß *et al.*, 2016)
mupA	Mupirocin resistance	(Strauß *et al.*, 2016)
qacA/C	Ethium bromide and QAC resistance	(Strauß *et al.*, 2016)
sat	Streptothricin resistance	(Strauß *et al.*, 2016)
sdrM	Multidrug efflux pump	(Strauß *et al.*, 2016)
tet(K)	Tetracycline resistance	(Strauß *et al.*, 2016)
tet(M)	Tetracycline resistance	(Strauß *et al.*, 2016)
vanA/B/Z	Vancomycin/Teicoplanin resistance	(Strauß *et al.*, 2016)
vatA	Streptogramin resistance	(Strauß *et al.*, 2016)
vatB	Streptogramin resistance	(Strauß *et al.*, 2016)
vga(A)	Streptogramin resistance	(Strauß *et al.*, 2016)
ORF-CM14	Enterotoxin homologue; host injury function	(Stefan Monecke *et al.*, 2013)
arcA-D	Arginine catabolic mobile element; persistence function	(Shore *et al.*, 2011b)

Continued overleaf

Gene	Gene product or function	Reference
aur	Aureolysin; immune evasion function	(Laarman *et al.*, 2011)
bap	Biofilm formation; adhesion function	(Strauß *et al.*, 2016)
cap1H/J/K	Capsular polysaccharide synthesis enzyme 1; immune evasion function	(Strauß *et al.*, 2016)
cap5H/J/K	Capsular polysaccharide synthesis enzyme 5; immune evasion function	(Strauß *et al.*, 2016)
cap8H/I/J/K	Capsular polysaccharide synthesis enzyme 8; immune evasion function	(Strauß *et al.*, 2016)
chp	Chemotaxis inhibitory protein; immune evasion function	(Strauß *et al.*, 2016)
ebpS	Elastin binding protein; adhesion function	(Strauß *et al.*, 2016)
edinA/B/C	Epidermal cell differentiation inhibitor; adhesion function	(Messad *et al.*, 2013)
eno	Enolase; adhesion function	(Strauß *et al.*, 2016)
etA/B/D	Exfoliative toxin; host injury function	(Strauß *et al.*, 2016)
fib	Fibrinogen-binding protein; adhesion function	(Strauß *et al.*, 2016)
hl/I	Haemolysin; adhesion function	(Alba *et al.*, 2015)
hlIII	Putative haemolysin; adhesion function	(Strauß *et al.*, 2016)
hla	Haemolysin; adhesion function	(Strauß *et al.*, 2016)
hlb-intact	Haemolysin; adhesion function	(Strauß *et al.*, 2016)
hlgA/B/C	Haemolysin gamma; adhesion function	(Gouaux *et al.*, 1997)
icaA/B/C	Intercellular adhesion protein; adhesion function	(Foster *et al.*, 2014)
clfA/B	Clumping factor; adhesion function	(Foster *et al.*, 2014)
cna	Collagen binding adhesin; adhesion function	(Foster *et al.*, 2014)
ebh	Extracellular matrix-binding protein homologue; adhesion function	(Strauß *et al.*, 2016)
fnbA/B	Fibronectin-binding protein; adhesion function	(Foster *et al.*, 2014)
hysA	Hyaluronate lyase; adhesion function	(Strauß *et al.*, 2016)
map	Extracellular adhesive protein; adhesion function	(Strauß *et al.*, 2016)
sasG	*S. aureus* surface protein G; adhesion function	(Foster *et al.*, 2014)
sdrC/D/E	Serene-aspartate repeat protein; adhesion function	(Barbu *et al.*, 2014)
vwb	von Willebrand factor-binding protein; adhesion function	(Viela *et al.*, 2019)
isaB	Immunodominant antigen B; adhesion function	(Mackey-Lawrence *et al.*, 2009)
isdA	Iron-regulated surface protein A; host injury function	(Foster *et al.*, 2014)
lukD/E/PV/PV83/M/PV/X/Y	Leucocidin; host injury function	(Strauß *et al.*, 2016)
sak	Staphlyokinase; host injury function	(Strauß *et al.*, 2016)

Continued overleaf

Gene	Gene product or function	Reference
seb	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
scn	Staphylococcal complement inhibitor; host injury function	(Strauß *et al.*, 2016)
sec	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sed	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
see	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
seg	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
seh	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sei	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sej	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sek	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sel	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sem	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
sen	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
seo	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
seq	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
ser	Enterotoxin; host injury function	(Strauß *et al.*, 2016)
setB1/2/3/C	Exotoxin; host injury function	(Strauß *et al.*, 2016)
seu	Enterotoxin homologue; host injury function	(Strauß *et al.*, 2016)
splA/B/E	Serine protease; host injury function	(Strauß *et al.*, 2016)
ssl1-11	Staphylococcal superantigen like gene; host injury function	(Strauß *et al.*, 2016)
sspA	Glutamyl endopeptase; host injury function	(Rice *et al.*, 2001)
sspB	Staphopain B; host injury function	(Rice *et al.*, 2001)
sspP	Staphopain A; host injury function	(Laarman *et al.*, 2012)
tst1	Toxic shock toxin; host injury function	(Strauß *et al.*, 2016)
*ccrA*1-4	Cassette chromosome recombinase A; resistance function	(Strauß *et al.*, 2016)
*ccr*AA/B1/ B2/B3/B4	Cassette chromosome recombinase AA; resistance function	(Strauß *et al.*, 2016)
mecA	Penicillin binding 2a production, conferring methicillin resistance	(Strauß *et al.*, 2016)
mecI	*mecA* gene regulator; resistance function	(Strauß *et al.*, 2016)
mecR-intact	*mecA* gene regulator; resistance function	(Strauß *et al.*, 2016)
mecR-truncated	*mecA* gene regulator; resistance function	(Strauß *et al.*, 2016)
ugpQ	Glycerophosphoryl diester phosphodiesterase; resistance function	(Strauß *et al.*, 2016)

Abbreviations: MLS_B, macrolides, lincosamides and streptogramin; QAC, quarternary ammonium compounds.

[1] *In silico* detection of virulence and resistance genes was undertaken for all 62 *S. aureus* isolates that had undergone whole-genome sequencing. Assembled contigs were first

exported from BioNumerics (v7.6) in GenBank flat file format, and then uploaded to Ridom SeqSphere (version 7.0; Ridom GmbH, Würzburg, Germany).

4.2.8 Analysis of isolate relatedness

The BioNumerics minimum spanning tree function was used to visually represent inter-isolate relationships based on wgMLST data. For the purpose of this work, isolates were considered related if they differed by ≤ 24 allelic variations (Schürch *et al.*, 2018).

4.2.9 Comparison of genes or sequences

Sequences or genes of interest were compared using Clustal Omega (Madeira *et al.*, 2019). First, the isolates were compared using the BioNumerics (v7.6) alignment function. The FASTA sequence of interest was first identified within the assembled genome by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The FASTA sequence, and predicted translated protein sequence, were then imported to the Clustal Omega alignment web form (https://www.ebi.ac.uk/Tools/msa/clustalo/). The output was in the form of an alignment file with annotations indicating where aligned sequences were incongruous.

Detection of known genes in the sequenced isolates

To determine the presence of a gene within the sequence isolates that was not included in the Seqsphere genotyping analysis (as described in Chapter 2, Section 2.6), the publicly available FASTA sequence was obtained using the ‘nucleotide’ search function in Pubmed (https://www.ncbi.nlm.nih.gov/nucleotide/). The presence of the gene or sequence was then searched for in the assembled sequence in BioNumerics (v7.6).

4.3 Results

The 62 *S. aureus* isolates were sequenced successfully, and the WGS data met all quality assurance measures required, so were all included in WGS data analysis (Table 4.4).

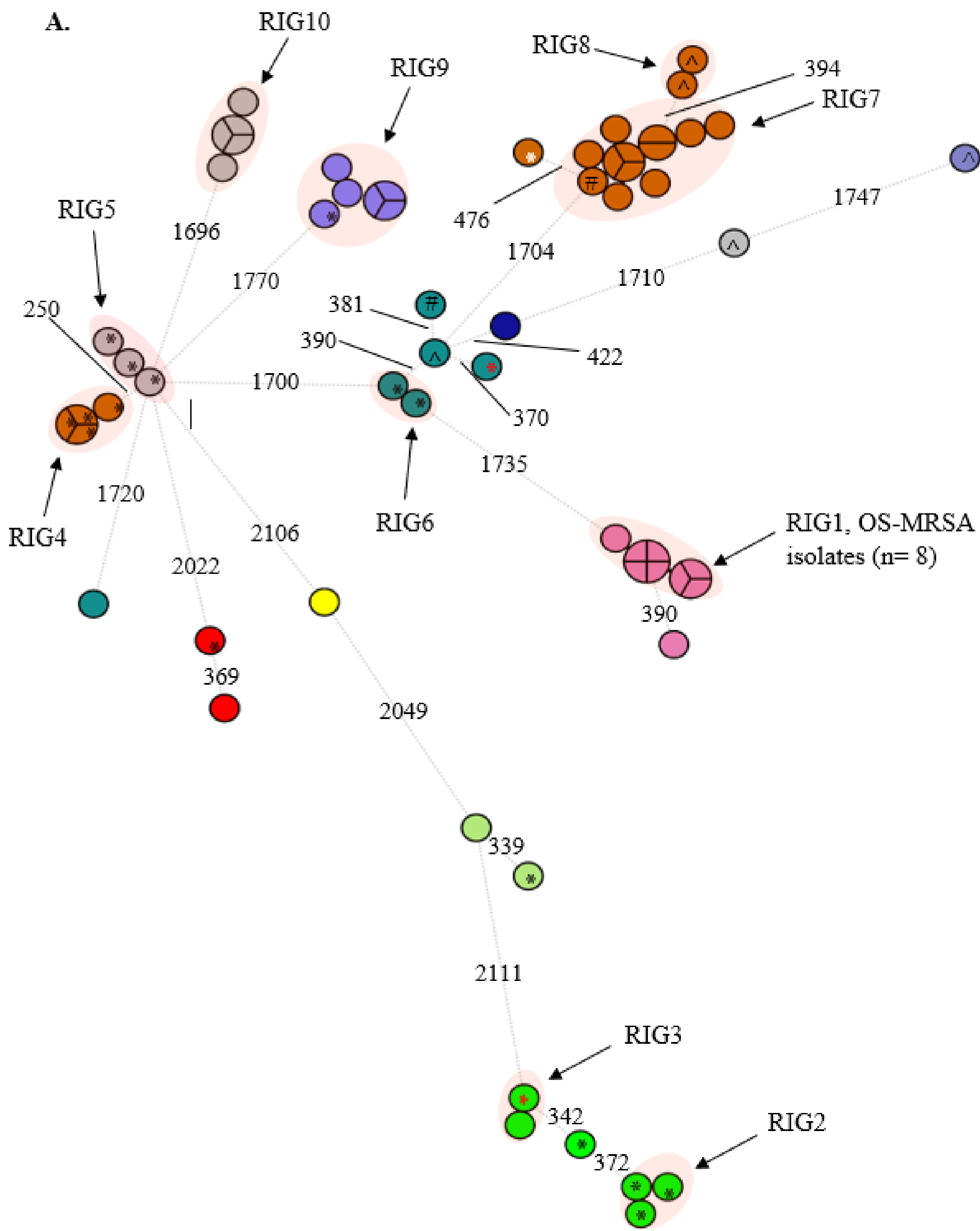
4.3.1 Population structure of *S. aureus* isolates investigated

Among the 62 isolates investigated, 13 STs were identified, and these were assigned to 11 MLST clonal complexes (CCs). The distribution of study isolates within these CCs and the associated assigned STs and *spa* types are shown in Table 4.5.

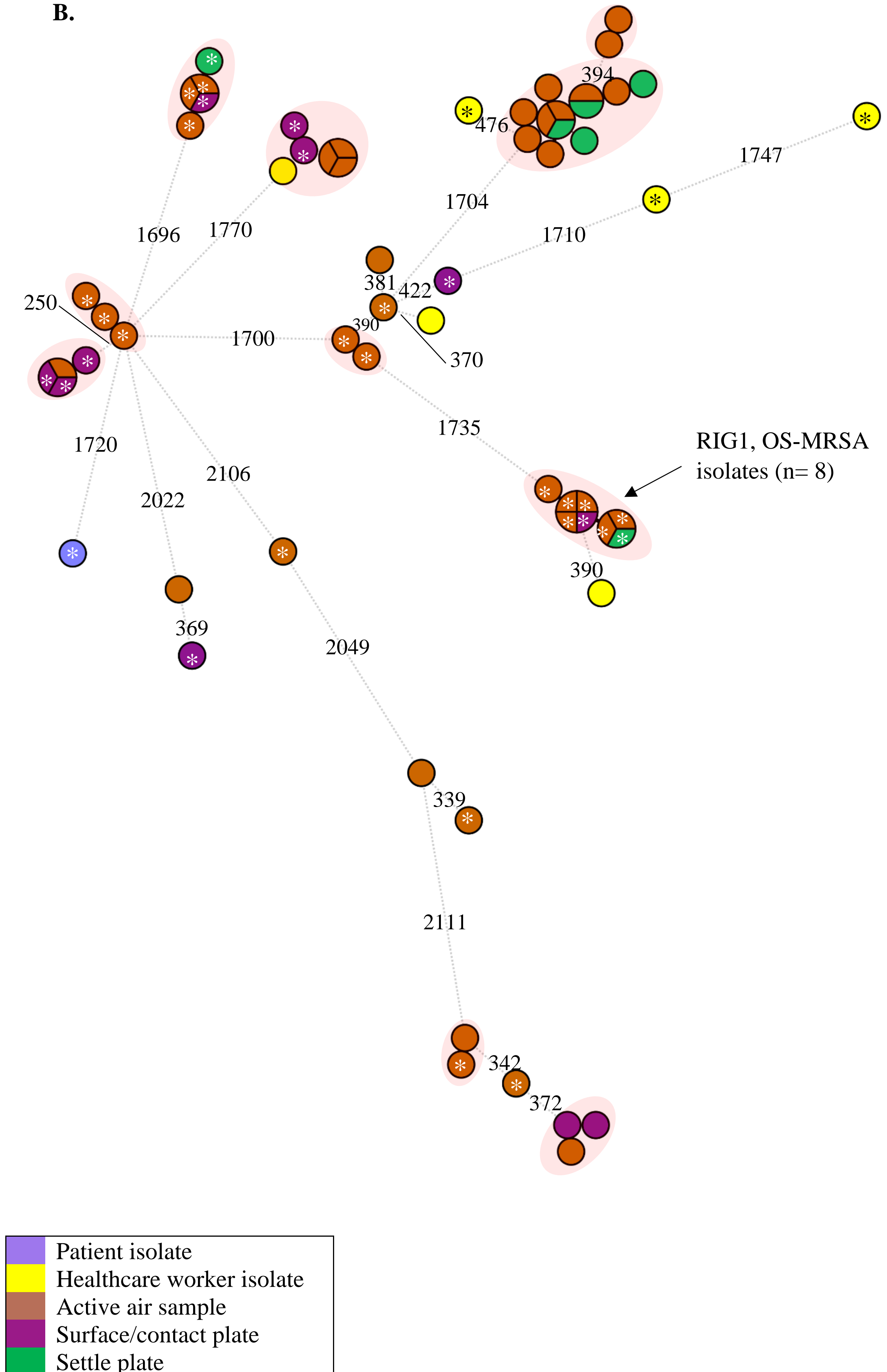
The most frequently identified CC was CC1 (22/62, 35.5%), with 22 isolates assigned comprising 19 ST1 isolates and three ST109 isolates. The remaining 40 isolates were assigned to CC8 (9/62, 14.5%), CC15 (7/62, 11.3%), CC5 (6/62, 9.7%), CC45 (6/62, 9.7%), CC97 (5/62, 8.1%;), CC22 (2/62, 3.2%), CC30 (2/62, 3.2%) and one each (1/62, 1.6%) to CC12, CC398 and CC672.

With regard to MLST types assigned, ST1 was most prevalent, representing 30.6% (19/62) of sequenced isolates, followed by ST8 of which 14.5% (9/62) isolates were assigned. The remaining isolates were assigned ST45 (9.7%; 6/62), ST5 (9.7%; 6/62), ST15 (9.7%; 6/62), ST97 (8.1%; 5/62), ST109 (4.8%; 3/62), ST22 (3.2%; 2/62), ST30 (3.2%; 2/62) and one each (1.6%; 1/62) to ST12, ST398, ST582 and ST672 (Fig. 4.1).

In-silico spa typing was undertaken and assigned 59 isolates to 24 *spa* types. Three isolates (A0801-17, A1501-13b and HN0534.1) were not assigned to any *spa* type and these were submitted to the curator at https://spa.ridom.de/submission.html for assignment. At the time of writing *spa* types for these isolates had not yet been assigned, so this will be referred to as 'NTA', or 'no type assigned'. The most prevalent *spa* type assigned was t127 to which 11/62 (17.7%) of isolates were assigned. The prevalence of *spa* types assigned among the remaining isolates included: t025 (9/62; 14.5%), t053 (5/62; 8.1%),



*	ST45 (t230)		ST22 (t223)	*	ST1 (t209)	*	ST5 (t9057)
	ST45 (t620)	*	ST22 (NTA)	^	ST1 (t177)		ST5 (t053)
*	ST45 (NTA)			#	ST1 (t114)		
			ST398 (t1451)	*	ST1 (t922)		ST15 (t701)
	ST30 (t021)				ST1 (t127)	*	ST15 (NTA)
*	ST30 (t8839)		ST582 (t084)			*	ST15 (t491)
						^	ST15 (t254)
*	ST109 (t693)		ST8 (t025)			#	ST15 (t1361)
	ST97 (t865)						
^	ST12 (t160)	^	ST672 (t3841)				



B.
1696
1770
394
476
1747
1704
1710
381
422
250
390
370
1700
1735
1720
2106
2022
RIG1, OS-MRSA
isolates (n= 8)
390
369
2049
339
2111
342
372
Patient isolate
Healthcare worker isolate
Active air sample
Surface/contact plate
Settle plate

Fig 4.1 Minimum spanning tree (MST) based on whole-genome multilocus sequencing typing (wgMLST) analysis showing the relationships between the 62 *S. aureus* isolates recovered during the present study. A total of 62 *S. aureus* isolates were recovered from patients, HCWs and environmental sites in two multi-bed hospital wards in an Irish teaching hospital. All isolates underwent Illumina MiSeq whole-genome sequencing. Isolates were assigned to conventional MLST types, and also classified by *spa* typing based on the sequence output data (A.). In diagram 'A.', isolates are represented by these MLST types with *spa* types indicated, both as described in the figure legend. Isolates were deemed to be related if they exhibited ≤ 24 allelic differences, as described by Schurch and colleagues (2018). Pairs or clusters of isolates that met this criteria, so were considered related, were assigned to Related Isolate Groups (RIGs). In the present study, 46 *S. aureus* isolates were assigned to 10 RIGs. These RIGs are indicated by red shading over the nodes within the RIG. The numbers on each branch represents the allelic differences exhibited between isolates, which are represented as nodes. Diagram 'B' represents the same 62 isolates, but indicated based on the isolate source (i.e. patient sample, HCW sample or environmental sample). Isolates are further classified based on the ward that they were obtained from. Isolates obtained from Ward B are annotated with an asterisk symbol within the node that represents each isolate. The majority of isolates in the present study were MSSA (54/62, 87.1%). Eight isolates were identified as OS-MRSA (8/62, 12.9%), and these were all assigned ST8 and identified as a related group of isolates (RIG1). These 8 isolates are labelled in both diagrams 'A' and 'B'.

Table 4.4 Whole-genome sequencing quality data for all 62 study isolates

Isolate ID	Average read coverage	N50	Number of contigs	Length	Percent of core genome present
A0801-01	97x	1053325	9	2749254	99
A0801-04	139x	996462	9	2742705	99
A0801-05	66x	170848	46	2803614	98
A0801-06	116x	614778	10	2741732	99
A0801-07	41x	653958	20	2748262	99
A0801-09	130x	996414	11	2744015	99
A0801-10	48x	340904	23	2718408	98
A0801-13	78x	675587	11	2742056	99
A0801-14	207x	1045569	8	2742620	99
A0801-18a	144x	606264	16	2713135	98
A0801-18b	220x	1053325	8	2749383	99
A0801-19	304x	347346	24	2842236	99
A0801-22	250x	1046130	8	2742873	99
A0801-8C	115x	1046130	8	2742760	99
A0801-17	25x	413389	25	2750132	98
A0901-0721	117x	653958	18	2747917	99
A0901-0836	132x	1046130	8	2742599	99
A0901-0850	118x	437206	14	2692013	99
A0901-1103a	60x	653958	22	2750609	99
A0901-1151	139x	252293	19	2716982	100
A1501-05	72x	485320	34	2856821	99
A1501-08b	100x	654830	19	2721076	99
A1501-09	128x	656105	15	2722103	99

Continued overleaf

Isolate ID	Average read coverage	N50[1]	Number of contigs[2]	Length[3]	Percent of core genome present
A1501-11	84x	484977	35	2856606	99
A1501-13a	131x	150314	49	2844977	98
A1501-14	247x	351126	33	2857020	99
A1501-13b	56x	170716	44	2834993	98
A1501-8A	138x	357581	25	2789861	99
A1601-01	48x	320744	39	2855612	99
A1601-03	41x	299202	42	2747271	99
A1601-05	110x	250119	21	2677085	99
A1601-08	42x	320744	40	2856652	99
A1601-09	59x	484977	48	2882421	99
A1601-10b	40x	321031	22	2722271	98
A1601-10c	65x	295538	22	2741765	98
A1601-18	122x	664601	23	2750362	98
A1601-13	37x	301553	27	2790041	99
A1601-16	71x	660830	33	2825447	99
C104	81x	654189	20	2748492	99
C122	64x	655823	15	2720757	98
C123	28x	654189	20	2748492	99
C37a	66x	606264	16	2713189	98
C52	162x	606264	16	2713343	98
C75	86x	500746	15	2722409	99
C78	52x	309926	27	2841205	99
C87	57x	290743	41	2868462	99
C89	37x	174117	37	2762081	99
C93	45x	347346	25	2841828	99

Continued overleaf

Isolate ID	Average read coverage	N50	Number of contigs	Length	Percent of core genome present
C94	34x	309926	28	2840857	98
HN0534.1	41x	252759	29	2757243	100
HN0538.1	37x	199123	36	2847018	99
HN0572.1	30x	334948	22	2754635	99
HN0574.1	78x	681954	12	2744739	99
HO0536.1	102x	230286	19	2731880	99
PN0621	116x	329582	25	2821608	99
S0801-0718	35x	541383	12	2742235	99
S0801-0821	631x	542050	9	2742125	99
S0801-1018	278x	1046130	8	2743150	99
S0901-0918	92x	837057	10	2742442	99
S1601-0912	127x	351126	32	2857680	99
S1601-1010	89x	656105	16	2722156	99

[1, 2, 3] Results are expressed to reflect base pair units.

Table 4.5 Population structure of the 62 *S. aureus* isolates recovered during the present study

Isolate	Isolate Source[1]	CC	ST	*spa* type
A0801-01	Air sample	CC1	ST1	t177
A0801-04	Air sample	CC1	ST1	t127
A0801-06	Air sample	CC1	ST1	t127
A0801-08c	Air sample	CC1	ST1	t127
A0801-09	Air sample	CC1	ST1	t114
A0801-13	Air sample	CC1	ST1	t127
A0801-14	Air sample	CC1	ST1	t127
A0801-18b	Air sample	CC1	ST1	t177
A0801-19	Air sample	CC1	ST1	t209
A0801-22	Air sample	CC1	ST1	t127
A0901-0836	Air sample	CC1	ST1	t127
A1501-08a	Air sample	CC1	ST109	t693
A1601-13	Air sample	CC1	ST109	t693
A1601-16	Air sample	CC1	ST109	t693
C78	Contact plate	CC1	ST1	t209
C93	Contact plate	CC1	ST1	t209
C94	Contact plate	CC1	ST1	t209
HN0574.1	HCW (anterior nares)	CC1	ST1	t922
S0801-07-18	Settle plate	CC1	ST1	t127
S0801-0821	Settle plate	CC1	ST1	t127
S0801-1018	Settle plate	CC1	ST1	t127
S0901-0918	Settle plate	CC1	ST1	t127
A0801-07	Air sample	CC5	ST5	t053
A0901-0721	Air sample	CC5	ST5	t053
A0901-1103a	Air sample	CC5	ST5	t053
C104	Contact plate	CC5	ST5	t053
C123	Contact plate	CC5	ST5	t053
HO0536.1	HCW (oropharynx)	CC5	ST5	t9057
A1501-05	Air sample	CC8	ST8	t025
A1501-11	Air sample	CC8	ST8	t025
A1501-14	Air sample	CC8	ST8	t025
A1601-01	Air sample	CC8	ST8	t025
A1601-08	Air sample	CC8	ST8	t025
A1601-09	Air sample	CC8	ST8	t025
C87	Contact plate	CC8	ST8	t025
HN0538.1	HCW (anterior nares)	CC8	ST8	t025
S1601-0912	Settle plate	CC8	ST8	t025
A0901-0850	Air sample	CC15	ST15	t254
A0901-1151	Air sample	CC15	ST15	t1361
A1601-03	Air sample	CC15	ST15	t491
A1601-05	Air sample	CC15	ST15	t491
C75	Contact plate	CC15	ST582	t084
HN0534.1	HCW (anterior nares)	CC15	ST15	NTA
PN0621	Patient (anterior nares)	CC15	ST15	t701

Continued overleaf

Isolate	Isolate Source	CC	ST	*spa* type
A0801-05	Air sample	CC30	ST30	t021
A1501-13a	Air sample	CC30	ST30	t8839
A0801-17	Air sample	CC45	ST45	NTA
A0801-18a	Air sample	CC45	ST45	t230
A1601-10c	Air sample	CC45	ST45	t230
A1601-18	Air sample	CC45	ST45	t620
C37a	Contact plate	CC45	ST45	t230
C52	Contact plate	CC45	ST45	t230
A1501-18b	Air sample	CC97	ST97	t865
A1501-09	Air sample	CC97	ST97	t865
A1601-10b	Air sample	CC97	ST97	t865
C122	Contact plate	CC97	ST97	t865
S1601-1010	Settle plate	CC97	ST97	t865
A0801-10	Air sample	CC398	ST398	t1451
HN0572.1	HCW (anterior nares)	CC672	ST672	t3841
HN0570.1	HCW (anterior nares)	CC12	ST12	t160
A1501-13b	Air sample	CC22	ST22	NTA
C89	Contact plate	CC22	ST22	t223

Abbreviations: CC, clonal complex; HCW, healthcare worker; ST, sequence type.

[1]Active air samples were taken using an Oxoid/Thermo Scientific model EM0100A air sampler (Oxoid Ireland) active air sampler. All *S. aureus* isolates were recovered on Colorex™ Staph Aureus chromogenic agar plates (Colorex).

t865 (5/62; 8.1%), t209 (4/62; 6.5%), t230 (4/62; 6.5%), t177 (2/62; 3.2%), t491 (2/62; 3.2%), t693 (2/62; 3.2%) and one each (1.6%) to t021, t084, t114, t1361, t1451, t160, t223, t254, t3841, t620, t701, t8839, t9057, t922 and t941.

4.3.2 Detection of genes encoding virulence factors and antimicrobial agent resistance

Virulence factors

All isolates harboured at least two immune evasion complex (IEC) genes (which include *chp*, *sea*, *sep*, *scn* and *sak*) and these were assigned IEC cluster types based on the combination of genes present (van Wamel *et al.*, 2006). IEC group D (27/62; 43.5%) comprising *chp, sak, scn* and *sea* was the most prevalent among the isolates investigated. IEC group B, comprising *chp*, *sak* and *scn,* was the second most prevalent (16/62; 25.8%) and was observed in slightly more isolates that IEC group E (12/62; 19.4%), which comprises *sak* and *scn*. Isolates belonging to IEC group C (6/62; 9.7%), which comprises *chp* and *scn*, and IEC group A (1/62; 1.6%), which comprises *chp*, *sak*, *scn* and *sea*, were also identified Genes encoding staphylococcal superantigen like (*ssl*) proteins were identified in all isolates. The prevalence of the *ssl* genes identified were: *ssl2* (61/62; 98.4%), *ssl7* (61/62; 98.4%), *ssl5* (60/62; 96.8%), *ssl9* (60/62; 96.8%), *ssl10* (60/62; 96.8%), *ssl1* (55/62; 88.7%), *ssl11* (49/62; 79.0%), *ssl8* (41/62; 66.1%), *ssl4* (39/62; 62.9%), *ssl3* (31/62; 50.0%) and *ssl6* (24/62; 38.7%). A complete *setB* gene cluster was identified in 59/62 (95.2%) of isolates.

Genes encoding cell-wall anchored proteins (CWAs) concerned with attachment and colonisation of potential hosts were relatively prevalent among all isolates. The genes encoding elastin binding protein (*ebps*) and *eno*, encoding laminin binding protein, were identified in 40/62 (64.5%) and 61/62 (98.4%) of isolates, respectively. The iron regulating surface protein A gene (*isd*A) was detected in 29/62 isolates (46.8%). Additional virulence

factors detected included *ica*D (62/62; 100%), *isa*B (61/62; 98.4%), *ica*A (60/62; 96.8%), *ica*B (60/62; 96.8%), *ssp*B (59/62; 95.5%), *ssp*P (59/62; 95.5%), *fib* (59/62; 95.2%), *set*C (49/62; 79.0%), *spl*A (42/62; 67.7%), *ssp*A (42/62; 67.7%), *spl*B (41/62; 66.1%) and *spl*E (31/62; 50.0%).

With regard to genes encoding toxin production, the enterotoxin gene complex (*egc*) was identified in 24/62 isolates (38.7%). The enterotoxin homologue, ORF CM14 was detected in one isolate only, and this was obtained from a swab of the anterior nares of a HCW- a nurse who worked on Ward B. The *sed* gene, encoding enterotoxin D protein was also detected in a single isolate- which was recovered from a nasal specimen obtained from a HCW- a healthcare assistant who worked on Ward B. *The tst1* gene encoding the toxic shock syndrome toxin TSST1 toxin was detected in an isolate recovered from a synthetic-cotton privacy curtain sampled with a contact plate on Ward B. Additional toxin genes identified included *hlg*B (62/62; 100%), *hlg*A (59/62; 95.2%), *hlg*C (58/62; 93.5%), *luk*X (58/62; 93.5%), *hla* (58; 93.4%), *luk*Y (53/62; 85.5%), *luk*D/E (42/62; 67.7%), *sek* (23/62; 37.1%), *seq* (15/62; 24.2%), *seh* (15/62; 24.2%), *seb* (11/62; 17.7%), *etA* (8/62; 12.9%), *sej* (7/62; 11.3%) and *sec/sel* (7/62; 11.3%). Details of all virulence factors detected among the study isolates are outlined in Table 4.6.

Antimicrobial agent resistance

A total of 34 genes encoding resistance to antimicrobial agents were detected in the 62 isolates investigated (Table 4.6). The most prevalent genes, and those that were detected in the majority of STs included *impR* (60/62; 96.8%), *blaI* (59/62; 95.2%), *bla*Z (57/62 (91.9%), *mprF* (57/62; 91.9%), *blaR* (54/62; 87.1%) and *sdrM* (49/62; 79.0%). In over half of isolates (32/62; 51.6%), *fosB* was detected, and these were among isolates assigned to ST8 (n=9), ST5 (n=6), ST15 (n=5), ST1 (n=4), ST109 (n=3), ST30 (n=2) and one each to

ST582, ST672 and ST12. In almost a quarter of isolates (15/62; 24.2%), *erm(A)* was detected, with these isolates assigned to ST5 (n=6), ST1 (n=4), ST109 (n=3), ST22 (n=1) and ST30 (n=1). A *fusC* gene was detected in 14/62 (22.6%) of isolates, all assigned to ST1 and all environmental isolates. The gene *qacC* was detected in 7/62 (11.3%) isolates, and these were assigned to ST8 (n=6) and ST582 (n=1). In 8/62 (12.9%) isolates, all assigned to ST8, *tet(K*) was detected. The *msrA* gene was detected in 5/62 (8.1%) of isolates, all assigned ST97. The macrolide resistance gene, *erm(C*) was recovered from a single isolate, and this was assigned ST8.

Detection of mecA

Phenotypic antimicrobial susceptibility testing of the 62 isolates investigated detected seven MRSA isolates (Chapter 3, Section 3.4.4). A *mecA* gene was detected these seven isolates by Seqshere *in-silico* genotyping, and also in one additional isolate (S-1601-09-12) which did not exhibit cefoxitin resistance and so was not included in further investigations of cefoxitin resistant isolates (Chapter 3, Section 3.4.2). This isolate was also tested for oxacillin susceptibility when carriage of the *mecA* gene was identified (as described in Chapter 2, Section 2.7.3), and found to be susceptible to oxacillin with an MIC of 0.5 μg/ml. Therefore, *mecA* was detected in: A1501-05, A1601-08, A1501-04, A1601-09, A1501-11, A1601-01, C87 and S-1601-09-12, all of which were assigned to ST8. Of these eight isolates, six were obtained by active air sampling (A1501-05, A1601-08, A1501-04, A1601-09, A1501-11, A1601-01), and one each by passive air sampling (S-1601-09-12) and surface sampling (C87). In all of these eight isolates, *fos*B, *Imr*P, *mpr*F and *tet*K were detected, with *erm*C also detected in C87. All eight isolates were obtained from Ward B on either SD3 (A1501-05, A1501-04, A1501-11, C87) or SD4 (A1601-08, A1601-09, A1601-01, S-1601-09-12).

Table 4.6 Virulence factors and resistance genes detected by *in-silico* genotyping of the 62 *S. aureus* isolates included in the present study

Study ID	Detected virulence factors	Detected resistance genes
HN0574.1	*ebpS, eno, fib, hl, hlgA, hlgB, hlgC, icaA, icaD, isaB, lukD, lukE, lukX, sak, scn, sea, seb, seh*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*
A0801-19	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, sak, scn, seg*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF*
C78	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, sak, scn, seg*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF*
C93	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, sak, scn, seg*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF*
C94	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, sak, scn, seg*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF*
A0801-09	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0801-8c	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*

Continued overleaf

Study ID	Detected virulence factors	Detected resistance genes
A0801-18b	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0801-13	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0801-14	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, fusC, lmrP, mprF, sdrM*
A0801-04	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0901-0836	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0801-06	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0801-01	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
A0801-22	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
S-0801-08-21	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
S-0901-09-18	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*

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Study ID	Detected virulence factors	Detected resistance genes
S-0801-07-18	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
S-0801-10-18	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seh*	*blaI, blaR, blaZ, fusC, lmrP, mprF, sdrM*
HO0536.1	*ebpS, eno, fib, hl, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF, sdrM*
A0801-07	*ebpS, eno, fib, hl, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF, sdrM*
A0901-1103a	*ebpS, eno, fib, hl, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF, sdrM*
A0901-0721	*ebpS, eno, fib, hl, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF, sdrM*
C104	*ebpS, eno, fib, hl, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF, sdrM*
C123	*ebpS, eno, fib, hl, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF, sdrM*
HN0538.1	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, icaA, icaC, icaD, isaB, lukX, lukY, sak, scn, sea, sed*	*blaI, blaR, blaZ, fosB, mprF, sdrM*
A1501-05	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, qacC, sdrM, tet(K)*
A1601-08	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, qacC, sdrM, tet(K)*

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Study ID	Detected virulence factors	Detected resistance genes
A1501-14	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, qacC, sdrM, tet(K*
A1601-09	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, sdrM, tet(K)*
A1501-11	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, qacC, sdrM, tet(K)*
A1601-01	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, qacC, sdrM, tet(K)*
C87	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, erm(C), fosB, lmrP, mprF, sdrM, tet(K)*
S-1601-09-12	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, fosB, lmrP, mprF, qacC, sdrM, tet(K)*
HN0570.1	*ORF-CM14, ebpS, fib, hl, hlIII, hla, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, sak, scn, sea-sep*	*fosB, lmrP, mprF, sdrM*

Continued overleaf

Study ID	Detected virulence factors	Detected resistance genes
HN0534.1	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, lukD, lukX, lukY, scn*	*blaI, blaR, blaZ, fosB, lmrP, sdrM*
PN0621	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, lukD, lukE, lukX, lukY, sak, scn, sea, seb*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*
A0901-0850	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, scn*	*fosB, lmrP, sdrM*
A0901-1151	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, fosB, lmrP, mprF, sdrM*
A1601-05	*chp, ebpS, eno, fib, hl, hlIII, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, scn*	*blaI, blaZ, fosB, lmrP, mprF, sdrM*
A1601-03	*chp, ebpS, eno, fib, hl, hlIII, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, scn*	*blaI, blaR, blaZ, fosB, lmrP, mprF, sdrM*
A1501-13b	*chp, ebpS, eno, hl, hlIII, hla, hlgA, hlgB, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sec, seg*	*blaI, blaR, blaZ, erm(A), lmrP, mprF, sdrM*
C89	*chp, ebpS, eno, hl, hlIII, hla, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, seg*	*blaZ, lmrP, mprF, sdrM*

Continued overleaf

Study ID	Detected virulence factors	Detected resistance genes
A1501-13a	*ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sea-sep*	*blaI, blaR, blaZ, erm(A), fosB, sdrM*
A0801-05	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sea, seg*	*blaI, blaR, blaZ, fosB, lmrP, mprF, sdrM*
A1601-10c	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, sak, scn, sec, seg*	*blaI, blaZ, lmrP, mprF, sdrM*
A0801-17	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sec, seg*	*blaI, lmrP, mprF, sdrM*
A0801-18a	*chp, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sec, seg*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*
C37a	*chp, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sec, seg*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*
C52	*chp, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sec, seg*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*
A1601-18	*chp, ebpS, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaC, icaD, isaB, isdA, lukX, lukY, sak, scn, sec, seg*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*

Continued overleaf

Study ID	Detected virulence factors	Detected resistance genes
A1601-10b	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, lmrP, mprF, msrA*
C122	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, lmrP, mprF, msrA*
A1501-08b	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, lmrP, mprF, msrA*
S-1601-10-10	*eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, lmrP, mprF, msrA*
A1501-08a	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, sak, scn, seg*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF*
A1601-13	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, sak, scn, seg*	*blaI, blaR, blaZ, erm(A), fosB, lmrP, mprF*
A1601-16	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, sak, scn, seg*	*blaI, erm(A), fosB, lmrP, mprF*
A0801-10	*chp, eno, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukX, lukY, scn*	*blaI, blaR, blaZ, lmrP, mprF, sdrM*
C75	*chp, ebpS, eno, etA, fib, hl, hlIII, hla, hlgA, hlgB, hlgC, icaA, icaC, icaD, isaB, isdA, lukD, lukE, lukX, lukY, scn*	*blaI, blaZ, fosB, lmrP, qacC, sdrM*
HN0572.1	*eno, hlIII, hlgA, hlgB, icaA, icaD, isaB, lukX, lukY, sak, scn*	*blaI, blaR, blaZ, fosB, lmrP*

4.3.3 Investigation of detected staphylococcal cassette chromosome elements

Isolates that were found to harbour genes associated with SCC*mec* (such as recombinases, *mec* complex or *ugpQ* genes) were examined for the presence and type of SCC*mec*. Elements of SCC*mec* were identified in 22/62 (34.5%) isolates, and all of these isolates were environmental involving air (n=16), settle plates (n=5) and one surface sample- obtained from a nightstand. Details of isolated in which elements of SCC*mec* were detected are provided in Table 4.7.

Fourteen isolates were assigned ST1 including A0801-09, A0801-8c, A0801-18b, A0801-13, A0801-14, A0801-04, A0901-0836, A0801-06, A0801-01, A0801-22, S-0801-08-21, S-0901-09-18, S-0801-07-18, S-0801-10-18. In all 14 isolates, *ccr*AB1 was detected, with no *mec* complex (no *mec* gene or *mec* regulatory genes present) and no detection of *ugp*Q. All fourteen isolates harboured *fus*C, encoding fusidic acid resistance. These isolates were therefore assigned CC1-MSSA-SCC*fus*.

SCC*mec* elements were detected in all 7 OS-MRSA isolates (as outlined in Section 4.3.3) and in S-1601-09-12. In each of these isolates, recombinases *ccr*AA and *ccr*C was detected, with *ugp*Q and *mec*A present also. As such, these isolates were assigned SCC*mec* type V (5C2&5).

4.3.4 Investigation of OS-MRSA isolates

Isolates harbouring a *mec*A gene but with *in-vitro* susceptibility to oxacillin (A1501-05, A1601-08, A1501-14, A1601-09, A1501-11, A1601-01, C87 and S-1601-09-12) were examined in further detail alongside a reference strain JCSC6944 (GenBank accession number: AB505629), which also harbours SCC*mec* typeV (5C2&5) and is MRSA. Guidance for clinical laboratories state that where a *mec* gene is identified, irrespective of

phenotypic resistance, the isolate should be reported as MRSA (CLSI, 2018). Therefore, these eight isolates were considered OS-MRSA.

*Comparison of mec*A *gene*

Using Clustal Omega, the FASTA sequence of the *mec* gene in all eight OS-MRSA isolates and the reference strain was found to be 100% identical.

Comparison of SCCmec structure

Comparison of the structure of the SCC*mec* element in all eight isolates and the reference strain revealed a high level of alignment (Fig. 4.2). The eight OS-MRSA SCC*mec* elements were identical, and notably there was no difference observed between the seven cefoxitin-resistant isolates and the single cefoxitin-susceptible isolate. . All isolates had intact recombinase and *mec* genes. The reference genome harboured *yozA*, an *AsrR* gene family transcriptional regulator, the sequence of which was not detected either in the OS-MRSA isolates.

Comparison of femXAB genes

The *femXAB* genes in all isolates in which *mecA* was detected were examined in detail using Clustal Omega. A reference strain from strain MRSA252 was used for comparison which was included in a publication investigating OS MRSA isolates (GenBank accession number BX571856) (Giannouli *et al.*, 2010). *FemXAB* genes encode protein structures which are essential for methicillin-resistance, and mutations in these genes have previously been reported in OS-MRSA isolates (Giannouli *et al.*, 2010; Brahma *et al.*, 2019).

Table 4.7 Isolates in the present study in which genes associated with SCC*mec* were detected

Sample ID	ST	Isolate type	Genes detected	Isolate/SCC*mec* type
A0801-01	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-04	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-06	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-09	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-13	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-14	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-18b	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-22	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0801-8c	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A0901-0836	1	Active air sample	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
S-0801-07-18	1	Settle plate	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
S-0801-08-21	1	Settle plate	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
S-0801-10-18	1	Settle plate	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
S-0901-09-18	1	Settle plate	*ccr*A1, *ccr*B1	CC1-MSSA-SCC*fus*
A1501-05	8	Active air sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
A1501-11	8	Active air sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
A1501-14	8	Active air sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
A1601-01	8	Active air sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
A1601-08	8	Active air sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
A1601-09	8	Active air sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
C87	8	Surface sample	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)
S-1601-09-12	8	Settle plate	*ccr*AA, *ccr*C, *mec*A, *ugp*Q	SCC*mec* type V (5C2&5)

Abbreviations: ST, sequence type; SCC, staphylococcal cassette chromosome.

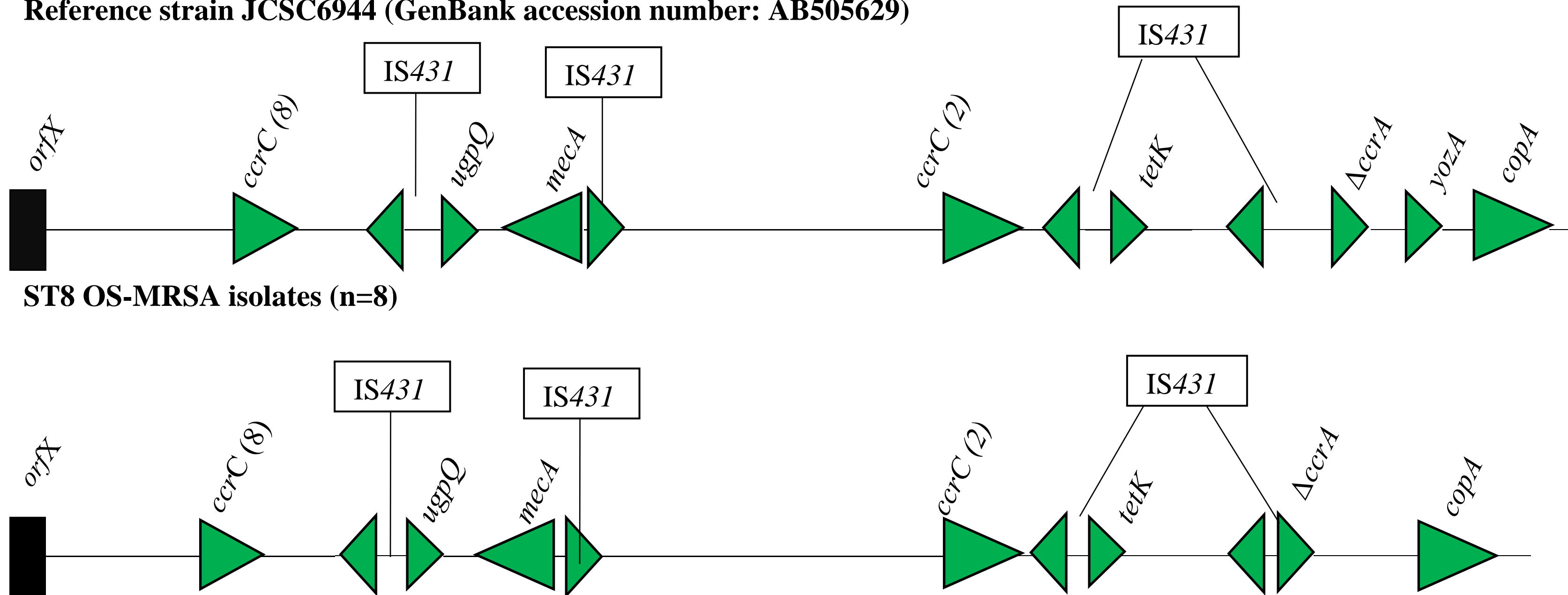

Figure 4.2 A schematic comparison of the SCC*mec* elements of eight OS-MRSA isolates recovered during the present study, compared to the SCC*mec* elements of the MRSA references isolate JCSC6944. The reference isolate was chosen as it is also assigned to SCC*mec* type V (5C2&5) and is phenotypically MRSA, whereas the study isolates were also assigned this SCC*mec* type but were oxacillin susceptible. A total of 62 *S. aureus* isolates were recovered from an Irish tertiary referral hospital, including from patients, HCWs and environmental sites. Phenotypic susceptibility testing

revealed seven isolates were resistant to cefoxitin, and these were identified as MRSA by the GeneXpert MRSA assay (Cepheid). These isolates were all susceptible to oxacillin by E-test (BioMérieux). All isolates were subjected to WGS, and analysis of this data revealed a *mec*A gene in eight isolates- the seven identified by their resistance to cefoxitin and one further cefoxitin-susceptible isolate that was also susceptible to oxacillin. The SCC*mec* elements of these eight isolates were compared with that of thr reference isolate JCSC6944, which was phenotypically MRSA. All the compared isolates were assigned SCC*mec* V (5C2&5). The genetic sequence of the SCC*mec* elements did not reveal any difference which would explain the differing resistances exhibited to oxacillin in the OS-MRSA and MRSA isolates. There was no difference in the sequences of the study isolates that were cefoxitin-resistant, and with the cefoxitin-susceptible isolate.

The output alignment generated by Clustal Omega in the analysis of *fem*A was first analysed. This revealed that the *femA* gene in the eight OS-MRSA isolates were identical to one another. However, eight point mutations in the nucleotide sequences were evident between the eight study isolates and the reference strain, and analysis of the predicted protein sequence revealed two amino acid changes (Fig. 4.3). The first was the replacement of tyrosine (in the reference strain) with phenylalanine in the eight study isolates, and this occurred at amino acid position 195 (Y195F). The second alteration to the FemA protein. sequence was observed at amino acid position 234, where glutamic acid (present in the reference strain), was replaced with aspartic acid in all eight study isolates (E234D).

Nine point mutations were observed between the *FemB* genes detected in the eight study isolates and the reference strain, and but these resulted in no changes to the predicted protein sequence, which was revealed to be 100% identical (Fig. 4.3).

Alignment of the *fem*X genes among the study isolates revealed these to be 100% identical. However, 12 point mutations were observed between the study isolates and the reference strain, and three alterations to the predicted amino acid sequence were observed as a result (Fig. 4.3). Asparagine in the reference strain was replaced with histidine in the study isolates at position 18 (N18H). Isoleucine was present in the reference strain at position 51 but in the study reference this had mutated to valine (I51V). Finally, glutamic acid was present at position 261 in the reference strain, but at this position in the study isolates was occupied by lysine (E261K).

4.3.5 Isolate relatedness

(a)

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MRSA252(femA)/1-420       1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
A150505(femA)/1-420       1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
A150511(femA)/1-420       1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
A150514(femA)/1-420       1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
A160109(femA)/1-420       1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
C87/1-420                 1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
A160101(femA)/1-420       1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
A160108/1-420             1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55
S16010912(femA)/1-420     1 MKFTNLTAKEFGAFTDSMPYSHFTQTVGHYELKLAEGYETHLVGIKNNNNEVIAA  55

MRSA252(femA)/1-420      56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
A150505(femA)/1-420      56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
A150511(femA)/1-420      56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
A150514(femA)/1-420      56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
A160109(femA)/1-420      56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
C87/1-420                56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
A160101(femA)/1-420      56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
A160108/1-420            56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110
S16010912(femA)/1-420    56 CLLTAVPVMKVFKYFYSNRGPVIDYENQELVHFFFNELSKYVKKHRCLYLHIDPY 110

MRSA252(femA)/1-420     111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
A150505(femA)/1-420     111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
A150511(femA)/1-420     111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
A150514(femA)/1-420     111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
A160109(femA)/1-420     111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
C87/1-420               111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
A160101(femA)/1-420     111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
A160108/1-420           111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165
S16010912(femA)/1-420   111 LPYQYLNHDGEITGNAGNDWFFDKMSNLGFEHTGFHKGFDPVLQIRYHSVLDLKD 165

MRSA252(femA)/1-420     166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRYLSEEELPIFRSFMEDTSESKAFADR 220
A150505(femA)/1-420     166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
A150511(femA)/1-420     166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
A150514(femA)/1-420     166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
A160109(femA)/1-420     166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
C87/1-420               166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
A160101(femA)/1-420     166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
A160108/1-420           166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220
S16010912(femA)/1-420   166 KTADDIIKNMDGLRKRNTKKVKKNGVKVRFLSEEELPIFRSFMEDTSESKAFADR 220

MRSA252(femA)/1-420     221 DDKFYYNRLKYYKERVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
A150505(femA)/1-420     221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
A150511(femA)/1-420     221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
A150514(femA)/1-420     221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
A160109(femA)/1-420     221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
C87/1-420               221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
A160101(femA)/1-420     221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
A160108/1-420           221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275
S16010912(femA)/1-420   221 DDKFYYNRLKYYKDRVLVPLAYINFDEYIKELNEERDILNKDLNKALKDIEKRPE 275

MRSA252(femA)/1-420     276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
A150505(femA)/1-420     276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
A150511(femA)/1-420     276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
A150514(femA)/1-420     276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
A160109(femA)/1-420     276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
C87/1-420               276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
A160101(femA)/1-420     276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
A160108/1-420           276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330
S16010912(femA)/1-420   276 NKKAHNKRDNLQQQLDANEQKIEEGKRLQEEHGNELPISAGFFFINPFEVVYYAG 330

MRSA252(femA)/1-420     331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
A150505(femA)/1-420     331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
A150511(femA)/1-420     331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
A150514(femA)/1-420     331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
A160109(femA)/1-420     331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
C87/1-420               331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
A160101(femA)/1-420     331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
A160108/1-420           331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385
S16010912(femA)/1-420   331 GTSNAFRHFAGSYAVQWEMINYALNHGIDRYNFYGVSGKFTEDAEDAGVVKFKKG 385

MRSA252(femA)/1-420     386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
A150505(femA)/1-420     386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
A150511(femA)/1-420     386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
A150514(femA)/1-420     386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
A160109(femA)/1-420     386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
C87/1-420               386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
A160101(femA)/1-420     386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
A160108/1-420           386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
S16010912(femA)/1-420   386 YNAEIIEYVGDFIKPINKPVYAAYTALKKVKDRIF                     420
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MRSA252(femX)/1-421        1 MEKMHITNQEHDAFVKSNPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEIQGVA  55
A150505(femX)/1-421        1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
A150111(femX)/1-421        1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
A150114(femX)/1-421        1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
A160109(femX)/1-421        1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
C87/1-421                  1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
A160101(femX)/1-421        1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
A160108(femX)/1-421        1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55
S16010912(femX)/1-421      1 MEKMHITNQEHDAFVKSHPNGDLLQLTKWAETKKLTGWYARRIAVGRDGEVQGVA  55

MRSA252(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
A150505(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
A150111(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
A150114(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
A160109(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
C87/1-421                 56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
A160101(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
A160108(femX)/1-421       56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110
S16010912(femX)/1-421     56 QLLFKKVPKLPYTLCYISRGFVVDYSNKEALNALLDSAKEIAKAEKAYAIKIDPD 110

MRSA252(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
A150505(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
A150111(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
A150114(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
A160109(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
C87/1-421                111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
A160101(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
A160108(femX)/1-421      111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165
S16010912(femX)/1-421    111 VEVDKGTDALQNLKALGFKHKGFKEGLSKDYIQPRMTMITPIDKNDDELLNSFER 165

MRSA252(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
A150505(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
A150111(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
A150114(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
A160109(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
C87/1-421                166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
A160101(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
A160108(femX)/1-421      166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220
S16010912(femX)/1-421    166 RNRSKVRLALKRGTTVERSDREGLKTFAELMKITGERDGFLTRDISYFENIYDAL 220

MRSA252(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMETSEKQAKKAQNMIN 275
A150505(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
A150111(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
A150114(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
A160109(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
C87/1-421                221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
A160101(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
A160108(femX)/1-421      221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275
S16010912(femX)/1-421    221 HEDGDAELFLVKLDPKENIAKVNQELNELHAEIAKWQQKMKTSEKQAKKAQNMIN 275

MRSA252(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
A150505(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
A150111(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
A150114(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
A160109(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
C87/1-421                276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
A160101(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
A160108(femX)/1-421      276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330
S16010912(femX)/1-421    276 DAQNKIAKNEDLKRDLEALEKEHPEGIYLSGALLMFAGSKSYYLYGASSNEFRDF 330

MRSA252(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
A150505(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
A150111(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
A150114(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
A160109(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
C87/1-421                331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
A160101(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
A160108(femX)/1-421      331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385
S16010912(femX)/1-421    331 LPNHHMQYTMMKYAREHGATTYDFGGTDNDPDKDSEHYGLWAFKKVWGTYLSEKI 385

MRSA252(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
A150505(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
A150111(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
A150114(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
A160109(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
C87/1-421                386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
A160101(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
A160108(femX)/1-421      386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
S16010912(femX)/1-421    386 GEFDYVLNQPLYQLIEQVKPRLTKAKIKISRKLKRK                    421
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Figure 4.3 The protein sequence alignment output of the *femA* and *femX* genes of eight ST8 OS-MRSA isolates and a reference strain (MRSA252) generated by Clustal Omega. In the present study, eight isolates exhibited oxacillin-susceptibility despite harbouring intact wild type *mec*A genes and complete SCC*mec* elements. Oxacillin MICs in these isolates ranged from 0.25-1 0 μg/ml, with isolates exhibiting MICs greater than 4 μg/ml considered resistant. Oxacillin-susceptible MRSA (OS-MRSA) isolates have been reported previously. Giannouli and colleagues (2010) detected mutations in the *femXAB* genes in OS-MRSA isolates and concluded that, due to the role of *femXAB* in the expression of methicillin-resistance, these mutations resulted in atypical responsiveness to oxacillin. Therefore, the DNA and protein sequences of the *femXAB* genes harboured by the eight OS-MRSA isolates were examined. The reference strain included in this figure was that MRSA strain used by Giannouli and colleagues (2010) for their investigations. Analysis of the *femA* gene in the present study (a) revealed two amino acid changes, at positions 195 and 234 in the predicted protein sequence. These are highlighted in the alignment output using red diamonds. (b) Analysis of *femX* revealed the following mutations: N18H, I51V, E261K- also denoted by a red diamond. Although nine point mutations were observed in the nucleotide sequence between *femB* in the reference strain and the study isolates, there were no changes to the amino acid sequence as a result.

For the purpose of this study, two or more isolates were considered to be related where ≤ 24 allelic differences were observed by wgMLST analysis. Previous studies have proposed that *S. aureus* isolates which exhibit ≤ 24 allelic differences should be considered related (Schürch *et al.*, 2018). On each occasion where two or more isolates were thought to be related using the above criterion, they were assigned to a Related Isolate Group (RIG). Each RIG was analysed with regard to the relatedness of isolates within the group. Using this criterion, 10 RIGs involving 46 isolates were identified among the 62 isolates investigated in the present study (Fig. 4.1). Each of the 10 RIGs involved one or more isolates recovered from an active air sample. Four RIGs consisted exclusively of isolates recovered from air samples, two RIGs comprised isolates from active air samples, settle plates and contact plate samples, one RIG involved isolates from an active air sample and contact plate and one involved isolates from active air samples, settle plates and a HCW. Details of the type of isolates involved in each RIG, their assigned ST and *spa* type and epidemiological information are provided in Table 4.8.

Related Isolate Group 1 (RIG 1)

RIG1 involved eight isolates recovered from Ward B on SD3 (n=4) and SD4 (n=4), with no more than 2 allelic differences observed between each of these isolates. All eight isolates were assigned ST8 and *spa* type t025. This RIG comprised the OS-MRSA isolates discussed in Section 4.3.3 (Fig. 4.3). The patient admitted to the bedspace associated with the nightstand isolate (C87) was sampled as part of the present study and was found not to be colonised with *S. aureus* either orally or nasally at the time. No patients were known to be colonised with *S. aureus* during SD3. On SD4 a patient was admitted to the Ward B during the study period who was subsequently found to be colonised with *S. aureus*, but the isolate (PN0621) recovered from this patient (Px0621) was assigned to ST15 and *spa*

Table 4.8 Isolates assigned to related isolate groups in the present study

RIG (No. isolates involved)	Isolate source (n)	SD recovered (No. isolates)	Ward (No. isolates)	wgMLST allelic differences (range)
RIG1 (8)	Active air sample (6), Settle plate[1] (1) Nightstand (1)	SD3 (4) SD4 (4)	Ward B (8)	0-2
RIG2 (3)	Active air sample (1) Patient notes folder (1) Privacy curtain (1)	SD1 (1) SD2 (2)	Ward A (3)	1-6
RIG3 (2)	Active air sample (2)	SD1 (1) SD4 (1)	Ward A (1) Ward B (1)	2[2]
RIG4 (3)	Active air sample (1) Privacy curtain (2)	SD1 (1) SD3 (2)	Ward A (1) Ward B (2)	0[3]
RIG5 (3)	Active air sample (3)	SD3 (1) SD4 (2)	Ward B (3)	2-11
RIG6 (2)	Active air sample (2)	SD4 (2)	Ward B (2)	4
RIG7 (12)	Active air sample (8) Settle plate (4)	SD1 (10) SD2 (2)	Ward A (12)	1-6
RIG8 (2)	Active air sample (2)	SD1 (2)	Ward A (2)	2
RIG9 (6)	Active air sample (3) HCW oropharynx (1) Patient notes folder (1) Privacy curtain (1)	SD1 (2) SD2 (2) SD4 (2)	Ward A (4) Ward B (2)	0-29
RIG10 (5)	Active air sample (3) Settle plate (1) Privacy curtain (1)	SD3 (2) SD4 (3)	Ward B (5)	0-3

[1]Settle plates were placed on the overbed table of each patient for a period of one hour.

[2]For RIGs containing two isolates only, no range could be provided, so this value represents the actual allelic differences between both isolates.

[3]Isolates that exhibited zero allelic differences were considered indistinguishable by wgMLST.

Abbreviations: TE, transmission event; SD, study day, RIG, related isolate group; wgMLST, whole-genome multi-locus sequence typing.

type t701 and was thus unrelated to the isolates involved in this RIG. No isolates within this RIG were related to those recovered from HCWs.

The settle plate sample that harboured *S. aureus* was collected between 09:00 and 10:00 from the over-bed table of Bed 12 during SD4. Two active air samples (A1601-08 and A1601-09) yielded *S. aureus* isolates (referred to as A1601-08 and A1601-09) that were indistinguishable to isolate S-1601-09-12 recovered from the settle plate. Air sample A-1601-08 was collected between 08:51-09:00 h and air sample A1601-09 was collected between 09:02-09:11 h. The settle plate sample likely reflects surface deposition from *S. aureus* shed into the air, as the sampling times overlap.

Related Isolate Group 2 (RIG2)

RIG2 involved three isolates exhibiting 1-5 allelic differences were recovered from Ward A over two sampling days (SD1 and SD2), and all were assigned ST45 and *spa* type t230. An isolate (A0801-18a) recovered from an active air sample collected during SD1 exhibited one allelic difference from an isolate (C52) recovered from a surface sample collected during SD2 (obtained from a synthetic cotton privacy curtain of Bed 20). Isolate C52 exhibited 5 allelic differences from an isolate (C37a) obtained from a plastic folder containing patient notes which was attached to Bed 18. Isolates C37a and C52 were recovered within hours of each another.

Related Isolate Group 3 (RIG3)

RIG3 involved two *S. aureus* isolates assigned to ST45 that were recovered from active air samples. The first of these isolates, A0801-17, was recovered on Ward A during SD1, and the second isolate, A1601-18, was recovered on Ward B during SD4. The isolates were

very closely related and exhibited only two allelic differences. Due to the break between sampling on Ward A (SD1 and SD2) and Ward B (SD3 and SD4) to facilitate sample processing in the laboratory, there was a gap of eight days between SD1 and SD4. Thus, the two isolates were recovered eight days apart on separate wards. These isolates were unrelated to all other isolates and were not linked to a colonised patient or HCW. As such, it cannot be definitively ascertained how both were recovered from the two separate locations. However, the period prior to the collection of both isolates was busy on the wards concerned, with visiting medical and surgical teams leading to increased room occupants.

Related Isolate Group 4 (RIG4)

RIG4 involved three indistinguishable *S. aureus* isolates: A0801-19, C93 and C94, all assigned to ST1 and *spa* type t209. A0801-19 was recovered from an active air sample recovered on Ward A on SD1. Isolates C93 and C94 were recovered from two contact plate samples in Ward B on SD4 (SD1 was undertaken eight days earlier than SD4). Isolates C93 and C94 were recovered from privacy curtains, with C93 separating bedspaces 13 and 14 and C94 surrounding the remaining surrounds of bed 14.

Related Isolate Group 5 (RIG5)

RIG5 involved three *S. aureus* isolates recovered from active air samples taken on Ward B. The isolates were assigned ST109 and *spa* type t693 and differed by 2-11 allelic differences. Isolate A1501-08a was recovered during SD3 and isolates A1601-13 and A1601-16 were recovered one day later on SD4 from air samples taken 30 minutes apart. There were no apparent links between the air samples that yielded these isolates. However,

all three samples were recovered during times of much activity in the ward, with patients washing and dressing and curtains being agitated. Sampling within the study area on both study days revealed *S. aureus* contaminating fomites, so potentially a contaminated object was agitated during this period of activity, resulting in recovery of *S. aureus* from active air samples.

Related Isolate Group 6 (RIG6)

RIG6 involved two *S. aureus* isolates exhibiting 4 allelic differences obtained by active air sampling on Ward B during SD4 (A1601-03 and A1601-05). Both isolates were assigned ST15 and *spa* type t491. Air sampling was undertaken continuously, with only short breaks of less than 90 s to facilitate decontamination of the air sampler headpiece and renewal of the agar plate. These two related isolates were obtained from air samples collected approximately 10 min apart; isolate A1601-03 was collected first and then the next consecutive air sample failed to yield *S. aureus* whereas the next air sample taken yielded A1601-05.

Related Isolate Group 7 (RIG7)

RIG7 involved a group of 12 isolates, all assigned to ST1 and *spa* type t127, all recovered from Ward A. During SD1, seven *S. aureus* isolates (A0801-04, A0801-06, A0801-08c, A0801-09, A0801-13, A0801-14 and A0801-22) involved in RIG7 were recovered from seven active air samples, three isolates involved in RIG7 (S-0801-0718, S0810-0821 and S-0801-10-18) were recovered from settle plates. During SD2 two isolates involved in this RIG were recovered from an active air sample (A0901-0836) and a settle plate (S-0901-09-

18), respectively. Isolates within this group were very closely related and differed by a median of 1 allelic difference, (range 0-6).

A review of where and when samples within this cluster were recovered indicated a potential link to an unsampled (and therefore colonisation status unknown) HCW, a member of non-clinical staff who worked on Ward A during both SD1 and SD2, and had cause to enter the sampling area on a number of occasions. Despite fluctuating numbers of occupants present within the room of SD1, the presence of this individual HCW consistently corresponded with *S. aureus* recovery from the environment, i.e. each time this HCW entered the room there was recovery of an isolate from a concurrent active air sample and often the subsequent sample also. Settle plates that were collected during periods when this HCW was present also harboured isolates from this RIG. No isolates from this RIG were obtained at any other points that did not correspond with this HCW entering the study ward, and no other patterns were evident in relation to other clinical activities or HCWs. This suggests that the HCW was potentially colonised with *S. aureus* relating to this RIG, although because this HCW declined to participate in the study, this cannot be proven. If this HCW was colonised at this time, the environmental sampling data suggests they were readily shedding *S. aureus* into the air upon entering the room, and this *S. aureus* then went on to settle on patient overbed tables.

Related Isolate Group 8 (RIG8)

Two active air samples collected on Ward A on SD1 yielded isolates A0801-01 and A0801-18b, which were assigned to ST1 and *spa* type t177. The isolates were very closely related and exhibited 2 allelic differences. Isolate A0801-01 was collected at 07:00 h whereas isolate A0801-18b was collected three hours later at 10:05 h, and no apparent link could be discerned based on ward activity or individuals present.

Related Isolate Group 9 (RIG9)

TE9 involved six *S. aureus* isolates recovered over the course of three study days (SD1, SD3 and SD4) and from both wards (HN0536.1, C104, C123, A0901-0721, A0901-1103a, A0801-07). All isolates were ST5 and five were assigned to *spa* type t053 with the remaining isolate assigned to *spa* type t9057. Three of the isolates (all t053) were recovered from active air samples from Ward A; isolate A0801-07 was recovered during SD1 and isolates A0901-0721 and A0901-1103a were recovered during SD2. All three isolates were indistinguishable (i.e. no allelic differences were observed between them). The two remaining t053 isolates were recovered during SD4 on Ward B. Isolate C104 was recovered from a notes folder associated with Ward B, Bed 11. Isolate C123 was recovered from the privacy curtain of Ward B, Bed 13. The t9057 (H00536.1) isolate was recovered during SD1 from an oropharyngeal rinse sample of an HCW, and this was the only day that this HCW was observed entering the study area.

The maximum allelic differences observed between isolates in this RIG is 29, which is greater than the cut-off of ≤ 24, as proposed by Schurch *et al.* (2018) and is being applied in this study, but it has been included to in this RIG due to its relatedness to C104 and relatively few differences above the cut-off (and known epidemiological links) to C123 (26 allelic differences) and three indistinguishable isolates (A0801-07, A0901-0721 and A0901-1103a) from which 29 allelic differences were observed . The HCW isolate (HO0536.1) exhibited 22 allelic differences from C104.)

In detail, an isolate obtained from an oral sample provided by a HCW (HO0536.1) exhibited 22 allelic differences from C104. C104 exhibited 4 allelic differences from C123, and 7 allelic differences from the three remaining isolates within the RIG (A0901-0721, A0901-1103a and A0801-07).

Related Isolate Group 10 (RIG10)

RIG10 involved five environmental isolates recovered from Ward B on SD3 (n=2) and SD4 (n=3). All five isolates were assigned to ST97 and *spa* type t865, and isolates exhibited 1-3 allelic differences. The isolates were recovered from a settle plate (S1601-10-10), three active air samples (A1501-08b, A1501-09 and A1601-10b) and a contact plate (C122). C122 was recovered from the privacy curtain of Bed 12. Three of these isolates were indistinguishable (A1501-08b, A1501-09 and C122), and these exhibited one and three allelic differences to A1601-10b and S-1601-10-10, respectively.

4.4 Discussion

This study investigated the population structure, diversity and relatedness of *S. aureus* isolates collected over four study days (involving a five-hour period of extensive environmental sampling) on two multi-bedded surgical wards. A collection of isolates that were obtained in January 2019 in a tertiary care hospital were subjected to WGS. Transmission of *S. aureus* in nosocomial settings is understood to occur mainly by patient-to-patient transfer, or via the contaminated hands of HCWs, but WGS studies have revealed the putative environmental role (Price *et al.*, 2014), in addition to outbreaks which have implicated contaminated environmental sites, specifically those associated with air (Wagenvoort *et al.*, 1993; Cotterill *et al.*, 1996; Kumari *et al.*, 1998).

Population structure of recovered isolates

The sampling method in the present study aimed to provide a snapshot of the circulating MSSA/MRSA clones in a relatively confined nosocomial environment over a short time period. As such, the study was not powered to reflect the overall population structure of

circulating Irish strains. In Ireland, the dominant MRSA clone reported to the NMRSARL is ST22-MRSA-IV, which has been attributed the largest proportion of invasive MRSA infections since the early 2000s (National MRSA Reference Laboratory, 2018). Other predominant MRSA clones include: ST5-MRSA-II, ST36-MRSA-II, ST1-MRSA-IV, ST5-MRSA-IV and ST8-MRSA-IV. Surveillance of MSSA is not routinely undertaken in the same systematic manner of that of MSSA, so less is known about the dominant circulating MSSA clones in Ireland. Investigations of European MSSA BSIs by *spa* typing have revealed that MSSA populations appear more genetically diverse than MRSA. Deasy *et al.* (2019) undertook a molecular epidemiological investigation of Irish MSSA BSIs from 2006-2017. Of the 252 MSSA isolates included in their investigation, almost have were assigned MLST CCs 45, 30 and 5, and these CCs were also among the most prevalent in the European PPS undertaken by Grundman et al. (2014). Almost a third of the isolates recovered in the present study were CC1 (22/62), which does not reflect the overall European or Irish trends for BSI isolates, but has predominated in other settings. In a study of clinical isolates obtained in Romania (which included BSI and SSTIs), CC1 was predominant (Monecke *et al.*, 2014). Of note, the isolates in this study were not infective, i.e. including either human colonisation or environmental contamination, further confounding efforts to apply the recovered population to a wider context.

The SCC*mec* types of eight OS-MRSA isolates included in the present study were classified as SCC*mec* type 5 (5C2&5). This SCC*mec* classification is characterized by a class C2 *mec* complex and two *ccrC* recombinase genes.

Continuous vs. intermittent air sampling

The results of the present study reveal the diverse population of *S. aureus* circulating in relatively small volumes of indoor hospital air. In Chapter 3, section 3.3.3.1, it was

outlined that 6 putatively discrete isolates (differing phenotypic colony appearance and non-identical antimicrobial resistance behaviours) were obtained from 3 cubic Ls of air. WGS revealed these isolates were unrelated (i.e. of different sequence types, *spa* types and differing by more than 40 allelic differences) to one another.

Studies that have investigated the role of the air in *S. aureus* transmission have been undertaken previously, but not outside of ICU settings (Moore *et al.*, 2015; Price *et al.*, 2017; Dancer *et al.*, 2019; Adams and Dancer, 2020). These studies have undertaken WGS on isolates obtained over varying periods. Dancer and colleagues (2019) report a study which included environmental sampling (of high-touch surfaces and air) which was undertaken on 10-study days over a period of ten months,with the sampling period lasting for two hours on each sampling day. The results of this study, which are subject to further analysis by Adams *et al.* (2020), outline that the air samples were obtained by moving the sampler to different sites within the ICU throughout the sampling period. Price *et al.* (2017) obtained 10 samples per month. Moore and colleagues (2017) have applied WGS technology to isolates collected in a previous study (Wilson *et al.*, 2011), the methods of which state that air samples were taken three times per sampling day (with 1,152 sampling days in total). As such, the present study is the first to the author's knowledge to undertake continuous active air sampling for *S. aureus* for a period of hours (with breaks in sampling only to renew the sampling plate), at a fixed location in an active clinical area.

Relatedness of recovered isolates

A threshold of $\leq$ 24 allelic differences between isolates subjected to wgMLST was applied to assess the relatedness of the 62 isolates included in the present study (Schürch *et al.*, 2018). No patient isolates were implicated in any RIGs, but this may reflect that only one

patient sampled as part of the study was found to harbour *S. aureus*. A HCW was included in one RIG. All remaining RIGs (n=8) involved at least one active air sample.

Five RIGs involved isolates obtained via surface sampling, and four involved synthetic cotton privacy curtains. Privacy curtains are not changed between patients, but are replaced when a bedspace is undergoing a terminal clean, if a ward has been 'deep-cleaned', such as in the case of an outbreak or quarterly. Privacy curtains were linked to isolates recovered from active air samples in all four RIGs in which they were included. In RIG2, a privacy curtain and patient notes folder were found to be related to an air sample that had been collected the previous day. In RIG4, two privacy curtains sampled on Ward B were linked to an active air sample collected on Ward A one week previously. This was similar to RIG9, where a notes folder and privacy curtain sampled on SD4 on Ward B was found to be related to a HCW who worked on Ward A during SD1. Three air samples collected on Ward A were also linked. RIG10 concerned a privacy curtain sampled on Ward B during SD4, and this also involved an air sample and settle plate collected on the same day, in addition to two active air samples collected the previous day. The liberation of *S. aureus* from agitated privacy curtains has been established and demonstrated previously (Noble, 1962), and a correlation has been observed between increased total viable airborne bacterial counts and curtain movement (Hathway *et al.*, 2013). Although contaminated curtains were linked with air samples recovered during this study, the directionality of transmission cannot be confidently inferred.

The involvement of privacy curtains in a number of RIGs may be a reflection of the degree of surface contamination on frequently touched sites. Patient notes folders, which were observed to be frequently handled by HCWs, were involved in 2/10 RIGs. Dancer *et al.* (2020) identified *S. aureus* transmission between a table and a cardiac monitor in an ICU, both items which have previously been shown to be frequently touched by the hands of HCWs in previous studies. A covert audit of the sequence of surfaces touched by the hands

of HCWs and relatives in an isolation/single room within NHS acute admissions unit identified that patient notes were the most frequently handled item during their study period. The putative transmission observed in the present study may have resulted from lapsed hand hygiene by a HCW, resulting in contamination of a touch site. The hands of HCWs were not sampled in the present study, as has been undertaken previously (Moore *et al.*, 2015; Kong *et al.*, 2016; Kpeli *et al.*, 2016; Dancer *et al.*, 2019). Observation of hand hygiene compliance identified that the hand hygiene event missed most commonly is following contact with the patient environment, with compliance only at 37% (FitzGerald *et al.*, 2013). Patient notes (which were contained together in a trolley in this setting) were frequently touched, second only to the patient bed. These data, in addition to the present study, suggest that hard-copy patient notes harbour bacteria (in this case *S. aureus*) and can serve as pathways for onward transmission in the ward environment, potentially via HCW hands. Furthermore, as notes folders are the remit of HCWs, they may not be viewed as an extension of the patient environment, thus HCWs will not be mentally prompted to perform hand hygiene after handling them (World Health Organization, 2013).

Settle plates were used in the present study with the aim of demonstrating surface deposition by *S. aureus*. Settle plates were placed on patient overbed tables, a site previously shown to be touched frequently in the general ward environment (Huslage *et al.*, 2010). In the course of this study, patients were observed to keep open fruit and other personal items on overbed tables. Settle plates were observed at all times during the study period, and were not handled by patients or HCWs, so it is felt that bacterial growth on settle plates is a true reflection of settling from air. Active air samples were linked to settle plates collected concurrently in RIG1, RIG7 and RIG10. As outlined in Chapter 3, section 3.3.3, settle plates yielded numerous colonies with varied phenotypic appearance, highlighting this transmission pathway for nosocomial bacteria. Inadequate cleaning and

decontamination a site in such close proximity to patients could increase the risk of resultant infection (Bogusz *et al.*, 2013).

Airborne S. aureus recovery from two differently ventilated wards

As discussed in chapter 2, section 2, Ward A is mechanically ventilated using a HVAC system with HEPA-filtration, whereas Ward B is naturally ventilated. Active air sampling recovered 19 *S. aureus* isolates from Ward A and 19 from Ward B. The isolates recovered from Ward A were assigned to six STs, including ST1 (n=11), ST5 (n=3), ST15 (n=2) and one each to ST30, ST398 and ST45. Isolates recovered from Ward B comprised seven STs, including ST8 (n=6), ST109 (n=3), ST97 (n=3), ST45 (n=2) ST15 (n=2) and one each to ST22 and ST30. Of the 18 isolates recovered from Ward A, six were assigned to RIGs; whereas 14/19 isolates recovered from the air of Ward B were found to related to one or more isolates.

The ventilation system serving Ward A is set to provide 15 air changes per hour, and it was confirmed by the technical services department in the hospital that the system was functioning to that effect during the sampling days. An equation to calculate a rate of purging airborne contaminants (Rhame, 1986) has been adapted by the CDC and states that a ventilation system providing 10 ACH (15 ACH per hour were provided in the present study) will have completely replaced the volume of air in a specific area within a 28-min period whilst functioning at 99% efficiency (Sehulster and Chinn, 2003). *Staphylococcus aureus* recovered from the air of Ward A was involved in six RIGs, less than half of those recovered from Ward B. This could potentially reflect a benefit of the ventilation system in Ward A, reducing putative transmission. However, this study was undertaken over a short-time period and did not account for patient infections, so it cannot be implied with sufficient power or confidence from this observational finding that mechanical ventilation

with the addition of HEPA-filtration will reduce *S. aureus* transmission, but further studies could be undertaken to investigate this.

Mechanisms underpinning oxacillin-susceptibility in mecA positive S. aureus isolates

Analysis of WGS data revealed eight isolates that were phenotypically susceptible to oxacillin (and thus were considered MSSA based on these criteria). All eight isolates harboured an intact *mec*A gene and were assigned SCC*mec* V (5C2&5). These isolates (all assigned to ST8) were thereafter referred to as OS-MRSA. These were closely related to one another (with no more than two allelic differences observed between the eight isolates. The CLSI recommend that in a clinical laboratory setting, where *mecA* is detected in an isolate but phenotypic oxacillin or cefoxitin susceptibility is exhibited, the isolate should be reported as MRSA, and treatment should be given as with a conventionally phenotypic MRSA (Cockerill, 2010)

Despite an increasing incidence in reports of OS-MRSA across a number of countries, the exact mechanisms underpinning this process are not yet fully understood. Point mutations in the *mec* gene (Proulx *et al.*, 2015; Goering *et al.*, 2019) have been reported, and revertant to wild type has been observed following in-vitro exposure to sub-inhibitory concentrations of oxacillin. Alterations to the structure of *fem*XAB have also been implicated in reducing oxacillin resistance in *mec*A positive *S. aureus* isolates (Giannouli *et al.*, 2010; Brahma *et al.*, 2019).

As outlined previously, Proulx and colleagues (2015) reported the inactivation of the *mec* gene by the insertion of the transposable element *IS*1181 into the *mec* sequence, in a clinical *S. aureus* isolate exhibiting 83% similarity to USA100 by PFGE. Western blot analysis demonstrated that the wild type protein encoded by *mec*A was not expressed in these isolates. In the presence of oxacillin, precise excision of *IS*1181 was observed (and

the resistance phenotype was restored). A second isolate was reported in the same study, also OS-MRSA, which was obtained from a different patient and *mecA* was amplified by PCR and the PCR products were sequenced. The sequence data from the *mecA* gene of this isolate revealed an altered reading frame within *mecA* (with a single nucleotide deletion observed), and due to an early stop codon, premature termination of *mecA* translation. Oxacillin-resistant derivatives of this isolate from this patient were also obtained *in-vitro* by growth in the presence of oxacillin, in which the wild type reading frame was restored by the insertion one or more nucleotides near the deletion site.

A similar frameshift mutation, and restoration of *mecA* function in the presence of sub-inhibitory concentrations of oxacillin was observed in a study of six non-epidemiologically linked and genetically diverse clinical OS-MRSA isolates obtained from six US states (Goering *et al.*, 2019). Oxacillin resistance was induced *in-vitro*, with primary mutations that had inhibited the function of *mecA* restored in the presence of sub-inhibitory concentrations of oxacillin by secondary mutation. All original isolates, and the resistant derivatives derived from them, were subjected to WGS. The WGS data revealed single-point of reading-frame mutations which are usually associated with tandem repeat sequences, and the authors report this were likely due to slip-strain mispairing during DNA replication. Exposure to sub-inhibitory concentrations of oxacillin induced *mecA* mutations that restored wild-type function. These findings demonstrated that instability in the *mecA* gene and resultant phenotypic susceptibility to oxacillin are not limited to a distinct lineage or isolate cluster. The authors report these *mecA*-positive isolates were PBP2a-negative when tested using latex agglutination, thus describing these types of isolates as ‘stealth MRSA’, due to their capacity to avoid detection using routine diagnostic methods, and their capacity to revert to resistance to oxacillin by reversal of point mutations that caused the oxacillin susceptible phenotype in the first place.

In the present study, the *mecA* gene sequence in the eight ST8 OS-MRSA isolates did not harbour any point mutations, insertions or deletions and was identical to the reference genome (strain: JCSC6944, GenBank accession number: AB505629) which was phenotypically MRSA. Therefore, mutations within *mec*A, could not be associated with the oxacillin susceptible phenotype of the OS-MRSA.

Mutations in Fem proteins have been observed in OS-MRSA isolates, and are known to influence the ability of isolates to exhibit resistance to oxacillin (Giannouli *et al.*, 2010; Phaku *et al.*, 2016). The term 'Fem' is an abbreviation for 'factors essential for methicillin resistance", and includes the enzymes FemA, FemB and FemX (FemX is now referred to as FmhB) encoded by the *femA*, *femB* and *femX* genes, respectively (Francklyn and Minajigi, 2010). Fem proteins have been shown to be integral to cell wall biosynthesis in MRSA, specifically through their role in the construction of a flexible pentaglycine interpeptide, which contributes to the integrity of the cell wall by providing a peptidoglycan cross-linking degree of up to 90% (Labischinski, 1992; Loskill *et al.*, 2014). Cross-linking of the pentaglycine bridges is undertaken by PBP2a (Srisuknimit *et al.*, 2017), and this ultimately provides a three-dimensional mesh structure which surrounds the cell. In MRSA, the pentaglycine interpeptide is synthesised by the FemXAB proteins, with glycyl-tRNA acting as a glycine donor. Glycine is then added to form the interpeptide in the following order: first glycine provided by FemX, second and third glycine by FemA, third and fourth glycine by FemB (Hübscher *et al.*, 2007). The construction of the pentaglycine interpeptide is dependent upon the position of Fem proteins in this specific order, and one cannot substitute the role of another in the synthesis process (Ehlert *et al.*, 1997). The action of PBP2a is vital for cell wall synthesis in the presence of beta-lactam antibiotics when native PBPs are inhibited, however PBP2a can crosslink only triglycyl or pentaglycyl interpeptides (Srisuknimit *et al.*, 2017). Mutations in FemXAB operons and

resultant reduction in the length of the interpeptide bridge have resulted in methicillin susceptibility and impaired cell growth previously (Strandén *et al.*, 1997).

In the present study, the DNA and predicted protein sequences of the eight ST8 OS-MRSA isolates were compared to a reference sequence obtained from strain MRSA252 (GenBank accession number BX571856). This reference strain was chosen as this was used to compare OS-MRSA isolates in a study that detected mutations in FemXAB previously (Giannouli *et al.*, 2010). Using *in-silico* protein modelling, Giannouli and colleagues determined that the presence of the accumulated amino acid mutations in their study isolates inhibited the function of Fem, and this was determined to have resulted in oxacillin susceptibility. The crystal structure of *S. aureus* FemA has been visualized by X-ray previously (Benson *et al.*, 2002). The functional structure of the two detected domains were examined, with one globular domain (domain 1) and one helical domain (domain 2). Two subdomains were revealed in domain 1, domain 1a (residues 1-110, 129-144 and 396-401) and domain 1b (residues 146-166, 189-245 and 308-395). Domain 1b was identified as integral to facilitate binding of the donor glycine and related substrates (Benson *et al.*, 2002). *In-silico* modelling by Giannouli and colleagues (2010) generated predicted structures from FemB and FemX based on the FemA visualisation undertaken by Benson *et al.* (2002).

The ST8 OS-MRSA isolates in the present study exhibited point mutations within *femA* and *femX*, resulting in amino acid subsitiutions at Y195F and E234D in FemA and (N18H, I51V and E261K) in FemX when compared with the reference strain MRSA252. No mutations were observed within *femB*. Both mutations in FemA occurred in the domain 1b, an integral region for the function of the protein. The mutation E234D was also present in the OS-MRSA isolates investigated by Giannouli *et. al* (2010), who also reported mutations at positions 210, 216, 346, 361. In FemX, mutations at N18H and I15V were identified, and these were identical to mutations in OS-MRSA isolates reported in a study

of isolates from India (Brahma *et al.*, 2019). Mutations of *femA* at Y195F and E243D were reported in nine ST8-MRSA recovered from the Democratic Republic of Congo (Phaku *et al.*, 2016). One potentially critical mutation was observed in E261K. Based on the structure of FemA visualised by Benson *et al.* (2002), this region was identified as being a platform that would contain an amino-acid charged tRNA molecule during the construction of the glycine cross-linking interpeptide. The region is coiled in structure, and the work of Benson and colleagues concluded that structural changes in this region that occur upon binding of the tRNA molecule would facilitate re-orientation of Fem to an area proximal to the glycine binding site. A mutation at position 263 was observed in the OS-MRSA isolates studies by Giannouli and colleagues (2010), and the authors concluded that mutations in this region could change the protein-tRNA reaction. They included two non-inducible OS-MRSA isolate in their analysis, and found two mutations in this region were well conserved, with the authors stating that this may not entirely inhibit the activity of FemX, as this would be critical to the viability of the cell.

In the present study, five mutations in *femA* and *fem X* were identified in in the ST8 OS-MRSA isolates, and three of these had been reported in OS-MRSA isolates previously (Giannouli *et al.*, 2010; Brahma *et al.*, 2019). The phenotypic susceptibility to oxacillin observed among this isolates in this study may be explained by Fem mutations, as mutations at the same points were also reported by other studies of OS-MRSA (Giannouli *et al.*, 2010; Brahma *et al.*, 2019). Seven of these OS-MRSA isolates exhibited resistance to cefoxitin despite their oxacillin susceptibility. This may reflect the differing mechanisms of action of oxacillin and cefoxitin. Oxacillin inhibits peptidoglycan production, resulting in cell auto-lysis, whereas cefoxitin inhibits cross-linking to decrease cell wall integrity (Williamson *et al.*, 1980). Therefore, mutations in Fem which could underpin oxacillin susceptibility may not affect the ability to withstand cefoxitin. Resistance to cefoxitin is variable in studies of OS-MRSA. In the two studies that reported similar *FemXAB*

mutations observed in the isolates in the present study, Giannouli and colleagues (2010) did not report cefoxitin testing, whereas Brahma and colleagues (2018) report resistance to cefoxitin in some of their OS-MRSA isolates but not all. In a report of 29 ST8 MRSA-V OS-MRSA isolates from São Tomé and Príncipe (STP) and Angola, all isolates were resistant to cefoxitin (Conceição *et al.*, 2015).

4.5 Conclusion

Whole-genome sequencing has revealed a dynamic population of *S. aureus* within mutli-bed patient rooms over four five-hour time periods. The application of wgMLST to the isolates recovered in the study afforded greater discrimination than would have been afforded if relying on MLST or *spa* types alone. Ten groups of related isolates were identified, and at least one air sample was involved in each of these, which may reflect the potentially under-estimated role of the air in the transmission of bacteria such as *S. aureus*. Fewer related isolates were recovered from the HEPA-filtered ward compared to the naturally ventilated,ward, which may reflect decreased transmission in such settings due to controlled air change rates. This benefit that this may have on patient infections in multi-bed wards has not been elucidated, but could be examined to determine whether there is a robust evidential underpinning for such ventilation systems in the prevention of *S. aureus* infection. Genotyping revealed one OS-MRSA isolate that exhibited resistance to neither oxacillin nor cefoxitin phenotypically and seven OS-MRSA that exhibited cefoxitin resistance but oxacillin susceptibility. Analysis of the *fem*XAB genetic sequence revealed mutations which may account for oxacillin susceptibility in the OS-MRSA, and these have been reported in OS-MRSA isolates previously. Further studies of OS-MRSA in Ireland are warranted.

Chapter 5

General Discussion

5.1 The potential of whole-genome sequencing to enhance hospital infection prevention and control

Infection prevention and control measures rely on information gathered through observation, investigation and surveillance. In Ireland, surveillance of invasive *S. aureus* infections is monitored via EARS-Net. This provides an overview of trends at a population level, allows contextualisation with other European countries and tracks rates of methicillin-resistance among reported isolates (European Centre for Disease Prevention and Control, 2019). The population of MRSA circulating in Ireland is monitored at a national level by the NMRSARL. Such surveillance monitors the clinical and public health implications of circulating clones, such as prevalence of significant virulence factors (e.g. Panton-Valentine leucocidin) and emerging resistance trends (such as monitoring high-level mupirocin resistance) (National MRSA Reference Laboratory, 2018). Clinical microbiology laboratories are concerned with providing data supporting clinical interventions, such as ensuring correct antimicrobial treatment protocols are commenced when an organism is identified and antimicrobial susceptibility results are made available. In the case of the significant hospital pathogen *S. aureus*, local outbreak investigations aim to suppress transmission of *S. aureus* and maintain patient safety and hospital operations. Each of these activities rely on the use of an array of culture-based and molecular methods in the clinical laboratory.

Genomic information, which is obtained by sequencing methods, including WGS, provides such information with far greater resolution than conventional molecular or phenotypic methods, enhancing the accuracy and effectiveness of infection prevention interventions (Ward *et al.*, 2019). From a surveillance perspective, WGS is increasingly being used routinely across the EU, most frequently to surveill population structures and outbreaks of *Neisseria meningiditis*, *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (European Centre for Disease Control, 2018). At the time of writing, the ECDC is

prioritizing the application of WGS to multi-drug resistant Gram-negative bacteria at a European level (European Centre for Disease Control, 2019). In Ireland, with regard to *S. aureus*, WGS remains limited to research studies, and is not yet used routinely in clinical laboratories (National MRSA Reference Laboratory, 2018).

Sequence-based typing methods applied to *S. aureus* have revealed a well-conserved core genome and a clonal population structure. Distinct ancestral lineages have been observed with geographical regions associated with predominant clones. For example, the ST22-MRSA-V clone has predominated in Ireland since the 1990s (Shore *et al.*, 2010). Conventional MRSA typing includes determination of MLST, *spa* and SCC*mec* types. Typing of MSSA using conventional methods is more challenging, relying on MLST and *spa* type, and without the reliably and relatively predictable clonal alignments observed in MRSA lineages and characteristics of SCC*mec*.

In the present study, all isolates were epidemiologically linked. That is, they were recovered from two hospital wards over four sampling days. If these isolates represented cases involved in an outbreak or cluster, different IPC interventions would have been employed based on the typing methods available at a local level in the hospital clinical laboratory. The application of wgMLST to the 62 isolates investigated in the present study identified 10 related isolated groups involving 46 isolates. In an outbreak investigation, such relatedness would infer transmission events.

The application of *spa* typing as a standalone measure would have assigned the 62 isolates to nine groups representing 44 isolates (Table 4.5). Three isolates were not assigned a *spa* type, with wgMLST identifying one of these isolates (an active air sample) to be related to another air sample included in the study by wgMLST. Comparison of wgMLST and *spa* typing revealed that wgMLST provided higher discrimination. Five isolates shared *spa* types with one or more isolates but were not deemed related by wgMLST. Furthermore,

spa typing did not identify relatedness of two isolates to others, where wgMLST did. Therefore, wgMLST identified 2/62 isolates involved in putative transmission that *spa* typing could not and eliminated five isolates from putative transmission links.

The application of a conventional MLST approach to the study isolates identified nine clusters of isolates (Table 4.5). However, concordance between MLST and wgMLST analysis was observed in just one of these groups. In the remaining clusters identified by conventional MLST, wgMLST analysis provided higher level discriminatory power, reducing the number of isolates involved in all putative transmission events based on MLST in all but one case. Such clarity can inform outbreak management, and ensure interventions are proportional and evidence-based.

The strengths of WGS with regard to outbreak investigations have been demonstrated previously (Durand *et al.*, 2018). Outbreak analysis by WGS has identified sub-clusters of transmission among collected isolates that were not apparent by conventional typing methods (Earls *et al.*, 2017; Madigan *et al.*, 2018). This can inform control interventions and provides an accurate representation of transmission dynamics if applied prospectively. Furthermore, the demands on IPC professionals in managing 'pseudo-outbreaks', where patients harbouring similar isolates by conventional typing are nursed in close proximity, could potentially be eliminated by the application of WGS as a prospective outbreak investigation tool due to increased resolution offered by WGS (Mellmann *et al.*, 2016; Ward *et al.*, 2019).

An integral element of IPC practice is antimicrobial stewardship, and in a wider context this practice strives to mitigate the increasing emergence of antimicrobial resistance. The "deep surveillance" of genotypic resistance traits afforded by WGS may ameliorate efforts to mitigate emerging resistance and facilitate precision treatment of sequenced pathogens (Hendriksen *et al.*, 2019). In the present study, chromogenic media failed to detect seven

OS-MRSA isolates which were identifiable by WGS. The extent of such resistance behaviours, described as 'stealth resistance' previously, which is not reliably identified by conventional culture approaches, may under-represent the circulating population of resistant or multi-drug resistant organisms (Goering *et al.*, 2019). Furthermore, such resistance mechanisms may confound conventional culture methods, and result in misclassification of isolates as methicillin-susceptible, when they are in-fact genotypically resistant, and can revert to functional resistance in the presence of antimicrobial agents (Proulx *et al.*, 2015). Treatment of MSSA and MRSA infection require different antimicrobial protocols and control measures, and failure to adequately identify an isolate as methicillin-resistant is associated with negative outcomes for affected patients (Duarte *et al.*, 2019).

The introduction of WGS to clinical laboratories will require optimisation of work-flows to minimise turnaround time and maximise the usefulness of the data generated. A trial implementation of WGS was undertaken in a tertiary care hospital in Germany to investigate the impact on IPC interventions and the financial implications (Mellmann *et al.*, 2016). Clinical and surveillance isolates were included of four species (MRSA, vancomycin-resistant *Enterococcus faecium*, MDR *E. coli*, and MDR *P. aeruginosa)* and subjected to Illumina MiSeq WGS. The application of WGS data in this study did not encompass predictions of virulence or resistance phenotypes but facilitated real-time evidence-based application of IPC interventions. Optimization of WGS workflows throughout the project decreased turnaround time, a vital metric of the usefulness of WGS in clinical settings. Avoided costs were calculated based on unnecessary patient isolations, and in this specific setting, these savings were greater than the amount spent subjecting the isolates to WGS. On a per isolate basis, the WGS cost was twice that of PFGE, but with similar turnaround times, and WGS is regarded has having superseded PFGE both in terms of the volume and quality of data generated (Stefani *et al.*, 2012). Rossen and colleagues

(2018) reviewed the practical issues that require consideration with regard to WGS integration in clinical laboratory settings, and cite the work of Mellmann *et al.* (2016), concluding that similar trials of the feasibility of WGS implementation should be undertaken to enable widespread access to WGS technology in clinical settings.

Overall, the potential offered by WGS as an IPC tool seems overwhelmingly promising. However, ongoing optimisation of workflows to decrease turnaround times and validate prediction of resistance phenotypes are needed, and the clinical laboratory workforce will need to be very significantly upskilled to include bioinformatics expertise (Rossen *et al.*, 2018). Education is a pillar of IPC. Whilst WGS technology is being optimised, results of studies which provide near to 'smoking gun' evidence of transmission should be actively communicated to clinical staff. We have known since the 1960s that poor hand hygiene is a driver of *S. aureus* transmission, for example, and yet hand hygiene in the hospital setting remains an ongoing and primary effort of IPC teams to improve compliance. Evidence of such transmission offered to clinical staff with the resolution of WGS could engage HCWs in a novel way, and could ultimately benefit patients. The usefulness of educational interventions including WGS-based evidence to improve IPC measures should be considered.

5.2 Detection of OS-MRSA

Current Irish guidance relating to the detection of MRSA for screening samples recommend the use of culture-based methods (Department of Health- An Roinn Sláinte, 2013). These guidelines acknowledge the 'gold standard' for culture-based identification would include a broth enrichment step but note the associated increased turn-around time as a disadvantage. Molecular methods are not advised outright, but the application of PCR for rapid diagnostic testing is acknowledged as a 'welcome development' and it is stated

that it could be introduced at local level for subsections of patients, such as admissions to ICU or in cases of emergency admissions. Chromogenic media is recommended for detection of MRSA from screening samples.

In the present study, seven *S. aureus* isolates that did not exhibit growth on chromogenic medium selective for MRSA were later identified as methicillin-resistant. In the case of six of these isolates, identification was indicated by the detection of phenotypic resistance to cefoxitin determined by disc diffusion. In a clinical setting, screening samples exhibiting no growth on selective chromogenic medium would be deemed 'MRSA-negative', as screening for MSSA is not routinely undertaken in Irish and many European hospitals (Department of Health- An Roinn Sláinte, 2013), so further processing (such as antimicrobial susceptibility testing) would not routinely be undertaken. All seven OS-MRSA were found, following WGS analysis, to harbour an identical SCC*mec* V (5C2&5) element with a wild type *mecA* gene. One further isolate that exhibited neither growth on Colorex^{TM} MRSA nor cefoxitin-resistance was subsequently found to harbour an identical SCC*mec* element to the previously identified OS-MRSA isolates following WGS analysis. All eight isolates exhibited susceptibility to oxacillin, and the GeneXpert MRSA assay identified all as MRSA. All eight isolates were deemed to be OS-MRSA, and were assigned to ST8. It has been estimated previously that the prevalence of OS-MRSA may be as high 3% of clinical *S. aureus* isolates (Proulx *et al.*, 2015), although the prevalence of OS-MRSA in Ireland is largely unknown.

Studies of OS-MRSA that have included culture with chromogenic media selective for MRSA report variable trends in detection. This may be due to differing mechanisms underlying oxacillin-susceptibility, with mutations in *FemXAB* and *mecA* independently reported previously (Giannouli *et al.*, 2010; Goering *et al.*, 2019). Furthermore, studies reporting OS-MRSA report variable resistance to cefoxitin (Conceição *et al.*, 2015).

Cefoxitin is generally acknowledged as superior to oxacillin for selection of MRSA by chromogenic culture methods (Perry *et al.*, 2004).

Differences, however subtle, in the composition of chromogenic agar appear to influence growth of OS-MRSA. In a report of two isolates from BSIs caused by OS-MRSA in the USA, differing growth was observed depending on the media used (Kumar *et al.*, 2013). Disc diffusion revealed one isolate to be cefoxitin-resistant and the other susceptible. The cefoxitin-resistant isolate grew on MRSASelect medium (Bio-Rad), but not ChromID MRSA (bioMérieux). The cefoxitin-susceptible isolate grew on neither media. In a study evaluating commercially available chromogenic media for the detection of MRSA, 12 OS-MRSA isolates were included in 161 MRSA isolates used for testing (Brennan *et al.*, 2016). Four such OS-MRSA isolates were not identified by MRSA Select II (n=1) and MRSA Brilliance 2 (n=3), whereas all OS-MRSA isolates grew on Colorex MRSA and ChromID.

In the present study, OS-MRSA isolates were confirmed by molecular methods. However, these are costly and rely on robust infrastructure and supply chains to ensure clinical demand is met. An advantage of chromogenic agar is its application to low-resource settings (Phaku *et al.*, 2016). Chromogenic agar remains reliable for the detection of the vast majority of MRSA (Brennan *et al.*, 2016). However, OS-MRSA prevalence may vary, meaning that where resources are more limited, chromogenic agar may be less reliable. Phaku and colleagues (2016) undertook a prevalence study of asymptomatic community-dwelling persons in the Democratic Republic of Congo, subjecting recovered isolates to WGS. They enrolled 753 people and a prevalence of *S. aureus* carriage of 13.3% was determined, yielding 100 isolates for WGS. Of these 100 isolates, 9% were identified as ST8 OS-MRSA and t1476. This may represent a local cluster of OS-MRSA, and the study was not powered to reflect OS-MRSA prevalence in the region. However, such a prevalence of OS-MRSA is higher than is typically reported in *S. aureus* prevalence

studies (Proulx *et al.*, 2015), potentially due to false-negative results resulting from atypical phenotypic traits. Phaku and colleagues (2016) comment that despite the advantages of WGS for detection of resistance traits, in resource-limited settings, culture-based susceptibility testing remains the most viable approach to the detection of MRSA.

Despite the massive potential of WGS to revolutionise diagnostic microbiology, immediate improvements are unlikely to reach many such settings for routine use in the near future (Balloux *et al.*, 2018). This may be due to poor infrastructure, lack of training opportunities or local logistical challenges. Standardization of sequencing and bioinformatics practices, in addition to the provision of training in bioinformatics remain significant concerns identified among surveyed end-users employed in clinical laboratories (Moran-Gilad *et al.*, 2015). As such, available conventional methods should be optimised for the detection of OS-MRSA. Immunochromotographic assays, such as those that detect PBP2a activity, are reported in studies of OS-MRSA with varying results. In a report of OS-MRSA due to *mecA* instability, with reversion to oxacillin resistance induced by exposure to sub-inhibitory oxacillin concentrations, PBP2a assays were negative before the reversion, but positive once *mecA* function had been restored (Goering *et al.*, 2019). Other studies report PBP2a assay positivity in oxacillin-susceptible isolates (Sharff *et al.*, 2012; Kumar *et al.*, 2013; Phaku *et al.*, 2016). Such assays are not recommended by current Irish guidelines for the detection of MRSA (Department of Health- An Roinn Sláinte, 2013).

An additional challenge in the detection of OS-MRSA is the dissemination of oxacillin-susceptibility within a diverse range of clonal lineages. Point mutations in *mecA* (Proulx *et al.*, 2015; Goering *et al.*, 2019) or *femXAB* have been identified as driving loss of methicillin resistance previously, such as in this study and others (Giannouli *et al.*, 2010; Phaku *et al.*, 2016; Brahma *et al.*, 2019). Where it is not feasible to undertake molecular diagnostic testing routinely, there is no emerging trend in OS-MRSA with regard to a specific lineage or region to facilitate optimisation of resources towards a specific clone.

The current prevalence of OS-MRSA in Ireland is not known. Retrospective or prospective testing should determine the national situation. Prospective testing would facilitate inclusion of MSSA isolates, which are not routinely screened for or stored, so likely to be prohibitively difficult to include in retrospective analysis. Such studies should include testing against a panel of chromogenic agars to determine the most specific test- this would be especially reassuring for settings relying solely on culture-based methods. A large dataset of OS-MRSA isolates would facilitate investigation of the prevalence of cefoxitin resistance, and if low, cefoxitin culture-based antimicrobial susceptibility testing could be optimised.

5.3 The air as a route of *S. aureus* transmission in hospitals

The role of the air in the transmission of bacteria (including *S. aureus*) is well accepted in certain hospital settings. Sophisticated ventilation systems are used to reduce the risk of surgical field contamination with *S. aureus* in operating theatres, for example, by replacing large volumes of air at regular intervals (Beggs *et al.*, 2008). Evidence underpinning the potential role of interventions targeting the air of multi-bed hospital rooms is sparse, despite these rooms housing the majority of admitted in-patients. Interventions to reduce airborne bacterial transmission have been classified into four categories, (1) pressure differential, such as negative pressure isolation rooms designed to house patients infected with transmissible organisms, i.e. *M. tuberculosis*, (2) dilution, such as control of ACH, (3) filtration, such as the HEPA-filtration incorporated into the AHU of Ward A in the present study and (4) purification, including the use of ultraviolet irradiation to inactivate airborne bacteria (Memarzadeh *et al.*, 2010). However, the efficacy of these interventions to mitigate *S. aureus* transmission has not been conclusively determined in multi-bed hospital rooms to date.

The primary intervention undertaken to reduce the overall environmental burden of *S. aureus* in multi-bed rooms is isolation of known colonised patients. Patients who are identified as MRSA colonised are isolated where possible, in order to mitigate dissemination of MRSA to other patients (Department of Health- An Roinn Sláinte, 2013). It is accepted that isolated patients colonised with MRSA shed the bacteria to surrounding surfaces resulting in contamination (Sexton *et al.*, 2006). The risk of transmission to other patients is managed by decontamination of equipment prior to leaving isolation rooms, adherence by HCWs to recommended PPE measures when caring for colonised patients and compliance with hand hygiene following care events or contact with the contaminated patient environment (World Health Organization, 2009). Isolation of patients with MSSA colonisation is not currently recommended in Irish guidance (Department of Health- An Roinn Sláinte, 2013). Patients colonised with OS-MRSA may not reliably be identified by current screening methods. However, the air can potentially circumvent these interventions, limiting the effectiveness of isolation.

Dansby et al. (2008), whilst investigating an outbreak of MRSA on a burns unit, sampled the air in the corridor outside MRSA-colonised patients rooms during dressing changes. They recovered MRSA from corridor samples linked to the isolated patients. In settings without controlled ventilation, inadvertent positive air pressure in isolation rooms can result in airborne *S. aureus* seeping outside the bounds of the isolation room. This route can be exploited by *S. aureus* and transmission of infection can occur, as was observed by Dansby and colleagues- despite physical isolation of patients in single room. In the case of multi-bed rooms, as investigated in the present study, there are no physical barriers to contain bacteria shed by patients, On three occasions in the present study, active air samples were found to be related to samples obtained from a concurrently collected settle plates. This represents deposition of airborne *S. aureus* to a frequently touched object in the near-patient environment. The patients occupying the bedspaces where the settle plates

were recovered were not known to be colonised with *S. aureus*. Such movement, via the air, provides an opportunity for onward trafficking to sites vulnerable to *S. aureus* colonisation or infection, and represents potential nosocomial transmission pathways.

A systematic review of bioaerosol concentrations in hospital settings concluded that the installation of functioning sophisticated ventilation systems minimises bioaerosols, but the impact that this may have on patient infections has not been evaluated (Stockwell *et al.*, 2019). This information, and evidence that *S. aureus* can cause illness via air (Kumari *et al.*, 1998) suggests that reducing airborne *S. aureus* could lessen infections in multi-bed rooms, and further studies should investigate this. In low-resource settings, such systems may not be feasible. Airborne liberation of *S. aureus* has previously been associated with certain activities, such as bed-making and curtain movement. These factors are typically not considered during routine hospital cleaning. Targeting cleaning of multi-bed wards following periods of such activity could decrease overall surface contamination, potentially decreasing the risk of nosocomial infection to patients. A shift from current cleaning practice, which does not consider such activities, could be evaluated as a low-technology but potentially efficient approach to the cleaning of hospitals, which may improve patient safety.

5.4 Concluding statement

Healthcare systems are dynamic, and patients cared for in acute hospitals have never been more vulnerable to infection due to increasingly complex surgeries and procedures. The ever-increasing prevalence of antimicrobial resistance places these individuals at even greater risk of negative outcomes. Preventing infection in the first instance could avoid exposure to antibiotic treatment, which will benefit patients on an individual level and the fight against antimicrobial resistance. By examining the exploitation of environmental

pathways in hospitals, such as air, potential links can be eliminated in the chain of infection. Engagement of HCWs is critical to this. Massive potential is offered by WGS from a surveillance perspective, but also as a tool for local IPC teams for investigative purposes and also for education of all HCWs. We should maximise the potential of this technology for the prevention of nosocomial infections.

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Appendix A: Ethical Approval

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

Principal Investigator: **Prof. H. Humphreys**
REC reference: **17/01**
Protocol Title: **An investigation of the role of *Staphylococcus aureus* colonization of healthcare workers in nosocomial transmission of *S. aureus* to patients in an MRSA-endemic setting using whole-genome sequencing**

Reviewed by Committee Chairperson & IRB Specialist in February 2017
Final Approval Date: **16th February 2017**

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

Documents Reviewed

Document and Date	Date Reviewed	Approved
Standard Application, RECSAF 5.6,		
Version 1, Signed H. Humphreys 29/11/16	16/2/17	Yes
Version 2	17/11/17	Yes
Patient Information Leaflet,		
V1, 25/11/16	16/2/17	Yes
6/11/17	17/11/17	Yes
V3, 2/1/19	26/2/19*	Yes
Patient Consent Form,		
V1, 25/11/16	16/2/17	Yes
V2, 6/11/17	17/11/17	Yes
V3, 3/1/19	26/2/19*	Yes
Patient Questionnaire, no version no.	16/2/17	Yes
Heathcare Worker Info Leaflet,		
V1, 25/11/16	16/2/17	Yes
V2, 3/1/19	26/2/19*	Yes
Healthcare Worker Consent Form,		
V1, 25/11/16	16/2/17	Yes
V2, 3/1/19	26/2/19*	Yes
Healthcare Worker Questionnaire, no version no.	16/2/17	Yes
Amendment:		
#1, 6/11/17 (look at healthcare records)	17/11/17	Yes
#2, 3/1/19 (update participant docs For purposes of GDPR)	26/2/19*	Yes
• Email – confirmation of CIS cover, 21/11/16	16/2/17	Yes
• CV: H. Humphreys	16/2/17	Noted
• Health Research Board Application, submitted 19/1/15	16/2/17	Noted

Dr. Peter Branagan
ERC/IRB Convenor's Signature
Approval # 3, dated 26th February 2019

Appendix B: Healthcare Worker Information Leaflet

Website: www.beaumont.ie

Ospidéal Beaumont

BEAUMONT HOSPITAL
P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

HEALTHCARE WORKER INFORMATION LEAFLET

Study title: An investigation of the role of *Staphylococcus aureus* colonisation of healthcare workers in the transmission of *S. aureus* to patients using whole-genome sequencing

Principal investigator's title name & title: Professor Hilary Humphreys, Beaumont Hospital and the RCSI

Telephone number of principal investigator: XX-XXXXXXX

Co-investigator's tite & name: Professor David Coleman & Dr. Anna Shore, Dublin Dental University Hospital and Trinity College Dublin

- You are being invited to take part in a research study to be carried out at Beaumont Hospital. Before you decide whether or not you wish to take part, you should take sufficient time to read the information below carefully and, if you wish, discuss it with your family, friends or doctor. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.
- You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as 'Informed Consent'.
- You don't have to take part in this study. If you decide not to take part you will not be penalised in any way and will not give up any benefits that you had before entering the study.
- You can change your mind about taking part in the study at any time. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, you will not be penalised.

Why is this study being done?

- *Staphylococcus aureus* is a germ, also known as MRSA or MSSA, that is carried by many people in the nose and throat/mouth, without causing them harm. However, it can also cause different types of infections which can be minor or very serious.

- Carriage of this germ is often higher among people working in hospitals. These healthcare workers often have close contact with patients and MRSA/MSSA from their nose and throat/mouth can be transferred to patients.
- This research study is taking place to find out how commonly and for how long MRSA/MSSA is carried in the nose and throat/mouth of healthcare workers and whether some of these are transferred to patients.

Who is organising and funding this study?

- This study is being carried out by researchers at Beaumont Hospital/RCSI and Trinity College Dublin and is funded by a Health Research Board (HRB) grant.
- No pharmaceutical companies are funding this study and we are not being paid to recruit patients.
- This research project has been approved by the Beaumont Hospital Research Ethics Committee.

Why am I being asked to take part?

- You are being asked to take part because you are a healthcare worker who is working on one of the wards in Beaumont Hospital where the study is taking place.
- You will not be asked to take part in the study if you are on antibiotics or have been on antibiotics during the previous month or if there is an outbreak of MRSA/MSSA on the hospital ward where you are currently mainly working on.

How will the study be carried out?

- All samples will be taken by a Research Nurse during a six-week period on each hospital ward. The samples will be taken by gently rubbing a cotton bud on the inside of your nose and using a liquid which you will be asked to use to rinse out your mouth with for 30 seconds and then return to the original container. These procedures will be painless and will take less than one minute.
- You will then be asked to fill in a short questionnaire asking about your age, country of origin, general health issues including any long-term medical conditions, any history of hospitaliation and other healthcare worker related issues. This information will remain confidential.
- The samples will be brought the The Dublin Dental University Hospital Microbiology Laboratory at Trinity College Dublin and will be analysed for the MRSA/MSSA germ by the Research Team.

- Any MRSA/MSSA isolates that we identify from your sample will be stored in a freezer and the cotton buds and rinse solutions will then be destroyed and will not be used for any other purpose.
- Anyone taking part in the study will be assigned a unique number which will be used to label the questionnaire and your samples. You will be sampled a total of four times i.e. every three months for one year. Each time the samples will be taken by the same Research Nurse using the same procedure but you will only need to complete the questionnaire the first time. Only your unique number will be recorded during these follow up screening sessions and not your name. The samples will be processed in the same way each time.
- We will also be seeking nose and mouth samples from patients.

What will happen to me if I agree to take part?

- If you decide to take part you will be asked to sign a consent form. You will then be given a unique code which will be used to label your samples and the questionnaire.
- Your nose and throat/mouth will be sampled by a Research Nurse four times over the next 12 months. Each sampling session will be performed during your normal working day at a time arranged with you and will take less than one minute. You will only be asked to fill in a short questionnaire the first time that the samples are taken and this will take less than 4 minutes.

What are the benefits?

- There are no direct benefits to you if you take part in this study and you will not be paid for your participation. The results of the study will not be made available to you and no treatment will be offered. Your participation in this study will provide research material for a study that will help to improve our understanding of the threat posed by the spread of these germs in hospitals.
- The results of this study will be discussed with Beaumont Hospital Infection Prevention and Control Team and with the Health Service Executive (HSE) and will be used to help in the prevention and control of the spread of this germ in hospitals and to reduce infections in patients.

What are the risks?

- There are no risks associated with sampling the nose using a cotton bud or rinsing the throat/mouth with this rinse liquid.
- The entire procedure, including sampling and completing the questionnaire, will take less than 5 minutes.

Is the study confidential?

- Yes, the study is confidential. Your identity will remain confidential. Your name will only be listed on the consent form. Your name will not be published and it will not be disclosed to anyone.
- Samples will be labelled with a number and the only details that will be recorded will be your age, country of origin and general health and work-related issues. These details will be stored in a password protected computer file and will not be linked to your name. Only the immediate research team will have access to the data. Consent forms and questionnaires will be shredded 1 year after the completion of the project.
- The samples from your nose and mouth will be destroyed once they have been tested for the germ. No genetic testing of your samples will be carried out. Only the germs identified will be kept for future research purposes.
- You will not receive any results from the study but they may be published and presented at scientific meetings. It will not be possible to identify you from any of the results or publications.

Where can I get further information?

- If you have any further questions about the study or if you want to opt out of the study, you can do so at any time and you will not give up any benefits that you had before entering the study.
- If you need any further information now or at any time in the future, please contact:

  Professor Hilary Humphreys XXXXXXXXX@XXXX.XXX

  Aoife Kearney (Research Nurse)XXXXXXXXXXXXX@XXXXXXXXXXXXX

Appendix C: Patient Information Leaflet

Website: www.beaumont.ie

Ospidéal Beaumont

BEAUMONT HOSPITAL
P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

PATIENT INFORMATION LEAFLET

Study title: An investigation of the role of *Staphylococcus aureus* colonisation of healthcare workers in the transmission of *S. aureus* to patients using whole-genome sequencing

Principal investigator's title name & title: Professor Hilary Humphreys, Beaumont Hospital and the RCSI

Telephone number of principal investigator: XX-XXXXXXX

Co-investigator's tite & name: Professor David Coleman & Dr. Anna Shore, Dublin Dental University Hospital and Trinity College Dublin

- You are being invited to take part in a research study to be carried out at Beaumont Hospital. Before you decide whether or not you wish to take part, you should take sufficient time to read the information below carefully and, if you wish, discuss it with your family, friends or doctor. Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision.
- You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as 'Informed Consent'.
- You don't have to take part in this study. If you decide not to take part you will not be penalised in any way and it will not affect in any way your future medical care.
- You can change your mind about taking part in the study at any time and any data or samples collected will not be used in the study. You don't have to give us a reason. If you do opt out, rest assured you will not be penalised.

Why is this study being done?

- *Staphylococcus aureus* is a germ, also known as MRSA or MSSA, that is carried by many people in the nose and throat/mouth, without causing them harm. However, it can also cause many different types of infections which can be minor or serious.
- Carriage of this germ is often higher among people who work in hospitals. These healthcare workers often have close contact with patients and MRSA/MSSA from their nose and throat/mouth can be transferred to patients.
- This research study is taking place to find out how commonly and for how long MRSA/MSSA is carried in the nose and throat/mouth of healthcare workers and whether some of these are transferred to patients.

Who is organising and funding this study?

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- No pharmaceutical companies are funding this study and we are not being paid to recruit patients.
- This research project has been approved by the Beaumont Hospital Research Ethics Committee.

Why am I being asked to take part?

- You are being asked to take part because you are a patient on one of the wards in Beaumont Hospital where the study is taking place.

How will the study be carried out?

- All samples will be taken by a Research Nurse. The samples will be taken by gently rubbing a cotton bud on the inside of your nose and using a liquid which you will be asked to use to rinse out your mouth with for 30 seconds and then return to the original container. These procedures will be painless and will take less than one minute.
- You will then be asked to fill in a short questionnaire asking about your age, country of origin and general health issues including any long-term medical conditions and history of hospitaliation. We will also seek permission to access your medical records to estimate the amount of contact time between you and healthcare workers during your hospital stay and if having more contact makes you more likely to carry *S. aureus.* This information will remain confidential.
- The samples will be brought to the The Dublin Dental University Hospital (DDUH) Microbiology Laboratory at Trinity College Dublin and will be analysed for the MRSA/MSSA germ by the Research Team.
- Any MRSA/MSSA germs that we identify from your sample will be stored in a freezer and the cotton buds and rinse solutions will then be destroyed and will not be used for any other purpose.
- If any MRSA/ MSSA germs have been stored in Beaumont Hospital laboratory from samples sent when you previously had an infection or if you currently have an infection, they will also be obtained to be include in this study and they will also be stored in a freezer at the DDUH.
- Everyone taking part in the study will be assigned a unique code which will be used to label their questionnaire and samples. On the day of sampling, your name will be used to link the samples we obtain from you with the germs stored when you previously had an infection. Once these germs are obtained from the hospital laboratory, your name will only be retained on the signed consent form.
- We will also be seeking nose and mouth samples from healthcare workers.

What will happen to me if I agree to take part?

- If you decide to take part your will be asked to sign a consent form. You will then be given a unique code which will be used to label your samples and the questionnaire.
- Your nose and throat/mouth will be sampled by a Research Nurse at a time arranged with you. This will take less than one minute. You will be asked to fill in a short questionnaire and this will take less than 4 minutes.

What are the benefits?

- There are no direct benefits to you if you take part in this study and you will not be paid for your participation. The results of the study will not be made available to you and no treatment will be offered. Your participation in this study will provide research material for a study that will help to improve our understanding of the threat posed by the spread of these germs in hospitals.
- The overall results of this study will be discussed with Beaumont Hospital Infection Prevention and Control Team and with the Health Service Executive (HSE) and will be used to help in the prevention and control of the spread of this germ in hospitals and to reduce infections in patients.

What are the risks?

- There are no risks associated with sampling the nose using a cotton bud or rinsing the throat/mouth with this rinse liquid.
- The entire procedure, including sampling and completing the questionnaire, will take less than 5 minutes.

Is the study confidential?

- Yes, the study is confidential. Your identity will remain confidential. Your name will be listed on the consent form and will be linked to your nose and mouth samples until the end of the day of sampling so that your clinical isolates can be obtained and your medical records accessed. Your name will then be replaced with your unqiue number and it will not be possible to link your samples to you. Your name will not be published in any scientific publications or other documents and it will not be disclosed to anyone.
- The only details that will be recorded will be your age, country of origin, history of hospitalisation and general health issues. These details will be stored in a password protected computer file and will not be linked to your name. Only the immediate research team will have access to the data. Consent forms and questionnaires will be shredded 1 year after the completion of the project.
- Your nose and mouth samples will be destroyed once they have been tested for the germ. No genetic testing of your samples will be carried out. Only the germs identified will be kept for future research purposes.
- You will not receive any results from the study but they may be published and presented at scientific meetings. It will not be possible to identify you from any of the results or publications.

Where can I get further information?

- If you have any further questions about the study or do not wish to participate you can rest assured that your routine care will not be affected in any way.
- If you need any further information now or at any time in the future, please contact:

  Name: **Professor Hilary Humphreys**

  Email: xxxxxxxxx@xxxxxxxx

Appendix D: Healthcare Worker Consent Form

Website: www.beaumont.ie

Ospidéal Beaumont

BEAUMONT HOSPITAL
P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Healthcare Worker Consent Form

Study title: *An investigation of the role of* Staphylococcus aureus *colonisation of healthcare workers in the transmission of* S. aureus *to patients using whole-genome sequencing*

I have read and understood the ***Information Leaflet*** *about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.*	***Yes □***	***No □***
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that by opting out I won't be penalised.	***Yes □***	***No □***
I have been given a copy of the Information Leaflet and this completed consent form for my records.	***Yes □***	***No □***
I am aware of the potential risks of this research study.	***Yes □***	***No □***

Storage and future use of information: *I give my permission for information collected about me to be securely stored or electronically processed for the purpose of scientific research and to be used in related studies or other studies in the future but only if the research is approved by a Research Ethics Committee.*	***Yes □***	***No □***

/ /

Healthcare Worker Name (Block Capitals) / Signature / Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above participant the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

/ / /

---------------*Name (Block Capitals)* / *Qualifications* /
Signature / *Date*

2 copies to be made: 1 for participant, 1 for PI.

Appendix E: Patient Consent Form

Website: www.beaumont.ie

Ospidéal Beaumont

BEAUMONT HOSPITAL
P. O. Box 1297 Beaumont Road Dublin 9
Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Patient Consent Form

Study title: *An investigation of the role of* Staphylococcus aureus *colonisation of healthcare workers in the transmission of* S. aureus *to patients using whole-genome sequencing*

I have read and understood the ***Information Leaflet*** *about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.*	***Yes*** □	***No*** □
I understand that I don't have to take part in this study and I understand that not taking part will not affect won't affect my future medical care.	***Yes*** □	***No*** □
I am aware of the potential risks of this research study.	***Yes*** □	***No*** □
I have been given a copy of the Information Leaflet and this completed consent form for my records.	***Yes*** □	***No*** □
Storage and future use of information: *I give my permission for information collected about me to be securely stored or electronically processed for the purpose of scientific research and to be used in related studies or other studies in the future but only if the research is approved by a Research Ethics Committee.*	***Yes*** □	***No*** □

/ /

Patient Name (Block Capitals) / Signature / Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

/ / /

---------------*Name (Block Capitals)* | *Qualifications* | *Signature* | *Date*

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

Appendix F: Patient Questionnaire

PATIENT QUESTIONNAIRE

An investigation of the role of *Staphylococcus aureus* colonisation of healthcare workers in the transnission of *S. aureus* to patients using whole-genome sequencing

TIME: DATE: WARD: BAY: BED NO:

1. Age range (Years):
   18-24 □ 25-34 □ 35-44 □
   45-54 □ 55-64 □ 65 or older □

2. Gender: Male □ Female □

3. Type of hospital room: Single side room □ Double side room □ Open ward bay □

4. Ward Type: ______________________

5. Country of Birth: ______________________

6. If born abroad, how long have you been living in Ireland:

   <1 year □ 1-5 years □ 6-10 years □ >10 years □

7. Length of current hospitalisation: 0-24 hours □ 24-48 hours □ 2-7 days □ 1-2 weeks □ >2 weeks □

8. Previous hospitalisation for more than 24 hours in the past year? Yes □ No □

9. If YES was that hospitalisation in:
   This hospital □ Another Irish hospital □ Nursing Home □

   Residential Unit □ Rehabilitation Unit □ A hospital abroad □

   If abroad, where: __________________________ , or Not Applicable □

10. Have you taken antibiotics during the last year? Yes □ No □

11. Have you taken steroids during the last year? Yes □ No □

12. Have you travelled abroad during the last year? Yes □ No □

If YES, where was that travel to? Within Europe □ Middle East □ Far East □ Australia and New Zealand □ Asia □ South America □ Central America □ USA □ Other________________

13. Have you had contact with farm animals in the last year? Yes □ No □

14. Do you have a history of the following? (Please answer 'yes', 'no', 'unknown'.)

a) Abscesses ________________________________
b) Boils ________________________________
c) Bone infections ________________________________
d) Septic arthritis ________________________________
e) Necrotizing pneumonia ________________________________
f) Cellulitis ________________________________
g) Other skin conditions ________________________________

Appendix G: HCW questionnaire

HEALTHCARE WORKER QUESTIONNAIRE

An investigation of the role of *Staphylococcus aureus* colonisation of healthcare workers in the transnission of *S. aureus* to patients using whole-genome sequencing

TIME: DATE: WARD: BAY: BED NO:

15. Age range (years):
18-24 □ 25-34 □ 35-44 □ 45-54 □ 55-64 □ 65 or older □

16. Gender: Male □ Female □

17. Country of birth: ____

18. If born abroad, how long have you been living in Ireland:
<1 year □ 1-5 years □ 6-10 years □ >10 years □

19. Role in hospital: ____

20. Number of hours of patient contact had per day: 0-30 mins □ 30 mins-1 hour □
1-2 hours □ 2-4 hours □ 4-8 hours □ 8-12 hours □ >12 hours □

21. Ward type: ____

22. Length of employment in Beaumont Hospital:
<1 year □ 1-5 years □ 6-10 years □ >10 years □

23. Previous hospital employment:
Yes □ No □

24. If YES was that in: Another Irish hospital □ A hospital abroad □
If abroad, where? __________________________

25. Have you been hospitalised overnight for any reason in the last year?
Yes □ No □

26. If yes, were you hospitalized in:

This hospital □ Another Irish hospital □ Nursing Home □

Residential Unit □ Rehabilitation Unit □ A hospital abroad □

If abroad, where: ________________________ , or Not Applicable □

27. Have you taken steroids during the past year? Yes □ No □

28. Have you taken antibiotics during the past year? Yes □ No □

29. Have you travelled abroad during the past year? Yes □ No □

30. If YES, where was that travel to?

Within Europe □ Middle East □ Far East □ Australia and New Zealand □

Asia □ South America □ Central America □ USA □

Other________________

31. Have you had contact with farm animals in the last year? Yes □ No □

32. Do you have a history of the following? (Please answer 'yes', 'no', 'unknown'.)

h) Abscesses ________________________________

i) Boils ________________________________

j) Bone infections ________________________________

k) Septic arthritis ________________________________

l) Necrotizing pneumonia ________________________________

m) Cellulitis ________________________________

n) Other skin conditions ____________________