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Molecular Characterisation of *Staphylococcus aureus* and *Staphylococcus epidermidis* Populations From Healthy and Diseased Oral Implant and Natural Tooth Sites in a Cohort of Patients With Periimplantitis

A thesis submitted to the University of Dublin, Trinity College, in fulfilment of the requirements for the degree of Doctor in Philosophy by

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June 2013

Declaration

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Phillipa Jane Cashin

(http://sepidermidis.mlst.net/) the majority of STs (19/22, 86.36%) belonged to the predominant clonal complex CC1. In comparison to the global *S. epidermidis* MLST database, the STs identified in this study exhibited no enrichment of or particular association with STs previously identified in healthcare environments, non-human, or environmental isolates, indicating the periimplantitis isolates reflected general carriage isolates. The population structure of *S. aureus* isolates from periimplantitis patients was investigated by subjecting all isolates recovered during the study (31 isolates from 25 patients, including 4 implant, 1 tooth, 5 nares and 21 oral rinse samples) to DNA microarray profiling. The 31 isolates were assigned to eight CCs (CC5, 7, 8, 9, 15, 22, 30 and CC101) and no evidence for enrichment for any CC was identified. This comparatively wide range of CCs suggested that the *S. aureus* recovered during this study represent general carriage isolates.

Microarray profiling of the 31 *S. aureus* isolates revealed no unusual patterns of virulence-associated genes and only a few antimicrobial resistance genes. One isolate harboured the fusidic acid resistance gene *fusB*. In contrast, microarray profiling of 43 *S. epidermidis* isolates from a range of sites from 24 patients revealed a variety of antimicrobial resistance genes. Four isolates harboured the methicillin resistance gene *mecA* (9.3%), four (9.3%) harboured the high-level mupirocin resistance gene *ileS2* and 11 isolates (25.6%) harboured *fusB*. Interestingly, the arginine catabolic mobile element ACME was identified in 24/43 (55.8%) of *S. epidermidis* isolates and was significantly associated ($p<0.025$) with subgingival isolates from periodontal or periimplant sites (13/17 ACME-positive, 76.5%), relative to isolates recovered from oral rinses (6/20 ACME-positive, 30%). The secondary arginine deiminase pathway encoded by ACME may enhance the ability of staphylococci to survive in the low oxygen and nutrient poor subgingival environment.

Studies were undertaken to investigate the disparity between the low prevalence of *S. aureus* from implant (5/353 samples, 1.4%) and tooth (1/449 samples, 0.2%) sites determined in this study by culture, in contrast to the previous CKB-based studies. Twenty-seven clinical samples from 14 periimplantitis patients from this study that were investigated for *S. aureus* by CKB as part of a separate study, were further assessed for *S. aureus* DNA using species-specific, real-time PCR (RT-PCR). The results revealed that 12/27 (44%) samples were CKB-positive for *S. aureus*, 2/27 (7.41%) were culture-positive for *S. aureus*, but all were *S. aureus*-negative by RT-PCR. These findings indicate lack of specificity with CKB, possibly due to cross-reactivity of the CKB *S. aureus* probe with DNA from other bacterial species or genera in the test samples.

The results of this study demonstrated that *S. aureus* is not prevalent at healthy or diseased implant or natural tooth sites. In contrast, *S. epidermidis* was prevalent at both healthy and diseased natural tooth and implant subgingival sites. The high prevalence of ACME in subgingival *S. epidermidis* isolates may be significant in the survival of these organisms in this nutrient poor and low oxygen environment. The prevalence of resistance genes and other mobile genetic elements among *S. epidermidis* further highlights its role as a reservoir of genetic determinants that can be transferred into *S. aureus*. This study demonstrates that *S. epidermidis* is a member of the oral flora and its role in the oral microbial ecosystem needs to be clearly defined by additional studies.

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Abbreviations

β	Beta
δ	Delta
γ	Gamma
<	Less than
=	Equals
>	Greater than
≤	Less than or equal to
≥	Greater than or equal to
µl	Microlitre
µM	Micromolar
16S rDNA	Genes encoding 16S ribosomal RNA component of the 30S ribosomal subunit
90°	Ninety degrees (angle)
ACME	Arginine catabolic mobile element
BD	Becton Dickinson
BLAST	Basic local alignment search tool
bp	Base pair(s)
BURST	Based upon related sequence types algorithm
CA-MRSA	Community acquired methicillin resistant *Staphylococcus aureus*
CA-MRSE	Community acquired methicillin resistant *Staphylococcus epidermidis*
CBA	Columbia blood agar
CC	Clonal complex
CCs	Clonal complexes
cfu	Colony forming unit
cfu/ml	Colony forming units per millilitre
cfu/sample	Colony forming units per sample (three paper points or a curette scraping)
CKB	Checkerboard DNA:DNA hybridisation
CoNS	Coagulase-negative staphylococci
Ct	Cycle threshold
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
DDUH	Dublin Dental University Hospital
dGTP	2'-deoxyguanosine 5'-triphosphate

DLV	Double locus variant
DLVs	Double locus variants
DNA	Deoxyribonucleic acid
dTTP	2'-deoxythymidine 5'-triphosphate
e.g.	*Exempli graita*; for example
eBURST	Implementation of BURST algorithm allowing formation of clonal complexes and the prediction of founder sequence types
eDNA	Extracellular DNA
EDTA	Ethylenediaminetetraacetic acid
egc	Enterotoxin gene complex
EMRSA	Epidemic methicillin resistant *Staphylococcus aureus*
et. al.	*Et alia*; and others
etc.	*Et cetera*; and the rest
FAM™	6-carboxyfluorescein
goeBURST	Global optimal eBURST
h	Hour
HA-MRSA	Hospital acquired methicillin resistant *Staphylococcus aureus*
HA-MRSE	Hospital acquired methicillin resistant *Staphylococcus epidermidis*
H_0	Null hypothesis
HOMb	Human Oral Microbiome project
HRP	Streptavidin-horseradish-peroxidase
i.e.	*Id est*; that is
ICE	International collaboration on endocarditis
IEC	Immune evasion complex
in vitro	"Within the glass"; performing a given procedure in a controlled environment outside of a living organism
in vivo	"Within the living"; performing a given procedure within a living organism
JREC	Joint research ethics committee
kbp	Kilo base pair(s)
L	Litre
LA	L-agar
Mbp	Mega base pair(s)
MGB	Minor groove binder
MGE	Mobile genetic element
MGEs	Mobile genetic elements

MH	Muller hinton agar
MIC	Minimum inhibitory concentration
min	Minute
ml	Millilitre
MLST	Multilocus sequence typing
MLVA	Multilocus variable number tandem repeat analysis
mm	Millimetre
mM	Millimolar
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MSA	Mannitol salt agar
MSCRAMM	Microbial surface components recognising adhesive matrix molecules
MSSA	Methicillin susceptible *Staphylococcus aureus*
MSSE	Methicillin susceptible *Staphylococcus epidermidis*
N/A	Not applicable
NA	Nutrient agar
NA	(Sample) Not available
NCBI	National centre for biotechnology information
Neg	Negative
^{o}C	Degrees Celsius
PCR	Polymerase chain reaction
PCRs	Polymerase chain reactions
PFGE	Pulse field gel electrophoresis
pH	Measure of acidity or alkalinity (hydrogen ion concentration)
pmol	pico-mol
R^2	Coefficient of determination
RFLP	Restriction fragment length polymorphism
rpm	Revolutions per minute
RT-PCR	Real time polymerase chain reaction
RT-PCRs	Real time polymerase chain reactions
s	Second
SCC	Staphylococcal cassette chromosome
SCC*mec*	Staphylococcal cassette chromosome *mec*
SCIN	Staphylococcal complement inhibitor
shPCR	Subtraction hybridisation polymerase chain reaction

SLV	Single locus variant
SLVs	Single locus variants
SNP	Single nucleotide polymorphism
SSR	Short sequence repeat
ST	Sequence type
STs	Sequence types
TBE	Tris-Boric acid-EDTA
TE	Tris-EDTA
TLV	Triple locus variant
TLVs	Triple locus variants
TMB	Tetramethylbenzidine
TSA	Trypticase soy agar
TSB	Trypticase soy broth
UK	United Kingdom of Great Britain and Northern Ireland
USA	United States of America
v/v	volume/volume
VIC™	TaqMan reporter dye VIC
VNTR	Variable number tandem repeat analysis
w/v	Weight/volume
x *g*	x Gravitational force
YEPD	Yeast extract peptone dextrose

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Chapter 1

Introduction

1.1 Oral implants and periimplantitis

1.1.1 Oral implants

Oral implants have been used to fix prosthetic teeth and other devices (bridges, partial or full dentures, etc.) in the oral cavity for the last 30 years [1, 2]. Implants may be used where teeth are lost due to trauma, disease or medical treatment, malformation, or hereditary predisposition. Oral implants consist of several parts depending on the design and the majority are manufactured from titanium or titanium alloys [3]. Most include a shaft that is implanted into the patient's mandibular or maxillary bone and an abutment onto which the prosthesis is loaded (e.g. a single artificial tooth, a bridge or denture clip) (Figure 1.1). Different implant designs have different methods for insertion. Many are physically screwed into the patient's bone, while others are hammered in. New bone should then form around the implant shaft, holding it firmly in place, a process termed osseointegration [2]. When osseointegration fails to occur, or when osseointegration does take place but there is subsequent bone loss around the implant site leaving the implant loose, implant failure often results [4, 5].

Oral implants are classified as indwelling medical devices according to the European Union Medical Devices Directive [6]. The implant is lodged in the bone, and soft tissue forms around the shaft of the implant partially sealing it off from the oral environment [2, 7]. Only the protruding abutment onto which the prosthesis is to be fixed is completely exposed to the oral environment. As with the natural teeth, there is a pocket between the shaft and the outer gingival tissue or gum [8, 9]. This pocket becomes colonised by commensal and potentially pathogenic bacteria and other microorganisms such as yeasts [9, 10].

1.1.2 Periimplantitis and perimucositis

Infections involving oral implants generally fall into two categories, perimucositis and periimplantitis [11]. Perimucositis is a condition where the gingival and mucosal lining becomes inflamed, often due to sensitivity or allergic reaction to the materials used in the implant [2, 3]. Periimplantitis involves the hard tissue, the bone that the implant has been inserted into. This can involve inflammation of the bone, or more commonly, re-absorption of the bone, so that the implant placement site shrinks away from the implant and there is a loss of attachment of either new implants, or previously osseointergrated implants.

Periimplantitis is one of the major causes of post-placement implant failure [12], and is the condition of interest in this study.

1.2 General oral microbiology

According to the Human Oral Microbiome project (HOMb) over 600 bacterial species have been identified in the oral cavity [13-15]. Many of these are bacteria that have only been identified through ribosomal DNA (16S rDNA) sequencing and are too fastidious to grow and isolate in the laboratory [14-16]. The general oral environment is quite varied, encompassing mucous membranes, the tongue, the gum surface, the tooth surface and periodontal pockets located in-between the tooth and gum, and many bacteria exploit the different niches [17-19]. Bacteria associated with dental plaque, tooth caries, and periodontal disease are commonly present as part of a mixed oral microbial biofilm which can enhance survival in a challenging environment [10, 20, 21].

1.2.1 Periodontal microbiology

As with most other sites in the human body the periodontal pocket is not sterile, playing host to many commensal and potentially pathogenic microorganisms. These have been referred to as the periodontal flora. Some may be permanently present in periodontal pockets, whilst others may be present transiently. The microbial flora (mostly bacterial species) associated with caries and with periodontal disease has been investigated at length over the past few decades [20, 22-29]. The majority of cultivable microbes present in the periodontal flora have been investigated for their associations with periodontal disease (periodontitis) and with other bacteria. Such studies have resulted in the well-known system of complexes consisting of groups of microorganisms to which many putative oral pathogens have been assigned (red, orange, green, yellow and purple) (Figure 1.2) [20]. These complexes were originally developed to describe patterns of microbial colonisation and biofilm formation over time, bacterial associations, as well as species associations with states of health and disease. As new laboratory techniques and methodology have developed, especially molecular technology including sensitive DNA sequencing methods, PCR panels, DNA probes, DNA microarrays, etc., many uncultivable species have been identified in addition to these complexes [14, 16, 20, 27, 30].

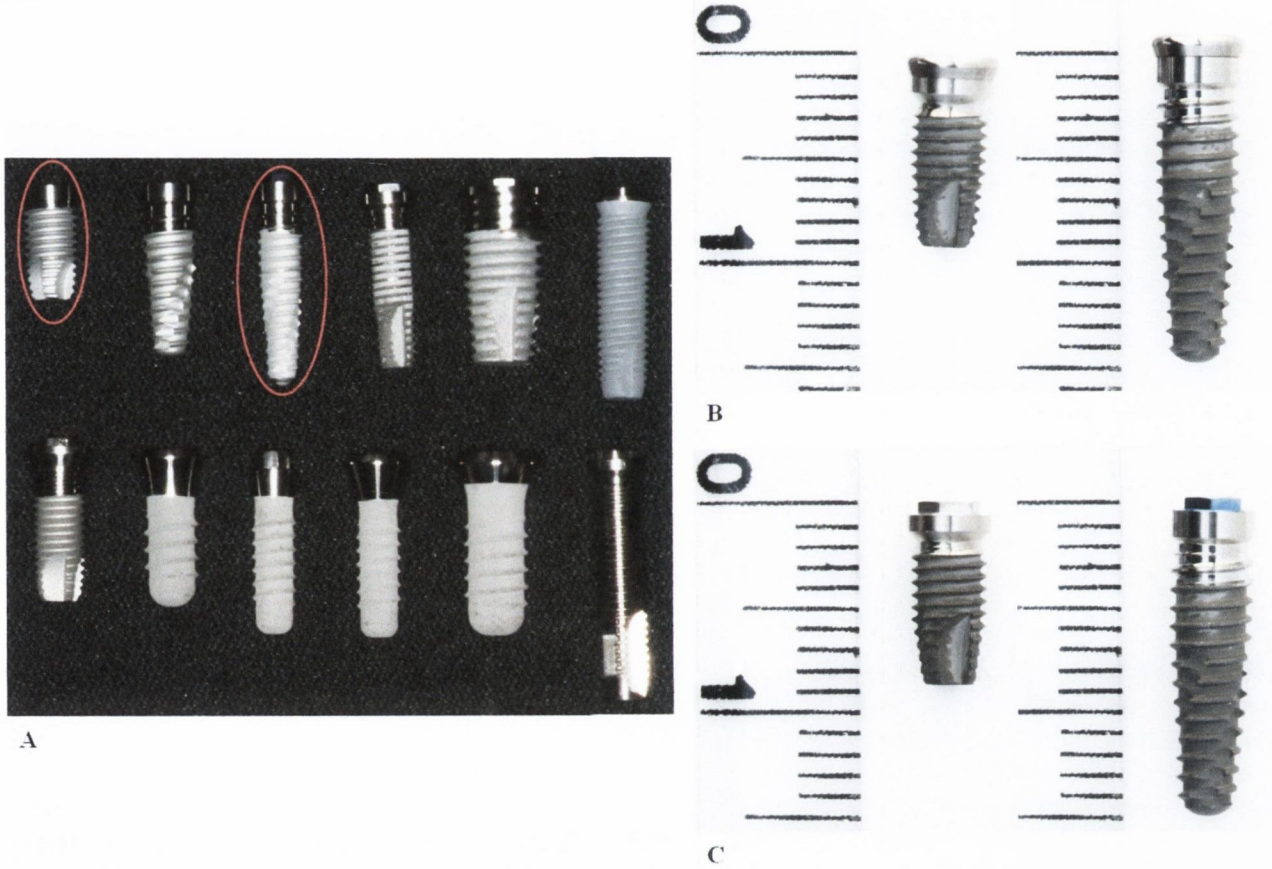

Figure 1.1. Examples of the variety of oral implants used at the Dublin Dental University Hospital (DDUH).

Panel A shows examples of different of oral implants that have been used to replace lost or missing teeth in patients treated at DDUH. The two implants circled in red and also presented in panels B and C are Biomet 3i Osseotite (BIOMET 3i, Palm Beach Gardens, Florida, USA) external connection hybrid implants (8.5 mm and 13 mm, respectively) that were the most commonly used implant in the patient cohort investigated in this study. Size, shape and surface texture of implants differ by brand and the site that the implant is designed for. Panels B and C show enlarged views of the 8.5 mm and 13 mm Biomet 3i Osseotite external connection hybrid implants. The scale to the left of each implant is in mm increments. Panel B shows the implants with temporary cover screws in place at the top of each implant. Panel C shows the same implants with the temporary cover screws removed revealing the abutment attachment site. Panel A is reproduced with the kind permission of Dr. I. Polyzois, Division of Restorative Dentistry and Periodontology, DDUH).

1.2.2 Oral implant microbiology

The microbiota present in the periimplant pocket may play a role in the continued viability or failure of an oral implant. So far many studies of periimplantitis associated with oral implants have concentrated on clinical presentation.

1.2.2.1 Studies of oral implant microbiology employing culture methods

Several of the microbiological studies (where bacterial cultures were grown from samples taken from implants) that investigated periimplantitis have been retrospective, post-failure, analyses of the flora of failed extracted and lost implants [5, 31]. These studies have found putative periodontal pathogens such as *Prevotella intermedia/Prevotella nigrescens*, *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans*, and *Actinomyces species*. Other bacteria associated with biofilm formation and caries formation were also found, including *Fusobacterium nucleatum*, *Streptococcus anginosus* (*milleri*), and *Parvimonas micra*. These studies also identified other species not usually thought to have a periodontal association, such as *Enterococcus* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp. yeasts (unspecified and *Candida albicans*) as well as *Staphylococcus epidermidis* [5, 31].

1.2.2.2 Studies of oral implant microbiology employing molecular methods

Other studies used a combination of methods (bacterial culture and identification, real time (RT) PCR, DNA-DNA checkerboard hybridisation and other DNA probing techniques) to observe the initial colonisation patterns of newly introduced oral implants [1, 8, 9, 32]. As with microbiological culture studies of failed implants a wide range of bacteria associated with periodontitis were identified including *F. nucleatum*. *P. intermedia*, *P. micra*, *Tanerella forsythia*, *A. actinomycetemcomitans* and *P. gingivalis* [1, 8, 9, 32]. However, in the studies quoted, putative periodontal pathogens were the organisms of interest and other organisms were not investigated.

Studies that used only molecular methods found similar organisms. The molecular methods employed allow a much wider range of bacteria to be identified from a single sample, but can only identify the species included on the testing panel used. Advanced molecular techniques that determine the nucleotide sequences of all 16S rDNAs present in a sample can be used to identify all microorganisms present, but this approach has not yet been applied to investigating the microbiome of oral implants.

Other studies have been conducted on implants that have been in place for a number of months, or even years, that showed signs of disease (periimplantitis or perimucositis) [11, 34-39]. Some of these studies contrasted results from implant sites with the corresponding flora present at healthy implant sites, or investigated the microbial flora pre- and post-treatment (mechanical or chemical intervention) for periimplantitis. The majority of these studies used molecular methods to identify the bacterial species present, and similar to the studies on initial implant colonisation, a wide range of putative periodontal pathogens were identified including *T. forsythia*, *T. denticola*, *Prevotella* spp., *Actinomyces* spp., *Streptococcus* spp., *Campylobacter* spp., *Fusobacterium* spp., as were *Pseudomonas aeruginosa*, *S. aureus*, *S. epidermidis* and *S. haemolyticus* when included on the testing panels. One study by Leonhardt *et al.* (2003) [37] that used media culture of clinical samples rather than molecular techniques identified several patients with enteric bacteria colonising the implants as well as a single patient with *S. aureus* present. Studies of successful implants (oral implants that have been in place for some time without showing signs of periimplantitis or perimucositis), again using molecular identification panels, also found a wide variety of periodontal bacteria [40, 41]. In one study, the majority of the bacteria found were oral streptococci [41].

1.2.3 Direct comparisons of oral implant microbiology with periodontal microbiology

As indicated previously, many studies that have been carried out on the microbiota of oral implants have made the assumption that the periimplant flora will be similar, if not identical to, periodontal flora. Several studies have been undertaken comparing the bacteria that are found at oral implants to the bacteria found at tooth sites tested in the same study [40, 42-47]. Again the majority of these studies used molecular detection methods to identify bacterial species directly from clinical samples. A study by Listgarten and Lai (1999) using bacterial culture from clinical samples and subsequent identification of isolates identified *T. forsythia*, *Fusobacterium spp.*, *P. micra* and *P. gingivalis* as the most frequently identified bacteria at periodontally diseased teeth and failing oral implants [44]. The same study also identified the presence of enteric bacteria, staphylococci and yeasts (although at low levels) [44]. A study by Mengel *et al.* (2001) which utilised DNA probes and dark field microscopy to identify bacteria directly from clinical samples found that there was no significant difference between the microbial composition of the periodontal and periimplant microbial flora for the species tested.

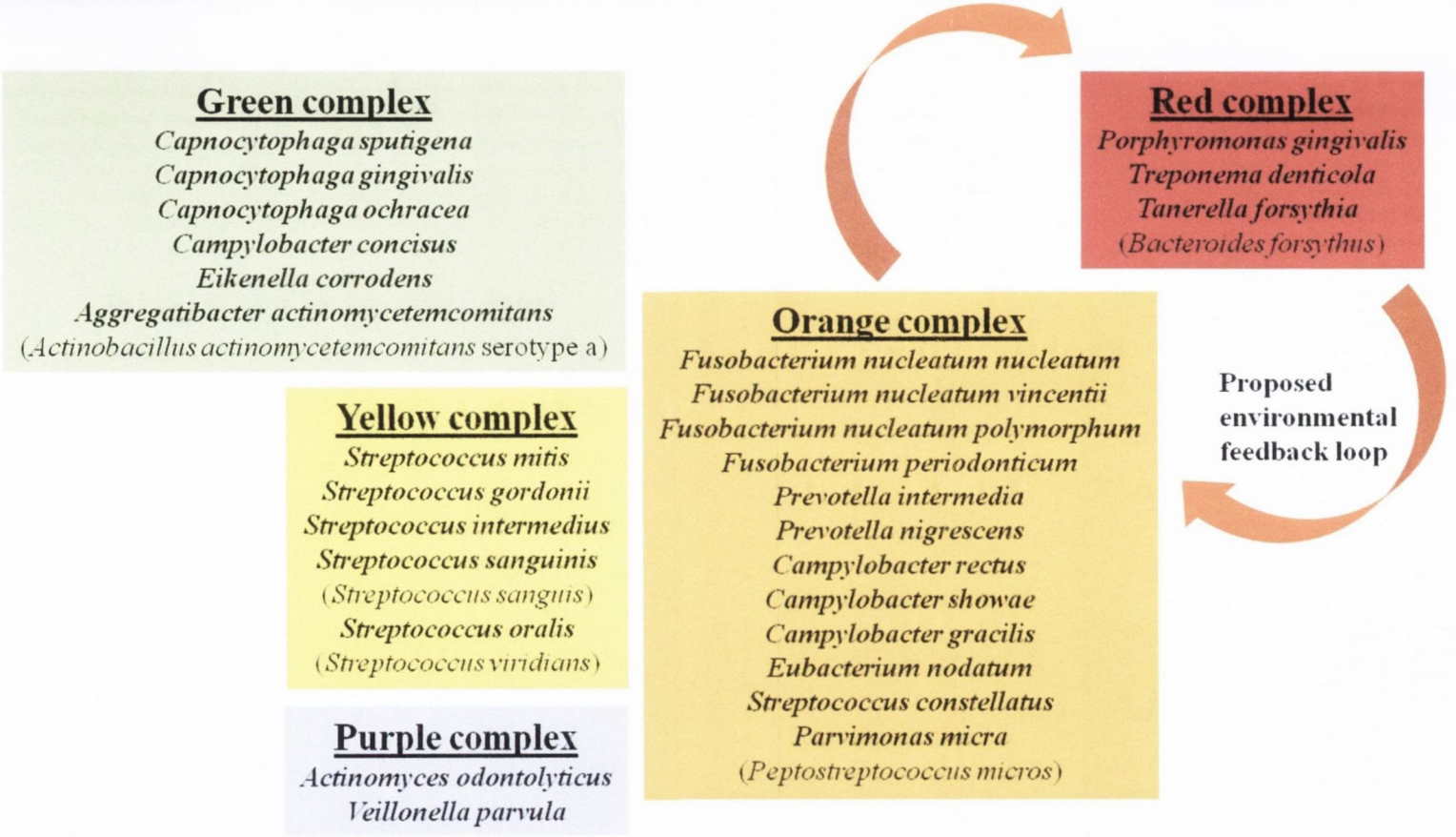

Figure 1.2. Microbial complexes in subgingival plaque

The figure above is adapted from Socransky *et al.*, 1998 [20] and includes the environmental feedback loop proposed by Socransky and Haffajee, 2005 [33]. Species names which may be found in older publications regarding these bacteria have been included in parenthesis below the currently accepted species name. The Yellow, Green and Purple complexes are thought to be early site colonisers, while members of the Orange complex have been associated with infections at non-periodontal sites. Members of the Red complex have been associated with periodontal disease [20, 33]. In the proposed feedback loop the presence of members of the Orange complex induce a habitable environment for the members of the Red complex, the increased presence of Red complex bacteria leads to a further change in habitat (including gingival inflammation) which in turn favours the proliferation of members of both the Orange and Red complexes [33].

In the study *A. actinomycetemcomitans*, *P. gingivalis* and *P. intermedia* were identified using DNA probes on selected samples, while all other samples were observed using dark field microscopy and the microbes observed were assigned to the following groupings: cocci, immotile rods, motile rods, large spirochetes, medium spirochetes, small spirochetes, filaments and fusiforms [45]. The same group (Mengel *et al.*, 2005) conducted an ongoing study of patients treated for generalised aggressive chronic periodontitis over three years (mentioned above) [46]. Listgarten *et al.*, 1999, used the same techniques (dark field microscopy and selected DNA probes) to undertake a paired control study of samples (received as part of the general work in a school of dental medicine) from failing oral implants with those received from patients diagnosed with adult periodontitis and patients with recurrent or refractory periodontitis [44]. All of these studies indicated that there was no difference between the bacteria observed in samples obtained from teeth or implants [44-46]. Several studies have been undertaken using checkerboard DNA:DNA hybridisation (CKB) to directly compare the presence and relative abundance of panels of putative periodontal pathogens at implant and tooth sites from the same patient. CKB (described in detail in section 1.4.3 and in Chapters 2 and 5) utilises a panel of total cellular DNA probes from selected bacterial species to identify and quantify bacterial species present in test samples. One study (Agerbaek *et al.*, 2006) investigated patients undergoing periodontal therapy (with no differentiation made between the state of health of each particular site) [42]. Another (Gerber *et al.*, 2006) observed patients not undergoing periodontal therapy without periimplantitis where both sites were assessed to be healthy [43]. While others (Renvert *et al.*, 2008, Salvi *et. al.*, 2008) observed patients that had had implants placed twelve months and seven years previously, respectively, who were undergoing periodontal therapy without differentiating between the state of health observed at each particular site [40, 47]. These last two both included *S. aureus* on their testing panels and will be discussed in detail in a later section. The checkerboard results were slightly more varied than those using dark field microscopy and selected DNA probes. Agerbaek *et al.'s* comparison between tooth and implant sites in patients undergoing periodontal therapy (state of health not stated) found no difference in the bacterial composition of the two different site types, but a higher total bacterial load was observed at implant sites [42]. The comparison between teeth and implants in patients not undergoing periodontal therapy and without periimplantitis where both sites were assessed to be healthy (Gerber *et al.*, 2006) revealed no difference in total estimated bacterial load between sites, though the proportions of *Streptococcus oralis* and *Fusobacterium periodonticum* were both significantly higher at tooth sites then at implant sites [43]. Salvi

et al.'s study, which used checkerboard DNA:DNA hybridisation (CKB) to investigate the colonisation patterns of implants at three time points (from 30 min following implantation up to one year post-implantation) including samples taken from the teeth adjacent to implants, showed a higher bacterial load at tooth sites than implant sites. Furthermore, 7/40 species tested (*Capnocytophaga sputigena*, *Actinomyces naeslundii*, *Campylobacter gracilis*, *Neisseria mucosa*, *Prevotella melaninogenica*, *Treponema socranskii* and *Veillonella parvula*) had significantly higher amounts of bacterial DNA (leading to higher estimated bacterial cell density) at tooth sites than at implant sites [47].

This study included *S. aureus* in its testing panel and found that if *S. aureus* was identified at a sampling site at the second sampling time point (twelve weeks post-implantation) then it was probable that *S. aureus* would be present at the last sampling point (12 months post-implantation). Similarly, the absence of *S. aureus* at the 12-week time point was indicative of the absence of *S. aureus* at the 12-month time point [47]. A later study that used CKB involved patients that had samples taken from implants and teeth (state of health not stated) who had oral implants in place for seven years. This study reported higher levels of *T. forsythia*, *C. sputigena*, *Actinomyces israelii* and *Lactobacillus acidophilus* on tooth surfaces compared to implant surfaces. *Staphylococcus aureus* was also included in the testing panel for this study and no differences in the frequency of identification of *S. aureus*, or the relative abundance of *S. aureus*, between the two types of sites sampled was observed [40]. Interestingly, the presence of *S. aureus* at a tooth site was found to be predictive of its presence at an implant site in the same patient [40].

1.3 Staphylococci

Staphylococci are Gram-positive cocci commonly found as commensal organisms colonising various parts of the human body [48, 49]. They have been known to be both commensal organisms and opportunistic pathogens for many years [49, 50]. Staphylococci are often grouped based on their expression of coagulase, as coagulase-positive (mainly *S. aureus*), or coagulase-negative (many other species, including *S. epidermidis*) [50-53]. Coagulase-negative staphylococci (CoNS) are generally considered less pathogenic than *S. aureus,* though they are well recognised as opportunistic pathogens of immunocompromised individuals, and have been particularly linked with infections associated with prostheses such as replacement hip joints and indwelling medical devices such as urinary and venous catheters [54-59]. The present study concentrated mainly on two species of staphylococci, *S. aureus* and *S. epidermidis*.

1.3.1 *Staphylococcus aureus*

Like *S. epidermidis*, *S. aureus* can be a commensal coloniser of humans, persistently carried by approximately 10-20%, and transiently carried by approximately 60% of the population in the anterior nares of the nose [49]. However, *S. aureus* is frequently pathogenic, which is associated with its ability to express a wide range of toxins and enzymes that can give rise to a range of disease syndromes, such as impetigo, scalded skin syndrome, toxic shock syndrome and skin and soft tissue infections as well as life-threatening bloodstream infections [60-62]. *Staphylococcus aureus* is known to be a significant pathogen of surgical wounds, can infect practically any tissue in the body including bone, heart and brain and has been thought to play a role in certain types of oral infections and inflammation [48, 54, 60, 63-66].

Different *S. aureus* strains or clones can encode genes for different sets of virulence and antibiotic resistance factors or determinants [67]. The presence and expression of these factors, combined with individual host responses, can influence whether or not a strain remains a harmless commensal or behaves as a harmful pathogen [62, 65, 66, 68-70]. Some of these factors can mediate evasion of host immune responses, others are involved in biofilm formation, others in cell adhesion and others (the toxins) can mediate cell and tissue damage [62, 69, 71-73]. Antibiotic resistance determinants allow a strain to persist despite exposure to the given antimicrobial agent. Resistance to clinically used antibiotics among *S. aureus* isolates has been a significant problem for decades and continues to cause difficulties today [67, 72, 74-76]. Methicillin resistant *S. aureus* (MRSA) encoding resistance to the majority of β-lactam antibiotics have been a major cause of nosocomial infections in hospitals worldwide for decades and more recently community-associated (CA)-MRSA have emerged as a significant cause of infections in the community [58, 72, 77].

1.3.2 *Staphylococcus epidermidis*

Staphylococcus epidermidis is generally non-pathogenic, commonly found as a commensal organism on human skin and is likely to be self inoculated into the mouth [48, 50]. Due to its status as a commensal *S. epidermidis* has commonly been regarded as a contaminant when found in clinical specimens [78]. However, *S. epidermidis* is frequently associated with opportunistic infections of surgical implant sites, such as artificial hip and knee joints [56, 57, 79, 80]. *Staphylococcus epidermidis* is adept at biofilm formation *in vivo* [81, 82]

and can be very difficult to eradicate once established around an infected prosthesis [54, 80, 83-86]. The identification of *S. epidermidis* in atherosclerotic plaques [87] and an increased incidence of *S. epidermidis* infections, such as catheter associated urinary tract infections, and ventilator associated respiratory infections, have highlighted its potential as an opportunistic pathogen [88, 89].

The ability to form biofilms is a significant feature of some *S. epidermidis* strains responsible for opportunistic infections, a property that enables the organisms to persist at particular sites (e.g. replacement hip joints) and resist mechanical removal or eradication by antibiotic treatment. Many *S. epidermidis* isolates harbour the biofilm-associated genes *icaA*, *icaD*, *icaB* and *icaC*, which can be prevalent among nosocomial *S. epidermidis* isolates [84, 90].

Considerable indirect evidence has accumulated over the last 10 years that CoNS species, and *S. epidermidis* in particular, function as a reservoir of mobile genetic elements (MGEs) for *S. aureus*. Many antibiotic resistance and some virulence genes found in *S. aureus* (especially MRSA) are prevalent in CoNS and are located on MGEs such as transposons, plasmids and bacteriophages. The staphylococcal cassette chromosome (SCC*mec*) element encoding resistance to methicillin has been identified in many *S. epidermidis* isolates [88, 91-99]. It has been proposed that of the many SCC*mec* types identified in MRSA, several may have arisen due to recombination events within *S. epidermidis* and subsequent gene transfer into *S. aureus* [76]. *Staphylococcus epidermidis* populations have not been characterised as thoroughly as *S. aureus*. Fortunately in recent years research interest in *S. epidermidis* has increased significantly and several methods have been used to characterise various *S. epidermidis* populations. Many of the investigations into *S. epidermidis* populations have been conducted retrospectively on isolate collections drawn from hospital or university reference laboratories, and as such have often included isolates derived from infections associated with indwelling medical devices (e.g. catheters, artificial joints, artificial heart valves and pacemakers) as well as blood isolates and isolates from atherosclerotic plaque [54, 55, 57-59, 84, 87, 88, 100]. A few wide ranging prospective population studies have recently been undertaken investigating the general carriage of *S. epidermidis* strains in distinct human populations [90, 92].

Staphylococcus epidermidis has an affinity for titanium, a lightweight, inert and extremely strong metal from which many prosthetic joints or their component parts are made from, as

are the shafts of the majority of commercial oral implants [8, 101, 102]. As mentioned briefly above, *S. epidermidis* has frequently been associated with infections of prosthetic joints and it is conceivable that *S. epidermidis* and/or other CoNS may have an affinity for oral implants and may even contribute to oral implantitis[54-59].

1.3.3 Staphylococcal mobile genetic elements

Staphylococci in general (and *S. aureus* and *S. epidermidis* in particular) play host to a large number of MGEs, some of which exist outside of the bacterial chromosome as plasmids while others such as insertion sequences, staphylococcal cassette chromosome (SCC) elements, transposons, pathogenicity islands, genomic islands and lysogenic prophages may be integrated into the genomic DNA [76, 103]. Often MGEs encode genes that provide a survival benefit such as antibiotic resistance, or enhance survival in challenging environments such as the arginine catabolic mobile element ACME [103]. These MGEs have been observed directly and through retrospective genetic analysis to transfer between the different staphylococcal species, such as *S. aureus*, *S. epidermidis* and other CoNS [104, 105].

Staphylococcal Cassette Chromosome *mec* (SCC*mec*) is probably the most well characterised staphylococcal MGE. SCC*mec* encodes the methicillin resistance determinant *mecA* and its regulatory genes along with others such as those that allow the incision and excision of the MGE (chromosome cassette recombinase, *ccr*, genes), genes that confer resistance to other antibiotics (such as β-lactamase antibiotics), or other efflux pumps (such as copper or mercury resistance) [103, 106-108]. Primarily associated with *S. aureus* (and extremely important in the clinical setting) SCC*mec* has been identified in many other CoNS [90, 93, 109, 110]. Several different SCC*mec* types have been identified in *S. epidermidis*, and transfer of SCC*mec* from *S. epidermidis* to *S. aureus* has been observed *in vivo* [88, 91-99, 111, 112].

1.3.4 Staphylococcal typing schemes

1.3.4.1 Pulse field gel electrophoresis

Pulse field gel electrophoresis (PFGE) is a highly discriminatory method of typing used to identify strains or clones present within a population for many species of microorganisms, especially bacteria such as *S. aureus*. In PFGE high molecular weight chromosomal DNA is embedded in a block of agarose gel and digested *in situ* using a restriction endonuclease that cleaves DNA sequences infrequently (e.g. *Sma*1) after which the gel block containing

the digested DNA is then transferred to an agarose gel and subjected to electrophoresis using an alternating electric field. This allows large DNA fragments to be clearly resolved as they slowly migrate along the gel, resulting in very distinct PFGE patterns which can be used to type bacterial isolates [113, 114]. While PFGE is generally regarded as the gold standard for typing organisms such as MRSA, especially in epidemic situations, it has several flaws when attempting a globally relevant population analysis. The reproducibility of PFGE patterns can vary greatly between laboratories, making the comparison of PFGE patterns obtained at different laboratories difficult [114, 115]. PFGE screening is highly dependent on the protocols followed, the reagents used and the skill of the individuals performing the technique.

1.3.4.2 Multi locus sequence typing (MLST)

Multi locus sequence typing (MLST) is an established technique used for typing of an isolate within a species and for grouping related isolates into sequence types (STs) and clonal complexes (CCs) [116, 117]. In MLST a set of highly conserved genes (often referred to as "housekeeping" genes) are amplified by PCR and sequenced. The sequences obtained are then compared to a baseline reference sequence for each gene, referred to as the consensus sequence. The *S. aureus* and *S. epidermidis* MLST schemes each employ seven housekeeping genes [118, 119]. The consensus sequences for *S. aureus* are those determined in the original *S. aureus* MLST study by comparison of the MSSA and MRSA isolates used [119], while the consensus sequences for *S. epidermidis* are based on *S. epidermidis* strain RP62A [71, 120, 121]. Unique point mutations away from the consensus sequence (alleles) are allocated their own specific numbers. The unique combination of a set of allele numbers is associated with an ST number [116, 117]. Sequence types are grouped in CCs of closely related strains by complex algorithms that attempt to determine the evolutionary relationships between the different STs based on their alleles [117, 122]. Unlike other typing methods, where there can be difficulties when attempting to directly compare results produced by different laboratories, MLST data is very transferable. Because MLST is a sequence-based technique, data gathered from MLST surveys can be easily shared among and compared between collaborating researchers and internationally.

Both *S. aureus* and *S. epidermidis* have dedicated international, internet-based MLST databases (http://saureus.mlst.net; http://sepidermidis.mlst.net). As a well-studied pathogen, the database for *S. aureus* contains many more entries (4703 isolates and 2595 STs on the 13th February 2013) from which a lot of information can be gathered. The *S.*

epidermidis database is substantially smaller (727 isolates and 472 identified STs when last updated in August 2012), but still provides valuable information about the global *S. epidermidis* population.

1.3.4.3 Multilocus variable number tandem repeat analysis (MLVA)

Like MLST, multilocus variable number tandem repeat analysis (MLVA), or fingerprinting, utilises nucleotide variations that occur at hypervariable loci within the genome under investigation [123, 124]. Based on variable number tandem repeat analysis (VNTR), a multiplex PCR targeting multiple hypervariable loci is performed (both *S. aureus* and *S. epidermidis* MLVA schemes employ five different loci) and the resulting PCR amplimers are visualised using agarose gel electrophoresis [123-125]. Loci with different repeat patterns yield different sized bands and the total band pattern created by all five loci can be assigned to different types (similar to restriction fragment length polymorphism (RFLP), or PFGE). MLVA is less discriminatory than PFGE, but is a more rapid method and does not require specialised equipment such as that required for PFGE [114, 124]. As with PFGE patterns, the protocols followed, the reagents used and the skill of the individuals performing the technique can result in differences in MLVA patterns between laboratories.

1.3.4.4 *Staphylococcus aureus* protein A (*spa*) typing

Typing of *S. aureus* isolates by sequencing the polymorphic X (or short sequence repeat, SSR) region of the protein A gene (*spa*) has been found to be an effective typing technique [115]. The SSR region is highly diverse, possibly due to the duplication and deletion of repeated units as well as point mutations within the repeated sequences. Because of the high degree of polymorphism within the region it has been suggested that the variation rate of the SSR may provide a high enough degree of discrimination to be employed in outbreak situations [115]. Because *spa* typing requires the analysis of a single polymorphic locus, it is much quicker to perform and cheaper than methods such as MLST, which requires the sequence of seven different loci to be determined. A comparable single polymorphic locus typing scheme similar to *spa* typing has not yet been developed for *S. epidermidis*.

1.3.4.5 Staphylococcal cassette chromosome *mec* typing

SCC*mec* elements consist of several discrete components, variations in each of which can be used to separate the strains in which they occur into different types. These include the

mec gene complex including the *mecA* gene (encoding resistance to methicillin) and its regulatory genes *mecI* and *mecR1*, the *ccr* complex encoding cassette chromosome recombinase (*ccr*) genes involved in site- and orientation-specific integration of SCC*mec* into *orfX* within the *S. aureus* chromosome, and the adjacent joining regions (J regions) [103, 106]. SCC*mec* typing utilises a range of specific PCRs targeted to detect a specific combination of genes and alleles, each adding an extra layer of information. Variations in the *mec* gene complex (the presence or absence of insertion sequences and regulatory genes) are used to assign the *mec* type (using roman numerals, currently from I-XI) [77, 106, 107]. The sequence of the J regions is used to determine the *mec* sub-type (reported using lower case alphabetic suffixes i.e. a, b, c, etc.) [106, 107]. Multiplex PCRs targeting sequences found in specific SCC*mec* types have been developed (targeting type-specific sequences in both the *mec* complex and J regions) so a simple PCR can give an indication of the SCC*mec* type without the requirement to sequence the element, although sequencing allows identification of SCC*mec* type variants that may not be detected by PCR [77, 106-108]. The *ccr* genes present in SCC*mec* can also be typed (through PCR, or sequencing) and reported on in addition to the SCC*mec* type and sub-type. Some *ccr* gene type combinations are generally associated with specific SCC*mec* types and variations in this may indicate recombination events, leading to novel SCC*mec* elements [106]. An international SCC*mec* typing nomenclature has been established to rationalise the naming of new SCC*mec* types, sub-types and variants [107].

1.3.4.6 *mec*-associated direct repeat unit (*dru*) typing

Another method that has been used to type MRSA is based on the *mec*-associated direct repeat unit (*dru*). The direct repeat units are a cluster of 40 bp sequences located adjacent to the SCC*mec* element component IS*431*. When present in MRSA (a few isolates have been identified that lack *dru* sequences) *dru* sequence locations are constant, regardless of the chromosomal SCC*mec* type. This allows consistent PCR amplification and DNA sequencing analysis to be conducted [126]. Single base pair variations from the consensus sequence for the repeated 40 bp sequence are each assigned a *dru* repeat number. Each pattern of these *dru* repeat numbers represents (and is assigned) a different *dru* type [126]. The *dru* types identified in epidemic MRSA (EMRSA) strains have been observed to remain stable over time with isolates of the same strain that were isolated at different time points exhibiting the same *dru* type [126]. Similar to MLST typing, *dru* typing has an internet-based database and curator, which make it useful for comparing isolates from different studies and geographic locations.

1.3.4.7 Combined typing schemes

As with most methods of identification, the more information that can be gathered from a given isolate, strain or clone, the more accurately it can be identified. Combinations of typing techniques are commonly used to enhance isolate discrimination, for example typing an isolate using both MLST and SCC*mec* typing in both *S. aureus* and *S. epidermidis*. In some situations such as when typing highly clonal epidemic or endemic staphylococci, a combination of several typing techniques can allow differentiation between seemingly indistinguishable isolates [127]. This can allow the creation of theoretical lineages of descent, or help to identify potential routes of transmission of very closely related hospital and environmental isolates from hospital outbreaks [127].

1.3.5 High-throughput DNA microarray profiling of staphylococci

In recent years, the availability of annotated whole genome DNA sequences for many *S. aureus* strains has revolutionised our understanding of the molecular biology of these organisms. The ready availability of many whole genome sequences has permitted the development of DNA microarrays that can be used for high-throughput screening of large numbers of clinical isolates for the presence of important virulence and antimicrobial resistance genes and typing markers. Such arrays have been used productively to gain valuable insights into the population biology of collections of *S. aureus* isolates and to rapidly and accurately type large numbers of isolates. One such array is the StaphyType DNA microarray developed by Alere Technologies (Dresden, Germany) [67, 128, 129]. This array contains oligonucleotide probes specific for 334 *S. aureus* gene sequences and alleles (including species-specific, antimicrobial resistance and virulence-associated genes, and typing markers) and accurately assigns the majority of isolates to the correct MLST ST and CC [107]. Such technology offers significant potential for screening *S. aureus* oral isolates relative to the global population of *S. aureus* [67]. Furthermore, because *S. epidermidis* and *S. aureus* frequently harbour antimicrobial resistance genes, and in some cases virulence genes (e.g. ACME), in common microarray profiling has potential for screening *S. epidermidis* isolates for the presence of such genes.

1.4 Staphylococci in oral microbiology

Until recently staphylococci were not frequently studied in oral microbiology. They have been reported in some studies of periodontal patients using direct culture of clinical specimens [130, 131], and also from implant patients [131]. Unfortunately, many studies of the microbiota of periodontal and implant pockets have not fully identified staphylococci

to the species level, often only relying on their appearance under dark field microscopy [44-46].

1.4.1 *Staphylococcus aureus* in oral microbiology

The presence of *S. aureus* in the oral cavity has been reported on several occasions [64, 132, 133]. There is evidence that *S. aureus* is trafficked directly from the nasal cavity to the oral cavity, as well as probable self inoculation via hand to mouth contact [134]. Systemic illness involving *S. aureus* thought to be instigated by dental extractions or oral surgery have also been reported [60]. *Staphylococcus aureus* has been found in bloodstream samples taken immediately after oral surgery, when samples taken prior to surgery were clear of all bacteria suggesting that *S. aureus* was introduced into the bloodstream as a result of the oral surgery [135]. Some of the more recent studies conducted using checkerboard DNA:DNA hybridisation investigated *S. aureus* in implants and natural teeth, identifying *S. aureus* as being present at each site type in some instances [8, 35, 40, 47]. These studies are discussed in detail in section 1.4.3 below. However, *S. aureus* is not generally considered to be part of the normal oral flora.

1.4.2 *Staphylococcus epidermidis* in oral microbiology

Staphylococcus epidermidis has also been identified by laboratory culture from clinical samples taken from tooth and implant sites [23, 130, 131]. *Staphylococcus epidermidis* has also been identified in bloodstream samples taken immediately following oral surgery [135]. The same study reported that the bacteria had been cleared from the bloodstream in samples taken several hours after surgery [135]. Due to its nature as a commensal organism, usually located on the epidermis, *S. epidermidis* has been to a large extent overlooked in oral microbiology.

1.4.3 Checkerboard DNA:DNA hybridisation studies investigating associations between staphylococci and periimplantitis

A majority of studies that investigated bacterial associations with oral implants have relied on molecular detection of a variety of particular bacterial species using checkerboard DNA:DNA hybridisation (CKB). CKB is a molecular technique that utilises total cellular DNA probes from 40-80 bacterial species (dependent on the research group and equipment being used) to identify and quantify bacterial species present in test samples. Whole sample DNA extracts are laid alongside a set of standards on a nylon membrane using a manifold that allows the samples to be dispersed in discrete rows across the membrane.

After the test samples have been bound to the membrane whole genome probes are introduced using a second manifold that allows the delivery of the probes in columns down the membrane at a 90° angle to the sample lanes, forming the 'checkerboard' pattern. After hybridisation and processing, chemiluminescent signals are captured and analysed. A comparison of the strength of the signal yielded by a probe from a test sample row to the strength of the signal yielded by the same probe on the standard rows permits a semi-quantitative estimate of the amount of target DNA present in the test sample [35, 136, 137]. The technique is extremely useful in that it allows a lot of information to be derived from a set of samples in a relatively short period of time. However, there are drawbacks. CKB analysis estimates the amount of DNA present in a test sample for each species included on the testing panel, but does not permit any estimate of the viable cell density present. Also, the possibility of cross hybridisation between whole genomic DNA probes where different species share genes in common must be guarded against. Often probes are not tested for cross-reactivity against species that have not been included in the CKB testing panel [35, 137]. If cross-reactions between species on the CKB panel are identified attempts are often made to limit cross reactivity by using subtraction hybridisation PCR (shPCR) to prepare probes or by setting up competitive hybridisation when running the probe hybridisation reactions [137]. However, when dealing with test samples made up of mixed microbial samples unknown bacteria that have not been tested for cross-reactivity with CKB probes may be present. The most commonly used CKB panels have been tested through continued use over time and have been compared to other methods [20, 137-139]. New test probes for species not usually included on the CKB testing panel are not as well established.

The results of four studies using CKB that included *S. aureus* on their testing panels all suggested that *S. aureus* was a significant coloniser of oral implants and may be involved in periimplantitis [8, 35, 40, 47]. Fürst *et al.* (2006) investigated early colonisation of oral implants, taking samples prior to implant surgery, immediately after suturing (at the completion of surgery), and at intervals of one, two, four eight and 12 weeks after surgical implant placement. Results of the study presented the proportion of sites tested with a positive result for each species investigated (defining a positive result as an estimated value of $\geq 1 \times 10^5$ microorganisms). They showed *S. aureus* to be present in 5.9% of sites sampled immediately post surgery, 11.1% of sites sampled at one week post surgery, 8.3% of sites sampled four weeks after surgery, 11.1% of sites sampled eight weeks after surgery and in 15% of sites sampled 12 weeks after surgery. Fürst *et al.* reported that *S. aureus* was

more commonly identified in samples than four bacterial species that are considered to be significantly associated with periodontal disease (*P. gingivalis*, *A. actinomycetemcomitans* serotype b, *T. forsythia* and *T. denticola*). Fürst *et al.* did not include other staphylococcal species on the CKB testing panel employed in the study and no consideration was given to potential cross-reactivity between species such as *S. aureus* and CoNS [8]. Salvi *et al.* (2007) [47] obtained additional clinical samples 12 months after surgery from the same patient cohort included in the study by Fürst *et al.* and undertook a CKB analysis of the Fürst *et al.* samples taken at 12 weeks after implant placement and the new samples taken 12 months after implant placement. Salvi *et al.* found that (using the same criteria as Fürst *et al.*) *S. aureus* was present at 18.9% of all sites sampled, 20% of implant sites and 25% of tooth sites sampled. Salvi *et al.* also determined that the presence (or absence) of *S. aureus* at a given site twelve weeks post-surgery was predictive of the presence (or absence) of *S. aureus* one year post-surgery. Salvi *et al.* [47] referred to an earlier study by Socransky *et al.*, 2004 [137], which outlined methods to determine the specificity of CKB whole genome probes and reduce cross-reactions between similar. However, as Salvi *et al.* [47] did not include any other staphylococci on the CKB testing panel, and did not state what precautions were taken against potential cross reactions between CKB probes and target DNA from related species present in test samples, it is unclear if the CKB methodology used was sufficient to prevent cross reaction between *S. aureus* and CoNS present in the clinical samples [47]. A separate study that used CKB analysis by Renvert *et al.*, 2008 [40] investigated the microbiological load of two brands of oral implant (Brånemark and AstraTech) seven years after placement. The patients involved in this study (n=54, 27 patients for each implant brand) had been clinically and radiographically examined one year post-implantation and again approximately seven years post-implantation, when the microbiological sampling took place. Renvert *et al.* reported on the percentage of subjects that returned a positive result for the species under investigation (defining a positive result as an estimated value of $\geq 1 \times 10^4$ microorganisms) at the tooth, Brånemark implant or AstraTech implant sampled. According to this study 44% of patients tested harboured *S. aureus* at a tooth site, 70.4% harboured *S. aureus* at a Brånemark implant site and 57.1% of patients harboured *S. aureus* at an AstraTech site. They also determined that the identification of *S. aureus* at a tooth site was predictive (85.7% probability) of the presence of *S. aureus* at an implant site (Brånemark or AstraTech) in the same patient. Like the studies of Fürst *et al.* [8] and Salvi *et al.* [47]. Renvert *et al.* did not include other staphylococcal species on the CKB testing panel employed nor did they indicate what, if any, precautions were taken against potential cross reactions between similar species [40].

Persson *et al.* (2010) utilised an expanded CKB panel including three *S. aureus* reference strains (ATCC25923, yellow strain GUH070921 and white strain GUH070922) and one reference strain each of *S. epidermidis* and *S. haemolyticus*. The investigation was a single-blinded randomised study assessing the effects of mechanical debridement, using either curettes or an ultrasonic device on the microbiota present in periimplantitis lesions [35]. Like Fürst *et al.* [8] Persson *et al.* collected samples prior to and immediately after debridement, one week, three months and six months after treatment, though detailed data was only presented for the baseline sample (immediately after debridement) and the sample taken six months post-debridement. Persson *et al.* reported the proportion of sites tested returning a positive result for the species under investigation, but chose to use two minimum values to determine a positive result, reporting both (proportion of sampling sites with an estimated value of $\geq 1 \times 10^4$ microorganisms and proportion of sampling sites with an estimated value of $\geq 1 \times 10^5$ microorganisms). At the baseline sample (prior to debridement) the *S. aureus* ATCC25923 whole genome probe hybridised with samples from 9.4% of sites, the *S. aureus* yellow strain whole genome probe hybridised with samples from 6.7% of sites and the *S. aureus* white strain whole genome probe hybridised with samples from 3.3% of sites when the cut off threshold for a positive *S. aureus* reading was determined to be $\geq 1 \times 10^5$ microorganisms. When the cut off threshold for a positive *S. aureus* reading was reduced to $\geq 1 \times 10^4$ microorganisms the values increased to 31.2%, 26.7% and 23.3% of sites sampled, respectively. The other two staphylococcal species included on the panel (*S. epidermidis* and *S. haemolyticus*) were each determined to be present at 3.3% of sites sampled when using $\geq 1 \times 10^5$ microorganisms as the cut off threshold for a positive reading, increasing to 20% and 26.7%, respectively, when the threshold was reduced to $\geq 1 \times 10^4$ microorganisms. Six months after debridement *S. aureus* was found to be present at 3.8% of sites sampled that had been debrided using an ultrasonic instrument at $\geq 1 \times 10^5$ microorganisms, increasing to 26.9% when the threshold for a positive result was lowered to $\geq 1 \times 10^4$ microorganisms. The *S. aureus* ATCC25923 whole genome probe hybridised with samples from 6.5% of sites sampled that had been debrided using a curette at $\geq 1 \times 10^5$ microorganisms, increasing to 29% when the threshold for a positive result was lowered to $\geq 1 \times 10^4$ microorganisms. No sites returned positive readings for the *S. aureus* yellow strain whole genome probe or the *S. aureus* white strain whole genome probes for either debridement method when assessed using $\geq 1 \times 10^5$ microorganisms as the cut-off threshold for a positive result. When the threshold was lowered to $\geq 1 \times 10^4$ microorganisms the *S. aureus* yellow strain probe hybridised with samples from 20% of sampling sites debrided using an ultrasonic instrument and 16.1% of

sites debrided using a curette, while the *S. aureus* white strain probe hybridised with samples from 16% of sampling sites debrided using an ultrasonic instrument and 35.5% of sites debrided using a curette.

None of the sites sampled that had been debrided using an ultrasonic instrument returned positive readings with the *S. epidermidis* or *S. haemolyticus* whole genome probes indicating failure to detect either of these CoNS species at the sites concerned at the threshold of detection for the probes. However, both species were each detected at 3.2% of sites sampled that had been debrided using a curette when assessed using $\geq 1 \times 10^5$ microorganisms as the cut-off threshold for a positive result with the *S. epidermidis* or *S. haemolyticus* probes. When the threshold was lowered to $\geq 1 \times 10^4$ microorganisms *S. epidermidis* was identified at 24% of sites debrided using an ultrasonic instrument and 29% of sites debrided using a curette, while *S. haemolyticus* was identified at 36% of sites debrided using an ultrasonic instrument and 32.2% of sites debrided using a curette [35]. Unlike the other three previous studies [83] Persson *et al.* stated that they routinely tested probes for cross-reactivity and stated that "our quality control results were consistent with those reported elsewhere (Socransky *et al.* 2004)."[35]. However, they did not report if any of the whole genome probes used cross-reacted with each other, nor did they outline any method used to overcome possible cross-reactivity. Importantly, by using two different minimum thresholds for a positive result ($\geq 1 \times 10^5$ and $\geq 1 \times 10^4$) Persson *et al.* showed what a large effect the baseline or standards used can have on the final results.

1.4.4 *Staphylococcus epidermidis* and periimplantitis

Staphylococci have been cultured from periodontal and periimplant pockets [130, 131], and *S. aureus* DNA has been identified in periimplant pockets [8, 35, 40, 47]. It has been suggested that *S. aureus* may play a role in periodontal disease, and may also be involved in periimplant disease [8, 40, 47, 130, 132]. However, the role of *S. epidermidis* in periodontal disease or periimplantitis has not been investigated. Like *S. aureus*, *S. epidermidis* is often associated with infections involving other types of implants and indwelling medical devices [56, 57, 79, 80]. As *S. epidermidis* is not usually thought to be involved in periodontal disease, it has been assumed that it will not be associated with periimplant disease either, and therefore ignored. Given the associations with other implant and indwelling medical device infections it is surprising that these two staphylococcal species have only been studied superficially with regard to oral implants and periodontal disease [131].

Previous studies investigating the microbiota of oral implants have reported a potentially significant association between the presence of *S. aureus* at an oral implant and periimplantitis [8, 35, 40, 47]. The authors of these studies suggested that *S. aureus* plays a role in the aetiology periimplantitis and ultimately in the failure of oral implants [8, 35, 40, 47]. However, these studies relied on molecular detection of *S. aureus* using CKB analysis using whole genome *S. aureus* probes to determine the presence of *S. aureus* DNA, rather than laboratory culture of staphylococci to investigate the microbiota associated with oral implants [8, 35, 40, 47]. Previous studies that investigated the microbiota of periodontal pockets using culture analysis identified the presence of several species of staphylococci including *S. epidermidis*, *S. capitis*, *S. hominis*, *S. warneri*, *S. aureus*, *S. cohnii*, *S. lugdunensis*, *S. intermedius*, *S. saprophyticus*, *S. haemolyticus*, *S. simulans* and other unclassified staphylococci, with *S. epidermidis* the most commonly identified species [23, 130, 131]. At the time of writing (to the best of my knowledge) only one previous study that used culture analysis to investigate the subgingival microbiota including staphylococci, also investigated samples taken from periimplantitis patients [131]. This study found that while a high proportion of the patients tested (9/13, 69.2%) harboured staphylococci including *S. aureus*, the majority of implants tested (13/20) yielded *S. epidermidis*, which has only been included in a single CKB study, where it was reported to be present at a lower proportion of sites than *S. aureus* [35]. To the best of my knowledge prior to the present study no published studies utilised a combination of culture, molecular analysis and population analysis to investigate associations between staphylococci and oral implant and periodontal pockets in both health and disease.

1.5 Aims of this project

1. To investigate whether subgingival staphylococci are significantly associated with diseased and healthy natural teeth and oral implants in a cohort of patients who had implants *in situ* for at least five years and who exhibited clinical symptoms of periimplantitis.
2. To compare staphylococcal populations from the same patient cohort before and following clinical treatment for periimplantitis.
3. To investigate oral *S. epidermidis* and *S. aureus* isolates from periimplantitis patients relative to their respective global population structures using species-specific MLST analysis.

4. To investigate the prevalence of virulence-associated and antimicrobial resistance genes in oral *S. epidermidis* and *S. aureus* isolates from periimplantitis patients using DNA microarray profiling.
5. To investigate the comparative prevalence of subgingival *S. epidermidis* and *S. aureus* from a subset of healthy and diseased natural tooth and oral implant sites in periimplantitis patients using laboratory culture, detection by species-specific real-time PCR and checkerboard DNA:DNA hybridisation analysis.

Chapter 2

Materials and Methods

2.1 Patient criteria

Forty three patients (18 male, 25 female) participated in this study (a preliminary power analysis based on previous studies indicated that a sample size of 34 patients was sufficient to show a significant difference between populations, e.g. if *S. aureus* was present in 70% of patients clinical samples taken from periimplantitis sites and 30% of patients clinical samples taken from healthy tooth sites). The patients ranged in age from 23-65 years (male range, 27–64 years; female range, 23-65 years), with a mean age of 43 years (both sexes had a mean age of 43 years). All patients enrolled in this study were partially dentate and had one or more oral implants in place for a minimum of five years, at least one of which showed clinical signs of disease at the time of inclusion in this study. Patients who were on antibiotic therapy, pregnant, or who were unable to provide informed consent were excluded from this study. The patient cohort in this study was recruited from patients attending clinics conducted by Dr. Rory Maguire at the Dublin Dental University Hospital (Lincoln Place, Dublin 2). Recruitment of patients that matched the criteria outlined above began in October 2007, the last patents were recruited in September 2009. Ethical approval for this study was granted by the St. James's Hospital and Federated Dublin Voluntary Hospitals Joint Research Ethics Committee (JREC) (including representatives from Trinity College Dublin) at the September sitting in 2007. All clinical samples were taken by Dr. Rory Maguire at the Dublin Dental University Hospital (Lincoln Place, Dublin 2) between October 2007 and September 2009.

2.2 Culture media

Media used for transport and storage of clinical samples, and growth of bacteria, were as follows: Yeast extract peptone dextrose (YEPD) broth: 10 g/L yeast extract (Merck, New Jersey, USA), 20 g/L peptone (Merck), 20 g/L glucose (Fisher Scientific, Massachusetts, USA). L-agar (LA): 10 g/L tryptone (Merck), 5 g/L yeast extract (Merck), 5 g/L NaCl (Fisher Scientific) 1.5 g/L agar #1 (Oxoid, Hampshire, UK). Mannitol salt agar (MSA): 1 g/L 'Lab-Lemco' powder, 10 g/L peptone, 10 g/L mannitol, 75 g/L NaCl, 0.025 g/L phenol red, 15 g/L agar (all from Oxoid). Nutrient agar (NA): 1 g/L 'Lab-Lemco' powder, 2 g/L yeast extract, 5 g/L peptone, 5 g/L NaCl, 15 g/L agar (all from Oxoid). Trypticase soy agar (TSA): 15 g/L pancreatic digest of casein, 5 g/L papaic digest of soybean meal, 5 g/L NaCl, 15 g/L agar (BD, Becton Dickinson, New Jersey, USA). Trypticase soy broth (TSB): 17 g/L pancreatic digest of casein, 3 g/L papaic digest of soybean meal, 5 g/L NaCl, 2.5 g/L K_2HPO_4, 2.5 g/L dextrose (BD). Muller Hinton agar base (MH): 2.0 g/L beef extract, 17.5 g/L acid hydrolysate of casein, 1.5 g/L starch, 17 g/L agar (BD). Columbia blood agar

(ready to use): 12 g/L pancreatic digest of casein, 1 g/L corn starch, 5 g/L peptic digest of animal tissue , 5 g/L sodium chloride, 3.5 g/L yeast extract, 13.5 g/L agar, 3 g/L beef extract, 5% defibrinated sheep blood (Oxoid). Agar plates contained 25 ml of agar in 94 mm diameter, triple vented, sterile plastic Petri dishes (Greiner Bio-One GmbH, Frickenhausen, Germany). Molten agar was poured under aseptic conditions in a Microflow Biological Safety Cabinet (Astec-Microflow, Bioquell UK Ltd., Hampshire, UK). Plates were allowed to cure for several hours before use, and were stored in sealed plastic bags at room temperature. Plates were usually used within one week of pouring. All plates were examined for contamination before use.

2.3 Clinical sample collection

All clinical samples were taken by Dr. Rory Maguire at the Dublin Dental University Hospital (DDUH) (University of Dublin, Trinity College Dublin, Lincoln Place, Dublin 2). Sampling sites included natural teeth, oral implants, and natural teeth adjacent to oral implants. Prior to sampling, saliva was removed from sampling sites with low volume suction. Sites were isolated using cotton wool rolls and the supra-gingival plaque removed using pellets of cotton wool. Separate samples were collected using three sterile paper points per site (Steri-cell, Coltene Whaledent, Altstätten, Switzerland) and a sterile disposable curette (Swede Dental AB, Örebro, Sweden, and, Hu-Friedy Europe, Rotterdam, the Netherlands) using general dental procedures. Paper point samples were taken by inserting them into the periodontal, or implant pocket, curette scrapings were taken from the periodontal, or implant pocket. Samples were placed immediately in sterile 2 ml conical base polypropylene screw cap microtubes (Starstedt AG & Co., Nümbrecht, Germany) containing 1 ml of YEPD broth. Three paper point samples taken from each site were placed in one tube, whereas a single curette scraping from each site was placed in a separate tube. Oral wash/rinse samples were taken by requesting patients to rinse their mouths with 10 ml of sterile distilled water provided in a sterile, lidded 100 ml plastic container (Starstedt) for approximately one min before returning it back into the sampling container. Samples were delivered to the DDUH microbiology laboratory by the collecting dentist as soon as possible after sampling and were stored at 4°C until processing (1-24 h depending on circumstances). Different clinical states of health were assigned for natural teeth and/or implants by the collecting dentist as healthy or diseased (periimplantitis, or periodontal disease). Each sample was assigned a unique, sequential, sample number as it arrived in the laboratory for processing, allowing samples to be processed blindly, with no bias. Each samples details (patient number, date of sampling, sampling method, tooth or

implant site, state of health etc.) were recorded with the sample number in a database (Microsoft Office Access 2003, Microsoft Corporation, Washington, USA) for future reference. Visit 1 was the initial appointment where treatment took place (post sampling). Subsequent visits (2+) were for post-treatment monitoring. Samples from the anterior nares of 12 patients were also collected using Copan Transystem® culture swab transport system (Starstedt).

2.3.1 Clinical sample processing and storage

On reception in the microbiology laboratory samples were stored at 4°C for 1-24 h until processing. As they were clinical in origin and some contained traces of blood, all samples were processed in a Microflow Biological Safety Cabinet. Each paper point and curette sample was vortexed for 40 s to 1 min using a Heidolph Reax vortex (Heidolph Instruments GmbH & Co., Schwanbach, Germany) at maximum speed to disperse microorganisms present in paper point and curette samples [23]. A 50 µl aliquot was taken using a 50-250 µl Gilson Pipetman pipette (Gilson Inc, Wisconsin, USA) fitted with a sterile 1-200 µl StarLab TipOne graduated filter tip (StarLab Ltd, Milton Keynes, UK). The aliquot was placed in the centre of a MSA plate and spread around the entire surface using a sterile disposable plastic spreader (Greiner Bio One). For each sample, duplicate aliquots were plated onto separate MSA plates. Plates were incubated in a Sayno static incubator (Sanyo E & E Europe, Biomedical division, Leicestershire, UK) at 37°C in aerobic conditions for approximately 48 h. Remaining material from each clinical sample was stored at -80°C. If it was not possible to process paper point and curette samples within 24 h of their receipt in the laboratory they were vortexed as above and stored at -80°C. These were thawed when required and processed as described above. Oral wash samples were vortexed at maximum speed for approximately 1 min and 1.5 ml aliquots were then transferred into sterile 1.5 ml Eppendorf Safe-Lock microfuge tubes (Eppendorf, Hamburg, Germany) and centrifuged at 16100 x *g* for 10 min using an Eppendorf 5415R bench top centrifuge. The supernatant was then drawn off and discarded and the pellets re-suspended in 1 ml YEPD by vortexing for approximately 1 min. Prior to plating the suspension was vortexed again for approximately 40 s to 1 min and processed as described above for paper point and curette samples. Plates were incubated at 37°C in aerobic conditions for approximately 48 h. The remainder of the 1.5 ml oral wash aliquots were stored at -80°C. If it was not possible to process oral wash samples within 24 hours of their receipt an aliquot was taken as above and stored at -80°C. When they were able to be processed they were thawed and treated as above.

2.3.2 Mean staphylococcal colony forming unit (cfu) counts from clinical samples recovered on MSA

Following incubation for 48 h at 37°C colonies on MSA plates were manually counted and plate counts were recorded in colony forming units (cfu) per plate. If all of the colonies appeared to be phenotypically identical the "Flash and Go" automatic plate reader (IUL Instruments SA, Barcelona, Spain) was used to determine a colony count The detection limit for the mean staphylococcal cfu counts was 10 cfu/ml sample (a single colony on one of the duplicate plates). When growth formed a confluent mass with few distinguishable colonies the plate count was recorded as either semi-confluent or confluent growth. Subsequently clinical samples that yielded confluent/semi-confluent growth on MSA were diluted 1/10 and 1/100 and again plated in duplicate and counted as described above. Plate counts were repeated after 7 days incubation at 37°C.

2.3.3 Subculture, isolation and storage of isolates from clinical samples

Individual colonies of each morphological type observed on primary isolation from each clinical sample were sub-cultured by streaking a single colony onto MSA followed by incubation at 37°C overnight, or for two to five days (as long as necessary to grow visible colonies). Subsequently, a single colony was then purified by subculture, preferentially on TSA (though also occasionally on NA, or LA), prior to being stored at -80°C on Microbank™ mixed microbial storage beads (Pro-Lab Diagnostics, Cheshire, UK) according to the manufactures instructions.

2.4 Buffers and solutions

Solutions used for DNA extraction, agarose gel electrophoresis and preparation of checkerboard DNA:DNA hybridisation samples were as follows: Tris-EDTA (TE), 10 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA) (both supplied by Sigma-Aldrich, Vale Road, Arklow, Wicklow) pH 7.6 [140]; Lysis Buffer, 0.05 mg lysostaphin, 0.02 *g* lysozyme in 1 ml TE; Tris-borate-EDTA buffer (TBE), 89 mM Tris, 89 mM boric acid, 2 mM EDTA (all supplied by Sigma) pH 8.0 [140].

2.5 Chemicals and molecular biology reagents

Analytical-grade or molecular-grade chemicals were purchased from Sigma-Aldrich. Oligonucleotide primers for universal bacterial 16S ribosomal DNA identification, multi locus sequence typing (MLST), and real-time PCR (RT-PCR) analysis (Table 2.1) were custom synthesised by Sigma-Aldrich. Oligonucleotide minor groove binding (MGB)

probes for real-time PCR (RT-PCR) were custom synthesised by Applied Biosystems (Inchinnan Business Park, Paisley, UK) (Table 2.1). PCR reagents, 10 x magnesium-free buffer, 25 mM magnesium chloride, dATP, dCTP, dTTP, dGTP and *Taq* DNA polymerase were all purchased from the Promega Corporation (Promega, Madison, Wisconsin, USA). 2 x TaqMan Fast Universal PCR Master Mix with AmpErase for RT-PCR was purchased from Applied Biosystems. 16S rDNA and MLST amplification reactions were made up to the desired volumes with sterilised ultrapure water from a MilliQ water system (Millipore Ireland BV, Carrigtwohill, Co. Cork, Ireland) or sterile ultrapure water (Sigma). *S. aureus*-specific and *S. epidermidis*-specific RT-PCRs utilised TaqMan Fast Universal PCR Master Mix with AmpErase supplied by Applied Biosystems. Reactions were made up to the desired volumes with the ultrapure water supplied in the TaqMan Fast Universal PCR kit.

2.6 Polymerase Chain reaction Master Mixes and reaction profiles

The PCR master mixes, and the thermocycler profiles used for the 16S rDNA and MLST amplification reactions are listed in Table 2.2. Primers are listed in Table 2.1. PCR reactions to confirm the presence of *ileS2* encoding high level mupirocin resistance in specific *S. epidermidis* isolates was performed by Ms. Orla Brennan from this laboratory according to the protocol outlined in Pérez-Roth *et al.* 2001(Tables 2.1 and 2.2) [141]. The RT-PCR master mixes, and the thermocycler profiles used, for the *S. aureus*-specific and *S. epidermidis*-specific RT-PCR reactions are listed in Table 2.3. Data collection took place during stage two of step three (30 s at 60°C).

Table 2.1. Oligonucleotide primers and probes

Oligonucleotide description	Primer/probe name	Primer sequence (5'-3')	Reference
Universal rDNA primers used for species identification			
Universal bacterial 16S ribosomal DNA (rDNA) forward primer	533F	AGAGTTTGATYMTGGCTCAG	[142]
Universal bacterial 16S ribosomal DNA (rDNA) reverse primer	142R	CGGYTACCTTGTTACGAC	[142]
***S. epidermidis* MLST primer set**			
S. epidermidis carbamate kinase (*arcC*) forward primer	arcC-F	TGTGATGAGCACGCTACCGTTAG	[118]
S. epidermidis carbamate kinase (*arcC*) reverse primer	arcC-R	TCCAAGTAAACCCATCGGTCTG	[118]
S. epidermidis shikimate dehydrogenase (*aroE)* forward primer	aroE-F	CATTGGATTACCTCTTTGTTCAGC	[118]
S. epidermidis shikimate dehydrogenase (*aroE*) reverse primer	aroE-R	CAAGCGAAATCTGTTGGGG	[118]
S. epidermidis ABC transporter (*gtr*) forward primer	gtr-F	CAGCCAATTCTTTTATGACTTTT	[118]
S. epidermidis ABC transporter (*gtr*) reverse primer	gtr-R	GTGATTAAAGGTATTGATTTGAAT	[118]
S. epidermidis DNA mismatch repair protein (*mutS*) forward primer	mutS-F3	GATATAAGAATAAGGGTTGTGAA	[118]
S. epidermidis DNA mismatch repair protein (*mutS*) reverse primer	mutS-R3	GTAATCGTCTCAGTTATCATGTT	[118]
S. epidermidis pyrimidine operon regulatory protein (*pyrR*) forward primer	pyr-F2	GTTACTAATACTTTTGCTGTGTTT	[118]
S. epidermidis pyrimidine operon regulatory protein (*pyrR*) reverse primer	pyr-R4	GTAGAATGTAAAGAGACTAAAATGAA	[118]
S. epidermidis pyrimidine operon regulatory protein (*pyrR*) forward primer	pyR_pc-F	TTTAGATGAGGCAGCGATACAA	This study [a]
S. epidermidis pyrimidine operon regulatory protein (*pyrR*) reverse primer	pyR_pcR	CGACTGCATTTCTATCGTCAA	This study[a]
S. epidermidis triosephosphate isomerase (*tpiA*) forward primer	tpi-F2	ATCCAATTAGACGCTTTAGTAAC	[118]
S. epidermidis triosephosphate isomerase (*tpiA*) reverse primer	tpi-R2	TTAATGATGCGCCACCTACA	[118]
S. epidermidis acetyl coenzyme A acetyltransferase (*yqiL*) forward primer	yqiL-F2	CACGCATAGTATTAGCTGAAG	[118]
S. epidermidis acetyl coenzyme A acetyltransferase (*yqiL*) reverse primer	yqiL-R2	CTAATGCCTTCATCTTGAGAAATAA	[118]

Continued overleaf

Table 2.1 continued. Oligonucleotide primers and probes

Oligonucleotide description	Primer or probe name	Primer sequence (5'-3')	Reference
Used to amplify a section of the *ileS2* gene encoding high level mupirocin resistance			
High level mupirocin resistance gene *ileS2* forward primer	MupA	TATATTATGCGATGGAAGGTTGG	[141]
High level mupirocin resistance gene *ileS2* reverse primer	MupB	AATAAAATCAGCTGGAAAGTGTTG	[141]
Species-specific RT-PCR primers and probes			
S. epidermidis superoxide dismutase (*sodA*) forward primer	Se_sodA-F	TCAGCAGTTGAAGGGACAGAT	[143]
S. epidermidis superoxide dismutase (*sodA*) reverse primer	Se_sodA-R	CCAGAACAATGAATGGTTAAGG	[143]
S. epidermidis superoxide dismutase (*sodA*) minor groove binding probe	S. epidermidis_sodA	FAM-TTAGATGGCACAC-MGB	[143]
S. aureus thermostable nuclease (*nuc*) forward primer	Sa_nuc-F	GATCCAACAGTATATAGTGC	This study[b]
S. aureus thermostable nuclease (*nuc*) reverse primer	Sa_nuc-R	TGACCTTTGTACATTAATTTAAC	This study[b]
S. aureus thermostable nuclease (*nuc*) minor groove binding probe	S. aureus_nuc	VIC-CACCATCAATCGCTT-MGB	This study[b]

[a] *S. epidermidis* pyrimidine operon regulatory protein gene (*pyrR*) forward and reverse primers pyR_pc-F and pyR_pc-R for MLST were designed using Serial Cloner 2.1 (F. Perez (Serial Basics), Paris, France, http://serialbasics.free.fr/Serial_Cloner.html) based on *S. aureus* RP62A and ATCC12228 *pyrR* sequences (National Centre for Biotechnology Information, NCBI) due to poor amplification and sequencing results when using pyR-F2 and pyR-R4 [118].

[b] *S. aureus* thermostable nuclease gene (*nuc*) forward and reverse primers and minor grove binding probes for RT-PCR were designed using AllelID7 (Premier Biosoft, Palo Alto, USA) based on previously published *nuc* sequences [144].

Table 2.2. PCR master mixes and thermocycler profiles for 16S rDNA and MLST amplification reactions

PCR and reagents used	Final concentration per reaction	Thermocycler profile	
		Temperature and time	No. reaction cycles
16S rDNA amplification reaction			
10x magnesium-free buffer	1 x		
Magnesium chloride	2.5 mM	94°C for 2 min	x 1
dATP, dCTP, dTTP and dGTP	200 µM each	94°C for 30 s	
Primers 533F and 142R	300 nM each	50°C for 30 s	} x 35
Taq DNA polymerase	2.5 units	72°C for 10 s	
1-10 µl total cellular DNA		72°C for 10 min	x 1
Sterile ultrapure water	Balance of volume up to 50 µl	4°C	Hold
MLST amplification reactions			
10x magnesium-free buffer	1 x		
Magnesium chloride	4 mM	95°C for 3 min	x 1
dATP, dCTP, dTTP and dGTP	200 µM each	95°C for 30 s	
Primers arcC-F and arcC-R	300 nM each	50°C for 1 min	} x 34
OR aroE-F and aroE-R		72°C for 1 min	
OR gtr-F and gtr-R		72°C for 10 min	x 1
OR mutS-F3 and mutS-R3		4°C	Hold
OR tpi-F2 and tpi-R2			
OR yqiL-F2 and yqiL-R2			
OR pyr-F2 and pyr-R4			
OR pyR_pc-F and pyR_pc-R			
Taq DNA polymerase	2.5 units		
4-10 µl total cellular DNA			
Sterile ultrapure water	Balance of volume up to 50 µl		
***ileS2* amplification reaction**[a]			
10x magnesium-free buffer	1 x	94°C 5 min	x 1
Magnesium chloride	3 mM	94°C 30 s	
dATP, dCTP, dTTP and dGTP	0.2 mM each	64°C 30 s	} x 10
Primers MupA and MupB	20 pmol each	72°C 45 s	
5 µl total cellular DNA		94°C 45 s	
Sterile ultrapure water	Balance of volume up to 50 µl	50°C 45 s	} x 25
		72°C 1 min	
		72°C 10 min	x 1
		4°C	Hold

[a]*ileS2* PCR undertaken by Ms. Orla Brennan (Microbiology Unit, Division of Oral Biosciences, Dublin Dental University Hospital).

Table 2.3. PCR master mixes and thermocycler profiles for *S. aureus*-specific and *S. epidermidis*-specific RT-PCRs

PCR reaction and reagents used	Final concentration/ reaction	Thermocycler profile	
		Temperature and time	No. reaction cycles
***S. aureus*-specific RT-PCR**			
2 x TaqMan Fast Universal PCR Master Mix with AmpErase	1 x		
Primer Sa_*nuc*-F	1800 nM	50°C for 2 min	x 1
Prime Sa_*nuc*-R	900 nM	95°C for 20 s	x 1
MGB *S. aureus_nuc* VIC probe	100 nM	95°C for 8 s	} x 40
2 µl clinical sample DNA		60°C for 30 s[a]	
***S. epidermidis*-specific RT-PCR**			
2 x TaqMan Fast Universal PCR Master Mix with AmpErase	1 x		
Primer Se_*sodA*-F	900 nM	50°C for 2 min	x 1
Primer Se_*sodA*-R	900 nM	95°C for 20 s	x 1
MGB *S. epidermidis_sodA* FAM probe	250 nM	95°C for 8 s	} x 40
2 µl clinical sample DNA		60°C for 30 s[a]	

[a]Data collection during this step

2.7 Clinical isolate identification by 16S rDNA sequence analysis

2.7.1 DNA extraction

DNA was extracted using a variation on the DDUH microbiology laboratory's standard method employing the Qiagen DNeasy Blood and tissue kit (Qiagen, West Sussex, UK) as described below. For the initial extraction attempts colonies of bacteria that were grown statically on agar plates were harvested for DNA extraction, but that provided inconsistent yields and quality. For subsequent extractions bacteria grown in liquid culture were used, and additional wash steps were added to the extraction process as described below.

Stored isolates were taken from -80°C, a single bead was removed from the storage vial (without allowing the vial to thaw/come up to room temperature), and placed on a TSA plate. Using a sterile wire loop the bead was used to inoculate a section of the plate that was then streaked across the remainder of the plate. Plates were incubated at 37°C in a static incubator (Sanyo) for as long as necessary for the culture to revive and visible growth to appear (usually 24-48 h, occasionally up to 72/96 h). Single colonies were taken and either, subcultured onto a second TSA plate, or inoculated into 5 ml TSB and incubated in an orbital shaking incubator at 200 rpm (Weiss Gallenkamp, Loughborough, UK) at 37°C overnight (15-24 h). Following incubation, 1.5 ml aliquots were transferred into 1.5 ml Safe-lock Eppendorf tubes and centrifuged at 5000 x *g* for 10 min (using an Eppendorf 54177 bench top centrifuge), as recommended in the Qiagen DNA extraction kit instructions. The supernatant was removed and the resulting pellet re-suspended in 1.5 ml of TE buffer [140]. The tube was centrifuged again for 10 min at 5000 x g, the supernatant was removed and the pellet re-suspended in 1 ml of TE buffer. Finally the tube was centrifuged for a third time as before, the supernatant discarded and the pellet re-suspended in 250 µl Lysis Buffer (section 2.4 above).

For DNA extraction from isolates grown on TSA plates two large loopfuls of bacterial growth were harvested using sterile disposable 1 µl loops (Greiner), and deposited in a 1.5 ml Safe-lock Eppendorf tube containing 250 µl Lysis Buffer (section 2.4 above), then vortexed to suspend the bacteria in the buffer. All samples that had been resuspended in lysis buffer (from broth or TSA plates) were then incubated in a 37°C water bath (Clifton, Nickel Electro Ltd, Weston-Super-Mare, UK) for 2-3 h, with vortexing approximately every 30 min. Following incubation 25 µl of Protein Kinase A solution and 200 µl of Buffer AL (both supplied with the Qiagen kit) were added, the samples were vortexed and incubated in a water bath at 70°C for 30 min. Following incubation, 200 µl of ice-cold 95%

(v/v) molecular-biology grade ethanol (Sigma-Aldrich) were added and the tubes were gently inverted several times to precipitate the DNA. The entire content of the tube was then transferred onto a mini-column provided in the Qiagen DNeasy Blood and tissue kit. From this point on extraction was performed as detailed in the kit manufacturer's instructions. DNA was eluted in 200 µl of Elution Buffer, and stored initially at 4°C. DNA samples were maintained at -20°C for long-term storage. Extracted DNA was visualised using agarose gel electrophoresis as described below in section 2.7.3.

2.7.2 PCR amplification of 16S rDNA for isolate identification by rDNA sequencing

Universal bacterial 16S ribosomal DNA (rDNA) primers 533F, and 142R (Table 2.1) were used to amplify isolate rDNA by PCR [142]. Each reaction consisted of 49 µl of PCR 'master mix', with 1 µl of DNA prepared as described in Table 2.2. PCR amplifications were performed in a G-Storm GSI thermocycler (Gene Technologies Ltd, Braintree, Essex, UK) using the parameters described in Table 2.2. PCR amplimers were purified using a Qiagen, QIAquick® 96 PCR Purification Kit following manufacturer's instructions.

2.7.3 Agarose gel electrophoresis

Extracted DNA and PCR amplification products were assessed for yield and quality by agarose gel electrophoresis in 0.8-1.7% (w/v) agarose gels (Sigma-Aldrich Type I) using 0.5 x TBE buffer [140], and containing either 0.5 µg/ml ethidium bromide (Sigma-Aldrich), or 0.02 µl/ml Gel Red (Biotium Inc, Hayward, California, USA). Prior to electrophoresis, a 5 µl aliquot of each sample was mixed with 1 µl of a solution of 6x loading dye (Promega) and loaded into gel wells. Reference size standards were included on each gel in adjacent wells consisting of 1 kbp ladders (Promega) for gels containing DNA extracted from bacterial isolates or 100 bp ladders (Promega) for gels containing PCR amplimers. Electrophoresis was performed using 0.5 x TBE buffer for between 40-60 min at 100V. Following electrophoresis, DNA bands were visualised on a UV transilluminator at a wavelength of 345 nm (Ultra Violet Products Ltd., Cambridge, UK) or an AlphaImager® Mini Analysis System (Alpha Innotech/Cell Biosciences, Santa Clara, California, USA) at 302 nm. Purified PCR products were visualised as described above using 1 µl of PCR amplimer reaction product and 5 µl of 1.2 x loading dye. The size and intensity of the bands were compared to bands of known size and concentration in the molecular ladders on either side of the samples to visually assess quality and estimate concentration.

2.7.4 DNA Sequencing

16S PCR amplimers were sequenced commercially by CoGenics Technologies (Hope End, Takeley, Essex, UK), or Source BioScience (Source BioSciences, Guinness Enterprise Centre, Dublin, Ireland) using the same universal bacterial 16S rDNA primers 533F and 142R used for amplification [142] (Table 2.1), using an ABI3730xl DNA Analyzer platform (Applied Biosystems, Foster City California, USA). MLST PCR amplimers were sequenced commercially by CoGenics, or Source BioScience. Samples submitted to CoGenics had a minimum of 500 ng per 10 µl sample (50 ng/µl) as estimated by agarose gel electrophoresis. Samples were submitted to Source Bioscience at a concentration of 1 ng/µl per 100 bp expected amplicon size, determined using a ThermoScientific NanoDrop 2000c UVvis spectrophotometer (Fisher Scientific Ireland, Ballycoolin, Dublin, Ireland). Sequence data and chromatographs were returned via e-mail and aligned using Bionumerics software, version 5.10 (Applied Maths NV, Sint-Martens-Lautem, Belgium).

2.8 Multilocus sequence typing of *S. epidermidis* clinical isolates

2.8.1 PCR amplification of target sequences for MLST

MLST master mixes were prepared as described in Table 2.2 using DNA extracted by the same method as that used for 16S DNA PCR amplification. Reactions were run in a G-Storm GSI thermocycler using the parameters described in Table 2.2. PCR products were visualised using agarose gel electrophoresis on 1.7% (w/v) agarose gels as described in section 2.7.3, then purified using a Qiagen, QIAquick® 96 PCR Purification Kit following the manufacturer's instructions. Purified amplimers were visualised using agarose gel electrophoresis on 1.7% (w/v) agarose gels, and quantified using a NanoDrop 2000c UVvis spectrophotometer.

2.8.2 MLST DNA Sequencing

PCR amplimers were sequenced commercially by CoGenics or Source Bioscience as described in section 2.7.4 using the same MLST primers used for PCR amplification (Table 2.1 and 2.2).

2.9 Alere DNA microarray profiling of selected *S. aureus* and *S. epidermidis* clinical isolates

DNA microarray profiling was undertaken using the Alere StaphyType Kit (Alere Technologies, Jena, Germany) according to the manufacturer's instructions, which have been described in detail previously [129]. The StaphyType kit consisted of a DNA

microarray chip adhered to each well of a microtitre strip and each chip consisted of 334 *S. aureus* genes targets including species-specific, antimicrobial resistance and virulence-associated genes, genes involved in attachment, adhesion and biofilm as well as typing markers. In addition, the ArrayMate software (Alere) can assign *S. aureus* isolates to sequence types (STs) and/or clonal complexes (CCs) by comparison of the DNA microarray results to those of a collection of previously characterised and MLST-typed strains in the ArrayMate database [107, 128, 129].

2.9.1 DNA Extraction

All clinical isolates from periimplantitis patients identified as *S. aureus* and a selection of clinical isolates identified as *S. epidermidis* by 16S rDNA sequencing had DNA extracted for StaphyType microarray profiling according to the instructions contained in the Alere StaphyType Kit instructions. Staphylococcal isolates stored on beads at -80°C had a single bead removed from the storage vial (without allowing the vial to thaw/come up to room temperature) and placed on a CBA plate. Using a sterile wire loop the bead was used to inoculate a section of the plate that was then streaked across the remainder of the plate. Plates were incubated at 37°C in a static incubator for 24 h. A single colony from each isolate was selected, inoculated on one half of a second CBA plate as a patch approximately 2 cm x 2 cm, and incubated at 37°C in a static incubator for 24 h. The lysis buffer supplied in the StaphyType kit was prepared by adding 200 µl of Lysis buffer A1 to the supplied reaction tubes containing Lysis enhancer A2 (reconstituted lysis buffer contains lysostaphin, lysozyme, ribonuclease A, Tris-HCl, EDTA and Triton X-100) [129]. The bacteria were scraped from the CBA plates using a sterile disposable 1 µl inoculation loop (Greiner) and one to two loopfuls of bacteria was added to the reconstituted lysis buffer. The solution was then mixed by vortexing and incubated at 37°C for 30-60 min in a shaking water bath. Following lysis 25 µl of proteinase K and 200 µl of buffer AL (both supplied with the Qiagen DNeasy kit) were added to the sample and mixed by vortexing. The tube was then incubated in a 70°C water bath (Clifton) for 30 min. Following incubation 100 µl of ice cold molecular biology grade ethanol was added to the tube and gently mixed by inversion. The entire contents of the tube (including any precipitate) was transferred into a Qiagen DNeasy mini spin column and centrifuged at 12800 x *g* for 1 min. The DNA extraction was completed as outlined in the Qiagen DNeasy kit instructions, with an additional final 20800 x *g* 3 min centrifugation prior to elution with 50 µl ultrapure water (Sigma). The eluted DNA was then incubated in a heating block (Grant Instruments, Cambridge, UK) at 70°C for 30 min with the tube cap left open to

evaporate any remaining traces of solvents used in the extraction[129]. After the final incubation extracted DNA samples were stored at 4°C, or transferred to -20°C for long term storage. DNA was visualised by agarose gel electrophoresis as described in section 2.7.3, and quantified using the NanoDrop 2000c UVvis spectrophotometer. If a second DNA extraction was required, i.e. if an initial microarray analysis could not be performed due to weak signals from the array, the isolate was cultured again from the stock of storage beads as outlined above, not subcultured from the previous CBA plates.

2.9.2 Linear PCR amplification and biotin labelling

DNA samples prepared as described in section 2.9.1 above were diluted to a concentration of 0.1-0.3 µg/µl with Sigma ultrapure molecular biology grade water. Linear PCR amplification and labelling was carried out on a G-Storm GSI thermocycler according to the StaphyType Kit instructions. The microarray amplification and labelling master mix, and thermocycler profile, is outlined in Table 2.4.

2.9.3 StaphyType microarray

The Alere StaphyType microarray was processed according to manufacturer's instructions. The well containing the microarray chip was washed with 200 µl of ultrapure water then 100 µl of hybridisation buffer (buffer C1 supplied with StaphyType kit) was added and the array was incubated in a thermomixer (Bioshake iQ, Quantifoil instruments Gmbh, Germany) at 55°C, 550 rpm, for 2 min. The C1 buffer in the array well was discarded and replaced with the labelled DNA which had been mixed with another 90 µl of buffer C1 ('hybridisation mixture', 100 µl total volume). The array well was capped and incubated in the thermomixer at 55°C, 550 rpm, for 1 h. After incubation the hybridisation mixture was discarded and the well containing the microarray chip was washed three times with 200 µl of washing buffer C2 (supplied with StaphyType kit). Next 100 µl of HRP-conjugate (1 µl of reagent C3, containing Streptavidin-Horseradish peroxidise (HRP) mixed with 99 µl of buffer C4) was added to the well and the microarray was incubated in the thermomixer set at 30°C, 550 rpm, for 10 min. The HRP-conjugate was discarded and the microarray washed once with 200 µl of washing buffer C5 (supplied with StaphyType kit). Finally, 100 µl of reagent D1, containing the HRP substrate tetramethylbenzidine (TMB), was added to the microarray well and incubated for 5 min at room temperature to allow precipitation. Reagent D1 was completely removed from the microarray well and the microarray was analysed using an Alere ArrayMate™ reader and software. Raw results were processed in Microsoft Excel as advised by Alere. Where 'staining' controls failed

Table 2.4. StaphyType DNA microarray amplification master mix and thermocycler profile

Reagents		Thermocycler profile	
		Temperature and time	No. reaction cycles
Solution B1 (2 x labelling buffer)	4.9 µl		
Solution B2 (DNA polymerase)	0.1 µl	Cover pre-heated to 110°C	
Sample DNA	5 µl	96°C for 5 min	x 1
		62°C for 30 s	} x 45
		72°C for 40 s	
		96°C for 1 min	
		4°C	Hold

microarray runs were repeated. Where *S. aureus* isolates returned weak signals the arrays were visually assessed and repeated. A single *S. aureus* isolate continually returned weak signals and was unable to be assessed using the array.

2.10 Confirmation of methicillin and mupirocin resistance indicated by microarray testing

When resistance to the antibiotics methicillin and mupirocin (the later employed in the nasal decolonisation of MRSA carriers) was indicated by microarray profiling, resistance was confirmed phenotypically.

2.10.1 Methicillin resistance testing

Methicillin resistance was confirmed by phenotypic testing at the National MRSA Reference Laboratory (NMRSARL), St. James's Hospital Dublin by disk diffusion using 10 µg and 30 µg cefoxitin disks (Oxoid) on MH agar, and 1 µg and 5 µg oxacillin disks (Oxoid) on CBA, as described previously [145, 146].

2.10.2 Mupirocin resistance testing

Mupirocin resistance testing was undertaken by Ms. Orla Brennan (Microbiology Unit, Division of Oral Biosciences, Dublin Dental University Hospital) using the disk diffusion method with 5 µg and 200 µg mupirocin disks (Oxoid) on MH agar, high level resistance was confirmed using E-Test strips (AB Biodisk, BioMérieux, Marcy-l'Étoile, France) on MH, both methods as described by Pérez-Roth *et al.* 2001 [141].

2.11 Real time polymerase chain reaction (RT-PCR)

2.11.1 Total genomic DNA extraction for RT-PCR

A 200 µl aliquot was taken from each clinical paper point sample to be tested and centrifuged at 16100 x *g* for 10 min, the supernatant was discarded and the pellet re-suspended in 250 µl Lysis Buffer. From then on the DNA extraction was performed according to the protocol described in section 2.7.1 above. Where a mixed sample was submitted for Checkerboard DNA:DNA Hybridisation by Dr. Maguire (equal volumes of clinical sample taken from the same site using paper point sampling and curette sampling) 100 µl aliquots of the appropriate samples were mixed then processed as above.

2.11.2 DNA standards for RT-PCR

Standard curves for RT-PCR were created using DNA extracted as described above (section 2.7.1) from staphylococcal reference strains *S. aureus* RN4220 [62] and *S. epidermidis* RP62A [71, 121]. Multiple DNA extractions were pooled and concentrated in a Centrivap Concentrator (Labconco Corporation, Kansas City, Missouri USA) at 42°C for approx 30 min. The genome mass for each species (*S. aureus* ≈ 2.8 mega base pairs (Mbp), *S. epidermidis* RP62A ≈ 2.6 Mbp) was used to calculate the appropriate genomic DNA concentrations needed for the desired cells/sample standards using the formula m=(n)(1.096e-21 g/base pair (bp)), where: m=mass, n=genome size (bp), e-21= $x10^{-21}$[147]. An 8 point logarithmic standard curve was set up to assess the reactions detection limits encompassing an estimated 10 to 1 x 10^8 copies of the target gene. Subsequent clinical sample tests were run using a 5 point standard curve as recommended by Applied Bioscience/from 1 x 10^4-1 x 10^8 cells, though the 1 x 10^4 standard used was not reliable in all subsequent reactions reducing the standard curve for *S. aureus* to a four point curve from 1 x 10^5-1 x 10^8 [147].

2.11.3 RT-PCR run protocol

Separate RT-PCRs were performed using an Applied Biosystems AB7500Fast cycler Thermocycler using the previously described *S. epidermidis* sodA primer and probe set [143] and the *S. aureus* nuc primer and probe set developed during the present study (Table 2.1, sections 2.5 and 2.6). The probes were initially designed to be combined in the same reaction and each probe contained a different fluorophore (each emitting light on a different wavelength). The *S. epidermidis sodA* probe was linked to the TaqMan reporter dye FAM™ (6-carboxyfluorescein) (5'FAM-TTAGATGGCACAC-MGB3') [143] while the *S. aureus nuc* probe was linked to the TaqMan reporter dye VIC® (5'VIC-

CACCATCAATCGCTT-MGB3', designed for this study based on *nuc* sequences previously published [144]), both were bound to a minor groove binder (MGB) quencher. The target specific probes bind to a single stranded copy of the appropriate DNA, and is cleaved as Fast *Taq* DNA polymerase moves along the strand, releasing the fluorophore from its proximity to the quencher and allowing fluorescence to occur. The thermocycler profile was set up and run according to the manufactures recommendations (Table 2.3), using MicroAmp®Fast Optical 96 well reaction plates and MicroAmp®Fast Optical 96 well adhesive covers, in a Applied Biosystems AB7500 Fast Real Time PCR system (all supplied by Applied Biosystems).

2.12 Checkerboard DNA:DNA hybridisation

Checkerboard DNA:DNA hybridisation (CKB) is a high throughput method for the identification and quantification of multiple bacterial species in a single sample. It has the capacity for multiple samples to be tested at the same time. The checkerboard DNA:DNA hybridisation test uses molecular probes created from total cellular genomic DNA extracted from reference strains of bacteria to identify the bacterial species present in a sample. The signal from the probes can be compared to a DNA standard of known quantity for each reference strain processed on the same membrane. This allows the quantity of a particular species' DNA present in a sample to be estimated. Briefly, samples are lysed with sodium hydroxide (NaOH), neutralised with ammonium acetate and laid onto a nylon membrane in rows using a slotted manifold alongside a pair of 10^5 and 10^6 cell standards, then fixed to the membrane using ultraviolet light [20, 36]. The membrane is then hybridised to whole genomic digoxigenin labelled probes, introduced using another manifold with "lanes" running at 90^o to the test sample rows. After hybridisation, washing (to remove unbound probe) and blocking, DNA is detected by incubation with an alkaline phosphatise-linked anti-digoxigenin antibody, followed by the introduction of a chemiluminescent substrate [20, 36]. Emissions are captured using an imaging system and emissions from samples are compared to emissions from standards in the same probe "lane" to estimate the amount of DNA present [20, 36]. Dr. Rory Maguire from this institution undertook an independent project assessing the microflora of the samples collected for the present study using CKB to determine the prevalence of 40 bacterial species. The panel of bacterial species included the two staphylococcal species *S. aureus* and *S. haemolyticus*. Aliquots of clinical samples were submitted to Professor Rutger Pearson's research group, (Department of Periodontology and Fixed Prosthodontics, Division of Oral Microbiology, University of Berne, Berne, Switzerland), as outlined

previously [8, 40, 47]. Dr. Maguire submitted a total of 164 samples taken from 29 periimplantitis patients. Results were provided in an excel spreadsheet giving the final estimated quantities (cells/sample) of target bacteria *S. aureus* present in each test sample.

Chapter 3

Investigation of cultured staphylococcal populations associated with diseased and healthy oral implants and natural teeth in periimplantitis patients

3.1 Introduction

Over 600 bacterial species have been identified as part of the human oral microbiome [14]. Of these the majority have not been cultured in the laboratory, but identified through a variety of molecular methods such as shotgun cloning libraries, ribosomal DNA sequencing, DNA microarrays, PCR panels, oligonucleotide probes and DNA:DNA hybridisation [14, 16, 20, 27, 30]. Staphylococci are a well recognised part of the human epidermal flora [48]. *Staphylococcus aureus*, the most pathogenic species of the genus *Staphylococcus*, is a frequent coloniser of mucosal tissues, such as the anterior nares of the nose [48, 49]. There have also been reports of nasal oral trafficking of staphylococci [134], as well as some previous evidence of staphylococcal species, in particular *S. aureus* and *S. epidermidis*, being associated with natural teeth [131, 148, 149].

As outlined in Chapter 1, both *S. aureus* and *S. epidermidis* can readily colonise medical implants and other indwelling medical devices to deleterious effect [54-59]. *Staphylococcus epidermidis* in particular has a strong affinity for many of the materials that medical implants are manufactured from (e.g. titanium and a wide variety of plastic materials), due in part to the ability of some strains to readily form biofilms. Such biofilms can be extremely difficult to completely remove using mechanical and antimicrobial treatments [83-86, 150]. Also, as outlined in Chapter 1, dental implants are not sealed within the body's tissues, but are partially exposed to the oral cavity and oral fluids enabling them to readily become colonised with microbial flora. Furthermore, the periimplant pocket created by implant insertion is not the same as a virgin periodontal pocket.

Given the ubiquity of staphylococci on human skin surfaces, the significant pathogenic potential of some staphylococcal species such as *S. aureus*, and the affinity of some staphylococci for artificial materials placed *in vivo*, it is surprising that few studies of the microbiota of oral implants have included staphylococci in their testing protocols. Previous studies of periimplant microbial flora have looked for (and in most cases found) bacteria already known to be associated with periodontitis [1, 9, 11, 32, 34, 36, 38, 39, 41-46, 151]. Several studies have shown that *S. aureus* can be present at natural tooth and at oral implant sites [8, 31, 35, 37, 40, 47, 131, 148]. Some of these studies have suggested that the presence of *S. aureus* may be associated with periodontitis, periimplantitis, and even eventual implant failure [8, 35, 40, 47]. In contrast, very few studies have reported on the presence of *S. epidermidis* at tooth and implant sites [35, 130, 131]. However, there has not

been a single study that has surveyed the population of staphylococci in the general oral cavity (through an oral rinse or swabbing), and at tooth and implant sites that are diseased (periimplantitis or periodontitis), and at healthy tooth and implant sites. Implants have been compared to implants (healthy, failed and those with associated periimplantitis), teeth have been compared to teeth (healthy and those with associated periodontitis), and diseased states have been compared (failed implants and periimplantitis to periodontitis) [1, 8, 9, 11, 31, 34, 38, 40-47], but not all together, and without comparative data from the general oral cavity (as determined by oral rinse sampling).

As outlined in Chapter 1, the majority of studies that investigated staphylococcal associations with oral implants utilised molecular identification of bacteria directly from clinical samples and did not include the culture and recovery of viable organisms. Potential problems associated with the sole reliance on molecular techniques for species identification have been outlined in Chapter 1 and are covered in more detail in Chapter 5. Briefly, all surveys of microbial ecology are limited by the methodology used either to recover viable microorganisms from samples or individual species detection probes included on testing panels and their specificity in molecular based techniques. Microbial species that are actually present at a clinical site or in a clinical sample but which cannot be cultured in the laboratory, or which are not tested for using molecular techniques will not be identified. Furthermore, DNA from dead bacteria may yield positive signals using molecular detection systems, which may well result in misleading findings regarding associations of particular species with oral implants and natural teeth. Thus studies that rely only on molecular identification of bacterial species directly from clinical samples run the risk of returning "false positive" detection where no or very few viable cells are present at the time of sampling. In biofilms, DNA is often present in excess of the amount of viable cells and is a component of the extracellular biofilm matrix [152].

The purpose of this part of the present study was to investigate the prevalence and relative abundance of individual staphylococcal species associated with healthy and diseased oral implants and healthy and diseased natural teeth in patients with clinically diagnosed periimplantitis. The approach used relied on laboratory culture of viable organisms to circumvent potential shortcomings associated with molecular detection systems. Clinical samples were first enriched by plating on Mannitol Salt Agar (MSA), which enables the selective growth of staphylococci and enterococci. Staphylococci have the ability to survive in a high salt environment, and *S. aureus* can also ferment the sugar mannitol [133,

134] resulting in a localised pH change and the indicative yellow colouration of the media surrounding presumptive *S. aureus* colonies (due to the reduction of the indicator phenol red in MSA) [153, 154]. Mannitol salt agar is a commonly used solid selective media for staphylococci isolation and enumeration employed with clinical specimens from various sites, including the oral cavity and periodontal pockets [64, 130, 155-157]. Though some enterococci and yeasts can survive in a high salt environment, very few bacteria thrive under these conditions. Chapter 2 (and section 3.2 below) outlines how MSA was used in this study to grow and isolate staphylococci for identification using 16S rDNA sequencing, and to estimate the cell density (colony forming units (cfu)/sample) of each identified staphylococcal species in each clinical sample.

The objectives of this part of the present study were to:

1. Determine whether particular staphylococcal species are significantly associated with failing oral implants.
2. To prospectively investigate staphylococcal populations, including species distribution and relative abundance, associated with diseased and healthy established oral implants, natural teeth and the general oral cavity prior to and following clinical treatment.
3. To investigate possible differences between the staphylococcal populations recovered from clinical specimens obtained using two different sampling methods; paper point sampling of the gingival fluid and curette scraping of the interior of the periodontal/periimplant pocket.
4. To relate the findings from laboratory culture of clinical samples on media selective for staphylococci to previous studies employing molecular detection of staphylococci directly from clinical specimens.

3.2 Materials and Methods

3.2.1 Patient cohort

The criteria used for patient selection for inclusion in the study are detailed in Chapter 2. Following primary clinical evaluation and sampling prior to treatment at clinical visit 1, not all periimplantitis patients attended at subsequent visits for treatment, and therefore the number of patients available at subsequent treatment visits (clinical visits 2, 3 etc.) for sampling was smaller than at visit one. Furthermore, following clinical visit 1, where full information was not available on sites sampled, state of disease or health relating to implants or natural teeth, then samples from these patients were excluded from further analysis.

3.2.2 Staphylococcal isolate collection

One of the main objectives of this part of the present study was to investigate staphylococcal populations associated with untreated diseased implants and therefore the staphylococcal populations recovered from the periimplantitis patients at visit one prior to treatment were used to determine whether periimplantitis was associated with particular staphylococcal species. As reviewed in Chapter 1, a number of previous studies have indicated that *S. aureus* is associated with periimplantitis. However, these studies relied on staphylococcal species detection based on DNA hybridization with "species-specific" probes using chequerboard analysis. In comparison, the approach used in the present study focused on recovery of viable staphylococcal isolates following primary enrichment on the high-salt-containing medium MSA agar as described in Chapter 2.

Staphylococci were recovered from a range of oral sites in patients with clinically diagnosed periimplantitis prior to treatment (clinical visit 1) and at a number of subsequent clinical visits following treatment (clinical visits 2, 3 etc.). The subgingival sites sampled included diseased implant sites, non-diseased implant sites (when available), healthy tooth sites (when available) and diseased tooth sites (when available). On submission to the laboratory samples taken from tooth sites (healthy and/or diseased) which were located adjacent to oral implants were labelled, but the state of health of the oral implant was not noted. Subgingival samples were taken consecutively using sterile paper points and sterile disposable curettes as described in Chapter 2. Paper point sampling was conducted first in each instance, as it is the less invasive and less disruptive technique, followed by curette sampling. Each patient also had an oral rinse sample taken as described in Chapter 2.

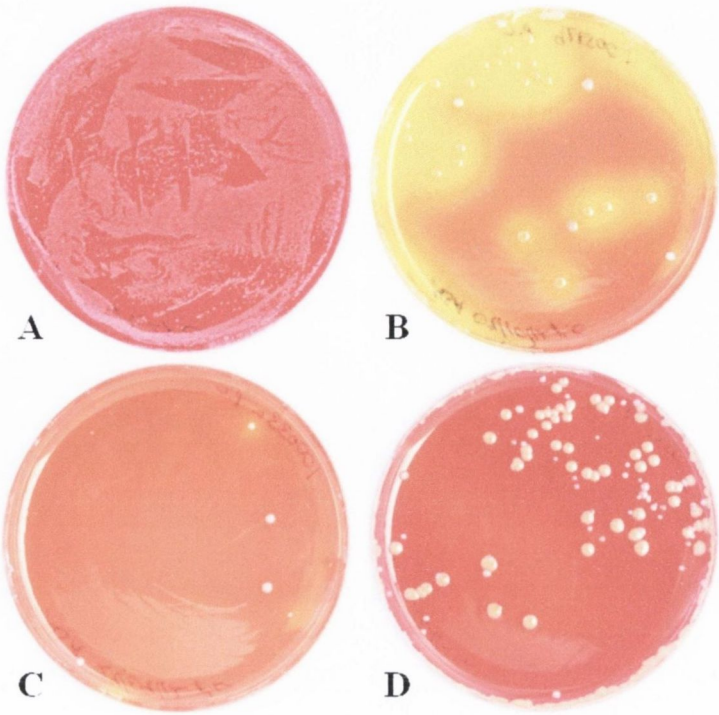

Figure 3.1. Examples of patterns of bacterial growth on MSA agar medium plated with samples from the oral cavity following 48 h incubation at 37°C.

Panel A shows an MSA plate with semiconfluent growth of *S. epidermidis*; panel B shows a plate with 29 cfu consisting of 25 colonies of *S. aureus* and four colonies of *S. epidermidis*; panel C shows a plate with six cfu consisting of two colonies of *S. pasteuri* and four of *S. epidermidis*; panel D shows a plate with 91 cfu consisting of 55 colonies of *S. aureus* and 36 colonies of *S. epidermidis*. Note that while colour conversion of MSA from red to yellow as observed on panel B is indicative of *S. aureus* it is not definitive. Panel C contains two colonies of *S. pasteuri* surrounded by small zones of yellow agar while panel D contains 25 golden colonies of *S. aureus* with no colour change. All isolates in this study were definitively identified using 16S DNA sequencing.

Fifty microlitre aliquots of each clinical sample were plated onto MSA plates in duplicate as described in Chapter 2.

Samples that yielded confluent, or semi-confluent, growth so that it was not possible to count individual colonies were serially diluted and re-plated in order that an accurate colony count could be determined. Examples of some of the patterns of growth on MSA agar plates obtained with samples from the oral cavity in the present study are shown in Figure 3.1. The relative abundance of each colony phenotype (including presence or absence of colour changes in the agar) present on MSA plates was recorded for each sample in cfu/ml of sample (three paper points or curette scraping) as described in Chapter 2. Examples of each colony phenotype observed were isolated and stored on Microbank™ mixed microbial storage beads for further analysis.

3.2.3 Staphylococcal isolate identification

Staphylococcal isolates selected for detailed study were all definitively identified by nucleotide sequence analysis of the small subunit ribosomal DNA (16S rDNA) gene following PCR amplification. DNA was extracted from clinical isolates, subjected to PCR amplification with 16S rDNA-specific primers and subsequently sequenced, all as described in detail in Chapter 2. 16S rDNA sequences were compared with the corresponding consensus sequences for all staphylococcal species in the GenBank nucleotide sequence database, as described in Chapter 2. Isolate identification was based on the highest BLAST score obtained (usually 100%) following interrogation of GenBank with the test sequence (Figure 3.2). In the case of some clinical samples, phenotypically distinct colonies were recovered on MSA but each yielded the same species identification following 16S rDNA sequencing. In such cases, the colony counts of each phenotype were combined when recording species prevalence.

3.2.4 Statistical analysis

Average colony forming units per sample (three paper points in 1 ml YEPD, a single curette scraping in 1 ml YEPD and 1 ml of oral rinse) values were estimated as described in Chapter 2 and statistical analysis was performed using IBM SPSS Statistics for Windows software version 19.0, released 2010. Non-parametric tests were used for all comparisons as the colony count data was found not to be normally distributed for any categories in the data set; oral implants with associated periimplantitis, healthy oral implants, teeth with associated periodontitis located adjacent to oral implants, healthy teeth

located adjacent to oral implants, teeth with associated periodontitis not located adjacent to oral implants, healthy teeth not located adjacent to oral implants. The one-sample Kolmogorov-Smirnov test was used to determine if the categories of species identified (including "no staphylococci recovered" as a category for the analysis) were normally distributed, the one-sample Chi-Squared test and one-sample Binomial test were used to compare the observed frequencies of each species identified to the expected frequencies if all species occurred at the same frequency within the sample population. The independent samples Mann-Whitney U test was used to compare the distribution of the cell densities recorded between sample populations with the null hypothesis (H_o) that the sample populations exhibit the same distribution, while the Independent samples median test was used to compare the medians with the null hypothesis (H_o) that the sample populations have the same median.

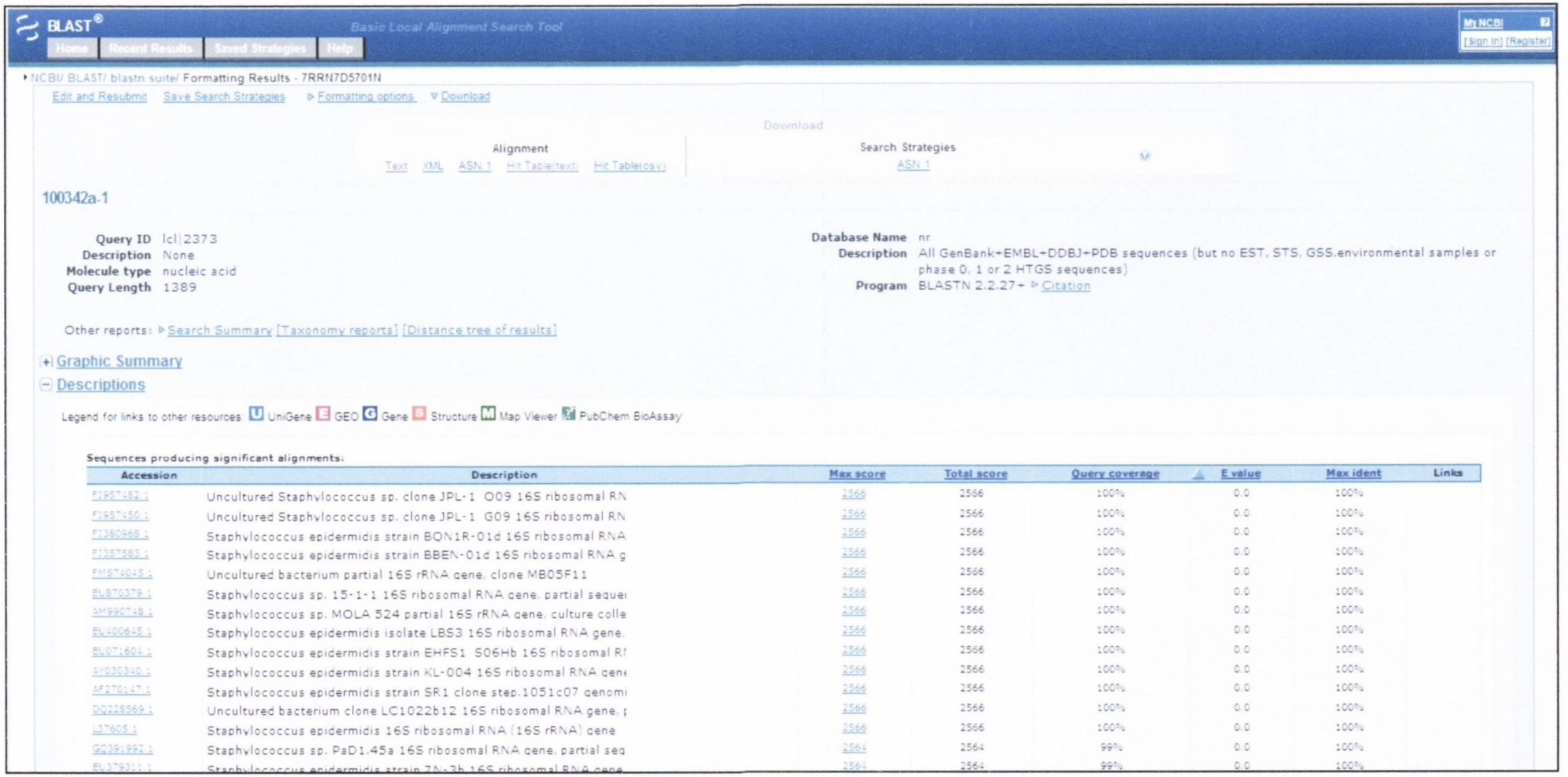

Figure 3.2. Example of BLAST results generated following interrogation of the National Centre for Biotechnology Information (NCBI) database with a 16S DNA sequence from an unknown staphylococcal isolate.

The concensus findings from the BLAST results indicated that the unknown isolate was *S. epidermidis* (100% identity).

3.3 Results

3.3.1 Patient samples and data presentation

For ease of analysis and data presentation, microbiological culture and species identification data obtained from clinical samples taken at each clinical visit from diseased or healthy implants and from diseased or healthy natural teeth have been broken down into separate sections and presented individually below. One of the criteria for patient inclusion in this study was that each patient had to have one or more failing (i.e. diseased) implants. The lack of a diseased implant sample on the first, or subsequent, visits in some cases was due to the sample not being clearly identified when submitted to the laboratory. When this occurred, sample processing continued as normal and the clinician responsible for taking the samples was contacted to provide further information. If the requested information was not available, the missing fields were recorded as unknown. All samples where the site type, or state of health, were recorded as unknown (33 samples from 9 patients) have been excluded from the following analysis. The state of health of each sampling site was assessed at each visit. Therefore, a site that was assessed as diseased by the clinician at visit one, may have been assessed as healthy at a subsequent visit. The clinical severity of disease at tooth or implant sites assessed as being periodontitis or periimplantitis associated was not reported to the laboratory. Due to sampling difficulties, and unclear identification on laboratory submission, samples from some patients were not available from all site types for each clinical visit attended. The initial patient intake was 43.

3.3.2 Overview of staphylococcal populations recovered from periimplantitis patients

For ease of data presentation and understanding, a summary overview of the total staphylococcal populations recovered from periimplantitis patients at all clinical visits and from all oral sites tested (i.e. healthy and diseased implants and healthy and diseased natural teeth and the general oral cavity sampled by oral rinsing) is presented in Table 3.1. A more detailed breakdown of the staphylococcal populations recovered from specific sites at each clinical visit is presented in sections 3.3.3 to 3.3.17 below.

Table 3.1. Summary data on staphylococcal species identified, percentage of patients with one or more samples positive for staphylococci and percentage of samples positive for staphylococci recovered from oral rinse samples, tooth and implant sites[a] in periimplantitis patients

No. of patients and samples and staphylococcal species recovered		Percentage of patients yielding one or more positive sample for staphylococci[b] and percentage of samples taken that were positive for a staphylococci												
		Sampling site												
		Diseased Implant		Healthy Implant		Diseased tooth adjacent to an implant		Healthy tooth adjacent to an implant		Diseased tooth not adjacent to an implant		Healthy tooth not adjacent to an implant		General oral cavity
		Sampling method												
		Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Oral rinse
Pre-treatment Clinical visit 1	**Patients n=42[c]**	**n=29**	**n=28**	**n=13**	**n=13**	**n=8**	**n=8**	**n=15**	**n=12**	**n=24**	**n=22**	**n=33**	**n=31**	**n=38**
	Samples n=388	**n=61**	**n=53**	**n=18**	**n=17**	**n=9**	**n=9**	**n=16**	**n=12**	**n=35**	**n=33**	**n=41**	**n=37**	**n=47**
Samples yielding	% Patients	82.8%	100.0%	92.3%	92.3%	62.5%	100.0%	93.3%	100.0%	83.3%	95.5%	84.8%	100.0%	34.2%
no staphylococci	% Samples	63.9%	92.5%	83.3%	88.2%	66.7%	100.0%	93.8%	100.0%	82.9%	87.9%	80.5%	91.9%	27.7%
S. aureus	% Patients	10.3%	3.6%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.2%	0.0%	0.0%	0.0%	21.1%
	% Samples	4.9%	1.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	0.0%	0.0%	0.0%	17.0%
S. epidermidis	% Patients	31.0%	3.6%	15.4%	15.4%	37.5%	0.0%	6.7%	0.0%	16.7%	13.6%	18.2%	8.3%	47.4%
	% Samples	19.7%	1.9%	11.1%	11.8%	33.3%	0.0%	6.3%	0.0%	11.4%	9.1%	14.6%	8.1%	38.3%
Other	% Patients	20.7%	7.1%	7.7%	0.0%	0.0%	0.0%	0.0%	0.0%	4.2%	4.5%	6.1%	0.0%	18.4%
staphylococci	% Samples	11.5%	3.8%	5.6%	0.0%	0.0%	0.0%	0.0%	0.0%	2.9%	3.0%	4.9%	0.0%	17.0%

Continued overleaf

Table 3.1 continued. Summary data on staphylococcal species identified, percentage of patients with one or more samples positive for staphylococci and percentage of samples positive for staphylococci recovered from oral rinse samples, tooth and implant sites[a] in periimplantitis patients

No. of patients and samples and staphylococcal species recovered		Percentage of patients yielding one or more positive sample for staphylococci[b] and percentage of samples taken that were positive for a staphylococci												
		Sampling site												
		Diseased Implant		Healthy Implant		Diseased tooth adjacent to an implant		Healthy tooth adjacent to an implant		Diseased tooth not adjacent to an implant		Healthy tooth not adjacent to an implant		General oral cavity
		Sampling method												
		Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Oral rinse
Post-treatment Clinical visit 2	**Patients n=21**	**n=14**	**n=14**	**n=14**	**n=14**	**n=7**	**n=7**	**n=8**	**n=8**	**n=11**	**n=11**	**n=16**	**n=17**	**n=17**
	Samples n=225	**n=27**	**n=24**	**n=20**	**n=21**	**n=7**	**n=7**	**n=9**	**n=9**	**n=16**	**n=16**	**n=23**	**n=24**	**n=22**
Samples yielding no staphylococci	% Patients	85.7%	85.7%	71.4%	92.9%	71.4%	71.4%	87.5%	87.5%	72.7%	90.9%	75.0%	82.4%	22.7%
	% Samples	63.0%	75.0%	55.0%	81.0%	71.4%	71.4%	88.9%	88.9%	62.5%	75.0%	73.9%	87.5%	22.7%
S. aureus	% Patients	0.0%	0.0%	7.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	4.5%
	% Samples	0.0%	0.0%	5.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	16.7%
S. epidermidis	% Patients	28.6%	21.4%	21.4%	21.4%	28.6%	28.6%	12.5%	12.5%	36.4%	27.3%	31.3%	11.8%	45.5%
	% Samples	37.0%	25.0%	35.0%	19.0%	28.6%	28.6%	11.1%	11.1%	37.5%	18.8%	21.7%	8.3%	45.5%
Other staphylococci	% Patients	0.0%	0.0%	7.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	9.1%	6.3%	5.9%	22.7%
	% Samples	0.0%	0.0%	5.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	6.3%	4.3%	4.2%	27.3%

Continued overleaf

Table 3.1 continued. Summary data on staphylococcal species identified, percentage of patients with one or more samples positive for staphylococci and percentage of samples positive for staphylococci recovered from oral rinse samples, tooth and implant sites[a] in periimplantitis patients

No. of patients and samples and staphylococcal species recovered		Percentage of patients yielding one or more positive sample for staphylococci[b] and percentage of samples taken that were positive for a staphylococci												
		Sampling site												
		Diseased Implant		Healthy Implant		Diseased tooth adjacent to an implant		Healthy tooth adjacent to an implant		Diseased tooth not adjacent to an implant		Healthy tooth not adjacent to an implant		General oral cavity
		Sampling method												
		Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Paper Point	Curette	Oral rinse
Subsequent post-treatment visits[d]	**Patients n=17**	**n=6**	**n=6**	**n=13**	**n=13**	**n=4**	**n=2**	**n=11**	**n=12**	**n=5**	**n=4**	**n=12**	**n=12**	**n=17**
	Samples n=300	**n=14**	**n=12**	**n=45**	**n=41**	**n=5**	**n=2**	**n=18**	**n=17**	**n=7**	**n=6**	**n=47**	**n=44**	**n=42**
Samples yielding	% Patients	100.0%	100.0%	92.3%	100.0%	100.0%	100.0%	90.9%	83.3%	80.0%	100.0%	100.0%	100.0%	29.4%
no staphylococci	% Samples	71.4%	75.0%	66.7%	92.7%	100.0%	100.0%	77.8%	82.4%	85.7%	100.0%	83.0%	97.7%	14.3%
S. aureus	% Patients	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	41.2%
	% Samples	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	16.7%
S. epidermidis	% Patients	16.7%	33.3%	53.8%	15.4%	0.0%	0.0%	27.3%	16.7%	20.0%	0.0%	41.7%	8.3%	94.1%
	% Samples	28.6%	25.0%	26.7%	7.3%	0.0%	0.0%	16.7%	11.8%	14.3%	0.0%	14.9%	2.3%	57.1%
Other	% Patients	0.0%	0.0%	15.4%	0.0%	0.0%	0.0%	9.1%	8.3%	0.0%	0.0%	8.3%	0.0%	29.4%
staphylococci	% Samples	0.0%	0.0%	6.7%	0.0%	0.0%	0.0%	5.6%	5.9%	0.0%	0.0%	2.1%	0.0%	11.9%

[a] Samples from teeth and implants were taken using both paper points and curettes as described in section Chapter 2. The oral cavity was sampled by oral rinse sampling. Species were identified based on the 16S rDNA nucleotide sequence Chapter 2.

[b] Individual patients may have had samples taken from multiple locations of the same sampling site type and state of health (e.g. diseased implant) at each visit. Where those multiple samples yielded different results e.g. one had no staphylococci whilst another yielded *S. epidermidis* the individual patient would have been counted in each category resulting in total percentages of >100 for some site types and states of health.

[c] Due to sampling difficulties and unclear identification upon submission to the laboratory samples from some patients were not available from all site types for each clinical visit attended, therefore the numbers of patients listed in each sample site type, state of health and collection method may differ from the total number of patients that attended each clinical session.

[d] Due to the low number of patients that attended post-treatment clinics subsequent to the initial post-treatment clinical visit (visit 2), and for ease of presentation, all subsequent visits 3-6 have been pooled.

3.3.2.1 *Staphylococcus aureus*

Overall the majority of samples collected from diseased or healthy implants and diseased or healthy natural teeth throughout the study did not yield any staphylococci (285/341 samples at visit 1 (83.6%), 149/203 samples at visit 2 (73.4%), 216/258 samples at subsequent visits, 83.7%) (Table 3.1).

In contrast, the majority of oral rinse samples did yield staphylococci (34/47 samples at visit 1 (72.3%), 17/22 samples at visit 2 (77.3%) and 36/42 samples at subsequent visits, 85.7%). Furthermore, the majority of patients had one or more samples that did not yield any staphylococci collected from each sampling site type and state of health (excepting oral rinse samples), including samples taken prior to clinical treatment (clinical visit 1), and from samples taken after clinical treatment (clinical visit 2 and subsequent visits) (Table 3.1). *Staphylococcus aureus* was isolated infrequently from all samples tested from both pre-treatment (i.e. clinical visit 1) and post-treatment clinical visits (Table 3.1). Prior to clinical intervention for periimplantitis *S. aureus* was only recovered from three diseased implants by paper point sampling (10.3% of patients sampled) and from one diseased implant by curette sampling (3.6% of patients sampled) (Table 3.1). *Staphylococcus aureus* was not recovered from any diseased implant site following clinical treatment (Table 3.1). *Staphylococcus aureus* was also isolated from one or more paper point samples collected from diseased tooth sites adjacent to an oral implant in 4.2% of patients (2.9% of all paper point samples collected from diseased tooth sites adjacent to an oral implant) (Table 3.1). At the initial clinical intake (prior to treatment) *S. aureus* was primarily isolated from oral rinse samples. Just over 21.1% of patients yielded *S. aureus* from their oral rinse samples (17% of all oral rinse samples taken throughout the study) (Table 3.1). These findings revealed conclusively that *S. aureus*, as determined by laboratory culture, was not significantly associated with untreated failing oral implants in the patient cohort studied.

Staphylococcus aureus was recovered from healthy implant sites in 7.1% of patients (5% of all samples taken from healthy implant sites) at the initial post-treatment clinical visit (visit 2) (Table 3.1). *Staphylococcus aureus* was isolated at a lower frequency from oral rinse samples (4.5% of all oral rinse samples) at the second clinical sampling. Furthermore, at subsequent post-treatment visits *S. aureus* was only isolated from oral rinse samples (16.7% of all oral rinse samples collected at subsequent post-treatment visits) (Table 3.1).

Overall, the results of this study demonstrated that *S. aureus* was not significantly associated with diseased or healthy oral implants or with diseased or healthy natural teeth.

3.3.2.2 *Staphylococcus epidermidis*

Where patients yielded staphylococci from one or more samples from a particular site type and state of health the majority of those patients yielded *S. epidermidis* (from each, and all, sampling site type and state of health) (Table 3.1). This was observed both in samples taken prior to treatment (visit 1), and from samples taken after clinical treatment (visit 2 and subsequent visits). *Staphylococcus epidermidis* did not appear to be particularly associated with any site type, or state of health, in the patient cohort that samples were obtained from in this study.

The oral rinse samples provided an overview of the staphylococcal prevalence and staphylococcal loads within the oral cavity in general at the time of sample collection. Unlike the majority of the paper point and curette samples collected from tooth and implant pockets (both healthy and diseased), the majority of oral rinse samples did yield staphylococci. The most frequently identified species was *S. epidermidis* at all clinical visits. *Staphylococcus epidermidis* was also the most abundant species. This suggests that a proportion of the patient population investigated in this study (47.4% at visit 1, 45.5% at visit 2 and 94.1% of visits 3-6 combined) harboured *S. epidermidis* as a transient or general member of their oral microbial flora. The higher incidence of patients testing positive for *S. epidermidis* in oral rinse samples when data from multiple time points (clinical visits 3-6) is pooled, compared to the incidence of patients testing positive for *S. epidermidis* in oral rinse samples from isolated time points (clinical visits 1 and 2) suggests that *S. epidermidis* is a transient organism in the oral cavity.

3.3.2.3 Other staphylococcal species

Although *S. epidermidis* was the predominant staphylococcal species recovered from the periimplantitis patients and *S. aureus* was recovered in some cases, 10 other staphylococcal species were identified from samples collected in this study including *Staphylococcus warneri* (20 isolates from 12 patients), *Staphylococcus pasteuri* (11 isolates from five patients), *Staphylococcus capitis* (four isolates from three patients), *Staphylococcus haemolyticus* and *Staphylococcus cohnii* (two isolates from two patients for each species), *Staphylococcus auricularis* and *Staphylococcus lugdunensis* (two isolates from one patient

for both species), *Staphylococcus caprae*, *Staphylococcus equorum* and *Staphylococcus hominis* (one isolate from one patient for each species). These species were each found at a far lower frequency and abundance than *S. epidermidis* (Table 3.1). As the frequency of isolation of these species was so low, these species were grouped together as "other staphylococci".

3.3.2.4 Paper point versus curette sampling

Paper point samples appeared to yield more staphylococci (higher frequency and density) than curette samples at the majority of sampling sites and states of health investigated, although statistical analysis revealed that this difference was not significant. A matched pairs analysis (Wilcoxon signed-rank test) was conducted using data from pairs of paper point and curette samples collected from the same site at the same visit. The Wilcoxon signed-rank test can be used to determine whether the median of the observed differences between the matched data points deviate enough from zero to indicate a significant difference between the two different groups from which the pairs were drawn [158] (in this case the two different sampling methods; paper point and curette). This test can be used with non-parametric data sets where at least one group has outliers [158]. The statistical analysis was performed separately for the pre-treatment clinical assessment (visit 1), the initial post-treatment clinical assessment (visit 2), and for all subsequent visits combined (visits 3-6). In all cases, bar one, the matched pairs analyses indicated that there was no significant difference between the staphylococcal density recovered by each collection method, for every combination of sample site type and state of health ($P>0.05$). The test indicated a significant difference between the staphylococcal density recovered by paper point and curette samples collected from healthy teeth that were non-adjacent to oral implants in the pooled "subsequent visits" group ($P<0.05$). This is understandable as no curette samples collected from healthy teeth that were non-adjacent to oral implants in the pooled "subsequent visits" group yielded any staphylococci.

One interesting observation was that there was a statistically significant increase in the staphylococcal density recovered between the pre-treatment, and post-treatment clinical sampling sessions (visit 1 and visit 2) at healthy and diseased implant sites and diseased teeth non-adjacent to oral implants for all staphylococci, and for all implant sites when the analysis was restricted to *S. epidermidis* only (Independent samples Mann-Whitney U test, $p < 0.05$).

Detailed microbiological culture and species identification data obtained from clinical samples taken at each clinical visit from diseased or healthy implants and from diseased or healthy natural teeth are presented separately in the following sections.

3.3.3 Oral staphylococci recovered from diseased implants in periimplantitis patients prior to treatment

Samples were collected from one or more clearly identified diseased or failing implant sites from 30 patients attending the first clinical visit (visit 1), prior to treatment (mechanical debridement of the implant site, and oral hygiene advice as described in Chapter 2) (Table 3.2).

3.3.3.1 Paper point and curette sampling

The majority of these patients (18/29, 62% paper point, 23/28, 82.14% curette) had no staphylococci recovered from any implant site sampled (Table 3.2). Nine of the 29 patients that had paper point samples collected (Patients 7, 13, 16, 21, 22, 25, 27, 31 and 36) yielded *S. epidermidis* (9/29, 31%) while only one of the 28 patients that had curette samples collected (Patient 36) yielded *S. epidermidis* (1/28, 3.57%). The mean average *S. epidermidis* density for patients positive for *S. epidermidis* at diseased implant sites recovered from paper point samples was 73 cfu per sample (range 10 to 400 cfu per sample). The single curette sample from which *S. epidermidis* was isolated yielded 1.7 x 10^4 cfu per sample.

Staphylococcus aureus was recovered from paper point samples from three of the patients (Patients 3, 25 and 36) that diseased implant samples were obtained from (3/29, 10.34%) (Table 3.2). One of these patients (Patient 36) also had *S. aureus* recovered from a curette sample (1/28, 3.57%). The mean average *S. aureus* density recovered from paper point samples for patients positive for *S. aureus* at diseased implant sites was 296.7 cfu (range 10-480 cfu) and 170 cfu for the single curette sample. *Staphylococcus epidermidis* and *S. aureus* were co-isolated from the same paper point sample taken from a diseased implant site from Patient 25 along with *S. pasteuri*. Although *S. epidermidis* and *S. aureus* were both recovered from a curette sample from patient 36, the samples came from two different diseased implants (sampling sites D and E) (Table 3.2).

Staphylococci other than *S. epidermidis* and *S. aureus* were recovered from paper point samples from diseased implants for from six patients (Patients 3, 7, 9, 20, 25 and 26) (6/30,

20%) (Table 3.2). Five species (*S. lugdunensis S. cohnii, S. auricularis*, *S. pasteuri* and *S. haemolyticus*) were recovered from a single patient, whereas *S. warneri* was recovered from two patients. Apart from *S. haemolyticus*, which yielded 920 cfu per sample from two patients, the density of these staphylococcal species recovered was very small in each case (average 45 cfu per paper point sample, the *S. haemolyticus*-positive sample excluded, and 75 cfu/ml oral rinse). Staphylococci other than *S. epidermidis* and *S. aureus* were recovered from curette samples from two patients (Patients 20 and 23) (Table 3.2). Patient 20 yielded *S. auricularis* at 10 cfu per sample (at a different sampling site to that which yielded *S. auricularis* in a paper point sample), and Patient 23 yielded *S. warneri* at 10 cfu per sample. Statistical analysis of the staphylococcal recovery data from diseased implant sites (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi-Squared test, $p < 0.05$). This reflects the findings, that the majority of samples had no staphylococci recovered, with *S. epidermidis* being the most prevalent staphylococcal species when staphylococci were isolated.

Table 3.2. Staphylococcal species and cell density recovered from[a] diseased implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Implant site**	**Paper Point**	**Curette**	**Oral rinse[c]**
1	A	0	0	0
3	A	0	0	90 *S. aureus*
	B	10 *S. cohnii*	0	-
	C	0	0	-
	D	0	0	-
	G	40 *S. aureus*	NA	-
4	C	0	0	0
7	C	40 *S. epidermidis*	NA	120 *S. epidermidis*
	D	10 *S. epidermidis*	NA	-
	E	10 *S. epidermidis* 10 *S. warneri*	NA	-
8	B	0	NA	0
9	A	0	0	30 *S. aureus*
	D	10 *S. warneri*	0	-
10	A	NA	0	990 *S. epidermidis* 15 *S. warneri*
	B	NA	0	-
	C	NA	0	-
11	A	0	0	60 *S. epidermidis*
13	C	10 *S. epidermidis*	0	210 *S. epidermidis*
	D	0	0	-
14	A	0	0	345 *S. epidermidis* 195 *S. aureus*
	B	0	0	-
16	C	70 *S. epidermidis*	NA	60 *S. epidermidis*
	D	20 *S. epidermidis*	0	-
17	A	0	0	0
18	A	0	0	2,775 *S. aureus*
19	C	0	0	15 *S. epidermidis*
	D	0	0	-
20	A	0	10 *S. auricularis*	1,785 *S. epidermidis* 1,605 *S. aureus*
	B	10 *S. aureus* 10 *S. auricularis*	0	-
21	G	0	0	1,155 *S. aureus*
	H	10 *S. epidermidis*	0	-
	I	0	0	-
	J	0	0	-
22	A	0	0	30 *S. epidermidis*
	B	10 *S. epidermidis*	0	-

Continued overleaf

Table 3.2 continued. Staphylococcal species and cell density recovered from[a] diseased implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Implant site**	**Paper Point**	**Curette**	**Oral rinse[c]**
23	A	0	0	0
	B	0	10 *S. warneri*	
24	B	0	0	375 *S. aureus*
	C	0	0	-
25	A	400 *S. epidermidis* 480 *S. aureus* 20 *S. pasteuri*	0	360 *S. epidermidis* 60 *S. pasteuri*
26	A	0	0	0
	B	0	0	-
	C	920 *S.* haemolyticus	0	-
27	A	110 *S. epidermidis*	0	105 *S. epidermidis*
29	F	0	0	90 *S. lugdunensis*
30	A	0	0	0
31	B	10 *S. epidermidis*	0	450 *S. epidermidis*
32	F	0	0	0
36	A	0	0	15 *S. epidermidis*
	B	0	0	-
	C	0	0	-
	D	0	170 *S. aureus*	-
	E	50 *S. epidermidis*	1.7×10^4 *S. epidermidis*	-
37	A	0	0	345 *S. epidermidis*
41	A	0	0	0
	B	0	0	-
	C	0	0	-
42	A	0	0	0

[a] Samples from diseased implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only one oral rinse sample was taken at the first clinical evaluation prior to treatment, whereas in some cases several diseased implants were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased implant samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.3.2 Oral rinse sampling

One third of the patients who had samples collected from diseased implants at the primary clinical evaluation (10/30, 33.33%) yielded no staphylococci from their oral rinse samples. Of these 10 patients, nine did not yield any staphylococci from their diseased implant sites; one patient had *S. warneri* recovered from a single curette sample (Patient 23, 10 cfu per sample). Almost half of the thirty patients that had samples collected from diseased implant sites on the first clinical visit (14/30, 46.67%) yielded *S. epidermidis* from their oral rinse samples with a mean average density of approximately 343 cfu per/ml oral rinse (range 15-1.7 x 10^3 cfu/ml oral rinse). Only seven of these oral rinse *S. epidermidis*-positive patients (Patients 7, 13, 16, 22, 25, 27, 31 and 36) also had *S. epidermidis* recovered from diseased implant samples (Table 3.2). Seven of the 30 patients (23.33%) where diseased implant samples were taken yielded *S. aureus* from their oral rinses (Patients 3, 9, 14, 18, 20, 21, 24), with a mean average density of 889 cfu/ml of oral rinse (range 30-2.7 x 10^3 cfu/ml of oral rinse). Only two of these oral rinse *S. aureus*-positive patients (Patients 3 and 9) also had *S. aureus* recovered at a diseased implant site. Two patients (Patients 14 and 20) had *S. epidermidis* and *S. aureus* co-isolated from their oral rinse samples. Only three of the 30 patients yielded staphylococci other than *S. epidermidis* or *S. aureus* from oral rinse samples. Patient 10 yielded *S. warneri* (15 cfu/ml of oral rinse), Patient 25 yielded *S. pasteuri* (60 cfu/ml of oral rinse) and Patient 29 yielded *S. lugdunensis* (90 cfu/ml of oral rinse). Patient 25 also had *S. pasteuri* recovered from a diseased implant site along with *S. epidermidis* and *S. aureus*), but the other two patients did not have any staphylococci recovered from their diseased implant samples. Patients 10 and 25 both also had *S. epidermidis* recovered from their oral rinse samples.

3.3.4 Oral staphylococci recovered from healthy implants in periimplantitis patients prior to treatment

3.3.4.1 Paper point and curette sampling

Thirteen patients had samples collected from oral implants assessed to be 'healthy' at the first clinical visit (pre-treatment). The majority of these (10/13, 76.9% paper point samples, 11/13, 84.6% curette samples) did not have any staphylococci recovered from the healthy implant samples by either paper point or curette sampling (Table 3.3). Two patients (2/13, 15.38%) (Patients 27 and 34) yielded *S. epidermidis* from paper point samples with cell densities of 100 and 10 cfu/sample, respectively (mean average density of 55 cfu/sample). One of these patients, Patient 27, also yielded *S. epidermidis* from a curette sample as did

another patient, Patient 35, (2/13, 15.38%) with cell densities of 10 and 40 cfu/sample, respectively (mean average density or 25 cfu/sample) (Table 3.3). *Staphylococcus aureus* was not recovered from any of the healthy implant sites sampled at the first clinical visit. Only one healthy implant site (1/13, 7.7%) yielded staphylococci other than *S. epidermidis*. Patient 9 yielded *S. haemolyticus* from a paper point sample at a density of 20 cfu/sample (Table 3.3). Statistical analysis of the staphylococcal recovery data from healthy implant sites (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi-Squared test, $p < 0.05$).

3.3.4.2 Oral rinse sampling

Two of the patients that had samples taken from healthy implants at the first clinical visit did not have oral rinse samples taken. Of the remaining eleven patients that had samples taken from both healthy implant sites and oral rinses, two (Patients 1 and 4) did not yield any staphylococci (2/11, 18.18%) (Table 3.3). Five patients (5/11, 45.45%) (Patients 10, 27, 34, 35 and 40) yielded *S. epidermidis* from their oral rinse samples with a mean average cell density of 243 cfu/ml of oral rinse (range 15 to 990 cfu/ml of oral rinse). While *S. aureus* was not recovered from any healthy implant sites on the first clinical visit, it was recovered from the oral rinses of 3/11 patients (27.3%) that had healthy implant samples taken with a mean average density of 1.5×10^3 cfu/ml of oral (range 30 to 2.7×10^3 cfu/ml of oral rinse) (Table 3.3). Staphylococci other than *S. epidermidis* and *S. aureus* were recovered from 5/11 (45.45%) oral rinse samples for patients who also had samples collected from healthy implants (Patients 10, 15, 29, 34 and 40). Three different species were recovered. *Staphylococcus warneri* was recovered from three patients (Patients 10, 15 and 34) with a mean average density of 35 cfu/ml of oral rinse (range 15 to 60 cfu/ml of oral rinse). Two patients (Patients 40 and 34) yielded *S. pasteuri* at cell densities of 15 and 1.3×10^3 cfu/ml of oral rinse, respectively. *Staphylococcus lugdunensis* was found in the oral rinse sample of a single patient (Patient 29) at 90 cfu/ml of oral rinse (Table 3.3).

Table 3.3. Staphylococcal species and cell density recovered from[a] healthy implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Implant site**	**Paper Point**	**Curette**	**Oral rinse[c]**
1	A	0	0	0
4	D	0	0	0
9	C	20 *S. haemolyticus*	0	30 *S. aureus*
10	A	0	0	990 *S. epidermidis* 15 *S. warneri*
	B	0	0	-
	C	0	0	-
15	C	0	0	30 *S. warneri*
18	B	0	0	2,775 *S. aureus*
27	D	0	0	105 *S. epidermidis*
	E	100 *S. epidermidis*	10 *S. epidermidis*	-
29	C	0	0	90 *S. lugdunensis*
33	B	0	0	NA
34	B	10 *S. epidermidis*	0	90 *S. epidermidis* 60 *S. warneri* 1,335 *S. pasteuri*
35	C	0	40 *S. epidermidis*	15 *S. epidermidis* 375 *S. aureus*
39	A	0	0	NA
40	A	0	0	15 *S. epidermidis* 15 *S. pasteuri*
	D	0	0	-

[a] Samples from healthy implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only one oral rinse sample was taken at the first clinical evaluation prior to treatment, whereas in some cases several diseased implants were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased implant samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.5 Oral staphylococci recovered from teeth with associated periodontitis located adjacent to oral implants in patients with periimplantitis prior to treatment

Eight patients had samples taken from teeth assessed to be diseased (associated periodontitis) that were adjacent to oral implants on their first clinical visit prior to treatment.

3.3.5.1 Paper point and curette sampling

The majority of patients (5/8 62.5% of paper point samples, 8/8 100% of curette samples) did not yield any staphylococci from any diseased teeth adjacent to oral implants (Table 3.4). *Staphylococcus epidermidis* was the only staphylococcal species recovered from paper point samples. Three (3/8, 37.5%) of the patients yielded *S. epidermidis* (Patients 3, 24 and 27) with a mean average cell density of 23.3 cfu/sample (range 10 to 40 cfu/sample). No staphylococci were recovered from any curette samples taken. Statistical analysis of the staphylococcal recovery data from tooth sites with associated periodontitis located adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi-Squared test, $p < 0.05$).

3.3.5.2 Oral rinse sampling

Two (patients 4 and 17) of the eight patients that provided samples from diseased teeth adjacent to oral implants at the first clinical visit did not have any staphylococci recovered from their oral rinse samples (2/8, 25%). Three patients (3/8; 37.5%) (Patients 11, 19 and patient 27) yielded *S. epidermidis* from their oral rinse samples with a mean average density of 60 cfu/ml of oral rinse (range 15 to 105 cfu/ml of oral rinse) only one of which (Patient 27) also yielded *S. epidermidis* from the diseased tooth adjacent to an oral implant (Table 3.4). Another two patients, Patients 3 and 24, (2/8, 25%) yielded *S. aureus* from oral rinses at cell densities of 90 and 375 cfu/ml, respectively. A single patient (Patient 29) (1/8, 12.5%) yielded *S. lugdunensis* from their oral rinse sample (Table 3.4).

Table 3.4. Staphylococcal species and cell density recovered from[a] teeth with associated periodontitis adjacent to implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse**
3	F	20 *S. epidermidis*	0	90 *S. aureus*
4	A	0	0	0
11	B	0	0	60 *S. epidermidis*
17	B	0	0	0
19	B	0	0	15 *S. epidermidis*
24	D	40 *S. epidermidis*	0	375 *S. aureus*
27	B	10 *S. epidermidis*	0	105 *S. epidermidis*
29	B	0	0	90 *S. lugdunensis*

[a] Samples from teeth with associated periodontitis adjacent to implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

3.3.6 Oral staphylococci recovered from teeth with associated periodontitis located non-adjacent to oral implants in patients with periimplantitis prior to treatment

Twenty-five patients had samples taken from teeth assessed to be diseased (associated periodontitis) that were located non-adjacent to oral implants on their first clinical visit prior to treatment.

3.3.6.1 Paper point and curette sampling

The majority of patients (19/25, 76% paper point samples, 19/22, 86.36% curette samples) did not yield any staphylococci from any diseased teeth non-adjacent to oral implants (Table 3.5). Four of the 25 patients (Patients 2, 6, 16 and 42) yielded *S. epidermidis* from paper point samples (4/25, 16%) with a mean average cell density of 760 cfu per sample (range 10 to 3 x 10^3 cfu per sample). One patient (Patient 24) (1/25, 4%) yielded *S. aureus* from a paper point sample (20 cfu per sample) (Table 3.5). A single patient (Patient 9) yielded *S. capitis* from a paper point sample at a density of 20 cfu per sample. Three patients (Patients 2, 6 and 16) yielded *S. epidermidis* from curette samples (3/22, 13.63%) with a mean average density of 263.3 cfu per sample (range 10 to 770 cfu per sample). *Staphylococcus aureus* was not recovered from any curette samples collected from diseased teeth that were non-adjacent to oral implants. A single patient (Patient 2) yielded *S. capitis* from a curette sample at a density of 10 cfu per sample (Table 3.5). Statistical analysis of the staphylococcal recovery data from tooth sites with associated periodontitis located non-adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi-Squared test, $p < 0.05$).

Table 3.5. Staphylococcal species and cell density recovered from[a] teeth with associated periodontitis located non-adjacent to implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse[c]**
1	A	0	0	0
2	A	0	770 *S. epidermidis*	NA
	C	10 *S. epidermidis*	10 *S. capitis*	-
	D	0	0	-
	E	0	0	-
6	B	10 *S. epidermidis*	0	45 *S. warneri*
	C	0	0	-
	D	0	10 *S. epidermidis*	-
	E	0	0	-
7	A	0	NA	120 *S. epidermidis*
9	E	40 *S. capitis*	0	30 *S. aureus*
10	F	NA	0	990 *S. epidermidis* 15 *S. warneri*
11	A	0	0	60 *S. epidermidis*
12	C	0	NA	NA
13	B	0	0	210 *S. epidermidis*
14	C	0	0	345 *S. epidermidis* 195 *S. aureus*
15	B	0	NA	30 *S. warneri*
16	G	3,010 *S. epidermidis*	10 *S. epidermidis*	60 *S. epidermidis*
19	E	0	0	15 *S. epidermidis*
21	B	0	0	1,155 *S. aureus*
	E	0	0	-
23	C	0	0	0
	D	0	0	-
24	E	20 *S. aureus*	0	375 *S. aureus*
25	A	0	0	360 *S. epidermidis* 60 *S. pasteuri*
26	D	0	0	0
27	F	0	0	105 *S. epidermidis*
29	B	0	0	90 *S. lugdunensis*
	G	0	0	-
31	D	0	0	450 *S. epidermidis*
32	D	0	0	0
38	B	0	0	0
	C	0	0	-

Continued overleaf

Table 3.5 continued. Staphylococcal species and cell density recovered from[a] teeth with associated periodontitis located non-adjacent to implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse[c]**
38	B	0	0	0
	C	0	0	-

[a] Samples from teeth with associated periodontitis located non-adjacent to implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only one oral rinse sample was taken at the first clinical evaluation prior to treatment, whereas in some cases several diseased implants were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased implant samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.6.2 Oral rinse sampling

Oral rinse samples were not collected for 3/25 patients that had samples taken from diseased teeth non-adjacent to oral implants at the first clinical visit. Of the 22 that patients that had oral rinses taken, six (6/22, 27.27%) did not yield any staphylococci. Ten patients yielded *S. epidermidis* (10/22, 45.45%) with a mean average cell density of 271.5 cfu per ml of oral rinse (range 15 to 990 cfu per ml of oral rinse). Four (Patients 9, 14, 21 and 24) (4/22, 18.18%) yielded *S. aureus* from their oral rinse samples with a mean average cell density of 438.75 cfu per ml of oral rinse (range 30 to 1.1 x 10^3 cfu per ml of oral rinse) . Five (Patient 6, 10, 15, 25 and 29) of the 22 patients that oral rinse samples taken yielded staphylococci other than *S. epidermidis* or *S. aureus*. Patients 6, 10 and 15 all yielded *S. warneri* at a mean average cell density of 30 cfu per ml of oral rinse (range 15 to 45 cfu per ml of oral rinse) (Table 3.5). Patient 25 yielded *S. pasteuri* at a cell density of 60 per ml of oral rinse and Patient 29 yielded *S. lugdunensis* at a cell density of 90 cfu per ml of oral rinse.

3.3.7 Oral staphylococci recovered from healthy teeth located adjacent to oral implants in patients with periimplantitis prior to treatment

Fifteen patients had samples collected from teeth assessed to be healthy that were located adjacent to oral implants at the first (pre-treatment) clinical visit.

3.3.7.1 Paper point and curette sampling

Of those fifteen patients the majority did not have any staphylococci recovered from the tooth site (14/15, 93.3% of paper point samples and 12/12, 100% or curette samples). A single patient (Patient 13) yielded *S. epidermidis* from the paper point sample only (230 cfu per sample) (Table 3.6). Statistical analysis of the staphylococcal recovery data from healthy tooth sites located adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi-Squared test, $p < 0.05$).

Table 3.6. Staphylococcal cell density and species recovered from[a] healthy teeth adjacent to implants and from the oral cavity by oral rinse sampling in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse**
3	E	0	0	90 *S. aureus*
8	A	0	NA	0
9	B	0	0	30 *S. aureus*
12	B	0	NA	NA
13	A	230 *S. epidermidis*	0	210 *S. epidermidis*
15	A	0	0	30 *S. warneri*
16	B	0	0	60 *S. epidermidis*
18	C	0	0	2,775 *S. aureus*
20	C	0	0	1,785 *S. epidermidis* 1,605 *S. aureus*
22	C	0	0	30 *S. epidermidis*
31	A	0	0	450 *S. epidermidis*
33	C	0	NA	NA
35	A	0	0	15 *S. epidermidis* 375 *S. aureus*
39	A	NA	NA	NA
	C	0	0	NA
42	B	0	0	0

[a] Samples from healthy teeth adjacent to implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

Abbreviations: NA, sample not available.

3.3.7.2 Oral rinse sampling

Oral rinse samples were collected from twelve of the fifteen patients from whom samples were collected from healthy teeth that were adjacent to oral implants. Of those twelve patients two yielded no staphylococci (2/12, 16.67%), six (Patients 13, 16, 20, 22, 31 and 35) yielded *S. epidermidis* (6/12, 50%) ranging from 15 to 1.7 x 10^3 cfu/ml oral rinse (mean average density 425 cfu/ml oral rinse). Five patients (Patient 3, 9, 18, 20 and 35) had *S. aureus* recovered from their oral rinses range 30 cfu/ml to 2.7 x 10^3 cfu/ml oral rinse (mean average density 975 cfu/ml oral rinse). *Staphylococcus warneri* was recovered from the oral rinse of Patient 15 (30 cfu/ml oral rinse) (Table 3.6).

3.3.8 Oral staphylococci recovered from healthy teeth located non-adjacent to oral implants in patients with periimplantitis prior to treatment

Thirty-four patients had samples taken from teeth assessed to be healthy that were non-adjacent to oral implants at the first (pre-treatment) clinical visit, 33 were sampled using paper points and 31 using curettes.

3.3.8.1 Paper point and curette sampling

The majority of patients did not have any staphylococci recovered from the healthy tooth samples non-adjacent to oral implants (27/33, 81.82% paper points, 28/31, 90.32% curette). Six patients (Patient 9, 16, 23, 25, 31 and 34) yielded *S. epidermidis* from paper point samples (6/33, 18.18%) ranging from 20 to 230 cfu (mean average density 83.33 cfu). Three of the patients (Patient 4, 6 and 9) yielded *S. epidermidis* from curette samples (3/31, 9.68%) range 10 to 30 cfu (mean average density 16.66 cfu) (Table 3.7). Only one of the patients yielded *S. epidermidis* from paper point and curette samples at the same site. *Staphylococcus aureus* was not recovered from any of the healthy tooth sites, non-adjacent to oral implants, that were sampled at the first visit. Four other staphylococcal species (other than *S. epidermidis* and *S. aureus*) were recovered from the patients that had samples taken from healthy teeth, non-adjacent to oral implants, at the first clinical visit. *Staphylococcus warneri* and *S. capitis* were recovered from paper point samples (Patients 34 and 9 respectively at 10 cfu and 20 cfu) (Table 3.7). Statistical analysis of the staphylococcal recovery data from healthy tooth sites located non-adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test,

Table 3.7. Staphylococcal species and cell density recovered from[a] healthy teeth located non-adjacent to implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse[c]**
1	A	0	NA	0
2	B	0	0	NA
4	B	0	10 *S. epidermidis*	0
6	A	0	30 *S. epidermidis*	45 *S. warneri*
7	F	0	NA	120 *S. epidermidis*
8	D	0	NA	0
	E	0	NA	-
9	F	230 *S. epidermidis* 20 *S. capitis*	10 *S. epidermidis*	30 *S. aureus*
10	D	0	0	990 *S. epidermidis* 15 *S. warneri*
	F	0	0	-
	G	NA	0	-
11	A	0	0	60 *S. epidermidis*
12	A	0	0	NA
13	E	0	0	210 *S. epidermidis*
	F	0	0	-
14	D	0	0	345 *S. epidermidis* 195 *S. aureus*
15	D	0	0	30 *S. warneri*
16	F	90 *S. epidermidis*	0	60 *S. epidermidis*
17	E	0	0	0
	F	0	0	-
18	D	0	0	2,775 *S. aureus*
19	A	0	0	15 *S. epidermidis*
20	D	0	0	1,785 *S. epidermidis* 1,605 *S. aureus*
22	D	0	0	30 *S. epidermidis*
23	D	20 *S. epidermidis*	0	0
24	A	0	0	375 *S. aureus*
25	A	120 *S. epidermidis*	0	360 *S. epidermidis* 60 *S. pasteuri*
	B	0	0	-
26	E	0	0	0
27	C	0	0	105 *S. epidermidis*
29	H	0	0	90 *S. lugdunensis*
30	A	0	0	0

Continued overleaf

Table 3.7 continued. Staphylococcal species and cell density recovered from[a] healthy teeth located non-adjacent to implants and from the oral cavity in periimplantitis patients at clinical visit 1 prior to treatment

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse[c]**
31	C	20 *S. epidermidis*	0	450 *S. epidermidis*
33	A	0	0	NA
34	A	20 *S. epidermidis* 10 *S. warneri*	0	90 *S. epidermidis* 60 *S. warneri* 1,335 *S. pasteuri*
35	B	0	0	15 *S. epidermidis* 375 *S. aureus*
38	A	0	0	0
39	D	NA	0	NA
40	C	0	0	15 *S. epidermidis* 15 *S. pasteuri*
	E	0	0	-
42	C	0	0	0

[a] Samples from healthy teeth located non-adjacent to implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only one oral rinse sample was taken at the first clinical evaluation prior to treatment, whereas in some cases several diseased implants were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased implant samples, where appropriate.

Abbreviations: NA, sample not available.

$p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi-Squared test, $p < 0.05$).

3.3.8.2 Oral rinse sampling

Of the thirty-four patients that had samples taken from healthy teeth that were non-adjacent to oral implants oral rinse samples were collected from twenty-nine. Of those twenty-nine patients nine did not yield any staphylococci (9/29, 31%). Fifteen patients yielded *S. epidermidis* (15/29, 51.72%) ranging from 15 to 1.7 x 10^3 cfu/ml oral rinse (mean average density 310 cfu/ml oral rinse). Six patients (Patients 9, 14, 18, 20, 24 and 35) yielded *S. aureus* in their oral rinse samples (6/29, 20.69%) range 30 to 2.7 x 10^3 cfu/ml oral rinse (mean average density 892.5 cfu/ml oral rinse). Seven of the twenty-nine patients where oral rinse samples were available yielded staphylococci other than *S. epidermidis* and *S. aureus*. Patients 6, 10, 15 and 34 yielded *S. warneri* at a range of 15 to 60 cfu/ml oral rinse (mean average density 37.5 cfu/ml oral rinse). Patients 25, 34 and 40 yielded *S. pasteuri* at a range of 15 to 1.3 x 10^3 cfu/ml oral rinse (mean average density 470 cfu/ml oral rinse). Patient 29 yielded *S. lugdunensis* at 90 cfu/ml oral rinse (Table 3.7).

3.3.9 Oral staphylococci recovered from oral rinse samples from patients with periimplantitis at clinical visit 1 prior to treatment

An additional eight periimplantitis patients not included in the data analysis described in section 3.3.4 above because key samples were not taken, not available, or not clearly identified also had oral rinse samples taken. In total 38 patients had oral rinses collected at the first (pre-treatment) clinical visit. Of these, 12 (12/38, 31.5%) did not yield any staphylococci from oral rinse samples (Table 3.8). Eighteen patients (18/38, 37.37%) yielded *S. epidermidis*, with densities ranging from 15 to 1.4 x 10^4 cfu per ml of oral rinse (mean average density 1 x 10^3 cfu per ml of oral rinse). Eight patients (8/38, 21%) yielded *S. aureus*, with densities ranging from 30 to 2.7 x 10^3 cfu per ml of oral rinse (mean value 825 cfu per ml of oral rinse). Seven patients yielded staphylococci other than *S. epidermidis* and *S. aureus*. Patients 6, 10, 15 and 34 yielded *S. warneri* (range 15 to 60 cfu per ml of oral rinse; mean average density 37.5 cfu per ml of oral rinse). Patients 25, 34 and 40 yielded *S. pasteuri*, (range 15-1.3 x 10^3 cfu per ml of oral rinse; mean average density 470 cfu per ml of oral rinse). Patient 29 yielded *S. lugdunensis*, 90 cfu per ml of oral rinse.

Table 3.8. Summary table showing staphylococcal species[a] and cell density recovered from oral rinse samples of patients[b] with periimplantitis at clinical visit 1 prior to treatment

Patient	Staphylococcal species and cell density in cfu/ml of oral rinse
1	0
3	90 *S. aureus*
4	0
5	0
6	45 *S. warneri*
7	120 *S. epidermidis*
8	0
9	30 *S. aureus*
10	990 *S. epidermidis*; 15 *S. warneri*
11	60 *S. epidermidis*
13	210 *S. epidermidis*
14	345 *S. epidermidis*; 195 *S. aureus*
15	30 *S. warneri*
16	60 *S. epidermidis*
17	0
18	2,775 *S. aureus*
19	15 *S. epidermidis*
20	1,785 *S. epidermidis*; 1,605 *S. aureus*
21	1,155 *S. aureus*
22	30 *S. epidermidis*
23	0
24	375 *S. aureus*
25	360 *S. epidermidis* ; 60 *S. pasteuri*
26	0
27	105 *S. epidermidis*
28	14,280 *S. epidermidis*
29	90 *S. lugdunensis*
30	0
31	450 *S. epidermidis*
32	0
34	90 *S. epidermidis*; 60 *S. warneri*; 1,335 *S. pasteuri*
35	15 *S. epidermidis*; 375 *S. aureus*
36	15 *S. epidermidis*
37	345 *S. epidermidis*
38	0
40	15 *S. epidermidis*; 15 *S. pasteuri*
41	0
42	0

[a] Species were identified based on the 16S rDNA nucleotide sequence as described in Chapter 2.
[b] Oral rinse samples were not available from four patients (patients 2, 12, 33 and 39).

Table 3.9. Summary data on staphylococcal species identified and average cell density recovered[a] by oral rinse, paper point and curette sampling for implant and natural tooth sites investigated in periimplantitis patients at clinical visit 1 prior to treatment

Category	Staphylococcal species identified	Paper points			Curette			Oral rinse		
		Cell density (cfu)[b]			Cell density (cfu)[b]			Cell density (cfu)[b]		
		No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean
Diseased implant	None	18/29 (62)	0	0	23/28 (82.1)	0	0	10/30 (33.3)	0	0
	S. epidermidis	9/29 (31)	10-400	61.7	1/28 (3.6)	N/A	1.7×10^4	14/30 (46.7)	15 to 1.7×10^3	343
	S. aureus	3/29 (10.3)	10-480	296.7	1/28 (3.6)	N/A	170	7/30 (23.3)	30 to 2.7×10^3	889.3
	Other species	6/29 (20.7)	10-920	161.7	2/28 (7.1)	N/A	10	3/30 (10)	15-90	55
Healthy implant	None	10/13 (76.9)	0	0	11/13 (84.6)	0	0	2/11 (18.2)	0	0
	S. epidermidis	2/13 (15.4)	10-100	55	2/13 (15.4)	10-40	25	5/11 (45.5)	15-990	243
	S. aureus	0/13			0/13			3/11 (27.3)	30 to 2.7×10^3	1.5×10^3
	Other species	1/13 (7.7)	N/A	20	0/13			5/11 (45.5)	15 to 1.3×10^3	169.5
Diseased tooth adjacent to an implant	None	5/8 (62.5)	0	0	8/8 (100)	0	0	2/8 (25)	0	0
	S. epidermidis	3/8 (37.5)	10-40	23.3	0/8			3/8 (37.5)	15-105	60
	S. aureus	0/8			0/8			2/8 (25)	90-375	232.5
	Other species	0/8			0/8			1/8 (12.5)	N/A	90
Diseased tooth not adjacent to an implant	None	19/25 (76)	0	0	3/22 (13.6)	0	0	6/22 (27.3)	0	0
	S. epidermidis	4/25 (16)	10 to3 x 10^3	760	3/22 (13.6)	10-770	263.3	10/22 (45.5)	15-990	271.5
	S. aureus	1/25 (4)	N/A	20	0/22	0	0	4/22 (18.2)	30 to 1.1×10^3	438.7
	Other species	1/25 (4)	N/A	40	1/22 (4.5)	N/A	10	5/22 (2.5)	15-90	48

Continued overleaf

Table 3.9 continued. Summary data on staphylococcal species identified and average cell density recovered[a] by oral rinse, paper point and curette sampling for implant and natural tooth sites investigated in periimplantitis patients at clinical visit 1 prior to treatment

Category	Staphylococcal species identified	Paper points			Curette			Oral rinse		
		Cell density (cfu)[b]			Cell density (cfu)[b]			Cell density (cfu)[b]		
		No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean
Healthy tooth adjacent to an implant	None	14/15 (93.3)	0	0	12/12 (100)	0	0	2/12 (16.7)	0	0
	S. epidermidis	1/15 (6.7)	N/A	230	0/12	N/A	N/A	6/12 (50)	15 to 1.7 x 10^3	425
	S. aureus	0/15	N/A	N/A	0/12	N/A	N/A	5/12 (41.7)	30 to 2.7 x 10^3	975
	Other species	0/15			0/12			1/12 (8.3)	N/A	30
Healthy tooth not adjacent to an implant	None	27/33 (81.8)	0	0	28/31 (90.3)	0	0	9/29 (31)	0	0
	S. epidermidis	6/33 (18.2)	20-230	83.33	3/31 (9.7)	10-30	16.66	15/29 (51.7)	15 to 1.7 x 10^3	310
	S. aureus	0/33	N/A	N/A	0/31	N/A	N/A	6/29 (20.7)	30 to 2.7 x 10^3	892.5
	Other species	2/33 (6.1)	10-20	15	0/31			7/29 (24.1)	15 to1.3 x 10^3	136.07
Oral rinse[d]	None							12/38 (31.5)	0	0
	S. epidermidis							18/38 (37.4)	15 to 1.4 x 10^4	1 x 10^3
	S. aureus							8/38 (21)	30 to 2.7 x 10^3	825
	Other species							7/38 (18.4)	15 to1.3 x 10^3	136.07

[a] Samples from implants and teeth were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.
[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide sequence (Chapter 2).
[c] Due to sampling difficulties, and unclear identification on laboratory submission, samples from some patients were not available from all category of site type, state of health and collection method. Total number of patients sampled in each category is represented by the denominator in the No. patients column.
[d] Oral rinse data for total number of patients in all categories.
Abbreviations: NA range not applicable (single sample, or all samples at the same value).

3.3.10 Summary of pre-treatment (visit 1) oral staphylococci and further statistical analyses

For all sites sampled (implant or tooth, assessed as healthy or diseased) the majority of patients yielded no staphylococci (Table 3.9). Sixty percent of diseased implant sites, 76.9 % of healthy implant sites, 62.5 % and 76 % of diseased teeth adjacent to and non-adjacent to oral implants, respectively, and 93.3% and 76.47% of healthy teeth adjacent to and non-adjacent to implants, respectively, yielded no staphylococci whatsoever (Table 3.9). A lower proportion of oral rinse samples were negative for staphylococci (31.5%).

3.3.10.1 Paper point samples

Staphylococcus epidermidis was present in 31% of patients that had samples taken from diseased oral implants, 37.5% and 16% of patients that had samples taken from diseased teeth adjacent, and non-adjacent, to oral implants, respectively. *Staphylococcus epidermidis* was present in paper point samples in 15.4% of patients that had samples taken from healthy implant sites and 6.7% and 18.2% of patients that had samples taken from healthy teeth adjacent, and non-adjacent, to oral implants, respectively, (Table 3.9). The abundance of *S. epidermidis* at these sites (mean and range per sample) were as follows: diseased implants 73 cfu/sample (range 10 to 400 cfu/sample), diseased teeth adjacent, and non-adjacent to oral implants 23.3 cfu/sample (range 10 to 40 cfu/sample) and 760 cfu/sample (range 10 to 3 x 10^3 cfu/sample), respectively. *Staphylococcus epidermidis* was found at healthy implant sites at a mean of 55 cfu/sample (range 10 to 100 cfu/sample), and at healthy teeth adjacent to oral implants at 230 cfu/sample from a single sample and at healthy teeth non-adjacent to oral implants at a mean of 83.33 cfu/sample (range 20 to 230 cfu/sample).

3.3.10.2 Curette samples

Staphylococcus epidermidis was present in 3.6% of patients that had samples taken from diseased oral implants, 13.6% of patients that had samples taken from diseased teeth that were non-adjacent to oral implants, and none from patients that had samples taken from diseased teeth that were adjacent to oral implants. *S. epidermidis* was present in curette samples from 15.4% of patients that had samples taken from healthy oral implants, 9.7% of patients that had samples taken from healthy teeth that were non-adjacent to oral implants, and no curette samples from patients that had samples taken from healthy teeth that were adjacent to oral implants harboured *S. epidermidis* (Table 3.9).

A comparison of the different states of health showed that there was no significant difference in the distribution of *S. epidermidis* cell densities across the different states of health sampled for each sampling site type (Independent Samples Mann-Whitney U test, $p > 0.05$), with no significant difference in the estimated average colony count median (Independent samples median test, $p > 0.05$). Similarly a comparison of the different site types showed that there was no significant difference in the distribution of *S. epidermidis* cell densities across the different sampling site types across at the various states of health sampled (Independent samples Kruskal Wallis test, $\alpha = 0.5$ $p > 0.05$), with no significant difference in the estimated average colony count median (Independent samples median test, $p > 0.05$).

Staphylococcus aureus was not recovered as often as *S. epidermidis*. *Staphylococcus aureus* was only found at diseased implants (present in paper point samples of 10.3% of patients, and curette samples from 3.6% of patients), diseased teeth non-adjacent to oral implants (4% of patients paper point samples) and oral rinse samples (21% of oral rinse samples) (Table 3.9). *Staphylococcus aureus* was not found at any 'healthy' tooth or implant sampling site (clearly identified on submission to the laboratory) at the first (pre-treatment) clinical visit. The estimated *S. aureus* cell densities within each sampling category were determined to have non-normal distributions therefore non-parametric statistical tests were employed. A comparison of the different sampling site types for the state of health 'diseased' (*S. aureus* was not recovered from any healthy sites) showed that there was no significant difference in the distribution of *S. aureus* cell densities (Independent Samples Mann Whitney U Test, $p > 0.05$). Similarly a non-parametric comparison of the median *S. aureus* cell densities across the different sampling site types (Independent samples median test, fishers exact significance $p > 0.05$) found no significant difference between the two populations. As *S. aureus* was not found at both states of health a comparison of distributions and means was not undertaken.

3.3.11 Oral staphylococci recovered from diseased implants in periimplantitis patients post-treatment

Fourteen patients had samples collected from implants classed as diseased on their second (post-treatment) clinical visit.

3.3.11.1 Paper point and curette sampling

The majority of patients had no staphylococci recovered from the implant sites sampled (10/14, 71.42% paper point, 11/14, 78.51% curette). Four of the patients (4/14, 28.57%) had *S. epidermidis* recovered from paper point samples (ranging from 40 to 3.3 x 10^6 cfu/sample with a per patient mean of 3.9 x 10^5 cfu/sample). Three of the same four patients (3/14, 21.43% of the patients sampled) were also *S. epidermidis*-positive at curette sites (ranging from 1 x 10^4 to 4.9 x 10^6 estimated cfu/sample with a mean value of 1.5 x 10^6 cfu/sample). No *S. aureus* isolates were recovered from any of the diseased implant sites (Table 3.10). Statistical analysis of the staphylococcal recovery data from periimplantitis sites (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the two categories of staphylococcal species present ("No staphylococci recovered" and *S. epidermidis*) did not occur with equal probabilities of 0.5 (One-sample Binomial test, $p < 0.05$).

3.3.11.2 Oral rinse sampling

Fewer patients yielded no staphylococci then on the initial clinical (pre-treatment) visit, 3/11 (27.27%). Seven patients yielded *S. epidermidis* from oral rinse samples (7/11, 63.64%) ranging from 30 to 1.3 x 10^3 cfu/ml oral rinse, mean 475.7 cfu/ml oral rinse. Interestingly the only patient who's oral rinse *S. epidermidis* load was anywhere near the paper point and curette recoveries (Patient 30) did not have any *S. epidermidis* recovered from their diseased implant samples (Table 3.10). No *S. aureus* isolates were recovered from any oral rinses samples of patients with diseased implants, on the second (post-treatment) clinical visit. Three other staphylococcal species were recovered from the oral rinse samples. *Staphylococcus warneri* was recovered from two patients (at 60 and 195 cfu/ml oral rinse, mean 127.5 cfu/ml oral rinse), *S. capitis*, *S. caprae* and *S. pasteuri* were found from single patients (at 15 cfu/ml oral rinse, 210 cfu/ml oral rinse and 45 cfu/ml oral rinse, respectively) (Table 3.10).

Table 3.10. Staphylococcal species and cell density recovered from[a] diseased implants and from the oral cavity in periimplantitis patients post-treatment at clinical visit 2

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient[c]	**Implant site**	**Paper Point**	**Curette**	**Oral rinse[d]**
8	B	0	0	0
9	A	8.6×10^4 *S. epidermidis*	1.4×10^4 *S. epidermidis*	195 *S. warneri*
	D	2.6×10^5 *S. epidermidis*	1×10^4 *S. epidermidis*	-
10	A	150 *S. epidermidis*	0	NA
	B	120 *S. epidermidis*	0	-
	C	40 *S. epidermidis*	NA	-
16	C	1.2×10^5 *S. epidermidis*	1×10^5 *S. epidermidis*	540 *S. epidermidis* 15 *S. caprae*
	D	7.5×10^4 *S. epidermidis*	9.7×10^4 *S. epidermidis*	-
17	A	0	0	NA
21	D	0	0	30 *S. epidermidis*
	F	0	0	-
23	A	0	0	45 *S. epidermidis*
25	C	0	0	0
29	C	0	0	0
	A	0	0	-
30	A	0	0	1,305 *S. epidermidis* 60 *S. warneri* 210 *S. capitis*
	B	0	0	-
31	B	0	0	570 *S. epidermidis*
32	F	0	0	NA
36	A	5.9×10^5 *S. epidermidis*	0	165 *S. epidermidis*
	D	3.3×10^6 *S. epidermidis*	4.9×10^6 *S. epidermidis*	-
	E	4,830 *S. epidermidis*	4.2×10^6 *S. epidermidis*	-
	I	0	0	-
40	A	0	0	60 *S. epidermidis* 45 *S. pasteuri*
	D	0	0	-

[a] Samples from diseased implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide sequence (Chapter 2).

[c] Only patients who presented at the second clinical visit with diseased implants were sampled.

[d] Only one oral rinse sample was taken, whereas in some cases several diseased implants were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased implant samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.12 Oral staphylococci recovered from healthy implants in periimplantitis patients post-treatment at clinical visit 2

Fourteen patients had samples collected from an implant described as healthy on the second (post-treatment) clinical visit. The majority (9/14 64.28% paper point, 11/14, 78.57% curette) had no staphylococci recovered from those implant samples (Table 3.11).

3.3.12.1 Paper point and curette sampling

Three of the patients (Patients 7, 9 and 36) had *S. epidermidis* recovered from paper point samples (3/14, 21.43%), ranging from 20 to 1.1 x 10^7 cfu/sample, with a mean value of 2.5 x 10^6 cfu/sample. The same three patients (3/14, 21.43%) also yielded *S. epidermidis* from curette samples ranging from 210 to 7.2 x 10^6 cfu/sample (mean across patients 1.8 x 10^6 cfu/sample). One patient had *S. aureus* present in a paper point sample (Patient 26, 30 cfu/sample). *Staphylococcus warneri* was recovered from a paper point sample from a single patient (Patient 34, 20 cfu/sample) (Table 3.11). Statistical analysis of the samples taken from healthy implant sites (including sites that did not yield staphylococci) indicated that the distribution of the estimated cfu/sample values was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi squared test, $p < 0.05$).

3.3.12.2 Oral rinse sampling

Staphylococcus epidermidis was recovered from oral rinse samples of five patients (5/11, 45.45%) with a range of 15 to 165 cfu/ml oral rinse, mean 75 cfu/ml oral rinse. *Staphylococcus aureus* was recovered from a single patient (Patient 11, 855 cfu/ml oral rinse). Other staphylococci were recovered from three patients, two patients yielded *S. warneri* (60-195 cfu/ml oral rinse, mean 127.5) and a single patient yielded *S. pasteuri* at 45 cfu/ml oral rinse (Table 3.11).

Table 3.11. Staphylococcal species and cell density recovered from[a] healthy implants and from the oral cavity in periimplantitis patients post-treatment at clinical visit 2

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient[c]	**Implant site**	**Paper Point**	**Curette**	**Oral rinse[d]**
7	A	11.7 x 10^6 *S. epidermidis*	7.2 x 10^6 *S. epidermidis*	0
	C	7.2 x 10^6 *S. epidermidis*	0	-
	E	6.3 x 10^4 *S. epidermidis*	210 *S. epidermidis*	-
9	C	2.5 x 10^4 *S. epidermidis*	9.1 x 10^4 *S. epidermidis*	195 *S. warneri*
10	C	0	0	NA
11	A	0	0	855 *S. aureus* 60 *S. warneri*
19	D	0	0	15 *S. epidermidis*
23	B	0	0	45 *S. epidermidis*
25	A	0	0	0
26	A	30 *S. aureus*	0	0
	B	0	0	-
	C	0	0	-
29	F	0	0	0
32	A	0	0	NA
	C	NA	0	-
34	D	20 *S. warneri*	0	NA
36	B	2 x 10^6 *S. epidermidis*	0	165 *S. epidermidis*
	C	1.4 x 10^6 *S. epidermidis*	0	-
	J	20 *S. epidermidis*	1.7 x 10^6 *S. epidermidis*	-
39	B	0	0	105 *S. epidermidis*
40	B	0	0	60 *S. epidermidis* 45 *S. pasteuri*

[a] Samples from healthy implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only patients who presented at the second clinical visit with healthy implants were sampled.

[d] Only one oral rinse sample was taken, whereas in some cases several diseased implants were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased implant samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.13 Oral staphylococci recovered from teeth with periodontitis located adjacent to oral implants in patients with periimplantitis post-treatment at clinical visit 2

Samples from teeth that were assessed to be in a diseased state at the second (post-treatment) clinical visit, located adjacent to oral implants, were collected from seven patients. Of these the majority (5/7, 71.43% paper point, 5/7, 71.43% curette) yielded no staphylococci.

3.3.13.1 Paper point and curette sampling

The remaining two patients, patients 7 and 16, (2/7, 28.57%) both yielded *S. epidermidis* from the paper point and curette samples. The ranges were 960 to 9 x 10^4 cfu/sample (mean 4.5 x 10^4 cfu/sample) for paper points and 2.4 x 10^6 to 3.6 x 10^4 cfu/sample (mean 1.2 x 10^6 cfu/sample) for the curette samples. No other staphylococci were recovered from the tooth sites (Table 3.12). Statistical analysis of the staphylococcal recovery data from tooth sites with associated periodontitis located adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the two categories of staphylococcal species present ("No staphylococci recovered" and *S. epidermidis*) occurred with equal probabilities of 0.5 (One-sample Binomial test, $p > 0.05$).

3.3.13.2 Oral rinse sampling

Three patients (Patients 16, 23 and 31) had *S. epidermidis* recovered from their oral rinses (3/5, 60%) ranging from 45 to 570 cfu/ml (mean 385 cfu/ml oral rinse). A single patient, patient 11, (1/5, 20%) yielded *S. aureus* from the oral rinse sample (855 cfu/ml oral rinse). Two other staphylococci were recovered from a single patient each, *S. warneri* (60 cfu/ml oral rinse) and *S. caprae* (15 cfu/ml oral rinse) (Table 3.12).

Table 3.12. Staphylococcal species and cell density recovered from[a] teeth with associated periodontitis adjacent to implants and from the oral cavity in periimplantitis patients post-treatment at clinical visit 2

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse**
7	D	960 *S. epidermidis*	2.4×10^6 *S. epidermidis*	0
10	F	0	0	NA
11	B	0	0	855 *S. aureus* 60 *S. warneri*
16	B	9×10^4 *S. epidermidis*	3.6×10^4 *S. epidermidis*	540 *S. epidermidis* 15 *S. caprae*
17	B	0	0	NA
23	C	0	0	45 *S. epidermidis*
31	A	0	0	570 *S. epidermidis*

[a] Samples from teeth with associated periodontitis were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

Abbreviations: NA, sample not available.

3.3.14 Oral staphylococci recovered from teeth with periodontitis located non-adjacent to oral implants in patients with periimplantitis post-treatment at clinical visit 2

Samples from teeth that were assessed to be in a diseased state at the second (post-treatment) clinical visit, that were not located adjacent to oral implants, were collected from twelve patients. Of these a slim majority (7/11, 63.63% paper point, 6/11, 54.54% curette) yielded no staphylococci.

3.3.14.1 Paper point and curette sampling

Four patients (Patients 10, 16, 30 and 31) yielded *S. epidermidis* from their paper point samples (4/11, 36.36%) ranging from 10 to 2.1 x 10^5 cfu/sample (mean 5.6 x 10^4 cfu/sample). Three patients (Patients 8, 10 and 12) had *S. epidermidis* recovered from curette samples (3/11, 27.27%) ranging from 10 to 80 cfu/sample (mean 36.6 cfu/sample). No *S. aureus* was recovered from any tooth sample (Table 3.13). *Staphylococcus equorum* was recovered from a single curette sample (10 cfu/sample). Statistical analysis of the staphylococcal recovery data from tooth sites with associated periodontitis located non-adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi squared test, $p > 0.05$).

3.3.14.2 Oral rinse sampling

Three patients did not yield any staphylococci from their oral rinse samples (3/11, 27.27%). Seven patients (Patients 12, 16, 21, 23, 30, 31 and 40) did yield *S. epidermidis* from their oral rinse samples (7/11, 63.63%) ranging from 30 to 1.3 x 10^3 cfu/ml oral rinse (mean 407.14 cfu/ml oral rinse). A single patient (Patient 11) yielded *S. aureus* in the oral rinse (1/11, 9.09%) at 855 cfu/ml oral rinse. Four other staphylococci were recovered from four patients. Two patients yielded *S. warneri* (both at 60 cfu/ml oral rinse). *Staphylococcus caprae, S. capitis and S. pasteuri* were recovered from one patient each (at 15 cfu/ml oral rinse, 210 cfu/ml oral rinse and 45 cfu/ml oral rinse respectively) (Table 3.13).

Table 3.13. Staphylococcal species and cell density recovered from[a] teeth with associated periodontitis located non-adjacent to implants and from the oral in periimplantitis patients post-treatment at clinical visit 2

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse[c]**
7	G	0	0	0
8	C	0	80 *S. epidermidis*	0
10	E	80 *S. epidermidis*	NA	NA
	F	30 *S. epidermidis*	NA	-
	G	0	10 *S. epidermidis*	-
11	C	0	0	855 *S. aureus* 60 *S. warneri*
12	C	NA	20 *S. epidermidis* 10 *S. equorum*	300 *S. epidermidis*
	D	NA	0	-
16	G	2.1 x 10^5 *S. epidermidis*	NA	540 *S. epidermidis* 15 *S. caprae*
21	A	0	0	30 *S. epidermidis*
	C	0	0	-
23	D	0	0	45 *S. epidermidis*
26	D	0	0	0
	E	0	0	-
30	D	6,390 *S. epidermidis*	0	1,305 *S. epidermidis* 60 *S. warneri* 210 *S. capitis*
31	D	10 *S. epidermidis*	0	570 *S. epidermidis*
40	E	0	0	60 *S. epidermidis* 45 *S. pasteuri*

[a] Samples from teeth with associated periodontitis were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only one oral rinse sample was taken, whereas in some cases several diseased tooth sites were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased tooth site samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.15 Oral staphylococci recovered from healthy teeth located adjacent to oral implants in patients with periimplantitis post-treatment at clinical visit 2

Samples from teeth that were assessed to be healthy at the second (post-treatment) clinical visit, located adjacent to oral implants, were collected from nine patients The majority of these (7/8, 87.5% paper points, 7/8, 87.5% curettes) yielded no staphylococci.

3.3.15.1 Paper point and curette sampling

A single patient (Patient 9) yielded *S. epidermidis* from both paper point and curette samples (1/8, 12.5% paper point 1/8, 12.5% curette) at 1.4×10^5 cfu/sample and 7×10^3 cfu/sample, respectively. No other staphylococci were recovered from the tooth samples (Table 3.14). Statistical analysis of the staphylococcal recovery data from healthy tooth sites located adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the two categories of staphylococcal species present ("No staphylococci recovered" and *S. epidermidis*) did not occur with equal probabilities of 0.5 (One-sample Binomial test, $p < 0.05$).

3.3.15.2 Oral rinse sampling

Three of the patients did not yield any staphylococci from their oral rinse samples (3/11, 42.86%). Another three patients (Patients 12, 19 and 39) yielded *S. epidermidis* from their oral rinse samples (3/7, 42.86%) ranging from 15-300 cfu/ml oral rinse (mean 140 cfu/ml oral rinse), though the single patient that had *S. epidermidis* present at the tooth sites was not one of them. A single patient had *S. warneri* recovered from an oral rinse sample at 195 cfu/ml oral rinse (Table 3.14).

Table 3.14. Staphylococcal species and cell density recovered from[a] healthy teeth adjacent to implants and from the oral cavity in periimplantitis patients post-treatment at clinical visit 2

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse**
8	A	0	0	0
9	B	1.4×10^5 *S. epidermidis*	7,000 *S. epidermidis*	195 *S. warneri*
12	B	0	0	300 *S. epidermidis*
17	D	0	0	NA
19	C	0	0	15 *S. epidermidis*
25	B	0	0	0
29	D	0	0	0
32	E	0	NA	NA
39	C	NA	0	105 *S. epidermidis*

[a] Samples from healthy teeth adjacent to implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

Abbreviations: NA, sample not available.

3.3.16 Oral staphylococci recovered from healthy teeth located non-adjacent to oral implants in patients with periimplantitis post-treatment at clinical visit 2

Samples from teeth that were assessed to be healthy at the second (post-treatment) clinical visit, that were not located adjacent to oral implants, were collected from seventeen patients. The majority of these (10/16, 62.5% paper points, 14/17, 82.35% curette) did not yield any staphylococci from the tooth sites.

3.3.16.1 Paper point and curette sampling

Five patients (Patients 7, 9, 16, 31 and 39) yielded *S. epidermidis* from paper point samples (5/16, 31.25%) ranging from 10-1.4 x 10^5 cfu/sample (mean 4.8 x 10^4 cfu/sample). Two patients (Patients 9 and 16) had *S. epidermidis* recovered from curette samples (2/17, 11.6%) at 4.2 x 10^5 cfu/sample and 8.6 x 10^4 cfu/sample respectively (mean 2.5 x 10^5 cfu/sample). No patients yielded *S. aureus* at the tooth sites (Table 3.15). A single patient had *S. warneri* recovered from a paper point sample (2.1 x 10^5 cfu/sample), another patient had *S. pasteuri* recovered from a curette sample (10 cfu/sample). Statistical analysis of the staphylococcal recovery data from healthy tooth sites located non-adjacent to oral implants (including sites that did not yield staphylococci) indicated that the staphylococcal density distribution in cfu/sample was not normal (One-sample Kolmogorov-Smirnov test, $p < 0.05$) and that the staphylococcal species present did not occur with equal probabilities (One-sample Chi squared test, $p < 0.05$).

3.3.16.2 Oral rinse sampling

Four of the thirteen patients that oral rinse samples were available for yielded no staphylococci (4/13, 30.78%). *Staphylococcus epidermidis* was recovered from seven patients, Patients 12, 16, 19, 23, 31, 39 and 60, (7/13, 53.85%) ranging from 15-540 cfu/ml oral rinse (mean 233.57 cfu/ml oral rinse). One patient had *S. aureus* recovered from the oral rinse (1/13, 7.69%) at 855 cfu/ml oral rinse. Three other staphylococcal species were recovered from four patients. *Staphylococcus warneri* was recovered from two patients at 60 cfu/ml oral rinse and 195 cfu/ml oral rinse (mean 127.5 cfu/ml oral rinse). *Staphylococcus caprae* and *S. pasteuri* were recovered from a single patient each at 15 cfu/ml oral rinse and 45 cfu/ml oral rinse, respectively (Table 3.15).

Table 3.15. Staphylococcal species and cell density recovered from[a] healthy teeth located non-adjacent to implants and from the oral cavity in periimplantitis patients post-treatment at clinical visit 2

		Staphylococcal species and cell density in cfu[b] recovered by three different sampling methods		
Patient	**Tooth site**	**Paper Point**	**Curette**	**Oral rinse[c]**
7	F	40 *S. epidermidis*	0	0
8	D	0	0	0
9	F	1.4×10^5 *S. epidermidis*	4.2×10^5 *S. epidermidis*	195 *S. warneri*
10	D	NA	0	NA
11	D	0	0	855 *S. aureus* 60 *S. warneri*
12	A	0	0	300 *S. epidermidis*
16	F	8.6×10^4 *S. epidermidis*	8.6×10^4 *S. epidermidis*	540 *S. epidermidis* 15 *S. caprae*
17	E	0	0	NA
	F	0	0	-
19	A	0	0	15 *S. epidermidis*
	E	0	0	-
23	D	0	0	45 *S. epidermidis*
25	D	0	0	0
	E	0	0	-
29	E	NA	0	0
	G	2.1×10^5 *S. warneri*	0	-
	H	0	0	-
31	C	10 *S. epidermidis*	0	570 *S. epidermidis*
32	C	0	NA	NA
	G	0	0	-
34	B	0	10 *S. pasteuri*	-
39	D	10 *S. epidermidis*	0	105 *S. epidermidis*
	E	0	0	-
40	C	0	0	60 *S. epidermidis* 45 *S. pasteuri*

[a] Samples from healthy teeth located non-adjacent to implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

[c] Only one oral rinse sample was taken, whereas in some cases several healthy tooth sites were sampled. Therefore, only one data value is provided for oral rinse sampling for each patient and a dash symbol (-) is included in the oral rinse column opposite the second and subsequent diseased tooth site samples, where appropriate.

Abbreviations: NA, sample not available.

3.3.17 Oral staphylococci recovered from oral rinse samples from patients with periimplantitis post-treatment at clinical visit 2

Seventeen patients had oral rinse samples taken at a second (post-treatment) visit. Of those five (29.41%) had no staphylococci recovered. Ten patients (Patients 12, 16, 19, 21, 23, 30, 31, 36, 39 and 40) yielded *S. epidermidis* (10/17, 58.82%) ranging from 15 to 1.3 x 10^3 cfu/ml oral rinse (mean 313.5 cfu/ml oral rinse). Five patients yielded a total of four other staphylococcal species. *Staphylococcus warneri* was recovered from three patients range 60 to 195 cfu/ml oral rinse (mean 105 cfu/ml oral rinse). *Staphylococcus caprae, S. capitis and S. pasteuri* were recovered from one patient each (at 15 cfu/ml oral rinse, 210 cfu/ml oral rinse and 45 cfu/ml oral rinse, respectively) (Table 3.16).

3.3.18 Summary of initial post-treatment staphylococcal data and further statistical analyses

The results from clinical visit two revealed a vast increase in the density of *S. epidermidis* recovered from paper point and curette samples (Tables 3.9 and 3.17). In contrast, the recovery of *S. epidermidis* from oral rinse samples did not increase that dramatically. A single patient did yield a very high *S. epidermidis* load from an oral rinse sample, and also from a diseased tooth adjacent to an implant, but not at any diseased implant sites (no other sites were returned). Despite the increase in the density of *S. epidermidis* recovered, the percentage of patients in each category that harboured the bacteria did not differ greatly from the corresponding data obtained from patients at clinical visit 1 prior to treatment (Tables 3.9 and 3.17). The percentage of patients that yielded *S. aureus* at tooth or implant sites decreased between the pre-treatment clinical visit (visit 1) and the initial post-clinical visit (visit 2). A single sample (which had been taken from a healthy implant site) yielded *S. aureus*. The proportion of patients yielding *S. aureus* from oral rinse sample also decreased from the pre-treatment visit. *Staphylococcus aureus* was recovered from a single oral rinse sample, from a different patient to the one from whom *S. aureus* was recovered at an implant site.

A comparison of the different states of health showed that there was no significant difference in the distribution of *S. epidermidis* cell densities across the different states of health sampled (Independent samples Mann-Whitney U test, $p > 0.05$) or the sampling site

Table 3.16. Summary table showing staphylococcal species[a] and cell density recovered from oral rinse samples of patients with periimplantitis post-treatment at clinical visit 2

Patient	Staphylococcal species and cell density in cfu/ml of oral rinse
7	0
8	0
9	195 *S. warneri*
11	855 *S. aureus* 60 *S. warneri*
12	300 *S. epidermidis*
16	540 *S. epidermidis* 15 *S. caprae*
19	15 *S. epidermidis*
21	30 *S. epidermidis*
23	45 *S. epidermidis*
25	0
26	0
29	0
30	1,305 *S. epidermidis*; 60 *S. warneri*; 210 *S. capitis*
31	570 *S. epidermidis*
36	165 *S. epidermidis*
39	105 *S. epidermidis*
40	60 *S. epidermidis*; 45 *S. pasteuri*

[a] Species were identified based on the 16S rDNA nucleotide Sequence and BLAST analysis as described in Chapter 2.

type (Independent Samples Kruskal-Wallis Test, $p > 0.05$), with the exception of diseased sites where teeth non-adjacent to oral implants had a different distribution to all other sampling sites (Independent Samples Kruskal-Wallis Test, $p < 0.05$). However, on the second visit samples were only collected from three diseased sites located adjacent to an oral implant, from two patients resulting in a normal distribution across the three sample sites. Similarly a non-parametric comparison of the median *S. epidermidis* cell densities across the different sample states of health showed no significant difference (Independent Samples median test, $p > 0.05$), nor was there any statistically significant difference in the median *S. epidermidis* cell density across the different sampling site types (Independent Samples median test, $p > 0.05$).

As *S. aureus* was only recovered from a single (non-oral rinse) site/state of health, no statistical tests were performed.

As noted earlier there was an increase in the staphylococcal load at the initial post-treatment clinical visit, visit 2 (Tables 3.9 and 3.17). An overall comparison of the *S. epidermidis* populations confirmed that this was statistically significant (Independent samples Mann-Whitney U test, $P<0.05$, Independent Samples median test, $p < 0.05$). A comparison of the *S. aureus* populations pre- and post-treatment found no significant differences (Independent samples Mann-Whitney U test, $p > 0.05$, Independent Samples median test, $p > 0.05$), despite the low sample size. Breaking down the two visit populations into their sub-categories (healthy and diseased implants, teeth adjacent to implants and teeth not adjacent to implants), the only significant difference in the populations appears in implants (both healthy and diseased) (Independent samples Mann-Whitney U test, $p < 0.05$), with samples collected at clinical visit two yielding higher *S. epidermidis* densities (in cfu/sample).

Table 3.17. Summary data on staphylococcal species identified and average cell density recovered[a] for implant and natural tooth sites investigated in periimplantitis patients at clinical visit 2 post-treatment

Category	Staphylococcal species identified	Paper points Cell density (cfu)[b]			Curette Cell density (cfu)[b]			Oral rinse Cell density (cfu)[b]		
		No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean
Diseased implant	None	10/14 (71.4)	0	0	11/14 (78.5)	0	0	3/11 (27.3)	0	0
	S. epidermidis	4/14 (28.6)	40 to3.3 x 10^6	3.6 x 10^5	3/14 (21.4)	1 x 10^4-4.9 x 10^6	1.5 x 10^6	7/11 (63.6)	30 to 1.3 x 10^3	475.7
	S. aureus	0/14			0/14			0/7		
	Other species	0/14			0/14			4/7 (57.1)	15-210	97.5
Healthy implant	None	9/14 (64.3)	0	0	11/14 (78.6)	0	0	4/11		
	S. epidermidis	3/14 (21.4)	20 to1.1 x 10^7	2.5 x 10^6	3/14 (21.4)	210 to 7.2 x 10^6	1.8 x 10^6	5/11 (45.5)	15-165	75
	S. aureus	1/14 (7.1)	N/A	30	0/14			1/11 (9.1)	N/A	855
	Other species	1/14 (7.1)	N/A	20	0/14			3/11 (27.3)	45-195	100
Diseased tooth adjacent to implant	None	5/7 (71.4)	0	0	5/7 (71.4)	0	0	1/5 (20)	0	0
	S. epidermidis	2/7 (28.6)	960 to 9 x 10^4	4.5 x 10^4	2/7 (28.6)	3.6 x 10^4-2.4 x 10^6	1.2 x 10^6	3/5 (60)	45-570	385
	S. aureus	0/7			0/7			1/5 (20)	855	855
	Other species	0/7			0/7			2/5 (40)	15-60	37.5
Diseased tooth not adjacent to implant	None	7/11 (63.6)	0	0	6/11 (54.5)	0	0	3/11 (27.3)	0	0
	S. epidermidis	4/11 (36.4)	10 to 2.1 x 10^5	5.6 x 10^4	3/11 (27.3)	10-80	36.6	7/11 (63.6)	30 to1.3 x 10^3	407.14
	S. aureus	0/11			0/11			1/11 (9.1)	855	855
	Other species	0/11			1/11 (9.09)	N/A	10	4/11 (36.4)	15-210	63.75

Continued overleaf

Table 3.17 continued. Summary data on staphylococcal species identified and average cell density recovered[a] for implant and natural tooth sites investigated in periimplantitis patients at clinical visit 2 post-treatment

Category	Staphylococcal Species identified	Paper points Cell density (cfu)[b]			Curette Cell density (cfu)[b]			Oral rinse Cell density (cfu)[b]		
		No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean	No. patients[c] (%)	Range	Mean
Healthy tooth adjacent to implant	None	7/8 (87.5)	0	0	7/8 (87.5)	0	0	3/7 (42.9)	0	0
	S. epidermidis	1/8 (12.5)	1.4×10^5	1.4×10^5	1/8 (12.5)	N/A	7×10^3	3/7 (42.9)	15-300	140
	S. aureus	0/8			0/8			0/7		
	Other species	0/8			0/8			1/7 (14.3)	N/A	195
Healthy tooth not adjacent to implant	None	10/16 (62.5)	0	0	14/17 (82.4)	0	0	4/13 (30.8)	0	0
	S. epidermidis	5/16 (31.25)	10 to 1.4×10^5	4.8×10^4	2/17 (11.8)	8.6×10^4-4.2×10^5	2.5×10^5	7/13 (53.8)	15-540	233.57
	S. aureus	0/16			0/17			1/13 (7.7)	855	855
	Other species	1/16 (6.25)	N/A	2.1×10^5	1/17 (5.9)	N/A	10	4/13 (30.8)	15-195	78.75
Oral rinse[4]	No staphylococci							5/17 (29.4)		
	S. epidermidis							10/17 (58.8)	15 to 1.3×10^3	313.5
	S. aureus							1/17 (5.9)	N/A	855
	Other species							5/17 (29.4)	15-210	90

[a] Samples from diseased implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.
[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide sequence (Chapter 2).
[c] Only patients who presented at the second clinical visit with diseased implants were sampled.
[d] Oral rinse data for total number of patients in all categories.
Abbreviations: NA range not applicable (single sample, or all samples at the same value).

3.3.19 Oral staphylococci recovered from periimplantitis patients at post-treatment clinical visits subsequent to clinical visit 2

Sampling was carried out on those patients who continued to attend clinical consultations subsequent to the initial post-treatment clinical visit (visit 2). Only 16 patients attended for a third assessment and clinical sampling, and far fewer for a fourth, fifth and sixth. The majority of patients had samples collected from oral implants assessed to be healthy, and teeth that were adjacent to oral implants, also assessed as healthy (rather than sites diagnosed with periimplantitis or associated periodontitis). It is possible that the subsequent visit sample population has been self-selected to bias patients with ongoing periimplantitis or periodontal disease (causing the patients discomfort and pain, necessitating multiple return visits to the attending dentist). Patients who were not experiencing any symptoms may have declined to attend further clinics. However, if that was the case then clinical samples from periimplantitis and periodontal sites were not adequately identified on submission to the laboratory (and thus were removed from the sample set) as they are in the minority of sampling sites in this population. Due to the low numbers of patients that attended clinical visits subsequent to the initial post-treatment visit (visit 2) data collected from visits 3-6 were pooled into one data set, a summary of which is presented below (Table 3.18). As with the pre-treatment (visit 1), and initial post-treatment (visit 2), sampling *S. epidermidis* was the most prevalent species recovered from all sites sampled. At one visit or another *S. epidermidis* was present in the oral rinses of 16/17 patients that attended, and in over half of all patients from whom paper point samples were taken from healthy implants. Due to the sparse numbers of samples for each sampling category the mean values may have been skewed by high or low values, but in general it would appear that when *S. epidermidis* was isolated, it was recovered at abundances slightly lower than the initial post-treatment visit (clinical visit 2). None of the categories of sampling site and state of health tested had a normal distribution of bacterial cfu/sample (all One-sample Kolmogorov-Smirnov test, $p < 0.05$). None of the categories of sampling site and state of health tested had an equal probability of each species occurring (all One-sample Chi-Squared tests, or One-sample Binomial tests, $p < 0.05$).

Table 3.18. Summary data on staphylococcal species identified and average cell density recovered[a] for implant and natural tooth sites investigated in periimplantitis patients at subsequent clinical visits post-treatment

Category	Staphylococcal species	Cell density (cfu)[b]								
		Paper Point			Curette			Oral rinse[c]		
		No. patients (%)[d]	Range	Mean	No. patients (%)[d]	Range	Mean	No. patients (%)[d]	Range	Mean
Diseased Implant										
	None	6/6 (100.0%)			6/6 (100.0%)					
	S aureus	0/6			0/6					
	S epidermidis	1/6 (16.7%)	10-50	28	2/6 (33.3%)	10-430	150			
	Other staphylococci	0/6			0/6					
Healthy Implant										
	None	12/13 (92.3%)			13/13 (100.0%)					
	S aureus	0/13			0/13					
	S epidermidis	7/13 (53.8%)	10 to 8×10^5	7×10^4	2/13 (15.4%)	10 to 9×10^5	3×10^5			
	Other staphylococci	2/13 (15.4%)	10 to 20	13	0/13					
Diseased tooth adjacent to an implant										
	None	4/4 (100.0%)			2/2 (100.0%)					
	S aureus	0/4			0/2					
	S epidermidis	0/4			0/2					
	Other staphylococci	0/4			0/2					

Continued overleaf

Table 3.18 continued. Summary data on staphylococcal species identified and average cell density recovered[a] for implant and natural tooth sites investigated in periimplantitis patients at subsequent clinical visits post-treatment

Category	Staphylococcal species	Cell density (cfu)[b]								
		Paper Point			Curette			Oral rinse[c]		
		No. patients (%)[d]	Range	Mean	No. patients (%)[d]	Range	Mean	No. patients (%)[d]	Range	Mean
Healthy tooth adjacent to an implant										
	None	10/11 (90.9%)			10/12 (83.3%)					
	S aureus	0/11			0/12					
	S epidermidis	3/11 (27.3%)	10 to 7×10^4	2×10^4	2/12 (16.7%)	5×10^4 to 3.9×10^6	2×10^6			
	Other staphylococci	1/11 (9.1%)	N/A	10	1/12 (8.3%)	N/A	20			
Diseased teeth not adjacent to implants										
	None	4/5 (80.0%)			4/4 (100.0%)					
	S aureus	0/5			0/4					
	S epidermidis	1/5 (20.0%)	N/A	300	0/4					
	Other staphylococci	0/5			0/4					

Continued overleaf

Table 3.18 continued. Summary data on staphylococcal species identified and average cell density recovered[a] for implant and natural tooth sites investigated in periimplantitis patients at subsequent clinical visits post-treatment

Category	Staphylococcal species	Cell density (cfu)[b]								
		Paper Point			Curette			Oral rinse[c]		
		No. patients (%)[d]	Range	Mean	No. patients (%)[d]	Range	Mean	No. patients (%)[d]	Range	Mean
Healthy teeth not adjacent to implants										
	None	12/12 (100.0%)			12/12 (100.0%)					
	S aureus	0/12			0/12					
	S epidermidis	5/12 (41.7%)	10 to 5.8×10^5	8×10^4	1/12 (8.3%)	N/A	5×10^4			
	Other staphylococci	1/12 (8.3%)	N/A	10	0/12					
Oral rinses[c]										
	None							5/17 (29.4%)		
	S aureus							7/17 (41.2%)	15 to 5,670	1,127
	S epidermidis							16/17 (94.1%)	15 to 2,130	209
	Other staphylococci							5/17 (29.4%)	15 to 1,470	357

[a] Samples from teeth and implants were taken using both paper points and curettes. The oral cavity was sampled by oral rinse sampling.

[b] Staphylococcal cell density in cfu recovered from three paper point samples, one curette sample and from 1 ml of oral rinse sample. Species were identified based on the 16S rDNA nucleotide sequence (Chapter 2).

[c] Oral rinse data for total number of patients in all categories.

[d] Individual patients may have had samples taken from multiple locations of the same sampling site type and state of health (e.g. diseased implant) at each visit. Where those multiple samples yielded different results e.g. one had no staphylococci whilst another yielded *S. epidermidis* the individual patient would have been counted in each category resulting in total percentages of >100 for some site types and states of health and ranges and means of cfu/sample where a species was only present in one patient but at multiple sampling sites.

Abbreviations: NA range not applicable (single sample, or all samples at the same value).

3.4 Discussion

3.4.1 Staphylococci associated with implants

As with all other sampling sites (except for oral rinse samples) the majority of periimplantitis sampling sites yielded no staphylococci by culture (63.9% of paper point and 92.5% of curette samples prior to treatment at visit 1, 63% of paper point and 75% of curette samples post-treatment at visit 2, and 71.4% of paper point and 75% of curette samples at the pooled post-treatment visits 3-6, (Table 3.1). When the data was analysed by patient rather than by sample site the results were the same. The majority of patients that had samples taken from one or more periimplantitis site(s) yielded no staphylococci at one or more of those sites (Table 3.1).

Staphylococcus epidermidis was the staphylococcal species recovered with the highest frequency from periimplantitis sampling sites (present at 19.7% of paper point and 1.9% of curette samples prior to treatment at visit 1, 37% of paper point and 25% of curette sites post-treatment at visit 2, 28.6% of paper point and 25% of curette samples from the pooled post-treatment visits 3-6, Table 3.1). *Staphylococcus epidermidis* was also the most frequently identified staphylococcus at periimplantitis sites when assessed by patient, being identified from one or more paper point samples in 31%, 28.6% and 16.7% of patients at pre treatment visit 1, post-treatment visit 2 and pooled post-treatment visits 3-6, and in one or more curette samples in 3.6%, 21.4% and 33.3% of patients at pre treatment visit 1, post-treatment visit 2 and pooled post-treatment visits 3-6, respectively (Table 3.1). The density of *S. epidermidis* recovered varied from visit to visit (see section 3.4.3 below). The cell densities ranged from 10 to 400 cfu/three paper points (mean 61.7 cfu cfu/three paper points) and 1.7×10^4 cfu/curette scraping at the pre treatment visit (visit 1) to 40 to 3.3×10^6 cfu/three paper points (mean 3.6×10^5 cfu/three paper points) and 1×10^4 to 4.9×10^6 cfu/curette scraping (mean 1.5×10^6 cfu/curette scraping) scraping at the initial post-treatment visit (visit 2).

There was no significant difference between the presence of *S. epidermidis* at implant sites described as healthy and those with periimplantitis at each clinical visit. Neither was there any statistically significant difference between the abundance of *S. epidermidis* when present at implant sites described as healthy and those with periimplantitis at each clinical visit. It has been suggested previously that *S. aureus* may be associated with periimplantitis and oral implant failure [8, 35, 40, 47, 159]. *Staphylococcus aureus* was identified at periimplantitis sites so infrequently in the present study (recovered from 4 sites by paper

points , with a range of 10 to 480 cfu/three paper points (mean 296.7 cfu/three paper points) and one curette site, with an abundance of 170 cfu/curette scraping, at the first visit only, Table 3.2), that this is unlikely.

3.4.2 Staphylococcal populations

The majority of samples taken from oral implants and from teeth (regardless of their state of health) yielded no staphylococci. Similarly approximately one third of oral rinse samples yielded no staphylococci. When staphylococci were recovered *S. epidermidis* was the most commonly isolated species and also the most abundant both pre- and post-treatment. *Staphylococcus aureus* was recovered far less frequently than *S. epidermidis*, but on occasion the estimated cfu/sample matched (or superseded) that of *S. epidermidis*. Other staphylococcal species (*S. auricularis, S. capitis, S. cohnii, S. haemolyticus, S. pasteuri, S. warneri* and *S. lugdunensis*) were also recovered from the various sampling sites, but nowhere near the same frequency of abundance as *S. epidermidis* (Tables 3.1-3.18). Statistical analysis did not indicate any difference in the range of staphylococcal species identified at each different sampling site type or state of health within each visit (the majority of all site types yielded no staphylococci, with *S. epidermidis* as the most commonly identified species). Nor was there any statistical significantly difference in the staphylococcal cell densities recovered from the different sampling site types, or state of health, at each visit. This suggested that, although staphylococci were not ubiquitously present in all oral samples, they are not unusual or associated with a particular site type or state of health.

Both *S. aureus* and *S. epidermidis* have a direct access route to the oral cavity (via trafficking of staphylococci from the nasal passages [134]), and both were found in oral rinse samples in a relatively high proportion of patients (*S. epidermidis* in 47.4%, 45.5% and 94.1% and *S. aureus* in 21.1%, 4.5% and 41.2% of patients at pre treatment visit 1, post-treatment visits 2 and subsequent visits, respectively). In the present study, the proportion of patients that yielded *S. aureus* from oral rinse samples at the initial (pre-treatment) sampling stage (21.1%) is similar to that generally quoted for the percentage of the population that persistently carry *S. aureus* in their anterior nares [49, 61]. *Staphylococcus epidermidis*, as a commensal organism of the skin, also has more direct access to the oral cavity via introduction from the face and hands, similar to the proposed route of inoculation for *S. aureus* into the nose [48, 68].

These findings suggest that *S. epidermidis* should be considered a commensal organism of the oral cavity, and perhaps even of the subgingiva (periodontal and periimplant pockets). This is in line with the few studies using media culture of clinical specimens from teeth or oral implants that have reported on the presence of *S. epidermidis* [130, 131], where *S. epidermidis* accounted for between 42.5% and 64.3% of the total staphylococcal isolates. Indeed several staphylococci, including *S. aureus* and *S. epidermidis*, have previously been noted as being present as part of the oral flora [14, 64, 132, 134].

3.4.3 Pre-treatment and post-treatment staphylococcal populations

When patients were treated (by mechanically debriding the periimplant or periodontal pockets and offering oral hygiene advice) the percentage of patients that yielded *S. epidermidis* at tooth and implant sites increased and the organism was recovered more frequently from samples taken by curette as well as using paper points. At the initial post-treatment clinical visit (visit 2) the density of *S. epidermidis* recovered from each sample increased markedly. Again, there was no significant difference between the populations of *S. epidermidis* found at periimplantitis or healthy oral implant sites. In contrast, there was a statistically significant difference between the *S. epidermidis* populations isolated from implant sites at the pre-treatment and post-treatment visits (visit 1 and visit 2), supporting the initial observation of an apparent increase in *S. epidermidis* post-treatment, both in terms of frequency of isolation and density. *Staphylococcus epidermidis* can be a very resilient organism. It is a well described biofilm former, and can be difficult to remove using mechanical methods [160]. The results of the present study indicated that the treatment of periodontal and periimplant sites by debridement results in conditions that allow *S. epidermidis* to thrive. The elimination of other microorganisms and necrotic tissue by debridement may have contributed to this. It was also evident that samples from the initial post-treatment clinical visit did not yield the variety of staphylococci identified in samples from the initial (pre-treatment) visit (see sections 3.3.2 to 3.3.17). It is likely that the populations of other species of oral bacteria that were present in the periimplant and periodontal pockets at the time of treatment were similarly disrupted, removing potentially competing organisms. Noticing the lower frequency of staphylococci isolation from curette samples taken prior to treatment (section 3.3.3 to 3.3.9), and the general increase in *S. epidermidis* at the initial post-treatment clinical sampling visit (visit 2), it may even be possible that the act of removing the dental biofilm unwittingly inoculates *S. epidermidis* deeper into the periodontal and periimplant pockets than it would otherwise colonise. The increase in estimated cell density of *S. epidermidis* at implant sites in particular is a point

of interest. As outlined in the introductory section, *S. epidermidis* has an affinity for many other types of medical implants and indwelling devices such as catheters and shunts. *Staphylococcus epidermidis* biofilm mediated resistance to mechanical pressures makes it extremely difficult to eliminate, and that seems to be the case in the present study.

The results of the present study indicate that it is unlikely that *S. epidermidis* has any adverse effect on the health status of established oral implants in an otherwise healthy population. Further investigation is needed on the role *S. epidermidis* plays in the success or failure of recently placed oral implants, and its role in the success or failure of oral implants in patients who are immunocompromised or suffering from some other underlying condition [161].

3.4.4 Survival of *S. epidermidis* in anaerobic periimplantitis pockets

Results from the present study revealed no significant difference in the density of staphylococci recovered from curette and paper point samples using matched pairs of samples from the same sampling site. Staphylococci are generally aerobic and the recovery of so many from putatively anaerobic sites (i.e. periimplantitis and periodontal sites) was surprising. However, strictly anaerobic *S. epidermidis* isolates have been reported previously [54], though the aerobic culture conditions used in this study might suggest facultatively anaerobic strains. The presence of biofilms in periimplant and periodontal pockets may contribute to the survival of *S. epidermidis* under anaerobic or semi-anaerobic conditions. Microbial biofilms are complex ecosystems. As well as providing physical protection against antimicrobials they enable the survival of fastidious bacteria by providing nutrients that they themselves cannot produce [21]. It is possible that they also create microenvironments, allowing oxygen to penetrate into areas deep within the plaque biofilm in periodontal and periimplant pockets where an anaerobic environment would be expected, though anaerobic environments are themselves thought to be mediated by biofilm interactions [28]. It is important to emphasise that biofilm formation is a common attribute of clinical isolates of *S. epidermidis* [80, 83, 84, 86, 150, 162, 163].

3.4.5 Previous studies

As stated in the introduction to this thesis, and to this chapter, one of the aims of this study was to observe any relationship between *S. aureus* and periimplantitis. From the data gathered from the population of periimplantitis patients investigated, no causal link between the presence of *S. aureus* and periimplantitis was observed. In fact *S. aureus* was

isolated from clinical samples much less frequently than was expected, based on the frequency with which it was identified from tooth and implant samples in previous studies using molecular methods for identification [8, 35, 40, 47]. This will be dealt with in more detail in Chapter 5, but there are a few points that may be salient here. The methods employed by other researchers for identification of bacteria located in periodontal and periimplant pockets have made use of powerful molecular tests to identify very small amounts of genomic DNA present in clinical samples. Due to the nature of periodontal and periimplant pockets, the bacteria found therein tend to occur in biofilms [21, 28, 164]. DNA from lysed bacteria is an important component of the extracellular matrix of biofilms that is vital for the survival of microorganisms in biofilms. Previous studies have shown that if *S. aureus* is prevented from undergoing autolysis, biofilm formation is effectively blocked [152]. The approach used in the present study relied on the culture of viable organisms that have then been definitively identified using 16S DNA sequencing. It is possible that studies that relied exclusively on molecular methods of detection, identification and relative quantification directly from clinical samples may have overestimated the prevalence of *S. aureus* at periimplantitis sites. Firstly, studies that used chequerboard analysis for detection and semi-quantification of *S. aureus* in periimplantitis samples relied on the use of total cellular DNA from a reference *S. aureus* strain(s) as a molecular probe for detection of *S. aureus* DNA. Such probes by their very nature can not be species-specific as frequently genes that form part of the *S. aureus* accessory genome are also found in coagulase-negative staphylococci (CoNS) [71]. As mentioned earlier this will be dealt with in more detail in Chapter 5. Secondly, the persistence of DNA from dead bacterial cells in biofilms in periodontal and periimplant pockets may provide a false impression of the actual density of particular species present.

A study by Krönstrom *et al.* in 2000 [159] has been referred to in studies by other researchers that suggested *S. aureus* may be implicated in periimplantitis and subsequent implant failure [40, 47]. In these papers Krönstrom *et al.* 2000 has been cited as proof of the association of *S. aureus* with the failure of oral implants in an attempt to give weight to arguments that the presence of *S. aureus* at implant sites in their studies is of concern. [159]. However, Krönstrom *et al.* used the absence of humoral evidence of an immune response against *S. aureus* in failed implants to implicate *S. aureus* as a causative agent for implant failure [159]. The study did not directly confirm the presence of *S. aureus* by any other methods (such as growth of *S. aureus* on selective media, or direct molecular identification of *S. aureus* DNA or antigens) at the failed implant site, or on the extracted

implant. Thus the contention by other researchers that *S. aureus* may be associated with implant failure has little robust scientific evidence to support it. This view is supported by the findings of the present study.

3.4.6 Future research

The presence of *S. epidermidis* in the oral cavity in general, and it's apparent increase in abundance at periodontal and periimplant sites after biofilm disruption by mechanical debridement, raises an interesting question. *Staphylococcus epidermidis* has the potential to cause serious complications when introduced into wound sites around implants and other indwelling medical devices (artificial valves, joint implants, screws, shunts, catheters etc) [54, 79, 80, 84, 92, 100, 162, 165]. This did not appear to be the case in the periimplantitis patients investigated in the present study. The oral implants in the patient cohort studied had been placed a minimum of five years prior to the initial clinical sampling point of this study, and the patients were otherwise healthy. *Staphylococcus epidermidis* infections are more often associated with immunocompromised patients, or patients with underlying medical conditions [80, 88, 166]. Given that *S. epidermidis* is prevalent in the oral cavity (in general as well as in periodontal and established periimplant pockets) as suggested by the data generated in the present study, it would be surprising if recently placed implants were not colonised by *S. epidermidis*. Assuming this does occur, would early colonisation of oral implants by *S. epidermidis* have any effect on implant integration? Could *S. epidermidis* contribute to early implant failure in immunocompromised patients? If *S. epidermidis* were to play a role in early implant failure, and taking into account the difficulty in removing an established *S. epidermidis*-containing biofilm, would nasal decolonisation (similar to that used to rid carriers of methicillin resistant *S. aureus*, MRSA) prior to implant placement in at risk populations be appropriate or indeed beneficial? These questions and others may well provide fruitful avenues for future research on periimplantitis.

3.4.7 Conclusions

In response to the objectives of this part of the present study (outlined in section 3.1) it has been established that :

1. There is no evidence of an association of any particular staphylococcal species and failing oral implants.

2. The majority of samples from teeth and implants (healthy and diseased) yielded no staphylococci. *Staphylococcus epidermidis* was the most frequently identified staphylococcal species (also the most abundant staphylococcal species) at all sites in the oral cavity. There is no statistically significant difference in the range of staphylococcal species found between diseased and healthy established oral implants and natural teeth (those located adjacent to, and not adjacent to, oral implants). There was an increase in the cellular density of *S. epidermidis* recovered from tooth and implants (healthy and diseased) at the initial post-treatment visit (visit 2) compared to the pre-treatment clinical visit (visit 1). There is no evidence that *S. epidermidis*, or any other staphylococci, has any direct association with the development of periimplantitis in our sample population (patients who had oral implants placed five or more years ago, one of which was showing signs of periimplantitis at the beginning of the study). There is no evidence that *S. epidermidis*, or any other staphylococci, has any direct association with long standing implants that have successfully integrated and are healthy, nor with healthy teeth.

3. There is no statistically significant difference between the staphylococcal populations recovered from clinical specimens obtained using two different sampling methods; paper point sampling of the gingival fluid and curette scraping of the interior of the periodontal/periimplant pocket when tested using matched pairs.

4. The results of the present study clearly demonstrated that viable *S. aureus* cells were not significantly associated with either periodontal, or periimplant sites as determined by laboratory culture of viable staphylococci. These findings contrast starkly with previous studies that relied on molecular identification directly from clinical samples [40, 167, 168]. The results of the present study show that *S. aureus* does not have any direct (or causal) association with long standing oral implants that have developed periimplantitis. Viable staphylococcal cells were not present at the majority of sampling sites, excluding oral rinses, regardless of the type of site (periodontal or periimplant pocket), or state of health (healthy or diseased).

Chapter 4

Multilocus sequence typing of oral *Staphylococcus epidermidis* and DNA microarray analysis of oral *Staphylococcus aureus* from periimplantitis patients

4.1 Introduction

4.1.1 *Staphylococcus epidermidis* and *S. aureus* populations

As outlined in Chapter 1, *S. epidermidis* and *S. aureus* are both commensal organisms that can colonise the skin and mucosal surfaces of humans and animals. Both species can also be found in the oral cavity and in periodontal and periimplant pockets [8, 31, 35, 37, 40, 47, 130, 131, 148]. Though a significant proportion of the human population carry *S. aureus*, either transiently or persistently with no obvious ill effects, the significant pathogenic potential of *S. aureus* is well established [48, 54, 60, 63-66]. Infections caused by *S. aureus* can differ depending on the location of the infection, and the range of virulence factors such as enterotoxins and other toxins produced by the infecting strain [62, 66, 68-70]. Many *S. aureus* strains have also acquired mobile genetic elements (MGEs) including plasmids and transposons encoding resistance to a range of antibiotics which can hinder treatment of infections as well as efforts at prophylactic decolonisation of at risk individuals including MRSA carriers [75, 104].

Staphylococcus epidermidis is not usually considered to be significantly pathogenic. However, *S. epidermidis* has been recognised as an opportunistic pathogen and is a well known cause of infections of artificial joints, other medical implants, and indwelling medical devices such as catheters and artificial heart valves [84, 86, 88, 92, 165]. *Staphylococcus epidermidis* has also been recognised as a potential pathogen in patients who are immunocompromised, or who have other underlying medical conditions that can compromise innate immunity [80, 161, 163]. The general view that *S. epidermidis* is a relatively benign organism in most circumstances is one of the reasons that it has not been as comprehensively studied as *S. aureus*. Because there has been relatively little demand to track *S. epidermidis* strains in outbreak situations, it has taken longer for global typing schemes for this organism to be developed. Typing studies of *S. epidermidis* have been undertaken using PFGE and various multi locus sequence typing (MLST) schemes to investigate retrospective populations of *S. epidermidis* isolates from clinical laboratories that were recovered from infected implants or heart valves, or that have been identified as being resistant to particular antibiotics [88, 91, 93-96, 98, 165, 166, 169]. Fortunately, recent studies using the latest MLST scheme [118] have established a global database of *S. epidermidis* sequence types (STs) [170] including both nosocomial and environmental isolates recovered from the same geographic locations at the same time, or both clinical isolates and carriage isolates from the same population [90, 92, 97, 99].

Staphylococcus aureus is a significant pathogen and considerable effort has been expended over the last decade in characterising *S. aureus* populations, especially MRSA [71, 77, 123, 171]. A wide variety of different *S. aureus* typing methods and schemes have been developed, such as PFGE, MLST, Protein A (*spa*) typing SCC*mec* and SCC*mec* associated direct unit repeat (*dru*) typing, mentioned in Chapter 1, many of which can be used in combination [106, 114, 115, 119, 126, 127]. The choice and complexity of typing method is often dependent on the reasons typing is undertaken in the first place including the length of time it takes to type a single isolate, the cost in equipment, reagents and man hours, and the accuracy of the method must all be taken into consideration. Most of the more complex typing methods were originally developed to characterise MRSA isolates [106, 126, 127].

4.1.2 *Staphylococcus epidermidis* MLST

Multi locus sequence typing (MLST) is an established technique used for typing of an isolate within a species and for grouping related isolates into sequence types (STs) and clonal complexes (CCs) [116, 117]. First illustrated with *Neisseria meningitides* in 1998 [116], MLST utilises point mutations within a set of highly conserved genes (often referred to as "housekeeping" genes) to map the evolutionary distance between isolates of the target species. This can be used for anything from a basic population survey, as undertaken in the present study, to tracking the emergence and spread of epidemic clones within a population, tracking lineages and the evolution of identified clones [116, 117, 123, 172, 173]. MLST schemes generally utilise between six and eight housekeeping genes [117]. A baseline reference sequence is determined for each gene (referred to as the consensus or reference sequence), and unique point mutations that differ from the consensus sequence (alleles) are allocated their own specific numbers. The combination of different alleles for a given isolate is associated with an ST number [116, 117]. Sequence types are grouped in CCs of closely related strains [117, 122]. The *S. epidermidis* MLST scheme used in the present study is based on that of Thomas *et al.* [118], and uses a set of seven housekeeping genes as described in Chapter 2. Several previous MLST schemes have been proposed for typing *S. epidermidis*. Thomas *et al* (2007) compared the schemes proposed by Wang *et al* [120], Wisplinghoff *et al* [112] and an unpublished scheme. The seven most discriminatory alleles from the three schemes were combined and assessed and found to be a more discriminating MLST scheme. [118]. The scheme devised by Thomas *et al* is the scheme currently used by the international *S. epidermidis* MLST database [170].

Unlike other typing methods, where difficulties can arise comparing results from different laboratories, MLST data is very transferable. Because MLST is a sequence-based technique, data gathered from MLST surveys can be easily shared among and compared between collaborating researchers and internationally. The allele and ST variations found through MLST are discriminatory enough in some circumstances to be used in short term epidemiological studies, such as identifying endemic STs and undertaking population analysis, yet are not too detailed to be used in global and temporal distribution surveys [116, 117, 123, 172, 173]. In bacterial species where MLST is well established (i.e. *S. aureus*) and there are comprehensive databases including data from thousands of individual isolates and strains, MLST can be used predicatively. The characteristics of a newly typed clinical isolate can be predicted to a large extent based on the published characteristics of other isolates identified with the same ST or belonging to the same CC [67, 107]. In a species where MLST is not yet well established as a method for global population analyses (i.e. *S. epidermidis*) MLST can be used to observe patterns or variance within sample populations. Depending on the aim of the MLST survey, such as studies of the global distribution of STs or the distribution of STs within a population over time, the ST of each isolate may be used to identify a clone or determine the relationship between STs as an indicator of descent.

The computer program eBURST can be used to assess relationships between isolates from a sample population based on MLST data. This software is readily accessible through Imperial College London's MLST.net web interface (http://eburst.mlst.net/), or through the MLST.net *S. epidermidis* website (http://sepidermidis.mlst.net/). eBURST uses an algorithm (BURST: Based Upon Related Sequence Types) to determine genetically related groups within the submitted data and proposes founding members for those groups. The program estimates probable lines of descent based on relationships between the STs allelic profiles, which can be viewed pictorially as radial diagrams centred around the predicted founder(s). When constructing these diagrams the eBURST algorithm uses 'tiebreak' rules to determine likely ST lineages. Tiebreak rule 1 is based on the number of single locus variants (SLVs), tiebreak rule 2 uses the number of double locus variants (DLVs), tiebreak rule 3 uses the number of triple locus variants (TLVs) and tiebreak rule 4 uses the frequency of an ST in the dataset. A variation of eBURST, goeBURST (global optimal eBURST) developed by Francisco *et al.* [122] (http://goeBURST.phyloviz.net), allows MLST clustering and visualisation with a global optimum implementation of the BURST

rules. Like eBURST, goeBURST arranges sequence types in radial clusters based on the most likely pattern of mutations among the alleles, but contains a last tiebreak rule based on the ST number (tiebreak rule 5), assuming that with an increasing MLST database, with submissions from international studies, the most common STs will be profiled first and will have lower ST numbers, and that subsequent studies will add more, less common STs to the database [122]. The ST linkage lines drawn on a goeBURST diagram reflect the level of tiebreak rule used: black lines; no tiebreak rule necessary, blue lines; tiebreak rule 1, green lines; tiebreak rule 2, red lines; tiebreak rule 3, yellow lines; tiebreak rule 4 or 5. In a goeBURST diagram an ST node which is a predicted group founder is assigned a light green border or fill, subgroup founders are assigned a dark green border or fill and all other (common) ST nodes are assigned a light blue border or fill. Thus goeBURST can provide a valuable overview of the overall relationships between a wide range of STs within a global population of isolates. goeBURST has also been the algorithm employed in the most recently published studies investigating clonality across the global distribution of *S. epidermidis* MLST data [90, 92].

At the time of writing very few systematic studies have been reported that used MLST to investigate *S. epidermidis* populations from humans [90, 92]. The published studies that have applied MLST to examine *S. epidermidis* isolates have tended to use clinical isolates recovered from hospital inpatients or isolates from a mixture of environmental and clinical sources from different geographic locations over a number of years [91, 93-95, 98, 99, 169]. When clinical isolates from disease states were used they were often recovered from catheters, cardiac valves or were bloodstream isolates from septic patients [94, 118, 165, 169]. Furthermore, many studies that investigated *S. epidermidis* isolates by MLST (including the two most recent studies looking at carriage and post surgical infection, and community and nosocomial isolates, respectively, [90, 92]) first typed isolates using PFGE and then selected a subset of isolates from each PFGE type for MLST analysis [90, 92, 169]. PFGE is extremely discriminatory for *S. epidermidis* and isolates with the same MLST ST can exhibit different PFGE patterns, but PFGE profile data for the same isolates can differ from laboratory to laboratory [114, 115]. MLST does not differentiate between as many sub-groups within isolates as PFGE, but data obtained by MLST is more consistent. This lower level of discrimination has lead to the proposal that *S. epidermidis* MLST CCs should be split into subgroups based on PFGE patterns and SCC*mec* typing for isolates where practical [98]. Two recent studies investigated commensal and nosocomial *S. epidermidis* populations using MLST [90, 92]. Of these, Rolo *et al* (2012) is

the only one to have used commensal isolates from individuals with no recent hospital admissions (i.e. not admitted to hospital within the previous three months) [90]. All of the *S. epidermidis* isolates investigated by Gordon *et al* (2012) were obtained from patients due to undergo surgery to place ventricular assist devices and had a significant history of hospitalisation [92]. To date there have been no detailed population surveys of *S. epidermidis* from the oral cavity.

It has been suggested that *S. aureus* can transiently colonise the oral cavity as a result of nasal oral trafficking from the nares, but no similar studies have been undertaken to determine if *S. epidermidis* behaves in the same way [134]. From the results described in Chapter 3 of this thesis it is clearly evident that *S. epidermidis* is present in the oral cavity, though it is unclear whether its presence is persistent or transient.

4.1.3 Genetic exchange between *S. aureus* and *S. epidermidis*

Staphylococcus aureus and *S. epidermidis* share many genes, especially genes located on MGEs (i.e. plasmids and transposons) [71] and it has been suggested that *S. epidermidis* and many other coagulase negative staphylococcal species, function as reservoirs for many genes that have been identified in *S. aureus* [111, 174]. These include antibiotic resistance genes and some virulence factor genes (e.g. the arginine catabolic mobile element ACME) [69, 77, 93]. The transfer of a plasmid carrying *ileS2* (also known as *mupA*, formerly *mupR*) encoding high level resistance to the antibiotic mupirocin (used for nasal decolonisation of *S. aureus* carriers, particularly those harbouring MRSA) from *S. epidermidis* to (previously mupirocin-susceptible) *S. aureus* clones has been observed in the clinical setting [104]. Furthermore, *S. epidermidis* is known to harbour Staphylococcal Cassette Chromosome *mec* (SCC*mec*), including types II, III, IV, V, VIII and various sub types, which encode the methicillin resistance determinant *mecA* and its regulatory genes [88, 91-99]. Indeed several of the SCC*mec* elements present in various MRSA lineages are thought to have developed in *S. epidermidis* and may have been transferred between the two species on multiple occasions and, like *ileS2* above, *in vivo* transfer of *mecA* from *S. epidermidis* to *S. aureus* clones has been observed [111, 112].

4.1.4 DNA Microarray profiling

In recent years advances in whole genome sequencing technology have permitted the determination of the complete nucleotide sequence of many *S. aureus* strains. This has enabled the development of DNA arrays that can be used to screen clinical isolates for the

presence of a range of virulence-associated and antimicrobial agent resistance genes. The StaphyType DNA microarray is one such array developed by Alere Technologies GmbH (Jena, Germany). The StaphyType Kit consists of individual oligonucleotide microarrays mounted in 8-well microtiter strips that detect 334 *S. aureus* gene sequences and alleles including species-specific, antimicrobial resistance and virulence-associated genes, and typing markers. Array profiles are analysed using ArrayMate software (Alere Technologies) which can assign isolates to inferred MLST STs and/or CCs by comparing the DNA microarray profile results of test isolates to microarray profiles from a collection of reference strains stored in the ArrayMate database that have been previously typed by MLST [67, 107].

A complete list of all antimicrobial resistance, virulence-associated genes MSCRAMM and biofilm-associated genes detected by the StaphyType DNA microarray is shown in Appendix 1. The DNA microarray allows investigation of a population of *S. aureus* isolates relative to the global population by accurately assigning individual isolates to CCs [107, 171]. The microarray can also quickly and accurately detect the presence or absence of virulence and antimicrobial resistance genes in the test isolates under investigation [67, 171]. Because *S. epidermidis* and *S. aureus* frequently harbour the same antimicrobial resistance genes, and in some cases virulence genes (e.g. ACME), microarray profiling has potential for screening *S. epidermidis* isolates for the presence of such genes.

The objectives of this part of the present study were to:

1. To investigate the population structure of *S. epidermidis* isolates obtained from a range of oral sites and nasal swabs from periimplantitis patients using MLST analysis.
2. To compare the *S. epidermidis* MLST STs identified with the corresponding STs of the global population entries in the *S. epidermidis* MLST database.
3. To determine the CCs of selected *S. aureus* isolates recovered from oral sites and nasal samples from periimplantitis patients by DNA microarray profiling.
4. To investigate the antibiotic resistance, virulence associated, MSCRAMM, adhesion and biofilm associated genes present in *S. aureus* oral and nasal isolates recovered from periimplantitis patients.
5. To investigate whether microarray profiling using the StaphyType kit could be used to determine the prevalence of antibiotic resistance, virulence associated, MSCRAMM, adhesion and biofilm associated genes in a selection of oral (tooth,

implant and oral rinse) and nasal *S. epidermidis* isolates from periimplantitis patients.

6. To compare the antibiotic resistance, virulence associated, MSCRAMM, adhesion and biofilm associated genes present in a selection of groups of *S. epidermidis* and *S. aureus* isolates obtained from oral sites and nasal swabs obtained from the same patient using the Alere StaphyType DNA microarray.

4.2 Materials and Methods

4.2.1 MLST typing

The *S. epidermidis* MLST scheme developed by Thomas *et al.* [118] was used to investigate the population structure of selected oral isolates recovered from periimplantitis patients. This scheme utilises seven housekeeping genes; *arcC* (encoding carbamate kinase), *aroE* (encoding shikimate dehydrogenase), *gtr* (encoding ABC transporter), *mutS* (encoding DNA mismatch repair protein), *pyrR* (encoding pyrimidine operon regulatory protein), *tpiA* (encoding triosephosphate isomerise) and *yqiL* (encoding acetyl coenzyme A acetyltransferase). DNA was extracted from *S. epidermidis* isolates and MLST loci amplified using PCR as described in Chapter 2. Due to inconsistent amplification and poor nucleotide sequence traces of amplimers when using the *pyrR* primers described by Thomas *et al* [118] a second *pyrR* primer set was designed for use in this study (Table 4.1 and Chapter 2, Table 2.1). Amplimers were sequenced as described in Chapter 2 and aligned with consensus sequences using Bionumerics software, version 5.10 (Applied Maths NV, Sint-Martens-Lautem, Belgium). The Bionumerics program MLST plug-in was used to determine the allele numbers assigned to each sequence variation (the different point mutations away from the consensus reference sequences of *S. epidermidis* RP62A), as defined by (and regularly updated against) the *S. epidermidis* MLST.net website. Forty-seven clinical isolates of *S. epidermidis* recovered from 25 individual periimplantitis patients were subjected to MLST analysis. Where possible clinical isolates derived from multiple sites (including oral rinses and nasal swabs), and from samples taken at different time points from the same patient were included in the analysis (Table 4.2).

4.2.2 Microarray analysis

DNA microarray profiling was undertaken using the Alere StaphyType Kit (Alere Technologies, Jena, Germany). Thirty one *S. aureus* isolates recovered from 20 patients and 43 *S. epidermidis* isolates recovered from 24 patients were processed for microarray analyses according to the manufacturer's instructions, which have been described in detail previously [129, 171]. *Staphylococcus epidermidis* isolates that were co-isolated with *S. aureus* from the same clinical sample or from a different sample from the same patient who yielded *S. aureus* from another sampling site at the same or different sample times were selected for investigation. DNA was extracted using the Alere StaphyType DNA microarray protocol as described in Chapter 2 and stored at 4°C, or transferred to -20°C for long term storage. DNA was visualised by agarose gel electrophoresis, and quantified

using the NanoDrop 2000c UVvis spectrophotometer as described in Chapter 2. Linear PCR amplification and labelling was carried out on a G-Storm GSI thermocycler according to the StaphyType Kit instructions. The labelled sample DNA was hybridised to the 334 gene probes present on the Alere StaphyType microarray chip according to manufacturer's instructions. The microarray chips were washed to remove unbound sample DNA, leaving only the (labelled) DNA specific for the DNA probes present on the array bound to the chip. The labelled DNA was conjugated with Streptavidin-Horseradish Peroxidase (HRP) as per the manufacturer's instructions, unbound HRP was washed away and HRP substrate tetramethylbenzidine (TMB) was added. After a five minute incubation to allow the precipitate to form the TMB containing supernatant was removed and the microarray plates were analysed in the ArrayMate plate reader. If 'staining' controls failed microarray runs were repeated. If *S. aureus* isolates returned weak signals the arrays were visually assessed and repeated if necessary. Because the microarray was designed for use with *S. aureus*, hybridisation signals obtained from *S. epidermidis* isolates were generally weaker than those from *S. aureus* isolates.

4.2.2.1 Confirmation of high level mupirocin resistance

High level mupirocin resistance was confirmed by Ms Orla Brennan. The presence of *ileS2* was confirmed using PCR as described in chapter 2 [141]. Phenotypic mupirocin resistance was confirmed by disk diffusion at both low (5 μg) and high (200 μg) levels. Confirmation of high level resistance and minimum inhibitory concentration (MIC) was performed with E-test strips as described in Chapter 2 [141].

4.3 Results

4.3.1 MLST analysis of *S. epidermidis*

MLST analysis was applied to 47 clinical isolates of *S. epidermidis* from 25 individual periimplantitis patients using the MLST scheme devised by Thomas *et al.* (2007) (Chapter 2, Table 2.1) [118]. A total of 22 STs were identified among the 47 isolates. At the time of submission to the MLST database (http://sepidermidis.mlst.net) two of the 47 isolates subject to MLST analysis yielded novel combinations of alleles and three yielded novel allele point mutations, resulting in five new STs (STs 431, 432, 433, 471 and ST472) (Tables 4.1 and 4.2, and Figure 4.2). Multiple *S. epidermidis* isolates from 11/25 (44%) implantitis patients were subject to MLST analysis and seven of these (28% of all patients) yielded *S. epidermidis* with different STs from different oral sites or from the same oral site at different sampling periods (Table 4.2, patients 7, 9, 21, 25, 31 and 36,). In contrast, for several of the patients that had multiple isolates subject to MLST typing, isolates with the same ST were identified at different sampling sites (Table 4.2, patients 7, 16, 34 and 39) or at different sampling periods (Table 4.2, patients 10, 16, 30 and 31).

The numbers of isolates subject to MLST recovered from each state of health or disease (e.g. implantitis) in the periimplantitis patients and the type of sampling site (tooth, implant, oral rinse or nasal swab) were too small to determine if there were any significant associations between a particular site type or health state and a specific *S. epidermidis* ST. ST73 was most prevalent ST identified among the isolates investigated (17/47, 36.2%), followed by ST153 (5/47, 10.6%), ST256 (4/47, 8.5%) and ST14 (3/47, 6.4%) (Tables 4.2 and 4.3). When multiple isolates from the same patient with the same ST were discounted, ST73 remained the most frequently identified ST (11/25 patients, 44%). STs 153 (3/25 patients, 12%), 256 (3/25 patients, 12%) and 14 (2/25 patients, 8%) were also found in multiple patients (Table 4.2). All other STs (ST5, 17, 59, 170, 184, 190, 193, 200, 204, 218, 253, 284, 297, 431, 432, 433, 471 and ST472) were identified among single isolates from individual patients (Tables 4.2, 4.3 and 4.4).

Table 4.1. Allelic profiles for *S. epidermidis* STs identified using MLST

ST	Allelic profiles						
	Genes used in MLST						
	arcC	*aroE*	*gtr*	*mutS*	*pyrR*	*tpi*	*yqiL*
5	1	1	1	2	2	1	1
14	1	1	2	1	1	1	1
17	1	1	6	2	2	1	1
59	2	1	1	1	2	1	1
73	1	5	2	6	2	1	6
153	2	1	6	2	2	1	1
170	25	19	17	4	23	10	2
184	16	1	2	1	2	1	1
190	1	1	1	2	5	1	1
193	12	1	9	8	6	5	8
200	1	1	2	2	3	1	3
204	2	1	2	6	2	1	7
218	1	1	2	6	2	16	1
253	1	5	2	6	2	1	32
256	1	1	2	6	2	1	33
284	1	1	9	5	2	1	1
297	1	2	2	2	2	1	3
431[a]	53	1	2	2	2	1	1
432[b]	1	45	2	2	2	1	3
433[b]	1	46	7	6	2	1	4
471[c]	1	38	2	6	2	16	1
472[c]	1	5	2	6	4	1	6

Seven pairs of SLVs, seven pairs of DLVs and 16 pairs of TLVs identified.
[a] New allele at *arc*.
[b] Novel ST: new allele at *aroE*.
[c] Novel ST: New allele combination.

Table 4.2. Multilocus sequence types determined for selected *S. epidermidis* isolates recovered from periimplantitis patients

Patient	Visit[a]	ST	Isolate ID	Sampling site type	Sampling site state of health
4	1	190	DDUH894-2	Nasal swab	Nasal carriage
7	1	73	DDUH224-1	Oral rinse	Oral carriage
	2	153	DDUH312-1	Implant	Healthy
		153	DDUH318-1	Tooth (adjacent to implant)	Periodontitis
		153	DDUH320-1	Tooth (non-adjacent to implant)	Healthy
	3	472[d]	DDUH963-1	Tooth (adjacent to implant)	Healthy
		204	DDUH972-1	Nasal swab	Nasal carriage
8	2	73	DDUH858-1	Nasal swab	Nasal carriage
9	1	17	DDUH095-1	Tooth (non-adjacent to implant)	Healthy
	3	73	DDUH906-1	Implant	Healthy
	3	256	DDUH914-3	Oral rinse	Oral carriage
10	2	73	DDUH060-1	Implant	Periimplantitis
	4	73	DDUH560-1	Tooth (non-adjacent to implant)	Healthy
	5	73	DDUH873-2	Nasal swab	Nasal carriage
11	1	256	DDUH170-1	Oral rinse	Oral carriage
12	2	153	DDUH311-1	Oral rinse	Oral carriage
13	1	193	DDUH590-1	Implant	Periimplantitis
14	1	431[b]	DDUH042-1	Oral rinse	Oral carriage
16	1	256	DDUH184-1	Implant	Periimplantitis
	1	256	DDUH191-1	Tooth (non-adjacent to implant)	Healthy
	2	73	DDUH357-1	Oral rinse	Oral carriage
	3	73	DDUH456-2	Implant	Healthy
19	3	5	DDUH1000-1	Implant	Healthy
21	1	73	DDUH761-1	Implant	Periimplantitis
	1	297	DDUH764-3	Nasal swab	Nasal carriage
22	1	73	DDUH544-1	Implant	Periimplantitis
23	3	153	DDUH374-1	Tooth (non-adjacent to implant)	Healthy
24	1	200	DDUH124-1	Tooth (adjacent to implant)	Not recorded by clinician

Continued overleaf

Table 4.2 continued. Multilocus sequence types determined for selected *S. epidermidis* isolates recovered from periimplantitis patients

Patient	Visit[a]	ST	Isolate ID	Sampling site type	Sampling site state of health
25	1	184	DDUH135-1	Implant	Periimplantitis
	1	284	DDUH139-1	Tooth (non-adjacent to implant)	Healthy
	4	59	DDUH947-2	Nasal swab	Nasal carriage
27	1	433[c]	DDUH529-1	Implant	Periimplantitis
30	2	73	DDUH047-1	Tooth (non-adjacent to implant)	Periodontitis
	3	73	DDUH171-1	Tooth (non-adjacent to implant)	Healthy
31	1	73	DDUH661-1	Implant	Periimplantitis
	1	170	DDUH667-1	Tooth (non-adjacent to implant)	Healthy
	2	73	DDUH821-2	Tooth (non-adjacent to implant)	Periodontitis
		471[d]	DDUH826-1	Nasal swab	Nasal carriage
32	6	218	DDUH745-1	Implant	Periimplantitis
34	4	14	DDUH513-1	Implant	Healthy
	4	14	DDUH515-1	Tooth (adjacent to implant)	Healthy
35	1	73	DDUH613-1	Implant	Healthy
36	1	432[c]	DDUH116-2	Implant	Periimplantitis
	1	14	DDUH119-1	Oral rinse	Oral carriage
39	5	73	DDUH839-1	Implant	Healthy
	5	73	DDUH848-1	Nasal swab	Nasal carriage
42	1	253	DDUH619-1	Tooth (non-adjacent to implant)	Periodontitis

[a] Samples taken from tooth or implant sites at visit 1 were pre treatment (mechanical debridement and oral hygiene advice), subsequent visits (2-6) are post treatment.
[b] Novel ST: New allele at *arcC*.
[c] Novel ST: New allele at *aroE*.
[d] Novel ST: New allele combination.

The MLST allelic profiles of *S. epidermidis* isolates from periimplantitis patients generated in the present study were combined with the allelic profile data of all isolates previously uploaded in the MLST database. Both an eBURST and goeBURST analysis was undertaken. Data for this analysis was downloaded from the database in November 2012, at which time the most recent database update by its curators was the 2nd of August 2012. Both eBURST and goeBURST analyses divided the isolates obtained in this study into three major CCs with one singleton (ST433). Figure 4.1 shows a goeBURST analysis of the complete set of STs contained within the *S. epidermidis* MLST database (http://sepidermidis.mlst.net). The majority of the STs identified in the present study (19/22, 86.4%) belonged to a single CC, termed CC1 (Figure 4.2). The remaining STs were divided into two further CCs termed CC3 and CC4, respectively, with one singleton (ST433) (Figure 4.2). The founding ST for CC1 was determined to be ST2 using both eBURST and goeBURST. CC1, which contains the majority of STs in the global *S. epidermidis* MLST database, corresponds to a CC referred to as CC2 or CC5 in a number of previous MLST studies of *S. epidermidis* [91, 98, 99].

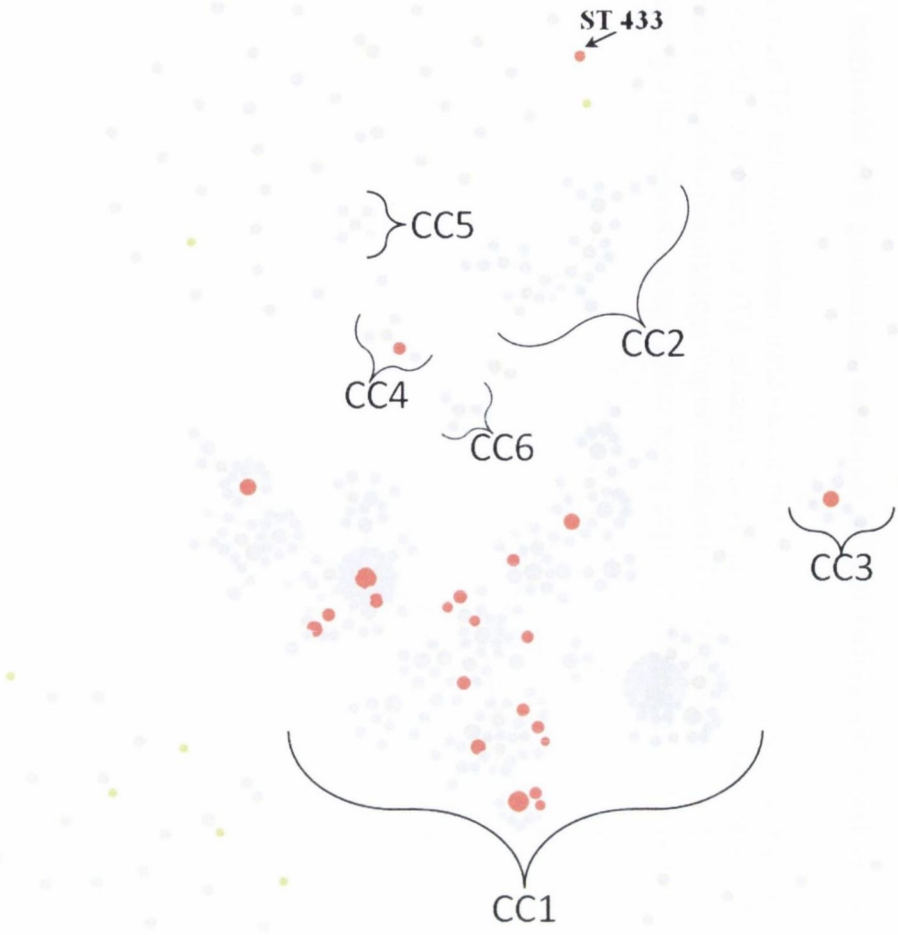

Figure 4.1. goeBURST analysis of the complete set of STs contained within the *S. epidermidis* MLST database (http://sepidermidis.mlst.net/).

The main clonal complexes (CCs) present in the population are shown. The singleton (ST443) outlier identified in the present study is indicated. STs identified in the present study are show in red. All other singleton STs present in the database are shown in shades of green or blue (see section 4.1.2 this chapter). The number of *S. epidermidis* MLST profiles submitted to the database at the time of analysis was 750 (accessed November 2012; database last updated 02 August 2012).

Figure 4.2. goeBURST analysis of *S. epidermidis* clonal complexes (CCs) from the global MLST database containing STs identified in the present study

The number of isolates belonging to each ST identified in this study (nodes highlighted in red) are listed in parenthesis underneath the ST number. ST numbers labelled in blue were novel STs identified in the present study. STs labelled in green are proposed founder STs for each CC. Details of the isolates from this study, and of other database entries with the same STs in the *S. epidermidis* MLST database (http://sepidermidis.mlst.net/) are listed in Table 4.2. ST node fill and outline colours are as follows: red node, ST identified in present study; light green node, group founder; dark green node, sub-group founder; all others, light blue node.

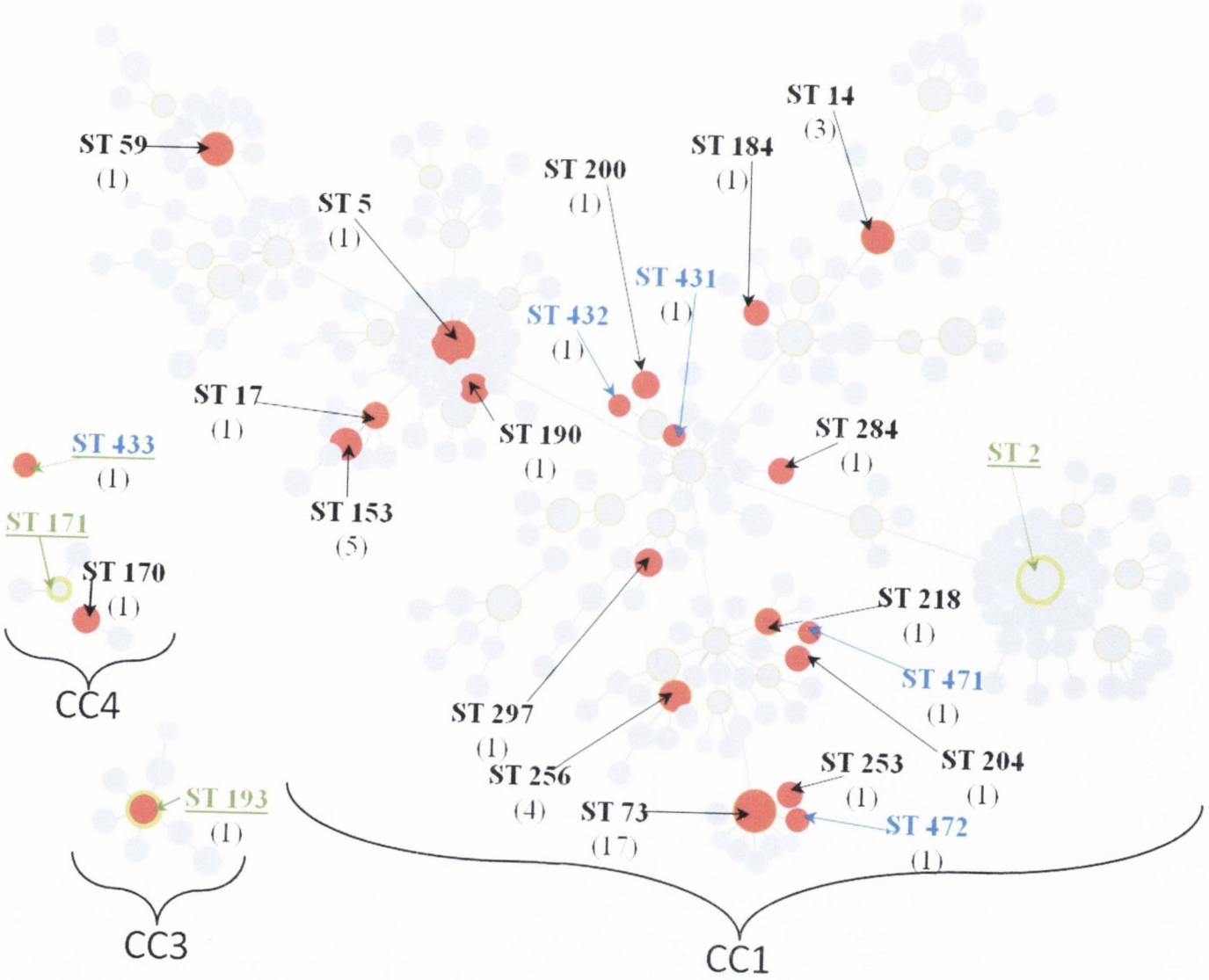
ST 59
(1)
ST 5
(1)
ST 200
(1)
ST 184
(1)
ST 14
(3)
ST 431
(1)
ST 432
(1)
ST 17
(1)
ST 190
(1)
ST 433
(1)
ST 153
(5)
ST 284
(1)
ST 2
ST 171
ST 170
(1)
CC4
ST 218
(1)
ST 471
(1)
ST 297
(1)
ST 256
(4)
ST 253
(1)
ST 204
(1)
ST 73
(17)
ST 472
(1)
ST 193
(1)
CC3
CC1

Table 4.3. *Staphylococcus epidermidis* MLST STs identified in the present study and data on isolates with similar STs obtained from the *S. epidermidis* MLST database[a]

ST[b]	Isolates from current study	Details of *S. epidermidis* isolates in the MLST database[b]
ST5	DDUH1000-1	9 isolates from Germany: 6 environmental isolates, 3 hospital environmental isolates, 2 bovine mastitis isolates 5 isolates from the USA: 2 IV catheter/blood isolates, 3 source not listed 2 inpatient colonisation isolates from Denmark: 1 blood isolate, 1 colonisation isolate 2 environmental isolates from Poland 2 isolates from the International Collaboration on Endocarditis (ICE) Collection: 1 prosthetic valve endocarditis, 1 natural valve endocarditis 1 inpatient colonisation blood isolate from Iceland 1 surgical inpatient infection isolate from Bulgaria 1 health care worker nasal colonisation from Cape Verde
ST14	DDUH513-1 DDUH515-1 DDUH119-1	1 isolate from the USA (source not listed) 1 inpatient colonisation blood isolate from Denmark 1 environmental isolate from Germany
ST17	DDUH095-1	1 inpatient nasal colonisation from Portugal
ST59	DDUH947-2	5 bovine mastitis isolates from Germany 1 skin isolate (external nares of an animal handler) from India
ST73	DDUH224-1 DDUH858-1 DDUH906-1 DDUH060-1 DDUH560-1 DDUH873-2 DDUH357-1 DDUH456-2 DDUH761-1 DDUH544-1 DDUH047-1 DDUH171-1 DDUH661-1 DDUH821-2 DDUH613-1 DDUH839-1 DDUH848-1	3 environmental isolates from Poland 1 inpatient nasal colonisation from Portugal 1 skin isolate (retroaricular crease) from the USA
ST153	DDUH312-1 DDUH318-1 DDUH320-1 DDUH311-1 DDUH374-1	1 nasal carriage from the USA

Continued overleaf

Table 4.3 continued. ***Staphylococcus epidermidis*** **MLST STs identified in the present study and data on isolates with similar STs obtained from the** ***S. epidermidis*** **MLST database**[a]

ST[b]	Isolates from current study	Details of *S. epidermidis* isolates in the MLST database[b]
ST170	DDUH667-1	1 environmental isolate from Germany
		1 skin isolate (retroaricular crease) from the USA
ST184	DDUH135-1	2 nasal isolates from the USA
ST190	DDUH894-2	2 nasal isolates from France 1 nasal isolate from Denmark
ST193	DDUH590-1	6 nasal isolates from Cambodia
ST200	DDUH124-1	2 nasal isolates from Algeria
ST204	DDUH972-1	1 nasal isolate from Denmark
ST218	DDUH745-1	1 blood isolate from Sweden 1 umbilicus isolate from the USA
ST253	DDUH619-1	1 skin isolate from Sweden
ST256	DDUH914-3 DDUH170-1 DDUH184-1 DDUH191-1	1 skin isolate from Sweden
284	DDUH139-1	1 environmental isolate from Poland
297	DDUH764-3	2 skin isolates from Sweden
431	DDUH042-1	No other isolates in MLST.net database
432	DDUH116-2	No other isolates in MLST.net database
433	DDUH529-1	No other isolates in MLST.net database
471	DDUH826-1	No other isolates in MLST.net database
472	DDUH963-1	No other isolates in MLST.net database

[a] http://sepidermidis.mlst.net.
[b]ST: MLST sequence type.

Table 4.4. Sources of isolates listed in the global *S. epidermidis* MLST database for CC1, CC2, CC3 and CC4

Clonal complex	Number of isolates in the *S. epidermidis* MLST database[a]	Source	Description
CC1	43	Animal	Animal isolates, mainly from bovine mastitis
CC1	44	Environment	Non-hospital associated isolates
CC1	19	Environment	Hospital associated isolates
CC1	17	Medical equipment	Intravenous lines, catheters or other "medical equipment"
CC1	58	Human	Nasal swabs from healthy community subjects
CC1	36	Human	Skin culture isolates from healthy community subjects
CC1	18	Human	Colonisation isolates from healthcare workers
CC1	26	Human	Inpatient colonisation isolates
CC1	64	Human	Blood culture isolate
CC1	43	Human	Wound, abscess or biopsy isolates
CC1	21	Human	Isolates from native valve endocarditis
CC1	10	Human	Isolates from prosthesis infections (including artificial valve endocarditis)
CC1	7	Human	pneumonia isolates
CC1	3	Human	Urinary tract infections
CC1	2	Human	Thoracic isolates
CC1	1	Human	Isolate from a neonates eye
CC1	22	Human	Isolates listed as "Infection"
CC1	1	Human	Faecal sample listed as "colonization"
CC1	70	Undefined	Undefined isolates
CC2	2	Environment	Non-hospital associated isolates
CC2	1	Environment	Hospital associated isolates
CC2	1	Human	Nasal swab from healthy community subject
CC2	1	Human	Skin culture isolate from healthy community subject
CC2	2	Human	Healthy subject, source of isolate unknown
CC2	7	Human	Inpatient colonisation isolates
CC2	2	Human	Colonisation isolates from healthcare workers
CC2	7	Human	Blood culture isolate
CC2	2	Human	Wound, abscess or biopsy isolates
CC2	1	Human	Cerebrospinal fluid listed as "infection"
CC2	3	Medical equipment	Intravenous lines, catheters or other "medical equipment"
CC2	2	Human	Human isolate, subjects state of health unknown
CC2	5	Undefined	Undefined isolates

Continued overleaf

Table 4.4 continued. Sources of isolates listed in the global *S. epidermidis* MLST database for CC1, CC2, CC3 and CC4

Clonal complex	Number of isolates in the *S. epidermidis* MLST database[a]	Origin	Description
CC3	2	Animal	Animal isolates all from bovine mastitis
CC3	1	Environment	Non-hospital associated isolate
CC3	9	Human	Nasal swabs from healthy community subjects
CC3	5	Human	Skin culture isolates from healthy community subjects
CC4	1	Environment	Hospital associated isolates
CC4	1	Environment	Non-hospital associated isolates
CC4	1	Human	Nasal swab from healthy community subjects
CC4	1	Human	Skin culture isolate from healthy community subjects
CC4	1	Human	Inpatient colonisation isolate
CC4	1	Human	Blood culture isolate

[a] http://sepidermidis.mlst.net, accessed November 2012, last updated 02 August 2012.

4.3.2 Microarray analysis of *S. aureus* and *S. epidermidis* isolates

All *S. aureus* isolates recovered from different oral sites and the nares in periimplantitis patients were subject to DNA microarray profiling using the Alere *S. aureus* StaphyType DNA microarray system. A single *S. aureus* isolate was subsequently excluded from the analyses as it continually returned weak readings, well below the quality control threshold of the *S. aureus* StaphyType DNA microarray. The Alere *S. aureus* StaphyType DNA microarray system permits *S. aureus* MLST STs/CCs to be inferred from microarray profile data as well determining the range of virulence-associated and antimicrobial agent resistance genes harboured by individual isolates. Selected *S. epidermidis* isolates from periimplantitis patients were also subjected to array profiling as this species and *S. aureus* often harbour similar antimicrobial resistance and genes (e.g. *mecA*) and some virulence genes (e.g. ACME element). Tables detailing all of the antimicrobial resistance, virulence-associated genes MSCRAMM and biofilm-associated genes present on the DNA microarray are listed in Appendix 1. The full microarray results (positive and negative results for all probes, for each isolate) for *S. aureus* and *S. epidermidis* isolates investigated are presented in Appendix 1.

4.3.2.1 *Staphylococcus aureus* clonal complexes and typing markers identified by DNA microarray profiling

Thirty one *S. aureus* isolates recovered from 20 separate periimplantitis patients were subject to DNA microarray profiling and were assigned to eight different CCs (CC5, 7, 8, 9, 15, 22, 30 and CC101) (Tables 4.5 and 4.6). Four CCs belonged to *agr* group I, three to group II and one to group III (Table 4.6) Half of the CCs exhibited capsule type 5 (CCs 5, 8, 9 and CC22) and the other half exhibited capsule type 8 (CCs 7, 15, 30 and CC101). The immune evasion complex (IEC) types present in many of the CCs were mixed. Two CC5 isolates and one CC30 isolate exhibited IEC type A, two CC22 isolates, one CC9 isolate and three CC30 isolates exhibited IEC type B, all seven CC15 isolates exhibited IEC type C, while two CC7 isolates and five CC8 isolates exhibited IEC type D. Four isolates were IEC negative (one each from CC8 and CC9 and two isolates from CC30). None of the isolates harboured *mecA*, or any other SCC*mec*-associated genes (Table 4.6).

4.3.2.2 *Staphylococcus aureus* antibiotic resistance genes identified by DNA microarray profiling

The majority (28/31 isolates, 90.3%) of the *S. aureus* isolates harboured *blaZ* encoding resistance to β-lactam antibiotics. The three isolates lacking *blaZ* all belonged to CC101

and all were originally isolated from the same sampling site in one patient. The majority (25/31 isolates, 80.6%) of isolates carried *fosB* encoding resistance to fosfomycin and bleomycin. The remaining *fosB*-negative isolates belonged to CC7 and CC22. The majority of isolates investigated (26/31 isolates, 83.3%) also harboured *sdrm* encoding a general efflux pump (Table 4.6). Along with those mentioned above, additional resistance genes including *msr*(A) (macrolide resistance) and *erm(A)* (combined macrolide, lincosamide, streptogramin resistance) were variably present in CC7, 8, 9 and CC30 isolates. The *qacC* gene encoding resistance gene to quaternary ammonium compounds was identified as being variably present in CC15 isolates. The chloramphenicol resistance gene *fexA* was identified in a single isolate belonging to CC9 and the fusidic acid resistance gene *fusB* was identified in a single CC15 isolate (Table 4.6).

4.3.2.3 *Staphylococcus aureus* virulence associated genes identified by microarray profiling

All isolates harboured leukocidin and haemolysin genes (combinations of the genes *luk* and *hl* present in all isolates) and aureolysin (*aur*) (two CC30 isolates returned ambiguous results) Table 4.6. All isolates harboured *sspA*, *sspB* and *sspP* (encoding glutamyl endopeptidase/V8-protease and staphopain B and A, respectively), while a combination of genes encoding serine proteases A, B and E (*splA*, *splB*, *splE*) was identified in all isolates except for those belonging to CC9 and CC22. The enterotoxin gene complex (termed *egc*) (*seg*, *sei*, *sem*, *sen*, *seo* and *seu*) was present in only 13/31 isolates (42%), all of which belonged to CCs 5, 9 and CC30 (Table 4.6). Four CC30 isolates (two from oral rinse samples, one from a nasal swab and one from a paper point sample taken from a healthy oral implant) recovered from three separate patients all harboured *tst* encoding the toxic shock syndrome toxin. None of the isolates investigated harboured genes for exfoliative toxins (*et*A, B and D), though a single CC9 isolate yielded an ambiguous result for *et*A.

Table 4.5. *Staphylococcus aureus* isolates subject to microarray profiling

Patient	Visit	Isolate ID	Site type	State of health	Clonal complex[a]
3	1	DDUH796a-1	Oral rinse	Oral carriage	CC9
7	3	DDUH972a-2	Nasal swab	Nasal carriage	CC7
8	2	DDUH858a-4	Nasal swab	Nasal carriage	CC30
9	1	DDUH097a-2	Oral rinse	Oral carriage	CC22
9	3	DDUH914a-1	Oral rinse	Oral carriage	CC22
10	5	DDUH873a-1	Nasal swab	Nasal carriage	CC30
11	2	DDUH508b-1	Oral rinse	Oral carriage	CC5
12	3	DDUH405a-1	Oral rinse	Oral carriage	CC15
14	1	DDUH042a-1	Oral rinse	Oral carriage	CC9
17	3	DDUH838a-2	Nasal swab	Nasal carriage	CC15
17	3	DDUH837b-1	Oral rinse	Oral carriage	CC15
18	1	DDUH559a-1	Oral rinse	Oral carriage	CC15
20	1	DDUH705b-1	Implant	Periimplantitis	CC15
20	1	DDUH712a-1	Oral rinse	Oral carriage	CC15
21	1	DDUH764a-1	Nasal swab	Nasal carriage	CC22
21	1	DDUH763a-3	Oral rinse	Oral carriage	CC22
24	1	DDUH130a-1	Oral rinse	Oral carriage	CC101
24	1	DDUH128b-2	Tooth (non-adjacent to implant)	Periodontitis	CC101
24	1	DDUH122b-1	Implant	Periimplantitis	CC101
26	2	DDUH974a-1	Implant	Healthy	CC15
26	2	DDUH975b-1	Implant	Healthy	CC30
30	3	DDUH183b-2	Oral rinse	Oral carriage	CC30
31	1	DDUH669a-2	Oral rinse	Oral carriage	CC7
32	1	DDUH011b-1	Oral rinse	Oral carriage	CC8
32	1	DDUH011b-5	Oral rinse	Oral carriage	CC8
32	1	DDUH011b-7	Oral rinse	Oral carriage	CC8
32	3	DDUH479a-1	Oral rinse	Oral carriage	CC8
33	5	DDUH703a-1	Oral rinse	Oral carriage	CC30
33	5	DDUH703a-2	Oral rinse	Oral carriage	CC30
34	4	DDUH517b-3	Oral rinse	Oral carriage	CC8
35	1	DDUH616b-1	Oral rinse	Oral carriage	CC8

[a] *S. aureus* clonal complex as assigned by the Alere StaphyType DNA microarray.

4.3.2.4 *Staphylococcus aureus* microbial surface components recognising adhesive matrix molecules (MSCRAMM) and adhesion and biofilm related genes identified by microarray profiling

A wide range of MSCRAMMs and biofilm associated genes were detected in the *S. aureus* isolates investigated by microarray screening (Table 4.6). The biofilm associated gene *icaA* (encoding intercellular adhesion protein A or N-glycosyltransferase) was detected in all isolates, *icaC* (intercellular adhesion protein C) was present in 29/31 (93.5%) isolates. The biofilm gene *icaD* (biofilm PIA synthesis protein D) was detected in 28/31 (90.3%) of isolates. The *bap* gene associated with a surface protein involved in biofilm formation was detected in a single CC8 isolate (Table 4.6).

4.3.2.5 Antibiotic resistance genes detected in *S. epidermidis* isolates by DNA microarray screening

Forty-three *S. epidermidis* isolates from periimplantitis patients were screened by microarray analysis, 22 of which had been typed by MLST typing (Table 4.7). These isolates were found to harbour a much wider array of antibiotic resistance genes than the corresponding *S. aureus* isolates tested by microarray profiling. The majority of *S. epidermidis* isolates (31/43, 72.1% of isolates) harboured *blaZ* encoding β-lactamase resistance. Four isolates (9.3%) were found to harbour the methicillin resistance gene *mecA*, including one CC1/ST5 isolate, one CC1/ST190 isolate and two isolates not typed by MLST (Table 4.8). All four of these isolates were confirmed to be phenotypically resistant to oxacillin when tested at the NMRSARL. Four isolates (9.3%) harboured the high-level mupirocin resistance gene *ileS2* including one CC1/ST5 isolate, both ST153 isolates and one *mecA*-positive isolate not subject to MLST typing (Table 4.8). All four of these isolates were confirmed to harbour the complete *ileS2* gene using PCR and all four expressed high level mupirocin resistance (all >1024 mg/L) following growth on mupirocin-containing agar (Orla Brennan, personal communication).

Table 4.6. Clonal complexes, *agr* and capsule types and antimicrobial resistance, virulence, MSCRAMM, adhesion factor and biofilm-associated genes identified among *S. aureus* isolates by DNA microarray profiling

Clonal complex[a]	Number of patients	Number of isolates	*agr* type	*Capsule* type	IEC type[b] (*n*)	Virulence associated genes (% isolates positive if <100%)	Antimicrobial resistance genes (% isolates positive if <100%)	MSCRAMMs/adhesion factors/ biofilm associated genes (% isolates positive if <100%)
CC5-MSSA	1	1	II	5	A (1)	*egc*[c]	*blaZ, sdrM, fosB*	*bbp, clfA, clfB, ebhl, ebpS, eno, fib, fnbA, fnbB, map, sdrC, vwb, sasG, icaA, icaC, icaD*
CC7-MSSA	2	2	I	8	D (2)		*blaZ, sdrM, msr*(A) (50.0%)	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, fnbB, map, sdrC, sdrD, vwb, icaA, icaC, icaD*
CC8-MSSA	3	6	I	5	Neg (1), D (5)		*blaZ, fosB, erm*(A) (16.7%), *sdrM* (83.3%)	*bbp, clfA, clfB, ebpS, eno, fnbA, fnbB, map, sdrC, sdrD, vwb, sasG, icaA, icaC, icaD, bap* (16.7%), *ebh* (83.3%), *fib* (83.3%), *bap* (16.7%)
CC9-MSSA	2	2	II	5	Neg (1), B (1)	*egc*[d]	*blaZ, sdrM, fosB, erm*(A) (50.0%), *msr*(A) (50.0%) *fexA* (50.0%),	*clfA, clfB, ebpS, eno, fib, fnbA, map, sdrC, sdrD, vwb, icaA, icaC, bbp* (50.0%),*ebh* (50.0%), *fnbB* (50.0%), *icaD* (50.0%)
CC15-MSSA	5	7	II	8	C (7)		*blaZ, sdrM, fosB, fusB* (14.3%), *qacC* (14.3%)	*bbp, clfA, clfB, ebh, ebpS, eno, fib, fnbA, map, sdrC, sdrD, vwb, sasG, icaA, icaC, icaD, fnbB* (85.7%)
CC22-MSSA	2	4	I	5	B (2)	*egc*[e]	*blaZ*	*clfA, clfB, cna, ebpS, eno, fib, fnbA, sdrC, vwb, sasG, icaA, icaD, fnbB* (50.0%), *sdrD* (50.0%)
CC30-MSSA	5	6	III	8	Neg (2), A(1), B(3)	*tst* (66.7%), *egc*[f], *seh* (16.7%)	*blaZ, sdrM, fosB, erm*(A) (16.7%)	*clfA, clfB, ebpS, eno, fib, fnbA, map, sdrC, vwb, icaA, bbp* (66.7%), *cna* (83.3%), *ebh* (66.7%), *sdrD* (66.7%), *icaC* (66.7%), *icaD* (66.7%)
CC101-MSSA	1	3	I	8	E (3)		*sdrM, fosB*	*bbp, clfA, ebh, ebpS, eno, fib, fnbA, fnbB, map, sdrC, sdrD, vwb, icaA, icaC, icaD, clfB* (66.7%)

[a] Clonal complex as determined by Alere StaphyType DNA microarray.
[b] Immune evasion cluster (IEC) type: A = *sea, sak, chp, scn*; B= *sak, chp, scn*; C= *chp, scn*; D= *sea, sak, scn*; E= *sak, scn.*
[c] *seg, sei, sem, sen, seo, seu.*
[d] *sei, sem, sen* and *seu* detected in all isolates; *seo* detected in 50% of isolates.
[e] *seg, sei, sem, sen, seu.*
[f] *seg* (83.3%), *sei* (83.3%), *sem* (83.3%), *sen* (66.7%), *seo* (16.7%), *seu* (66.7%).

Genes associated with resistance to quaternary ammonium compounds and divalent cations (*qacA* or *qacC*) were found in 11/43 (27.9%) isolates including CC1/ST5, 218, 204, 297 and 432 isolates and in seven isolates not typed by MLST. The fusidic acid resistance gene *fusB* was identified in 25.6% of isolates including CC1/ST5, 73, 153, 190, 204 and ST432 isolates and in three isolates not typed by MLST. Genes associated with macrolide, lincosamide and streptogramin resistance (*erm*(A), *erm*(B) or *erm*(C)) were identified in 10/43 (23.3%) isolates including the CC1/ST5, 218, 190 and ST204 isolates, and in six isolates not typed by MLST. A gene associated with streptogramin resistance (*vga*) was identified in 5/43 (11.6%) isolates including the CC1/ST17 isolate, both CC1/ST153 isolates, one CC1/ST73 isolates and a single isolate not typed by MLST. Genes associated with macrolide resistance (*msr*(A), or *mph*(C)) were identified in 4/43 (9.3%) isolates including all the CC1/ST218, 297 and CC1/ST432 isolates and in one isolate not typed by MLST. Genes associated with aminoglycoside (gentamicin, tobramycin) resistance were found in one CC1/ST73 isolate (*aadD*) and one isolate not typed by MLST. A gene associated with streptothricin resistance (*sat*) was identified in a single isolate not typed by MLST, as was a trimethoprim resistance gene (*dfrA*), which was also identified in a CC1/ST5 isolate. Tetracycline resistance genes (*tetK* and *tetM*) were identified in one isolate not typed by MLST and *tetM* was also identified in the CC1/ST 284 isolate (Table 4.8).

Table 4.7. *Staphylococcus epidermidis* isolates subject to microarray profiling

Patient ID	Visit	Isolate ID	Site type	State of health	MLST ST[a]
4	1	DDUH894a-2	Nasal swab	Nasal carriage	190
7	1	DDUH224a-1	Oral rinse	Oral carriage	73
7	2	DDUH312a-1	Implant	Healthy	153
7	2	DDUH318b-1	Tooth (adjacent to implant)	Periodontitis	153
7	3	DDUH963a-1	Tooth (adjacent to implant)	Healthy	73
7	3	DDUH972a-1	Nasal swab	Nasal carriage	204
8	2	DDUH858a-1	Nasal swab	Nasal carriage	73
9	1	DDUH095a-1	Tooth (non-adjacent to implant)	Healthy	17
10	2	DDUH060a-1	Implant	Periimplantitis	73
13	1	DDUH590b-1	Implant	Periimplantitis	193
19	3	DDUH1000a-1	Implant	Healthy	5
21	1	DDUH761b-1	Implant	Periimplantitis	73
21	1	DDUH764a-3	Nasal swab	Nasal carriage	297
24	1	DDUH124b-1	Tooth (adjacent to implant)	Unknown	200
25	1	DDUH139a-1	Tooth (non-adjacent to implant)	Healthy	284
30	2	DDUH047a-1	Tooth (non-adjacent to implant)	Periodontitis	73
32	6	DDUH745a-1	Implant	Periodontitis	218
34	4	DDUH513a-1	Implant	Healthy	14
34	4	DDUH515b-1	Tooth (adjacent to implant)	Healthy	14
35	1	DDUH613b-1	Implant	Healthy	73
36	1	DDUH119a-1	Oral rinse	Oral carriage	14
36	1	DDUH116b-2	Implant	Periimplantitis	432
6	1	DDUH816a-1	Oral rinse	Oral carriage	Untyped[e]
7	2	DDUH315a-1	Implant	Healthy	Untyped[e]
10	1	DDUH059b-1	Oral rinse	Oral carriage	Untyped[f]
13	1	DDUH598a-1	Oral rinse	Oral carriage	Untyped[f]
17	3	DDUH838a-1	Nasal swab	Nasal carriage	Untyped[c]
17	3	DDUH837a-1	Oral rinse	Oral carriage	Untyped[c]
19	3	DDUH1007a-1	Nasal swab	Nasal carriage	Untyped[b]
21	2	DDUH805b-1	Oral rinse	Oral carriage	Untyped[f]
25	1	DDUH141a-3	Oral rinse	Oral carriage	Untyped[c]
28	1	DDUH717a-2	Oral rinse	Oral carriage	Untyped[b]
29	4	DDUH585a-1	Oral rinse	Oral carriage	Untyped[c]
30	2	DDUH049b-1	Oral rinse	Oral carriage	Untyped[e]
30	3	DDUH183a-1	Oral rinse	Oral carriage	Untyped[d]
31	1	DDUH669a-1	Oral rinse	Oral carriage	Untyped[f]

Continued overleaf

Table 4.7 continued. *Staphylococcus epidermidis* isolates subject to microarray profiling

Patient ID	Visit	Isolate ID	Site type	State of health	MLST ST[a]
32	3	DDUH479a-2	Oral rinse	Oral carriage	Untyped[d]
33	5	DDUH703a-4	Oral rinse	Oral carriage	Untyped[f]
34	4	DDUH517a-1	Oral rinse	Oral carriage	Untyped[f]
35	1	DDUH616b-2	Oral rinse	Oral carriage	Untyped[e]
37	1	DDUH227a-1	Oral rinse	Oral carriage	Untyped[f]
39	5	DDUH847a-1	Oral rinse	Oral carriage	Untyped[c]
40	3	DDUH901b-1	Oral rinse	Oral carriage	Untyped[f]

[a] CC/ST assigned by MLST [118] and goeBURST analysis as described in this chapter.
[b] Presented in microarray results table as part of untyped group 1: *mecA* positive.
[c] Presented in microarray results table as part of untyped group 2: *mecA* negative but *ccr* positive
[d] Presented in microarray results table as part of untyped group 3: SCC*mec* negative and positive for all ACME genes.
[e] Presented in microarray results table as part of untyped group 4: SCC*mec* negative and positive for between 1 and 3 ACME genes.
[f] Presented in microarray results table as part of untyped group 5: SCC*mec* negative and ACME negative.

Table 4.8. Mobile genetic elements SCC*mec* and ACME, and antimicrobial resistance associated genes identified among *S. epidermidis* isolates by DNA microarray profiling

Clonal complex/ Sequence type[a]	Number of patients	Number of isolates	SCC*mec* type[b]	SCC*mec* genes[c] (% positive if <100%)	ACME (*n*)	ACME genes[c] (% positive if <100%)	Antimicrobial resistance genes[c] (% positive if <100%)
CC1/ST5- MRSE	1	1	IV	*mecA, δ-mecR1, ugpQ, dcs, ccrA-2, ccrB-2*	ACME[d]	*arcA, arcB, arcD*	*blaZ, erm*(C), *dfrS1, fusB, ileS2, qacA, qacC*
CC1/ST14- MSSE	2	3	.....	.....	ACME (1), ACME[d] (1), Neg (1)	*arcA* (66.7%), *arcB* (66.7%), *arcC* (33.3%), *arcD* (66.7%)	*blaZ* (66.7%),
CC1/ST17- MSSE	1	1	.....	.....	ACME	*arcA, arcB, arcC, arcD*	*blaZ, vga*
CC1/ST73-MSSE	6	7	.....	.....	ACME (2), ACME[d] (3), Neg (2)	*arcA* (71.4%), *arcB* (71.4%), *arcC* (28.6%), *arcD* (71.4%)	*blaZ, msr*(*A*) (14.3%), *vga* (14.3%), *aadD* (14.3%), *fusB* (42.9%)
CC1/ST153 MSSE	1	2	.....	*ccrA-2, ccrB-2*	ACME[e] (2)	*arcA, arcC, arcD*	*blaZ, msr*(*A*), *vga, fusB, ileS2*
CC1/ST190- MRSE	1	1	V & *ccrB*4	*mecA, ugpQ, ccrAA, ccrC, ccrB-4*	ACME	*arcA, arcB, arcC, arcD*	*blaZ, erm*(C), *fusB*
CC1/ST200- MSSE	1	1	.....	.....	ACME	*arcA, arcB, arcC, arcD*	*blaZ, tet*(K)
CC1/ST204- MSSE	1	1	.....	.....	Neg		*blaZ, erm*(C), *fusB, qacC*
CC1/ST218- MSSE	1	1	.....	*ccrA-2, ccrB-2*	ACME	*arcA, arcB, arcC, arcD*	*blaZ, erm*(B), *msr*(*A*), *mph*(*C*), *qacA*
CC1/ST284- MSSE	1	1	.....		Neg	*arcA, arcD*	*blaZ, tet*(M)
CC1/ST297- MSSE	1	1	.....	*ccrA-2, ccrB-2*	ACME	*arcA, arcB, arcC, arcD*	*msr*(*A*), *mph*(*C*), *qacA*

Continued overleaf

Table 4.8 continued. Mobile genetic elements SCC*mec* and ACME, and antimicrobial resistance associated genes identified among *S. epidermidis* isolates by DNA microarray profiling

Clonal complex/ Sequence type[a]	Number of patients	Number of isolates	SCC*mec* type[b,c]	SCC*mec* genes[c] (% positive if <100%)	ACME (*n*)	ACME genes[c] (% positive if <100%)	Antimicrobial resistance genes[c] (% positive if <100%)
CC1/ST432- MSSE	1	1	.....	.....	ACME	*arcA, arcB, arcC, arcD*	*msr(A), mph(C), fusB, qacA*
CC3/ST193- MSSE	1	1	.....	.....	Neg		*blaZ,*
Untyped group 1- MRSE	2	2	IV	*mecA, δ-mecR1, ugpQ, ccrA-2, ccrB-2, dcs (50%)*	ACME (1), Neg (1)	*arcA* (50%), *arcB* (50%), *arcC* (50%), *arcD* (50%)	*blaZ, qacA, erm*(A) (50%), *erm(*C) (50%), *aphA* (50%), *sat* (50%), *dfrA* (50%), *fusB (50%), ileS2 (50%), qacC (50%)*
Untyped group 2- MSSE	5	5	.....	*ccrA-2, ccrB-2*	ACME (2), ACME[d] (1), Neg (2)	*arcA* (60%), *arcB* (60%), *arcC (40%), arcD (60%)*	*blaZ* (40%), , *erm(*C) (20%), *msr(A)* (20%), *mph*C*(20%), fusB (20%%),* tet(K) (20%), *tet*(M) (20%), *qacA (40%)*
Untyped group 3- MSSE	2	2	.....	.....	ACME (2)	*arcA, arcB, arcC, arcD*	*blaZ* (50%), *erm*(B) (50%)
Untyped group 4- MSSE	4	4	.....	.....	ACME[d](2), Neg (2)	*arcA, arcC, arcD*	*blaZ* (75%), *erm*(B) (25%)
Untyped group 5- MSSE	8	8	.....	.....	Neg (8)		*blaZ* (50%), *erm(*C) (12.5%), *msr(A)* (12.5%), *mph*C (12.5%), *vga (12.5%), fusB* (12.5%), *qacA* (37.5%), *qacC* (12.5%)

[a] CC/ST assigned by MLST [118] and goeBURST analysis as described in this chapter.
[b] SCC*mec* type assigned after manual inspection of DNA microarray profiles, ... *mecA* not present therefore no SCC*mec* type assigned.
[c] ..... no genes detected.
[d] *arcC* ambiguous.
[e] *arcB* ambiguous.

4.3.2.6 Virulence associated genes identified in *S. epidermidis* by DNA Microarray profiling

The only significant virulence-associated genes identified in the *S. epidermidis* isolates subject to microarray profiling belonged to the staphylococcal arginine catabolic mobile element (ACME), *arcA*, *arcB*, *arcC* and *arcD*, which were identified in 24/43 (54%) of the isolates investigated (Table 4.8). Due to the generally weaker signals produced by *S. epidermidis* using the Alere StaphyType microarray an isolate was deemed ACME-positive if all four genes (*arcA*, *arcB*, *arcC* and *arcD*) yielded positive hybridisation signals (14/24 ACME-positive isolates), or if three of the four genes were positive and the fourth was ambiguous (10/24 ACME-positive isolates). If less than three ACME genes were detected the isolate was deemed ACME-negative (Table 4.8). The prevalence of ACME carriage by *S. epidermidis* isolates recovered from the general oral cavity by oral rinse sampling was 30% (6/20 isolates). In contrast, 76.4% (13/17) of isolates recovered subgingivally from implant (8/10) or tooth (5/7) sites were ACME-positive.

Other virulence factors were far less common. Staphopain A (*sspP*) was identified in both CC1/ST153 isolates investigated, a single CC1/ST73 isolates and two isolates not typed by MLST (Appendix 1). Genes encoding haemolysin gamma, component C (*lukS*), staphylokinase (*sak*) and staphylococcal complement inhibitor, or SCIN, (*scn*) were identified in three separate isolates not typed by MLST.

4.3.2.7 MSCRAMM, adhesion factor and biofilm associated genes identified in *S. epidermidis* by DNA Microarray profiling

MSCRAMM and adhesion factor genes were rarely identified in the *S. epidermidis* isolates using the microarray. The gene coding for serine-aspartic acid rich fibrinogen-binding, bone sialoprotein binding protein C (*sdr*C) was identified in the CC1/ST218, 284 and 432 isolates, two CC1/ST73 isolates and three isolates not typed by MLST. The gene coding for fibronectin binding protein A (*fnbA*) was identified in a single CC1/ST14 isolate, while the gene encoding fibronectin binding protein B (*fnbB*) was identified in one CC1/ST73 isolate and one isolate not typed by MLST (Appendix 1).

Interestingly, no biofilm-associated *ica* genes (encoding intercellular adhesion proteins A and C and biofilm PIA synthesis protein D) were detected in any of the *S. epidermidis* isolates subject to microarray profiling (Appendix 1).

4.3.2.8 Detection of SCC*mec* and *ccr* genes in *S. epidermidis* by DNA microarray profiling

Genes associated with Staphylococcal Cassette Chromosome *mec* (SCC*mec*) were identified in the four *S. epidermidis* isolates found to harbour *mecA* including a CC1/ST5 isolate, a CC1/ST190 isolate and two non-MLST typed isolates (Table 4.8). All four isolates also harboured the SCC*mec* gene *ugpQ* and exhibited variably positive results for other SCC*mec*-associated genes such as *mecR* and *dcs* (Table 4.8). All four *mecA*-positive isolates also carried a combination of SCC*mec* associated cassette chromosome recombinase *ccr* genes, the three SCC*mec* type IV were *ccrAB*2, while the SCC*mec* type V was *ccrB*4 (*ccrAA*, *ccrC*, *ccrB*4). However, *ccr* genes were also identified in nine additional *mecA*-negative isolates including isolates belonging to CC1/ST153, ST218, ST297 and six non-MLST typed isolates (all *ccrAB*2).

No evidence of shared antimicrobial resistance or virulence-associated genes in *S. aureus* and *S. epidermidis* isolates from the same patient determined by microarray profiling

Coagulase negative staphylococci are thought to be either the origin of, or a reservoir for, many mobile genetic elements that are found in *S. aureus*. The microarray results, for a selection of matched *S. epidermidis* and *S. aureus* isolates obtained from the same clinical site, or isolated from the same patient did not appear to show any patterns suggesting that *S. aureus* and *S. epidermidis* isolates from the same patient (or even site) shared common antimicrobial resistance or virulence-associated genes (Appendix 1).

4.4 Discussion

4.4.1 *Staphylococcus epidermidis* population analysis by multi locus sequence typing

MLST was selected as an appropriate tool to explore the population structure of a selection of *S. epidermidis* isolates recovered from healthy and diseased implant and tooth sites and nares samples in the periimplantitis patients investigated. MLST has been used extensively to investigate the population structure of *S. aureus* and many other bacterial and fungal species. The *S. aureus* MLST database is particularly well populated because of its importance as a nosocomial pathogen (http://saureus.mlst.net). Because MLST is a sequence-based typing method, data generated by different researchers can be readily shared over the internet and the establishment of international databases for MLST data has facilitated its widespread application.

In the present study the majority of *S. epidermidis* STs identified (19/22, 86.4%) were assigned to CC1, as were the majority of STs of other isolates in the international *S. epidermidis* MLST database (Figure 4.1). Indeed CC1 contained 69.5% (505/727) of all isolates present in the global *S. epidermidis* MLST database that were not derived from the current study. Table 4.4 provides a list of the sources of all CC1 *S. epidermidis* isolates in the global MLST database excluding isolates from the present study. Of the 505 isolates from the database assigned to CC1, 43 (8.5%) originated from animals, 63 (12.5%) were environmental isolates (19 of which were hospital-associated) and 17 isolates (3.4%) were taken from intravenous lines, catheters or other "medical equipment". The remaining 382 isolates from CC1 originated from human samples including 94 isolates (18.6%) from healthy community subjects, 18 (3.7%) colonisation isolates from healthcare workers and 27 (5.3%) inpatient colonisation isolates. There were 64 (12.7%) blood isolates, 109 isolates (21.6%) derived from wounds, biopsies, or other infections and the 70 (13.9%) remaining isolates were undefined (Table 4.4).

Of the three STs identified in the present study that were not assigned to CC1, one was assigned to CC3, one to CC4 and the last was a singleton. The MLST global database isolates, not from this study, in CC3 (n=17) included two (11.8%) bovine mastitis, one (5.9%) environmental and 14 (82.3%) healthy community subject isolates (Table 4.4). The MLST global database isolates, not from this study, in CC4 (n=6) included one hospital environmental, one non-hospital environmental, one inpatient colonisation, one blood culture and two healthy community isolates (Table 4.4).

None of the *S. epidermidis* isolates from periimplantitis patients in the present study were assigned to CC2, the second largest CC present in the global MLST database (n=36). MLST global database CC2 contains no isolates from animals and only three environmental isolates. Of the human isolates in CC2, very few were from healthy subjects in the general community. CC2 contains colonisation isolates from inpatients, colonisation isolates from healthcare workers, blood culture isolates, wound isolates, an isolate from cerebrospinal fluid listed as "infection", as well as isolates from catheters or "medical equipment". A further five isolates (13.9%) belonging to CC2 were undefined. On superficial inspection it would appear that CC2 may be enriched for hospital-associated isolates. However, further investigation revealed that 25% of the isolate profiles were all methicillin-resistant *S. epidermidis* (MRSE) isolates submitted by the same individual or research group, collected from Denmark (four isolates), Cape Verde (two isolates), Portugal (one isolate), Mexico (one isolate) and Greece (one isolate). Likewise another group of four inpatient carriage isolates, all from Sweden, were all submitted by another individual or group. Clonal complex 2 may be enriched for healthcare associated (and potentially more pathogenic) isolates but, because of the relatively small size of the global MLST database, the overall characteristics of isolates in CC2 are possibly unduly influenced by an abundance of isolates submitted by single studies, or from particular geographic areas.

The STs identified in our population of *S. epidermidis* isolates have been identified at a range of geographic locations (spanning Europe, North and South America and parts of Asia), possibly limited only by the lack of studies conducted into *S. epidermidis* populations to date. They have been associated with general carriage, environmental isolates, bovine mastitis as well as blood and surgical infections (Table 4.3).

ST73 was the most abundant ST identified among *S. epidermidis* isolates recovered from periimplantitis patients in the present study, accounting for 36.2% (17/47 isolates subject to MLST) of all isolates tested and recovered from 11/25 (31.4%) patients from whom isolates were typed (Table 4.2, Figure 4.2). This sequence type has previously been recovered from both clinical and environmental samples and belongs to CC1 (Table 4.3, Figure 4.2). Sequence type 73 was identified in one recent systematic study, with isolates obtained from both the hospital and community (information not yet entered into the MLST.net database) [90]. Sequence type 73 has also been identified among single isolates

selected from independent studies for MLST and in unpublished studies entered into the MLST.net database [118, 170]. Of the five ST73 entries currently in the MLST.net *S. epidermidis* database, three are environmental in origin, one is from a skin carriage site (behind the ear) and the other is from an inpatient nasal swab [99, 170, 175] (Table 4.3). A study by Rolo *et al.* did not specify the exact numbers of isolates typed as ST73, but have listed ST73 as being recovered from nasal swabs from both community and hospitalised subjects. The other frequently identified STs in this study, ST153, ST256 and ST14 have been identified in carriage isolates (skin and nasal colonisation), blood isolates and environmental isolates from Denmark, Germany, Sweden and the USA (Table 4.3).

The high prevalence of ST73 *S. epidermidis* isolates identified in the present study is unusual relative to the findings of other studies that used the same MLST typing scheme as used here (i.e. the MLST scheme of Thomas *et al.*). Several such previous studies identified ST2 as the predominant ST, which corresponds to ST27 using the Wisplinghoff *et al. S. epidermidis* MLST scheme [88, 90, 92, 118, 163, 166]. ST2 is thought to be very successful at colonising sites such as implants or catheters because all ST2 isolates previously characterised have formed biofilms (determined by physical testing or PCR analysis of biofilm associated genes) [88, 91, 94, 97]. The absence of ST2 from *S. epidermidis* isolates investigated in the present study may be due to differences between the patient populations or anatomical sites sampled in different studies, but this does not explain the relative abundance of ST73 in the present study. In the present study ST73 was recovered from all site types (nasal swabs, oral washes, healthy and diseased implants and teeth) and in around one third of the patients that isolates were typed from. Previous studies using MLST to systematically type clinical *S. epidermidis* isolates have been carried out in China, Portugal and the USA and the overall difference in human population profiles compared to the present study is noticeable. It is possible that the difference in ST abundance is due to the difference in the general geographic distribution of *S. epidermidis* strains. It is possible that ST73 is more predominant among *S. epidermidis* isolates from Irish individuals.

The *S. epidermidis* STs identified in the present study did not belong to any CCs that appeared to be enriched for isolates derived from human, animal or environmental sources. Furthermore, they do they belong to any CCs where there appears to be any enrichment for isolates derived from hospital or community sources. In total 14 other *S. epidermidis* isolates in the global MLST database originated from prosthesis infections (including both

prosthetic joints and artificial heart valves), 10 belonged to CC1, one to CC6 and the remaining three were either singletons or members of CCs containing only 2 STs. Thus it is evident that the *S. epidermidis* isolate STs from the periimplantitis patient cohort in the present study do not appear to represent any unusual sub-population of *S. epidermidis* STs. The majority are members of the largest CC within the global *S. epidermidis* population as represented by entries in the international MLST database, and are well distributed throughout it. However, the current population of *S. epidermidis* STs in the global MLST database is relatively limited (727 isolates and 472 identified STs when last updated in August 2012) compared to more established MLST population databases for bacterial species such as *S. aureus* (4703 isolates and 2595 STs as at 13th February 2013). Representations of the current global *S. epidermidis* population structure based on the current data set are likely to be deceptively simple. As more surveys of *S. epidermidis* are conducted, and the MLST database is expanded a more detailed picture of the relationship between various STs and their origins will become apparent. Based on our current understanding of the population structure of *S. epidermidis* determined by MLST relative to *S. aureus*, it would appear that the former is significantly less diverse than the latter. However, this may be just a reflection of the relative number of isolates in each MLST database and the relative diversity of isolate origins. Nonetheless, the prevalence of ST73 isolates among Irish periimplantitis patients is intriguing and warrants further investigation.

4.4.2 DNA microarray analysis of *S. aureus* and S. *epidermidis* populations from periimplantitis patients

Microarray profiling of a selection of 31 *S. aureus* isolates from 20 separate periimplantitis patients assigned the isolates to eight different clonal lineages including CC5, CC7, CC8, CC9, CC15, CC22, CC30 and CC101 (Table 4.6). All of the isolates were methicillin-susceptible *S. aureus* (MSSA) and lacked *mecA* and other SCC*mec*-associate genes. The relative diversity identified among the *S. aureus* isolates from the periimplantitis patients contrasts with the clonal nature of the ST22 MRSA-IV currently endemic in Irish hospitals [107]. Microarray profiling of the *S. aureus* isolates from the periimplantitis patients also showed that they did not harbour a significant number of antimicrobial agent resistance genes and could be predicted to be susceptible to most classes of antibiotic. A recent study by Shore *et al.* (2012) demonstrated that the correlation between isolate antimicrobial resistance phenotype and the presence of specific resistance genes in *S. aureus* detected by microarray profiling was >97% [107]. The *S. aureus* isolates from the periimplantitis

patients harboured virulence-associated and MSCRAMM genes typically found in *S. aureus* (Table 4.6). The majority harboured Immune Evasion Cluster (IEC) genes. These are usually encoded on lysogenic bacteriophages that integrate into the chromosomally located β-haemolysin gene *hlb* and are a typical feature of human *S. aureus* isolates [176, 177]. Interestingly, the enterotoxin gene complex *egc* was identified in only 42% of isolates, all of which belonged to CC5, CC9 and CC30 (Table 4.6). Four CC30 isolates recovered from one periimplantitis patient harboured the *tst* gene encoding toxic shock syndrome toxin. Overall, microarray profiling of the selected *S. aureus* isolates from periimplantitis patients revealed a diverse group of isolates that were generally susceptible to antimicrobial agents. Individual patterns of virulence-associated, adhesion, MSCRAMM and biofilm-associated genes were to some extent lineage-specific.

4.4.2.1 SCC*mec*

In contrast to the *S. aureus* isolates, microarray profiling of 43 selected *S. epidermidis* isolates from periimplantitis patients, 22 of which were also typed by MLST, revealed the presence of a wider variety of antimicrobial resistance genes (Table 4.8) many of which have been identified previously in the *S. aureus* isolates. Significantly, four of the *S. epidermidis* isolates from separate patients subject to microarray profiling were found to harbour SCC*mec* including one CC1/ST5 isolate (SCC*mec* IV, *ccrAB*2), one CC1/ST190 (SCC*mec* V, *ccrB*4) and two non-MLST typed isolates (both SCC*mec* IV, *ccrAB*2), (Table 4.8). These findings were not surprising as many previous studies have reported the relatively frequent detection of SCC*mec* elements in CoNS, particularly *S. epidermidis* [99]. Previous studies have shown that SCC*mec* IV is the most commonly identified SCC*mec* type in *S. epidermidis,* in agreement with the findings of the present study. A further nine *S. epidermidis* isolates (21%) from the periimplantitis patients were *mecA*-negative, but *ccrAB*2-positive. The *ccr* type *ccrAB*2 (also referred to as type 2) is generally associated with SCC*mec* type IV [91, 98, 106]. These findings also agree well with previous surveys of methicillin resistant *S. epidermidis* (MRSE) [91, 94, 95, 98, 99, 169]. Previous studies investigating methicillin resistance in *S. aureus* and CoNS have found that CoNS can harbour a wide range of SCC*mec* elements in common, but the different species may not exhibit the same resistance phenotype. For example a previous study has shown that *S. epidermidis* and *S. aureus* isolates containing the same SCC*mec* elements (SCC*mec* IV) have different oxacillin susceptibilities: *S. epidermidis* exhibiting a much lower average minimum inhibitory concentration (MIC), with a wider distribution of MICs, than the *S. aureus* isolates [93]. Another study found that of six *S. epidermidis* isolates that

tested positive for the *mec*A gene using PCR, only one was phenotypically resistant to oxacillin when tested using microdilution [178].

The complete absence of any SCC*mec* associated genes in the *S. aureus* population tested using the microarray is not totally unexpected, given the small sampling population, and must be seen as a good thing. While the isolates were obtained from patients within the dental healthcare system there was no evidence of hospital acquired (HA)-MRSA or community-associated (CA)-MRSA clones within the cohort of patients investigated. On the other hand the presence of two SCC*mec* types and associated genes in four of the *S. epidermidis* isolates from separate patients causes more concern. As well as reflecting the population of MRSE in the community to which our patients belong they may be a reservoir of SCC*mec* elements that could be transferred into MSSA giving rise to new MRSA strains.

No pattern was observed between the antibiotic resistance genes present in *S. aureus* and *S. epidermidis* isolates obtained from the same clinical sample, or same patient. Indicating that, though horizontal gene transfer was possible, it had not obviously occurred within the sample population investigated.

4.4.2.2 ACME

The staphylococcal MGE ACME was not identified in any of the *S. aureus* isolates tested by microarray profiling. In contrast, ACME was identified in 24/43 (54%) *S. epidermidis* isolates subject to microarray profiling, with ACME-associated *arc* genes (but not the full ACME element) detected in a further three (7%) isolates. There was a significant difference between the prevalence of ACME in *S. epidermidis* isolates derived from the general oral cavity (from oral rinse sampling) and *S. epidermidis* isolates obtained from periodontal and periimplant pockets (Chi squared $P<0.025$). The prevalence of ACME in *S. epidermidis* isolates recovered from the general oral cavity by oral rinse sampling was 30% (6/20 isolates). In contrast, 76.5% (13/17) of isolates recovered subgingivally from implant (8/10) or tooth (5/7) sites were ACME-positive. ACME is generally located adjacent to the SCC*mec* element and was first identified in the ST8 MRSA strain USA300 harbouring an SCC*mec* type IV element [76, 103]. It has been proposed that the ACME-*arc* cluster, encoding a full arginine deiminase pathway, enhances the ability of its host staphylococcus to survive and proliferate, allowing survival at low pH levels and low oxygen environments as well as conferring some protection against innate immune

responses involving nitric oxide [76]. The *arc* genes present in ACME resemble native *S. epidermidis arc* gene sequences, and it has been proposed that ACME originated in *S. epidermidis* and has since been spread through other CoNS species and into *S. aureus* [76]. ACME is often located adjacent to the SCC*mec* element and the two MGEs integrate into the same attachment site in the staphylococcal chromosome within *orfX* and similar to SCC*mec* elements, ACME is flanked by repeat sequences. SCC*mec*-encoded *ccr* genes catalyze integration and excision of ACME from the staphylococcal chromosome [76, 103]. ACME has been particularly associated with SCC*mec* type IV, the most commonly identified SCC*mec* type present in *S. epidermidis*, and the most common type identified in MRSE in the present study (Table 4.8) [76, 91, 94, 95, 98, 99, 103, 169]. ACME has been observed to be highly prevalent in *S. epidermidis* carriage isolates (range 58-67%) and neonatal *S. epidermidis* blood isolates (43%), while the element was only identified in 13% of isolates obtained from prosthetic joint infections [76, 110, 179]. The prevalence of ACME in the *S. epidermidis* isolates subject to microarray profiling in the present study is very similar to that reported in other groups of commensal isolates recovered from both healthy subjects not associated with a healthcare environment, and from subjects upon admission to hospital [76, 179].

4.4.2.3 Mupirocin resistance

The *ileS2* gene (also known as *mupA* and formerly *mupR*) encoding high level mupirocin resistance was identified in 4/43 (9.3%) of the *S. epidermidis* isolates subject to microarray profiling. These four isolates yielded *ileS2*-specific amplimers by PCR and all exhibited a high level mupirocin resistance phenotype (all >1024 mg/L) following testing using disk diffusion and E-test strips (O. Brennan, personal communication). Mupirocin is an antibiotic of the monoxycarbolic acid class originally isolated from the Gram-negative bacterium *Pseudomonas fluorescens*. Mupirocin is bacteriostatic at low concentrations and bactericidal at high concentrations. It is used topically, is effective against Gram-positive bacteria and commonly used to decolonise patients and healthcare workers who are colonised by MRSA. In staphylococci high level mupirocin resistance mediated by the *ileS2* gene is frequently encoded on large conjugative plasmids that can be readily transferred among different *S. aureus* strains and between *S. aureus* and CoNS [103, 104]. In the present study, the four *S. epidermidis* isolates found to harbour the *ileS2* gene were recovered from two patients, two isolates from each patient at the same clinical visit in each case, but from different sampling sites; a healthy implant site, and a tooth affected by periodontitis located adjacent to an implant, from patient 19 collected at the third clinical

visit, and from a healthy implant, and a nasal swab, from patient 7 collected at the second clinical visit (Table 4.7). Three of the four isolates were typed using MLST, one belonged to ST5 and the other two to ST153. The fourth isolate was not typed using MLST, but was also *mecA*-positive.

The presence of high level mupirocin resistance among *S. epidermidis* isolates, especially nasal carriage isolates is of some concern. Mupirocin resistance in commensal bacteria, such as *S. epidermidis*, on its own is not necessarily a problem as it is unlikely that the antibiotic would be used to eliminate such an organism. However, as mentioned above, mupirocin is commonly used to decolonise at risk patients prior to medical/surgical treatment and, more importantly in some cases, to decolonise patients and healthcare workers who are carriers of MRSA. The *ileS2* gene is usually located on conjugative plasmids and is easily transferred between CoNS and *S. aureus* and has been documented to do so in a clinical setting [104]. Should a patient or health worker carrying a mupirocin-resistant strain of *S. epidermidis* become colonised by MRSA there is the possibility that the *ileS2*-carrying plasmid could be acquired by the MRSA. This could result in MRSA decolonisation failure and the potential spread of the mupirocin-resistant MRSA amongst the patient's/health worker's close contacts, or within the hospital environment, resulting in the need for more extreme infection control measures.

4.4.2.4 Genes encoding resistance to other types of antimicrobial agents

Isolates of both *S. aureus* and *S. epidermidis* tested carried resistance genes for several other classes of antimicrobials and antibiotics including a gene associated with macrolide/lincosamide resistance (*msr*(A), or *mph*(C)) and streptogramin resistance (*vga*) along with genes associated with macrolide resistance (*msr*(A), *mph*(C), *erm*(A), *erm*(B) and *erm*(C)) and resistance to quaternary ammonium compounds (*qacC*). Resistance to these antibiotics and disinfection agents have all been recorded previously in both *S. epidermidis* [91, 92, 94, 154, 169] and *S. aureus* [55, 67, 156], as well as in mixed subgingival staphylococcal species [130]. That the majority of *S. aureus* isolates carried genes encoding fosfomycin and bleomycin resistance (*fosB*), was not that surprising as a high percentage of isolates containing the *fosB* gene has previously been reported, though some studies have found low incidences of phenotypic fosfomycin resistance within MSSA and MRSA [107, 180]. The identification of the chloramphenicol resistance gene *fexA* in a single *S. aureus* CC9 isolate is more unusual, but not unprecedented being reported as a gene that is variably present in several *S. aureus* CCs in a survey of antibiotic resistance genes in MRSA clones

screened with the DNA microarray used in the present study [67]. A fusidic acid resistance gene was only identified in a single *S. aureus* isolate (*fusB*). Fusidic acid resistance is relatively uncommon in *S. aureus*, but its incidence is increasing, possibly due to overuse or inappropriate use of topical fusidic acid monotherapy [67, 74, 181]. Interestingly, *fusB* was identified in 25.6% of the *S. epidermidis* isolates subject to microarray profiling. Fusidic acid is a very useful antibiotic for the treatment of staphylococcal infections particularly those which have developed resistance to other antibiotics such as MRSA and vancomycin-intermediate *S. aureus* (VISA) [74, 181]. The relatively high prevalence of *fusB* in the *S. epidermidis* isolates investigated is of concern because of the potential for its transfer to *S. aureus* where it could compromise the use of this antibiotic.

4.4.2.5 Virulence factors, other than ACME associated *arc* genes

As outlined above the majority of virulence factors present in *S. epidermidis* belonged to the MGE ACME. The *S. aureus* isolates were positive for a range of virulence genes including aureolysin, staphopain A and B and various combinations of serine proteases.

IEC genes were present in the majority of isolates, with only four isolates (13%) returning negative microarray signals for all IEC genes. The most common IEC types were C and D (26% of IEC positive isolates each), closely followed by type B (22.2% of IEC positive isolates). High population proportions of IEC types B and D have been reported before [107, 176]. All of the type C isolates in the present study belonged to the same CC (CC15), while the type B and D isolates were spread among several different CCs (Table 4.6). The *S. aureus egc* gene cluster encoding superantigens was present in 42% of isolates tested using the microarray. Previous studies have identified the *egc* in 37% of MRSA, and 57% of carriage *S. aureus* respectively [73, 107]. None of the virulence gene patterns identified by the DNA microarray in the *S. aureus* isolates tested were particularly unusual compared to other isolates tested using the same method [67, 107]. The only virulence factor that may be of concern was the presence of *tst* (toxic shock syndrome toxin) detected in four CC30 isolates, recovered from three separate patients. Three of the isolates were derived from general carriage samples (two from oral rinse samples, one from a nasal swab), while the fourth *tst*-positive isolate was recovered from a sample taken from a healthy oral implant.

4.4.2.6 MSCRAMM, and adhesion factor and biofilm associated genes

As noted in the results section the MSCRAMM, adhesion factor and biofilm associated genes identified in both *S. aureus* and *S. epidermidis* using the DNA microarray did not

return any unusual patterns of genes. The only surprise was the complete lack of biofilm-associated *ica* genes in *S. epidermidis*. These genes have been identified frequently in clinical isolates, though they have been shown not to be of significance as an indicator of hospital associated *S. epidermidis* clones [166].

4.5 Conclusions

1. *Staphylococcus epidermidis* isolates from periimplantitis patients typed using MLST belonged to a wide range of STs, the most prevalent of which was ST73, in contrast to previous studies where ST2 was the most prevalent. No particular STs were associated with isolates obtained from any particular sampling site type (tooth, implant, oral rinse or nasal swab) or state of health (healthy or diseased teeth or implants).
2. When combined with the *S. epidermidis* global MLST database entries, all of the STs identified in the periimplantitis patients, except for three, were assigned to CC1. The isolates were spread throughout the range of STs identified in other studies from varying geographic locations and types of isolate (environmental isolates, pathogenic isolates or carriage isolates). Apart from the predominance of ST73, it does not appear that the *S. epidermidis* isolates from the periimplantitis patients vary greatly from the general global *S. epidermidis* population.
3. The *S. aureus* isolates obtained in the present study were all MSSA, were diverse and belonged to eight different clonal complexes, the most commonly identified of which was CC15 (7 isolates from 5 patients), followed by CC30 (6 isolates from five patients) and CC8 (6 isolates from 3 patients).
4. The *S. aureus* isolates that were screened for *S. aureus* antibiotic resistance, virulence, MSCRAMM, adhesion factor and biofilm-associated genes by DNA microarray profiling did not harbour any unusual patterns of antibiotic resistance, virulence, MSCRAMM, adhesion factor or biofilm-associated genes suggesting they are carriage isolates.
5. The *S. epidermidis* isolates screened for staphylococcal antibiotic resistance, virulence, MSCRAMM, adhesion factor and biofilm associated genes by DNA microarray profiling contained a wider range of antibiotic resistance genes than the *S. aureus* isolates tested. Four isolates were MRSE and harboured SCC*mec*. The *S. epidermidis* isolates harboured far fewer *S. aureus* virulence genes than the *S. aureus* isolates tested. However, 55.8% of the isolates harboured the ACME

element associated with enhanced host colonisation and survival on skin and mucosal surfaces.

6. *Staphylococcus aureus* and *S. epidermidis* isolates obtained from the same, or different, sampling sites from a single patient did not show any similarity in the pattern of antibiotic resistance, virulence associated, MSCRAMM, adhesion or biofilm associated genes.

Chapter 5

Detection and quantification of *S. aureus* and *S. epidermidis* DNA present in clinical samples from periimplantitis patients using real-time PCR and checkerboard DNA:DNA hybridisation

5.1 Introduction

The majority of previous studies that investigated the microbiota associated with oral implants in general, and oral implantitis in particular, have assumed that there would be very little difference from the microbiota associated with periodontal areas and periodontitis. Relatively few studies have investigated oral staphylococcal populations in general, and even fewer have investigated staphylococcal populations around oral implants and natural teeth. Most staphylococci are generally regarded as epidermal bacterial species and are often considered to be contaminants when they are found in clinical specimens. Studies investigating the microbial flora of the oral cavity that have included staphylococci (particularly *S. aureus* and *S. epidermidis*) in their testing panels/isolation protocols have found staphylococci to be more prevalent than would be expected if they were just transient microbes recently imported from another site [23, 31, 130-132, 167, 182, 183]. Studies using molecular methods of concurrent identification and quantification have suggested that *S. aureus* may be an important pathogen involved in periimplantitis and oral implant failure [8, 35, 40, 47]. Other studies that utilised culture-based methods to quantify oral microbial species followed by laboratory identification showed that *S. epidermidis* was more than an "occasional contaminant" of clinical samples and may in fact be a member of the oral microbial flora [130, 131].

Previous studies that investigated associations between periimplantitis and staphylococci have primarily focused on the detection of *S. aureus* as a potential pathogen using molecular methods [8, 35, 40, 47]. However, molecular identification and quantification methods, while very sensitive and relatively specific, can have significant limitations. For example, molecular detection of a specific microorganism, in most cases, tests for the presence of DNA in the absence of detection of viable organisms. Furthermore, the majority of microorganisms in nature and in many types of human infections live as part of a biofilm community, and most biofilms contain significant amounts of free DNA released from lysed microbial cells. Interestingly, *S. aureus* mutant derivatives exhibiting reduced lysis form less adherent biofilms than wild type strains [152]. Thus the presence of DNA in biofilms could possibly result in incorrect interpretations of molecular detection and quantification test results. In such a scenario *S. aureus* may be identified as a current part of the microbial population from a particular clinical site such as a failing oral implant, when in reality it may have been superseded by other microorganisms and only its DNA remains in the biofilm matrix. Furthermore, in this scenario, the presence of a significant

density of *S. aureus* may be estimated by molecular detection and quantification tests, when in reality the actual level of viable bacteria may be significantly lower.

The recovery of viable staphylococci from diseased and healthy implant and natural tooth sites in the periimplantitis patient cohort investigated in the present study clearly demonstrated that *S. aureus* was not significantly associated with periimplantitis (Chapter 3). In contrast, several previous studies using checkerboard DNA:DNA hybridisation-based methods indicated that *S. aureus* was significantly associated with periimplantitis [8, 35, 40, 47]. These conflicting results could be due to the inherently different approaches used in viable culture versus molecular detection and quantification.

Checkerboard DNA:DNA hybridisation (CKB) is a molecular technique that utilises digoxigenin-labelled total cellular DNA probes from between 40 and 80 bacterial species (depending on the equipment being used) to identify and quantify the species present in clinical samples. DNA extracts from test samples are laid on a nylon membrane using a manifold that allows the samples to be dispersed in discrete rows across the membrane. After the test samples have been bound to the membrane by ultraviolet light crosslinking, the labelled whole genome probes are introduced using another manifold that allows the delivery of the probes in columns down the membrane, forming the 'checkerboard' pattern. Digoxigenin-labelled probes that hybridise to DNA from test samples yield a signal that is proportional to the amount of target DNA present yielding a semi-quantitative estimate of the amount of target DNA present in the test sample [35, 136, 137]. The technique is extremely useful in that it allows a lot of information to be derived from a set of samples in a relatively short amount of time. However there are drawbacks. As outlined in Chapters 1 and 3, CKB analysis estimates the amount of DNA present in a test sample for each species included on the testing panel, but does not permit any estimate of the viable cell density present. Furthermore, whole genomic DNA probes from individual bacterial species may well cross-hybridise with DNA from other species present in the test sample if different species share genes in common. Such is likely to be the case with *S. aureus* and CoNS, which frequently harbour similar or identical MGEs encoding antimicrobial agent genes (e.g. SCC*mec*) or virulence genes (e.g. ACME element) [71, 90, 103, 110, 112, 179, 184].

The high prevalence of *S. aureus* detection by CKB analysis at oral implant and tooth sites in several previous studies (see Chapter 1) is in stark contrast to the very low prevalence of

S. aureus detected by culture and 16S rDNA gene sequence species identification from oral implant and tooth sites in the present study (see Chapter 3). Furthermore, the only study to date that included *S. epidermidis* in the CKB analysis of the microbiota of oral implant and tooth sites (Persson *et al.* [35]) reported a low prevalence of *S. epidermidis* (3.3-3.8% of tooth and implant sites). This low prevalence of *S. epidermidis* detection by CKB analysis contrasts strongly with the relatively high prevalence of *S. epidermidis* detected by culture and 16S rDNA gene sequence species identification from oral implant and tooth samples from the majority of patients in the present study (present in 19-37% of paper point samples and 1.9-25% of curette samples taken from periimplantitis sites pre- and post-treatment, 33-37% of paper point and 9-28% of curette samples taken from diseased tooth sites pre- and post-treatment, 11-35% of paper point samples and 11-19% of curette samples taken from healthy implant sites pre- and post-treatment, 6-21% of paper point samples and 8-11% of curette samples taken from healthy tooth sites pre- and post-treatment) see Chapter 3. As mentioned briefly above, *S. aureus* and *S. epidermidis* are known to share a significant number of accessory genome genes (e.g. antimicrobial resistance genes and some virulence-associated genes) in common, especially genes encoded by MGEs such as plasmids and transposons [71]. A significant number of antibiotic resistance and virulence genes known to be carried on MGEs were present in many of the *S. epidermidis* isolates tested by microarray profiling in the present study (Chapter 4). Because *S. aureus* and CoNS can share a variety of genes in common it is probable if not likely that whole genome *S. aureus* probes will cross-hybridise with CoNS DNA present in test samples subject to CKB analysis.

In order to investigate the disparity between culture-based detection of *S. aureus* from oral sites in implantitis patients (as undertaken in the present study in Chapter 3) compared to molecular detection and quantification by CKB analysis as described in several previous studies [8, 35, 40, 47], this part of the present study investigated the presence of *S. aureus* and *S. epidermidis* DNA in selected oral samples from periimplantitis patients. This was determined by species-specific molecular detection using RT-PCR and compared to the corresponding culture data from the same samples as described in Chapter 3. RT-PCR and culture data were also compared with the corresponding data for *S. aureus* obtained by CKB analysis with the same samples.

5.1.1 Aims

1. To investigate the presence of *S. aureus* DNA in selected clinical samples from implantitis patients using RT-PCR in comparison to CKB data and culture data.
2. To investigate the presence of *S. epidermidis* DNA in selected clinical samples from implantitis patients using RT-PCR in comparison to culture data.

5.2 Materials and methods

5.2.1 RT-PCR for *S. aureus* and *S. epidermidis*

The methodology used for RT-PCR is described in detail in Chapter 2, sections 2.7 and 2.13. RT-PCR reactions were set up in quadruplicate for each clinical sample being tested using the *S. epidermidis sodA* primer and probe set [143] or the *S. aureus nuc* primer and probe set developed as part of the present study (see Chapter 2, section 2.7). Separate five point standard curves (ranging from 1 x 10^4-1 x 10^8 cells/sample) (Chapter 2) were set up in quadruplicate alongside the appropriate clinical samples on each RT-PCR plate run using staphylococcal reference strains *S. aureus* RN4220 and *S. epidermidis* RP62A. The thermocycler profile was set up and run according to the manufactures recommendations.

The primers and probes for each of the two RT-PCR reactions were optimised against DNA isolated from *S. epidermidis* RP62A [71, 121], and *S. aureus* RN4220 [62]. The two reactions were each tested for cross reactivity using the same DNA preparations. Initially it was hoped to run the *S. epidermidis* and *S. aureus* RT-PCRs together. However, during the reaction optimisation process it was found that the amplicon produced by the *sodA* primers in the presence of *S. epidermidis* template DNA inhibited signalling of the *nuc* probe for *S. aureus*. The most likely cause of this was competitive inhibition due to a region of homology of 8 bp between the *S. aureus* probe and the *S. epidermidis* antisense amplicon, combined with the lower cycle threshold (Ct) values (the number of amplification cycles needed for the fluorescent signal to clearly exceed background florescence levels) returned by the *S. epidermidis* RT-PCR. This was overcome by running the two reactions separately. Each RT-PCR run included a series of positive controls, that also acted as the five point standard curve from which quantitative estimations of the number of *S. aureus* or *S. epidermidis* cells in the original sample could be made. Both reactions were initially optimised to 1 x 10^4 cells/sample, but the *S. aureus* 1 x 10^4 standards were not consistently detected when clinical sample runs were undertaken, so the *S. aureus* standard curve was calculated using four points with 1 x 10^5 as the lowest.

5.2.1.1 Sample selection for RT- PCR

Twenty-seven oral samples from implantitis patients from the present study (that had previously had their staphylococcal loads assessed by culture on MSA and identification based on 16S rDNA amplimer sequencing, see Chapter 3) were subject to *S. aureus*-specific and *S. epidermidis*-specific RT-PCR analysis (Table 5.1). These samples were

selected on the basis of CKB analysis data on the same samples as part of another study from this institution undertaken by Dr. Rory Maguire. Dr. Maguire tested a total of 164 samples from 29 periimplantitis patients by CKB of which 31 samples from 16 separate patients were *S. aureus*-positive by CKB analysis (Table 5.2). Fifteen samples that were *S. aureus*-negative by CKB analysis and 12 samples were *S. aureus*-positive by CKB analysis (Table 5.1) were randomly selected for RT-PCR analysis. To randomise the selection process the clinical samples that were submitted for CKB analysis were split into CKB *S. aureus*-positive and CKB *S. aureus*-negative lists by ascending sample number, each clinical sample was assigned a randomly generated number and the lists were re-ordered using the assigned random number, again in ascending order. Clinical samples with sufficient volume remaining were selected for RT-PCR analysis. Unfortunately, due to the multiple submission of some samples for CKB analysis by Dr. Maguire, only 12 clinical samples which tested *S. aureus*-positive by CKB analysis had sufficient sample remaining for RT-PCR analysis.

5.2.2 CKB analysis

CKB analysis was undertaken on oral samples from implantitis patients used for culture analysis in the present study (Chapter 3) as part of a separate research project undertaken by Dr. Rory Maguire from this institution. CKB analysis was undertaken by staff at the laboratory of Professor Rutger Persson (Department of Periodontology and Fixed Prosthodontics, Division of Oral Microbiology, University of Berne, Berne, Switzerland) as described previously [8, 40, 47].

5.3 Results

5.3.1 *Staphylococcus aureus*-specific and *S. epidermidis-specific* RT-PCR analysis of oral samples from periimplantitis patients

Two RT-PCR reactions were performed separately on aliquots of DNA extracted from the same clinical sample using two different species-specific RT-PCR primer and probe combinations and DNA standards (one set for *S. aureus* and another for *S. epidermidis*). The standard curve for each was set up using a five point standard curve from 1 x 10^4 cells to 1 x 10^8 cells, but no readings were detected at the 1 x 10^4 cell level for *S. aureus*, reducing the curve to a four point curve from 1 x 10^5 cells to 1 x 10^8 cells, matching the minimum standard used routinely in CKB analysis (i.e. 1 x 10^5 cells).

Twenty-seven clinical samples from 14 separate periimplantitis patients that were initially cultured for staphylococci on MSA agar (Chapter 3) were tested in this part of the present study for the presence of *S. aureus* and *S. epidermidis* DNA using species-specific RT-PCR (Table 5.1). Twelve samples were *S. aureus*-positive by CKB analysis and 15 were *S. aureus*-negative by CKB analysis. The type of oral site and state of health of patients for each sample tested by RT-PCR is shown in Table 5.1). None of the 27 samples tested returned positive results for *S. aureus* DNA using the *S. aureus*-specific RT-PCR primer and probe set despite the fact that 12 samples had tested *S. aureus*-positive by CKB analysis (Table 5.1). Only 2/27 (7.4%) samples tested by RT-PCR (Patient 24, periodontitis tooth sample, and Patient 36, first periimplantitis sample) yielded *S. aureus* by culture as determined in the study described in Chapter 3 (see Tables 3.2 and 3.5), one of which was CKB-positive for *S. aureus* (estimated cell load of 0.85 x 10^5 cells/sample). The cell density recovered by culture on MSA for these two samples was 170 and 20 cfu/sample, respectively.

Six samples (6/27, 22.2%) from five patients (5/14, 35.7%) tested positive for *S. epidermidis* DNA using the *S. epidermidis*-specific RT-PCR primer and probe set (Table 5.1). Five of these samples (5/6, 83.3%) also yielded viable *S. epidermidis* isolates following culture on MSA (Table 5.1). In total, 9/27 (33.3%) samples tested by RT-PCR yielded viable *S. epidermidis*, by either paper point or curette sampling, following culture on MSA, four of which were RT-PCR-negative for *S. epidermidis* (Patient 7 first periimplantitis sample; Patient 9 first healthy tooth sample; Patient 31 periimplantitis sample and Patient 35 healthy implant sample, Table 5.1). The *S. epidermidis* cell density

in cfu for these latter four samples ranged from 5-230 cfu/sample. Only one (Patient 24, periodontitis tooth sample) of the 19/27 samples tested by RT-PCR that did not yield any *S. epidermidis* following culture on MSA was RT-PCR-positive for *S. epidermidis*. Two of the samples (Patient 24 periodontitis tooth sample and Patient 36 healthy implant sample) that were found to harbour *S. epidermidis* DNA by RT-PCR were *S. aureus*-positive when tested by CKB analysis (Table 5.1). One of these samples (Patient 36, healthy implant sample) yielded a very high density of *S. epidermidis* by culture on MSA (8.9×10^5 cfu/sample) but no detectable *S. aureus* by culture. The second sample (Patient 24, periodontitis tooth sample) was the only sample where RT-PCR indicated the presence of *S. epidermidis* DNA but where no *S. epidermidis* was recovered by culture on MSA, but which did yield low numbers of *S. aureus* (20 cfu/sample).

5.3.2 Checkerboard DNA:DNA hybridisation

Professor Persson's laboratory at Berne, Switzerland, that performed the checkerboard testing of clinical samples determined a sample as positive for *S. aureus* if it yielded CKB hybridisation signals equivalent to an estimated cell count of $\geq 0.5 \times 10^5$.

Checkerboard (CKB) DNA:DNA hybridisation was conducted on 164 samples taken from 29 periimplantitis patients (Chapter 3). Using this method, samples from 16/29 patients (55.2%) were assessed to harbour *S. aureus* DNA at one or more of the tooth or implant sites tested (Table 5.2). Of the 164 samples submitted for CKB analyses, only three (from three different patients) yielded *S. aureus* when cultured on MSA. Two of the *S. aureus* culture-positive samples (Patient 24 periodontitis tooth sample and Patient 36 third periimplantitis sample, Table 5.2) were assessed to harbour *S. aureus* DNA by CKB. CKB analysis indicated that the third sample did not harbour any *S. aureus* DNA (and therefore was not included in Table) which only presents the samples that returned CKB results indicating the presence of *S. aureus*). However, it was this CKB *S. aureus*-negative sample that yielded the highest MSA culture yield of all three samples, 480 cfu/sample (Table 3.2). Three other samples obtained from periimplant or periodontal pockets yielded *S. aureus* through culture (Tables 3.2, and 3.11), but were not submitted for CKB analysis.

Table 5.1. Comparative estimates of *S. aureus* and *S. epidermidis* density in oral samples from implantitis patients determined by species-specific RT-PCR, checkerboard DNA:DNA hybridisation and by culture on MSA[a]

Patient	Visit[b]	Sampling site type	Site state of health	Collection method	RT-PCR estimated cells/sample[c]		CKB estimated *S. aureus* cells/sample	Staphylococcal load cfu/sample estimated from growth on MSA[d]	
					S. aureus	*S. epidermidis*		Paper point sample	Curette sample
7	1	Implant	Periimplantitis	Paper point & Curette	0	0	1.05×10^5	40 *S. epidermidis*	0
7	1	Implant	Periimplantitis	Paper point	0	1.28×10^3	0	10 *S. epidermidis*	No sample taken
8	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point & Curette	0	0.72×10^3	0	0	80 *S. epidermidis*
9	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0	0[e]	1.48×10^5	20 *S. capitis* 230 *S. epidermidis*	10 *S. epidermidis*
11	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0	0	1.17×10^5	0	0
11	1	Implant	Periimplantitis	Paper point	0	0	0	0	0
13	1	Implant	Periimplantitis	Paper point & Curette	0	0.88×10^3	0	10 *S. epidermidis*	0
14	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	0	0	0.85×10^5	0	0

Continued overleaf

Table 5.1 continued. Comparative estimates of *S. aureus* and *S. epidermidis* density in oral samples from implantitis patients determined by species-specific RT-PCR, checkerboard DNA:DNA hybridisation and by culture on MSA[a]

Patient	Visit[b]	Sampling site type	Site state of health	Collection method	RT-PCR estimated cells/sample[c]		CKB estimated *S. aureus* cells/sample	Staphylococcal load cfu/sample estimated from growth on MSA[d]	
					S. aureus	*S. epidermidis*		Paper point sample	Curette sample
19	1	Implant	Periimplantitis	Paper point	0	0	0	0	0
19	3	Tooth (adjacent to implant)	Healthy	Paper point	0	0[f]	0	0	0
19	3	Tooth (non-adjacent to implant)	Healthy	Paper point & Curette	0	0	0	0	0
21	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point & Curette	0	0	1.05×10^5	0	0
21	1	Implant	Periimplantitis	Paper point & Curette	0	0	0.95×10^5	0	0
24	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0	0	0	0	0
24	1	Implant	Periimplantitis	Paper point	0	0	0	0	0
24	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	0	1.29×10^3	0.85×10^5	20 *S. aureus*	0
29	1	Implant	Healthy	Paper point	0	0	0	0	0
29	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0	0	0	0	0

Continued overleaf

Table 5.1 continued. Comparative estimates of *S. aureus* and *S. epidermidis* density in oral samples from implantitis patients determined by species-specific RT-PCR, checkerboard DNA:DNA hybridisation and by culture on MSA[a]

Patient	Visit[b]	Sampling site type	Site state of health	Collection method	RT-PCR estimated cells/sample[c]		CKB estimated *S. aureus* cells/sample	Staphylococcal load cfu/sample estimated from growth on MSA[d]	
					S. aureus	*S. epidermidis*		Paper point sample	Curette sample
30	2	Implant	Periimplantitis	Paper point	0	0	0	0	0
30	3	Tooth (non-adjacent to implant)	Periodontitis	Paper point	0	0	1.23×10^5	0	0
31	1	Implant	Periimplantitis	Paper point & Curette	0	0	0	10 *S. epidermidis*	0
35	1	Implant	Healthy	Paper point & Curette	0	0	0	0	40 *S. epidermidis*
36	1	Implant	Periimplantitis	Paper point & Curette	0	0	1.48×10^5	0	0
36	1	Implant	Periimplantitis	Paper point & Curette	0	0	1.2×10^5	0	0
36	1	Implant	Periimplantitis	Paper point & Curette	0	0	1.62×10^5	0	170 *S. aureus*
36	1	Implant	Periimplantitis	Paper point & Curette	0	1.03×10^3	0	50 *S. epidermidis*	1, 7020 *S. epidermidis*
36	2	Implant	Healthy	Paper point & Curette	0	1.63×10^7	2.14×10^5	20 *S. epidermidis*	1,797,892 *S. epidermidis*

[a] Twelve samples found to contain *S. aureus* DNA and 15 samples found not to contain *S. aureus* DNA using CKB analysis that MSA culture data was available for were assessed using RT-PCR.

[b] Visit 1 pre-treatment (mechanical debridement and oral health advice), all other visits are post treatment.

[c] 0 = undetectable levels of *S. aureus*, or *S. epidermidis* DNA.

[d] Staphylococcal isolates were identified to the species level by nucleotide sequence analysis of a segment of the 16 S rDNA gene (Chapter 3).

[e] Out of the four RT-PCR reactions run with this sample three had undetectable levels of DNA, one had a reading of 0.85×10^3 cells/sample.

[f] Out of the four RT-PCR reactions run with this sample three had undetectable levels of DNA, one had a reading of 0.63×10^3 cells/sample.

Table 5.2. Comparative estimates of staphylococcal cell density determined by culture on MSA in oral samples from implantitis patients that were checkerboard-positive for *S. aureus*[a]

Patient	Visit[b]	Sampling site type[c]	Site state of health[d]	Collection method	CKB estimated *S. aureus* cells/sample	Staphylococcal species[e] and density in cfu/sample determined from culture on MSA	
						Paper point sample	Curette sample
7	1	NRC	NRC	Paper point & Curette	1.05×10^5	40 *S. epidermidis*	0
9	1	Tooth (non-adjacent to implant)	Healthy	Paper point	1.48×10^5	20 *S. capitis* 230 *S. epidermidis*	10 *S. epidermidis*
9	3	Tooth (non-adjacent to implant)	Healthy	Paper point & Curette	0.98×10^5	0	0
11	1	Tooth (non-adjacent to implant)	Healthy	Paper point	1.17×10^5	0	0
14	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	0.85×10^5	0	0
16	1	Implant	Periimplantitis	Paper point	0.78×10^5	70 *S. epidermidis*	NA
16	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0.79×10^5	90 *S. epidermidis*	0
16	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	1.23×10^5	3,010 *S. epidermidis*	10 *S. epidermidis*
17	1	Tooth (adjacent to implant)	Periodontitis	Curette	2.34×10^5	No data available	No data available
19	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0.85×10^5	0	0
21	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point & Curette	1.05×10^5	0	0

Continued overleaf

Table 5.2 continued. Comparative estimates of staphylococcal cell density determined by culture on MSA in oral samples from implantitis patients that were checkerboard-positive for *S. aureus*[a]

Patient	Visit[b]	Sampling site type[c]	Site state of health[d]	Collection method	CKB estimated *S. aureus* cells/sample	Staphylococcal species[e] and density in cfu/sample determined from culture on MSA	
						Paper point sample	Curette sample
21	1	Implant	Periimplantitis	Paper point & Curette	0.95×10^5	0	0
23	1	Implant	Periimplantitis	Paper point & Curette	2.34×10^5	0	10 *S. warneri*
23	1	Tooth (non-adjacent to implant)	Healthy	Paper point	1.48×10^5	20 *S. epidermidis*	0
23	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	7.41×10^5	0	0
24	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	0.85×10^5	20 *S. aureus*	0
25	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	2.19×10^5	0	0
25	1	Implant	Periimplantitis	Paper point	5.62×10^5	10 *S. pasteuri*	0
25	1	Tooth (non-adjacent to implant)	Healthy	Paper point	0.91×10^5	120 *S. epidermidis*	0
25	1	Tooth (adjacent to implant)	NRC	Curette	0.98×10^5	0	0
25	3	Tooth (non-adjacent to implant)	Healthy	Paper point & Curette	1.29×10^5	0	0
29	1	Implant	Periimplantitis	Paper point	1.02×10^5	0	0
30	1	Tooth (non-adjacent to implant)	Healthy	Paper point	1.12×10^5	0	0

Continued overleaf

Table 5.2 continued. Comparative estimates of staphylococcal cell density determined by culture on MSA in oral samples from implantitis patients that were checkerboard-positive for *S. aureus*[a]

Patient	Visit[b]	Sampling site type[c]	Site state of health[d]	Collection method	CKB estimated *S. aureus* cells/sample	Staphylococcal species[e] and density in cfu/sample determined from culture on MSA	
						Paper point sample	Curette sample
30	1	Tooth (non-adjacent to implant)	Periodontitis	Paper point	1.35×10^5	0	0
30	1	Implant	Periimplantitis	Paper point	1.55×10^5	0	0
30	3	Implant	Periimplantitis	Paper point	1.38×10^5	0	0
30	3	Tooth (non-adjacent to implant)	Periodontitis	Paper point	1.23×10^5	0	0
30	5	Tooth (non-adjacent to implant)	Healthy	Paper point	1.05×10^5	0	0
32	3	Tooth (adjacent to implant)	Healthy	Curette	4.79×10^5	3,953,275 *S. epidermidis*	0
36	1	Implant	Periimplantitis	Paper point	1.48×10^5	0	0
36	1	Implant	Periimplantitis	Paper point & Curette	1.2×10^5	0	0
36	1	Implant	Periimplantitis	Paper point & Curette	0.91×10^5	0	0
36	1	Implant	Periimplantitis	Paper point & Curette	1.62×10^5	0	170 *S. aureus*
36	2	Implant	Healthy	Paper point & Curette	2.14×10^5	20 *S. epidermidis*	1.7×10^6 *S. epidermidis*
39	1	Tooth (non-adjacent to implant)	Healthy	Paper point & Curette	1.07×10^5	No data available	No data available

[a] All samples assessed to harbour *S. aureus* DNA by CKB analysis where MSA culture results were also available.
[b] Visit 1 pre-treatment (mechanical debridement and oral health advice), all other visits are post treatment.
[c] NRC = Sampling site type not recorded by clinician on submission to laboratory.
[d] NRC = Sampling site state of health not recorded by clinician on submission to laboratory.
[e] Staphylococcal isolates were identified to the species level by nucleotide sequence analysis of a segment of the 16 S rDNA gene (Chapter 3).

5.4 Discussion

5.4.1 Conflicting results for *S. aureus* detection by checkerboard DNA:DNA hybridisation and species-specific RT-PCR

Staphylococcus aureus-specific RT-PCR analysis of 27 oral samples from implantitis patients, 12 of which were *S. aureus*-positive (range 0.85-2.14 x 10^5 cells/sample) and 15 of which were *S. aureus*-negative by CKB analysis, showed no detectable *S. aureus* DNA in any of the samples tested (Table 5.1). Only two of the 27 samples (Patient 24, periodontitis tooth sample, and Patient 36, first periimplantitis sample, Table 5.1) yielded *S. aureus* by culture on MSA, yielding 20 and 170 cfu/sample, respectively. Patient 36, first periimplantitis sample was also CKB-positive for *S. aureus*. These conflicting findings indicated that overall the CKB analysis data appeared to be less reliable than the RT-PCR and culture data, which in the majority of cases showed excellent correlation (Table 5.1). The two samples that were *S. aureus*-negative by RT-PCR but *S. aureus*-positive by culture yielded low viable *S. aureus* cell densities. Lack of specificity in the CKB approach is likely to have been a major contributory factor in the significant disparity between the CKB and RT-PCR data.

The two step RT-PCR process (amplification by species-specific primers and binding by a targeted species-specific probe) offers greater specificity than CKB, which utilises whole genomic DNA probes. Furthermore, the DNA extraction and purification method used for the RT-PCR samples included the use of Qiagen DNeasy spin columns, which yield clean, high quality genomic DNA, while the CKB method utilises crude sample lysis and is less likely to yield sample template DNA of comparable quality. The negative RT-PCR findings obtained with 27 samples (26/27; 96.3%, were culture-negative for *S. aureus*), 12 of which were CKB-positive for *S. aureus*, suggests that DNA other than *S. aureus* DNA present in test samples hybridised with the CKB *S. aureus* whole genomic probe resulting in false positive results. As outlined in Chapter 1, *S. aureus* and CoNS, but especially *S. epidermidis*, can share a large number of accessory genes [71]. Mobile genetic elements (i.e. transposons, plasmids, SCC elements etc.) found in *S. aureus* are also frequently identified in CoNS [71, 103, 174, 179].

The whole genome probes used in CKB analysis should be tested for cross-reactivity against the reference strains used to create the probes of the other species included on the testing panel (which included *S. aureus* and the CoNS species *S. haemolyticus*), though no specific information was provided with regard to the protocol employed for the CKB panel

used to test the samples submitted by Dr. Maguire. To the best of my knowledge probes are not tested for cross-reactivity against species that have not been included in the CKB testing panel. If cross-reactions between species on the CKB panel are identified attempts could be made to limit cross reactivity by using subtraction hybridisation PCR (shPCR) to prepare probes or by setting up competitive hybridisation when running the probe hybridisation reactions. However, both of these techniques are only effective if the DNA sequences that are being bound by the probe for the target species are present in the reference strain of the non-target species that is being used as the agonist. If an MGE is present in a wild type strain that is missing from the reference strain it will still cross hybridise with the whole genome, or shPCR probe resulting in a false positive reading.

5.4.2 Comparison of *S. aureus* load estimations obtained by clinical sample culture on MSA and Checkerboard DNA:DNA hybridisation

The results obtained from the samples submitted for CKB analysis showed very poor correlation with the results obtained by culturing clinical samples on MSA (Table 5.1). The CKB analysis suggested that *S. aureus* was frequently present in the samples tested from implantitis patients, whereas the culture results (Chapter 3) suggested that *S. aureus* was seldom isolated from the same samples, including samples from periimplant and periodontal pockets. The culture results suggested that if *S. aureus* was identified in an oral sample from a patient it was more likely to be present in an oral rinse sample representing the general oral cavity, possibly trafficked from the nares, rather than a tooth or implant site. It has been suggested previously that studies of the microbiota of oral implant pockets utilising CKB provides a more accurate picture than culture due to differences between sampling techniques used, i.e. paper points for CKB and curettes for culture [35]. However, the present study used both sample collection methods (described in Chapter 2, culture results for both methods presented in Chapter 3) and samples taken using both collection methods were submitted for CKB analysis and culture, which yielded the same anomalous results (Table 5.1). It is unlikely that the sample collection method is the cause for the significant disparity between the CKB and culture results for *S. aureus*. As discussed above it is more likely that cross reactivity between *S. aureus* DNA from the whole genomic DNA probe used in the CKB analysis and other (non-*S. aureus*) DNA present in clinical samples.

5.4.3 Comparison of *S. aureus* and *S. epidermidis* load estimations obtained by clinical sample growth on MSA and RT-PCR

None of the 27 clinical samples tested using *S. aureus*-specific RT-PCR yielded detectable levels of *S. aureus* DNA, though two clinical samples (Patient 24, periodontitis tooth sample, and Patient 36, first periimplantitis sample) yielded low numbers of *S. aureus* cfu following culture on MSA (Table 5.1). It is possible that due to the low *S. aureus* yields obtained from culture on MSA that the quantity of *S. aureus* DNA present in the test samples following DNA extraction was too low to be detected by RT-PCR. The Ct values returned for the 1×10^8, 1×10^7, 1×10^6 and 1×10^5 cells/sample *S. aureus*-specific RT-PCR standards were slightly higher than the corresponding Ct values obtained from the *S. epidermidis*-specific RT PCR 1×10^8, 1×10^7, 1×10^6, 1×10^5 and 1×10^4 cells/sample standards, and the *S. aureus* standard curve exhibited a slightly steeper slope than the *S. epidermidis* standard curve indicating it was a less efficient reaction. However, both standard curves had similar R^2 values (mean 0.986 for the *S. epidermidis*-specific reactions, mean 0.991 for the *S. aureus*-specific reactions) indicating a similar level of confidence in the ability of the Ct values obtained to predict the presence and quantity of DNA present in the test sample. R^2 is a statistical term that indicates how good one value is at predicting another. An R^2 value >0.99 provides good confidence in correlating two values. It is possible that if the number of cycles in the amplification reaction (when florescence readings were taken) had been increased, lower levels of *S. aureus* DNA may have been detected. However increasing the number of amplification cycles, beyond the 40 currently used, risks non-specific amplification (leading either to false positive readings, or to competitive inhibition) and an increase in background signals.

The *S. aureus*-specific RT-PCR had a lower efficiency than the *S. epidermidis*-specific RT-PCR and it is possible that low levels of *S. aureus* DNA were not detected in some of the clinical samples tested. However, the lowest level of *S. aureus* detectable using the *S. aureus*-specific RT-PCR with the *S. aureus* RN4220 standards was 1×10^5 cells/sample and the standard curve would allow an estimation of results slightly below this level. The lowest standard for each species included on the CKB membrane was also 1×10^5 cells/sample, and 0.5×10^5 cells/sample was the lowest reading reported as *S. aureus* positive by the Swiss laboratory that conducted the analysis. Therefore, any of the samples tested harbouring *S. aureus* DNA around the same levels as detected by CKB should have been identified by RT-PCR. The *S. epidermidis*-specific RT-PCR findings showed that only one sample contained detectable *S. epidermidis* DNA in the absence of recovery of *S.*

epidermidis cfu following culture on MSA. In contrast, four samples that yielded low densities of *S. epidermidis* on MSA were RT-PCR-negative for *S. epidermidis* (Table 5.1).

Because the two methods of *S. epidermidis* detection used in the present study used a radically different rationale for detection (species-specific estimated cells/sample by RT-PCR versus cfu/sample by culture) a direct comparison of load data obtained with the two methods is not valid. However, some associations were apparent. While the *S. epidermidis*-specific RT-PCR was not as sensitive as would be hoped for in identifying the presence of *S. epidermidis* at low levels, samples that yielded high *S. epidermidis* cfu counts when cultured on MSA returned high *S. epidermidis* cell/sample estimates using RT-PCR (Table 5.1).

The estimated cells/sample determined using RT-PCR were not equivalent to the estimated cfu/sample yields obtained by growth on MSA. The discrepancies between the relative *S. epidermidis* cell densities estimated using RT-PCR and determined by culture on MSA are likely to be influenced by of the presence of DNA from non-viable *S. epidermidis* present in clinical samples. Because of the nature of the sites that the samples were collected from (natural biofilms around oral implant and natural tooth sites are likely to harbour DNA from lysed bacteria and other microbiota), the *S. epidermidis*-specific RT-PCR conducted in this study is probably best suited to determining the presence or absence of *S. epidermidis* DNA in a sample rather than attempting to extrapolate the density of *S. epidermidis* cells present in a sample.

The contrast between the *S. aureus*-specific RT-PCR estimations of the presence of *S. aureus* in the test samples (i.e. all *S. aureus*-negative) and the CKB estimations of *S. aureus* presence in the test samples indicated that it is unlikely that the whole genome *S. aureus* CKB probe actually hybridised with *S. aureus* DNA present in the sample, but rather returned a false positive result due to cross reaction with non-*S. aureus* DNA. The contrast between the *S. epidermidis*-specific RT-PCR estimations of the presence of *S. epidermidis* in the test samples (6/27, 22.2%) and the CKB estimations of *S. aureus* presence in the test samples (12/27, 44.4%) indicated that the whole genome *S. aureus* CKB probes were not solely cross-reacting with *S. epidermidis* DNA present in the test samples. These findings suggest that the CKB *S. aureus* whole genome probes cross-react significantly with other DNA targets present in the test samples, possibly with non-staphylococcal species or genera.

Protocols used to determine and minimise cross reactions between the *S. aureus* whole genome probes and DNA from other species and genera need to take into account the possibility of cross reactivity with the DNA of species not included on the CKB testing panel that may be present in biofilm present in periimplant and/or periodontal pockets.

5.5 Conclusions

1. No *S. aureus* DNA was identified in any of the 27 samples from periimplantitis patients tested using species-specific RT-PCR even though 12 of the samples were *S. aureus*-positive by CKB analysis. These findings indicate lack of specificity with CKB, possibly due to cross-reactivity of the CKB *S. aureus* probe with DNA from other bacterial species or genera in the test samples. *Staphylococcus aureus* was recovered by culture on MSA at low levels in 2/27 samples. It is probable that the level of *S. aureus* DNA present in these two samples was below the detection level of the RT-PCR.
2. *Staphylococcus epidermidis* was identified in 6/27 (22.2%) clinical samples from implantitis patients tested using *S. epidermidis*-specific RT-PCR, while 33.3% of the same sample set (9/27) yielded *S. epidermidis* isolates by culture on MSA. The disparity between RT-PCR and culture findings it is likely due to the low numbers of viable cells in individual samples below the detection level of the RT-PCR. In the case of a single sample that tested positive for *S. epidermidis* using RT-PCR, but yielded no *S. epidermidis* through culture on MSA, and it is likely that the RT-PCR was detecting extracellular, or non viable cellular *S. epidermidis* DNA.

Chapter 6

General discussion

6.1 Introduction

Several previous studies that investigated the microbiota surrounding oral implants reported a potentially significant association between the presence of *S. aureus* and oral implantitis suggesting that *S. aureus* plays a role in the aetiology of oral implantitis and ultimately in the failure of oral implants [8, 35, 40, 47, 159]. Many of these studies were authored by key opinion leaders in the field of dental implantology and their studies have been influential in the development of a consensus opinion regarding a possible role for *S. aureus* in oral implantitis. However, none of the above studies utilised laboratory culture of staphylococci to investigate the microbiota associated with oral implants, but either relied on molecular detection of *S. aureus* using checkerboard DNA:DNA hybridisation (CKB) analysis using whole genome *S. aureus* probes [8, 35, 40, 47, 159] or on serum antibody titres against *S. aureus*. One of the earliest of these studies investigated serum antibody titres against selected bacteria, including *S. aureus,* from patients with successfully osseointergrated oral implants and oral implants that failed to integrate adequately or subsequently failed following osseointegration. The authors concluded that high antibody titres against *S. aureus* were associated with patients with successfully osseointergrated oral implants and by inference that *S. aureus* was indicated in implant failure [5].

Previous studies that investigated the microbiota of periodontal pockets using culture analysis identified the presence of several species of staphylococci including *S. epidermidis*, *S. capitis*, *S. hominis*, *S. warneri*, *S. aureus*, *S. cohnii*, *S. lugdunensis*, *S. intermedius*, *S. saprophyticus*, *S. haemolyticus*, *S. simulans* and other unclassified staphylococci, with *S. epidermidis* the most commonly identified species [23, 130, 131]. At the time of writing (to the best of my knowledge) only one previous study that used culture analysis to investigate the subgingival microbiota including staphylococci, also investigated samples taken from periimplantitis patients [131]. This study found that while a high proportion of the patients tested (9/13, 69.2%) harboured staphylococci including *S. aureus*, the majority of implants tested (13/20) yielded *S. epidermidis*, the latter finding similar to the results of the present study. To the best of my knowledge prior to the present study no published studies utilised a combination of culture, molecular analysis and population analysis to investigate associations between staphylococci and oral implant and periodontal pockets in both health and disease. The present study used a combination of culture analysis and molecular analysis to undertake a comprehensive evaluation of the prevalence and species distribution of staphylococci in a cohort of periimplantitis patients.

Results from culture analysis revealed that of the few staphylococcal species identified, *S. epidermidis* was by far the most prevalent, recovered from all oral sites tested, including healthy and diseased implant and natural tooth sites and the general oral cavity (Chapter 3, Tables 3.1-3.18). In stark contrast to previous studies, *S. aureus* was rarely identified in or recovered from samples taken from healthy or diseased natural tooth or implant sites.

6.2 Disparity between detection of *S. aureus* by culture and CKB

The prevalence of *S. aureus* in clinical samples taken from tooth and implant sites in this study was far less than expected based on previous studies using molecular methods, principally CKB analysis [8, 35, 40, 47]. The possibility that *S. aureus* small colony variants (persistent strains of *S. aureus* that take a long time to grow on culture media and form much smaller colonies than typical *S. aureus* isolates on solid media [185, 186]) were present at subgingival sites around oral implants was considered. However, the results of this study indicated that this possibility was unlikely as an extended incubation period (performing colony counts at 48 h and 7 days incubation) was used when clinical samples were initially cultured on MSA and all colony phenotypes observed on the plates were identified by 16S rDNA sequencing, and not just those exhibiting the typical appearance of *S. aureus*.

6.2.1 Presence of DNA from non-viable bacteria or CKB probe cross reactivity

A more likely explanation for the discrepancy between the prevalence of *S. aureus* at tooth and implant sites determined in the present study by culture and previous studies using CKB analysis relates to the possible detection by the latter method of extracellular DNA (eDNA) from lysed bacteria in biofilms from implant and tooth sites [152]. To investigate this possibility a selection of samples from the present study for which both culture and CKB analysis data was available were analysed using a *S. aureus*-specific RT-PCR. None of the samples tested using the *S. aureus*-specific RT-PCR indicated the presence of *S. aureus* (Chapter 5), despite the fact that 44.4% of the samples were assessed to harbour *S. aureus* using CKB analysis at a level that should have been detected by the RT-PCR. A comparison of the species identified in the test samples by MSA culture and 16S rDNA species identification supported the lack of *S. aureus* detection by species-specific RT-PCR. These results strongly suggested that the CKB analysis data indicating the presence of *S. aureus* DNA (from viable bacteria, non-viable bacteria, or present as eDNA in biofilm) was unreliable.

The whole genome probes used in CKB analysis are usually tested for cross reactivity against the other bacterial species present on the CKB testing panel. They are not tested for cross reactivity against other microbial species which may be present in CKB test samples. In this regard, it is important to note that the microbiota found in periodontal and periimplant pockets usually exists as a complex mixed species microbial biofilm [10, 20]. The results of the present study demonstrated that *S. epidermidis* is frequently present in these types of oral sites. It was initially thought that the *S. aureus* whole genome probe used in CKB analysis may have been cross reactive with *S. epidermidis* DNA as the most predominant CoNS identified in the present study. However, for this to be true, it would have been expected that the *S. epidermidis*-specific RT-PCR would identify *S. epidermidis* DNA in all of the samples that were CKB-positive for *S. aureus* and not just those where *S. epidermidis* was identified through culture on MSA and 16S rDNA identification.

The whole genome probes employed in CKB analysis are hybridised against the total DNA extracted from test samples and the possibility of probe cross-reactivity with DNA from other species than the target organisms is likely to be significant. Previous studies have investigated the sensitivity and specificity of CKB whole genome probes for other species commonly included on the CKB hybridisation panel compared with bacterial growth on culture media, or species-specific PCRs [138, 139, 151]. Their results depend to some extent on which method was taken as the standard for comparison. One study (Haffajee *et al.*, 2009) took CKB to be the "gold standard" against the PCR method used (micro-IDent). This study determined that between the CKB and PCR methods there was mean agreement of 71.8% (±9.6), mean sensitivity of 57.8% (±21.7) and mean specificity of 72.6% (±15.3) [139]. This suggested that while the two methods were comparable in their detection of the 13 species tested for and in their specificity, that CKB was more sensitive overall. However, Siqueria *et al.* (2002) conducted a study using six species using CKB and PCR analysing the same data twice, once taking CKB as the 'gold' standard, then repeating the analysis taking PCR as the 'gold' standard. When CKB was taken as the standard the accuracy of the PCR (agreement with the CKB findings) was assessed to range from 60-74% (mean 68.6%), sensitivity was assessed to range from 0-100% (mean value 61.2%) with specificity between 50-88% (mean value 69.6%). When the roles were reversed and PCR was taken as the standard, the accuracy of the CKB (agreement with the PCR findings) was still assessed to range from 60-74% (mean value 68.6%), but sensitivity was assessed to range from 0-73% (mean value 35%) with specificity between 75-100% (mean value 89%) [138]. With each of these studies the individual species being tested for had

different sensitivities and specificities. Some probes/PCR reactions exhibited very similar sensitivity and specificity between the two methods, while others were wildly different. The study conducted by Barbosa *et al.* (2009) used both growth in culture and analysis by CKB to investigate leakage of bacteria (*Fusobacterium nucleatum*) seeded inside oral implant abutments immersed in nutrient media [151]. Unfortunately the authors did not test the same samples using both methods, instead choosing to split their samples into two groups, one of which was tested by each method. They reported that CKB was more sensitive than conventional culture, returning higher estimates of the absolute number of cells present (CKB immediately post inoculation: all samples 5.5 cells, culture immediately post inoculation: 0.21-0.62 cells, mean 0.419, CKB 14 days post-inoculation: 0-10 cells, mean 3.17 cells, culture 14 days post-inoculation: 0-0.23 cells, mean 0.05 cells). Again depending on which method you choose to use as the standard, either culture analysis underestimates the bacterial load due to lower sensitivity, or CKB analysis overestimates the bacterial load based on DNA from non-viable bacteria. These studies indicate that while CKB appears to be a very sensitive and specific molecular technique for some species of bacteria, it is less sensitive and specific when employed with others, and may not necessarily return accurate data on the viable bacterial load present at the site a sample was collected from. CKB may not be an appropriate technique for the identification and quantification of *S. aureus* in mixed microbial samples. Due to the many MGEs commonly present in *S. aureus* that have been found in other CoNS species, and the limited knowledge that we have of staphylococci as part of the oral milieu, cross reactions between whole genomic *S. aureus* probes and non-*S. aureus* DNA present in biofilm samples obtained from periodontal and periimplant pockets cannot be ruled out. Other techniques, such as the human oral microbe identification microarray (HOMIM) based on ribosomal DNA sequences present in the human oral microbiome study (HOMb), have become available for high throughput testing of mixed microbial samples and thought should be given to employing these techniques in future investigations of *S. aureus* in the oral cavity [14, 187].

6.3 *Staphylococcus epidermidis* survival in periimplant/periodontal pockets

The results of the present study demonstrated that *S. epidermidis* was prevalent in the oral cavity, isolated from 45-47% of oral rinse samples from periimplantitis patients pre- and post-treatment. *S. epidermidis* was also frequently recovered from both periodontal and periimplant pockets. In samples taken from implant sites *S. epidermidis* was present in 19-

37% of paper point samples and 1.9-25% of curette samples taken from periimplantitis sites pre- and post-treatment, and 11-35% of paper point samples and 11-19% of curette samples taken from healthy implant sites pre- and post-treatment. In samples taken from tooth sites *S. epidermidis* was present in 33-37% of paper point and 9-28% of curette samples taken from diseased tooth sites pre- and post-treatment, and in 6-21% of paper point samples and 8-11% of curette samples taken from healthy tooth sites pre- and post-treatment (see Chapter 3, Tables 3.1 and 3.8).

Staphylococcus epidermidis is generally an aerobic bacterium and its frequent recovery from the low oxygen environments associated with periodontal and periimplant pockets and subgingivally is intriguing. One previous study reported the isolation of a strictly anaerobic *S. epidermidis* strain from an infected artificial hip prostheses [54]. However, staphylococci are not commonly cultured under anaerobic conditions and all of the isolates recovered in the present study were isolated from aerobic culture. It is possible that some of the *S. epidermidis* isolates recovered in this study were facultatively anaerobic.

The microbial flora surrounding teeth and dental implants generally forms a mixed species microbial biofilm [10, 20]. Biofilms are highly complex microbial communities and both the physical architecture of the biofilm and the environmental conditions (pH levels, available nutrients etc.) existing in different parts of the biofilm can vary significantly [21]. It is conceivable that *S. epidermidis* in periimplant and/or periodontal pockets may exist in a niche environment with a slightly higher oxygen tension than other levels of the biofilm. Alternatively, it is possible that the presence of the ACME-*arc* element identified in 24/43 (55.8%) of *S. epidermidis* isolates from periimplant patients subject to DNA microarray profiling (Chapter 4, Table 4.8) was significant in enhancing the survival of these organisms. Previous studies have shown that the ACME-*arc* cluster encoding an alternative arginine deiminase pathway can enhance the ability of staphylococci to survive at low pH levels (allowing survival in low oxygen environments) as well as offering enhanced resistance to nitric oxide (generated as part of the innate immune response) [76]. The presence of the ACME among *S. epidermidis* isolates may also enhance the ability of these organisms present in subgingival pockets to metabolise nutrients prevalent in the gingival crevicular fluid, predominantly glycoproteins, proteins and amino acids but with low carbohydrate levels relative to saliva, allowing them to compete successfully with the predominantly asaccharolytic flora generally located at such sites [188]. The overall prevalence of ACME in the *S. epidermidis* isolates subject to microarray profiling (55.8%)

in the present study is in line with that reported by previous studies [76, 179]. However, detailed analysis of the prevalence of ACME in *S. epidermidis* isolates from specific oral sites revealed some significant findings that reflect the type of site sampled. There was a significant difference between the prevalence of ACME in *S. epidermidis* isolates derived from the general oral cavity (from oral rinse sampling) and *S. epidermidis* isolates obtained from periodontal and periimplant pockets ($P<0.025$). The prevalence of ACME in *S. epidermidis* isolates recovered from the general oral cavity by oral rinse sampling was 30% (6/20 isolates). In contrast, 76.5% (13/17) of isolates recovered subgingivally from implant (8/10) or tooth (5/7) sites were ACME-positive. These findings from subgingival samples were much higher than expected and may indicate that the presence of ACME allows the survival of *S. epidermidis* in potentially inhospitable environments. It is conceivable the presence of ACME could be advantageous to *S. epidermidis* present in a biofilm under low oxygen conditions in deep periodontal or periimplant pockets. However, 5/6 (83.3%) of *S. epidermidis* isolates from nasal swabs were also ACME-positive suggesting that ACME may also confer some survival advantage in this environment.

6.4 Population analysis of *S. epidermidis* and *S. aureus*

6.4.1 *Staphylococcus epidermidis*

The *S. epidermidis* population present in the cohort of periimplantitis patients enrolled in the present study was investigated using MLST on selected isolates. A wide range of STs (22 STs from 25 patients) was identified, the majority of which belonged to the predominant global clonal complex (CC) designated CC1 in this study. The *S. epidermidis* isolates recovered in the course of this study appeared to represent general carriage isolates. However, it must be noted that at the present time the global *S. epidermidis* MLST database is relatively small (compared to other, more established MLST databases) and as such the information that can be derived from such a database has some limitations. Comparison of the *S. epidermidis* STs identified in the periimplantitis patients with the corresponding STs of *S. epidermidis* isolates in the global *S. epidermidis* MLST database showed that there was no obvious enrichment of any ST that appeared to have any association with non-human or environmental isolates, or with STs associated with healthcare environments or infection identified in other studies. However, a single ST (ST73) was identified most often (in 17/47 isolates (36.2%) and 11/25 patients (44%)). Interestingly, of the *S. epidermidis* isolates identified as belonging to ST73 that were analysed using the microarray 5/7 (71.4%) harboured ACME. It is possible that ST73 is the predominant Irish ST, though a more detailed survey (including skin and nasal swabs) of a

larger cohort drawn from the general population would be needed to confirm that with any statistical validity. Likewise a larger study (as discussed below) would be able to show the nature of *S. epidermidis* colonisation in the oral cavity. While *S. epidermidis* is undoubtedly a part of the oral flora it is unclear whether it is a persistent or transient. Some STs were identified at the same sampling site, or in the same patient at multiple time points in this study, while others appeared to be superseded over time. This indicates the presence of multiple STs in the same individual and/or oral sites. However the number of clinical *S. epidermidis* isolates typed using MLST in the present study is too small to make any confident predictions. Studies with more statistical power (provided by larger cohorts), with sampling conducted at multiple time points and with multiple isolates, would be able to identify persistent as opposed to transient *S. epidermidis* STs in individual subjects.

6.4.2 *Staphylococcus aureus*

The 31 *S. aureus* isolates recovered from 20 separate periimplantitis patients that were typed using the DNA microarray belonged to a range of CCs (eight CCs from 20 patients) as determined by DNA microarray profiling. This relatively wide range of CCs suggests that the few *S. aureus* obtained in the course of this study represent general carriage of *S. aureus* and no evidence for enrichment with any particular ST or CC was obtained. In general, MSSA carriage isolates, like those identified in this study, tend to be more diverse in contrast to MRSA isolates from the same geographic region. In Ireland, nosocomial MRSA recovered over the past 30 years were highly clonal with specific clones predominating during particular time periods [67, 127].

6.4.3 Identification of SCC*mec* associated genes in *S. epidermidis*

DNA microarray profiling of a selection of *S. epidermidis* isolates from the present study showed that several (4/43, 9.3%) harboured SCC*mec* (three SCC*mec* IV, *ccrAB2* and one SCC*mec* V *ccrB4*), while others (9/43, 21%) harboured *ccr* genes, but no other detectable SCC*mec*-associated genes. As discussed in Chapter 4, the presence of SCC*mec* in *S. epidermidis* has been well documented and *S. epidermidis* and other CoNS may have played some role in the emergence and dissemination of some of the community acquired (CA) SCC*mec* types [76]. SCC*mec* elements have been identified in CoNS, particularly *S. epidermidis*, relatively frequently [99]. Previous studies have shown that SCC*mec* IV is the most commonly identified SCC*mec* type in *S. epidermidis* (SCC*mec* IV was the predominant SCC*mec* (3/4, 75%)) identified in the isolates characterised in this study and is generally associated with the *ccr* type *ccrAB2* [91, 98, 106].

The presence of *ccr* genes in *S. epidermidis* independent of any other SCC*mec*-associated genes is interesting. The *ccr* genes associated with SCC*mec* have also been associated with ACME and the two MGEs integrate into the same attachment site in the staphylococcal chromosome within *orfX*. SCC*mec*-encoded *ccr* genes are thought to catalyse integration and excision of ACME from the staphylococcal chromosome [76, 103]. Although the majority of isolates in which *ccr* genes were identified in the absence of SCC*mec* did harbour ACME, two of the isolates subject to microarray profiling carried *ccrAB2* in the absence of any other microarray-detectable SCC*mec*-or ACME-associated genes. This suggests that at some stage in the history of these two isolates that either SCC*mec* or ACME elements or other SCCs have been lost. One possible scenario might envisage the presence of two MGEs inserted adjacent to one another in the *S. epidermidis* chromosome with two sets of adjacent recombinase genes. Following MGE excision one set of *ccr* genes may have remained in the host genome. Alternatively, the detection of *ccr* genes in the two *S. epidermidis* isolates may be a reflection of the presence of novel SCC or composite elements in the isolates. Non-*mec* SCC's have been identified in several CoNS, and it is possible that one or more of these elements are present in the *S. epidermidis* population, and in particular in the two isolates that contained *ccr* in the absence of SCC*mec* or ACME [108, 109].

SCC*mec* elements in CoNS, particularly *S. epidermidis* have been reported previously [91, 94, 95, 98, 99, 169]. Previous studies have shown that SCC*mec* IV is the most commonly identified SCC*mec* type in *S. epidermidis,* in agreement with the findings of the present study, and it has been suggested that CoNS act as a reservoir of SCC*mec* and a source of novel SCC*mec* types in *S. aureus* [174]. Likewise ACME resembles native *S. epidermidis arc* genes, and it is thought that the element may have originated in *S. epidermidis* and have spread to *S. aureus* via other CoNS [76]. ACME has been particularly associated with SCC*mec* type IV, the most commonly identified SCC*mec* type present in *S. epidermidis*, and the most common type identified in MRSE in the present study (Chapter 4, Table 4.8) [76, 91, 94, 95, 98, 99, 103, 169]. Analysis of the genetic diversity of ACME within a diverse collection of *S. epidermidis* strains indicated that ACME had been acquired on at least 15 occasions by strains belonging to the same lineage [105].

6.4.4 Antibiotic resistance genes in *S. epidermidis*

It was not surprising that so many of the *S. epidermidis* isolates subject to microarray profiling returned positive results for antibiotic resistance associated genes commonly found in *S. aureus*. Many previous studies have reported the presence of a wide variety of such genes in *S. epidermidis* [77, 93, 189]. As outlined in Chapter 4, the two species share many genes in common as part of the variable genome, especially antimicrobial resistance genes, and gene transfer between the two species has been documented [104, 111]. However, as resistance was not confirmed phenotypically in the *S. epidermidis* the genes may not necessarily be functional. However, this is unlikely as a recent study by Shore *et al.* (2012) showed a very strong correlation (≥97%) between the detection of resistance genes by microarray profiling and expression of the corresponding resistance phenotype for a variety of MRSA recovered in Ireland [107].

As a commensal organism, usually found on the skin, *S. epidermidis* is under different selection pressures than pathogenic strains of *S. aureus*. There is less of a cost to the fitness of the organism to harbour a gene conferring resistance to a particular antimicrobial. Nonetheless, *S. epidermidis* will also be exposed to antibiotics targeted at eliminating other microorganisms when a host is treated with systemic or topical antibiotics. In these situations the ability to express even low level resistance may be beneficial. *Staphylococcus epidermidis* has also been considered as a reservoir of antibiotic resistance genes that can be transferred to different CoNS species as well as to *S. aureus* [90, 91, 104, 189]. In the present study, the majority of *S. epidermidis* isolates subject to microarray profiling were either co-cultured with *S. aureus* or were recovered from a patient who had also yielded an *S. aureus* isolate from a different clinical sample. The abundance of antibiotic resistance genes detected within the *S. epidermidis* population, especially those encoded on mobile genetic elements such as *fusB*, *ileS2*, *dfrA* etc., is of concern as these could easily be transferred into *S. aureus* (Chapter 4, Table 4.8) [103].

6.5 Further investigations

6.5.1 *Staphylococcus epidermidis* population studies

The results of the present study show that *S. epidermidis* is prevalent in the oral cavity and most interestingly, prevalent in subgingival sites around both healthy and diseased natural teeth and implants. The very high prevalence of ACME among subgingival isolates is also particularly interesting. All of these findings pose a range of questions that undoubtedly will provide fruitful avenues for future research to address fundamental questions about the

association of *S. epidermidis* with the human body and its role as a reservoir of MGEs encoding antimicrobial resistance and virulence determinants for its more pathogenic relative, *S. aureus*.

The present study does not show if a particular *S. epidermidis* clone is a permanent feature of an individual's oral flora, or if it is a transient coloniser, or if multiple strains persist at different oral sites. We do not know if the presence of oral implants influences the prevalence of *S. epidermidis* in the oral cavity, nor do we know if *S. epidermidis* has a role in the aetiology of periimplantitis. We do not know if *S. epidermidis* is trafficked into the oral cavity from the skin and/or nose. Many other questions spring to mind regarding oral *S. epidermidis*. Does ACME provide *S. epidermidis* with a survival advantage in subgingival sites? Do strains that are trafficked from subgingival sites to the general oral cavity lose ACME? Are ST73 *S. epidermidis* more common in the oral cavity than other body sites, or are they just more common among the Irish population? To address these questions a detailed prospective investigation of the oral *S. epidermidis* population of healthy individuals would be a good starting point. Such a study would involve taking multiple samples from several different sites within the mouth (for example cheek and tongue swabs, oral rinse samples and sampling from subgingival sites) along with nasal swabs and skin swabs (from the hands/wrist and around the mouth) from each subject. If *S. epidermidis* were present in any of the samples, multiple colonies from each sample should be isolated and typed using MLST, array profiling and perhaps a high resolution method such as PFGE or single nucleotide polymorphism (SNP) analysis. A comparison could then be made of the different *S. epidermidis* clones recovered at each different sampling site, and at the same sampling site over time. A detailed study such as this would address many of the questions mentioned above. Multiple isolates from the same samples, and multiple samples from the same individual would provide an indication of the clonality of any *S. epidermidis* present in the oral cavity and if single or multiple types are prevalent at the same or different sites. Sampling the same sites from the same subject at different time points enables observation of any transition, or of the persistence of particular types at the same site over time. This would also permit a determination of whether oral *S. epidermidis* clones are derived from the epidermal flora. Subsequent MLST typing of representatives of each type would allow further comparison, and placement within the global *S. epidermidis* population. Representatives of each *S. epidermidis* clone identified could be further characterised for the presence of MGEs, antibiotic resistance, biofilm and potential virulence genes. Once a pilot study on a small group of subjects has provided an indication

of the *S. epidermidis* population, and its fluidity, a larger study including implantitis patients with matched healthy controls could be undertaken. Enrolling a large number of patients with periimplantitis, and matching them with healthy control subjects, would allow a characterisation and statistically significant comparison of any association between the carriage of various *S. epidermidis* clones and particular states of health or treatment outcomes. If possible a similar study could be undertaken including periodontitis patients matched to healthy controls and/or periimplantitis patients.

6.5.2 Associations of *S. epidermidis* within oral biofilm

In this study some sampling sites yielded extremely high cell densities of *S. epidermidis*. Dental biofilm is very complex and the presence of a species in such high numbers is interesting. What role does *S. epidermidis* play? The present study was unable to divine any significant difference between the different sites sampled (healthy teeth and implants, periimplantitis and periodontitis associated sites). It would be interesting to try to determine if the presence (or absence) of *S. epidermidis* has any effect on the other species found in a biofilm and in turn if this had any effect on the clinically assessed state of health, or any treatment outcomes. A study such as that described above (a large scale comparison of the oral *S. epidermidis* populations of healthy and periimplantitis patients) may provide enough statistical power to determine any associations between *S. epidermidis* sampling sites (tongue or cheek, general oral cavity, periodontal or periimplant pocket), or states of health. However, it is important to remember that if there is an association between *S. epidermidis* and a particular state of health, *S. epidermidis* may not be acting directly. It may be that other species which are in turn associated with the presence (or absence) of *S. epidermidis* are responsible for any differences in states of health or treatment outcome observed. For example, if *S. epidermidis* is outcompeting a potentially perio-pathogenic bacteria it may have a protective effect, but if *S. epidermidis* is providing a more hospitable environment for a potentially perio-pathogenic bacteria its presence could be associated with a disease state. To study this, *in vivo* studies like the ones outlined above would need to determine what other microorganisms were present in the biofilm. The current method for high throughput analysis of the microorganisms present in dental biofilm is checkerboard DNA:DNA hybridisation analysis, which (as discussed above) is not the best method for all species of bacteria, and definitely not for staphylococci. Other choices include selective PCR of target organisms (creating the problem: which organisms to include on the panel of PCRs) and 16S rDNA microarray analysis, which may not be economically viable for a large scale study as suggested above. A simpler initial approach

may be needed. There are many *in vitro* biofilm models that may be useful in studying the associations between *S. epidermidis* and other oral bacteria. Confocal microscopy (with *S. epidermidis*-specific probes) of biofilms seeded with saliva would enable an examination of *S. epidermidis* location within the biofilm structure, while the use of probes for other species of bacteria also found in the oral biofilm could help determine what species particularly co-locate with *S. epidermidis*. As would a study of *in vitro* biofilm cultures with duplicate culture set ups where one set of samples have been treated to prevent the growth of *S. epidermidis* so that the biofilm structure and microbial composition can be compared to samples with *S. epidermidis*. Further *in vivo* (or *in situ*) studies, utilising removable appliances or collection devices that can be fixed in various locations in the mouth as substrates for biofilm formation (enabling the retrieval of intact oral biofilms) could be used to confirm structural associations observed *in vitro* [190, 191].

6.6 Conclusions

The results of the present study clearly demonstrate that *S. epidermidis* is prevalent in the oral cavity of the cohort of periimplantitis patients investigated and that *S. aureus* is not significantly associated with healthy or diseased natural teeth or implants. *S. epidermidis* was unexpectedly found to be prevalent subgingivally at healthy and diseased natural tooth and implant sites, sites that are usually anaerobic or have low oxygen tension. The very high prevalence of ACME among *S. epidermidis* from such sites may contribute to their ability to survive in deep subgingival sites. The prevalence of antimicrobial resistance genes and in some cases SCC*mec* and possibly other SCC elements among oral *S. epidermidis* further highlights the role of *S. epidermidis* as a reservoir of genetic determinants that can be transferred into *S. aureus*, potentially giving rise to new MRSA strains and/or to MSSA strains that are resistant to clinically important antibiotics such as mupirocin. The results of this study raise many additional questions regarding the role of staphylococci in the oral cavity and highlight several productive avenues of future research.

Chapter 7

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Appendix

Appendix Table 1. Antibiotic resistance, virulence, adhesion factors and biofilm associated genes detected by the Alere StaphyType microarray and the associated expected expression products or phenotype

Hybridisation (gene probe)	Expected expression product/phenotype
SCC*mec*-Typing	
mecA	Resistance to methicillin, oxacillin and all β-lactams, defining **MRSA**
mecR	Signal transducer protein *mec*R1
mecI	Methicillin-resistance regulatory protein
ugpQ	Glycerophosphoryl-diester-phosphodiesterase (gene adjacent to *mec*A)
ccrA-1	Cassette chromosome recombinase A, type 1
ccrA-2	Cassette chromosome recombinase A, type 2
ccrA-3	Cassette chromosome recombinase A, type 3
ccrAA	Cassette chromosome recombinase A, type ZH47
ccrA-4	Cassette chromosome recombinase A, type 4
ccrB-1	Cassette chromosome recombinase B, type 1
ccrB-2	Cassette chromosome recombinase B, type 2
ccrB-3	Cassette chromosome recombinase B, type 3
ccrB-4	Cassette chromosome recombinase B, type 4
ccrC	Cassette chromosome recombinase C
merA	Mercuric reductase (SCC*mec* type III)
merB	Alkylmercury Lyase (SCC*mec* type III)
kdpA-SCC	Potassium-transporting ATPase A chain
kdpB-SCC	Potassium-transporting ATPase B chain
kdpC-SCC	Potassium-transporting ATPase C chain
kdpD-SCC	Sensor histidine kinase (Sensor protein located in kdp operon)
kdpE-SCC	KDP operon transcriptional regulatory protein (DNA-binding response regulator)
plsSCC	Plasmin-sensitive surface protein
Q9XB68-dcs	Hypothetical protein historical name: CN050 Synonyms: dcs
xylR	Pseudogene of xylose repressor
Hybridisation (gene probe)	**Expected expression product/phenotype**
agr-Typing	
agrI	Accessory gene regulator - Type 1
agrII	Accessory gene regulator - Type 2
agrIII	Accessory gene regulator - Type 3
agrIV	Accessory gene regulator - Type 4
Hybridisation (gene probe)	**Expected expression product/phenotype**
Capsule	
capsule-1	Capsule Type 1
capsule-5	Capsule Type 5
capsule-8	Capsule Type 8
capH1	Capsular polysaccharide synthesis enzyme *capH* Capsule type
capJ1	O-antigen polymerase *capJ* Capsule Type 1
capK1	Capsular polysaccharide biosynthesis protein *capK* Capsule Type 1
capH5	Capsular polysaccharide synthesis enzyme *capH* Capsule type 5
capJ5	O-antigen polymerase *capJ* Capsule Type 5
capK5	Capsular polysaccharide biosynthesis protein *capK* Capsule Type 5
capH8	Capsular polysaccharide synthesis enzyme *capH* Capsule type 8
capI8	Capsular polysaccharide synthesis enzyme *capI* Capsule type 8
capJ8	O-antigen polymerase *capJ* Capsule Type 8
capK8	Capsular polysaccharide biosynthesis protein capK Capsule Type 8
Hybridisation (gene probe)	**Expected expression product/phenotype**
Resistance genotype	
mecA	Resistance to methicillin, oxacillin and all β-lactams, defining **MRSA**
blaZ	β-lactamase resistance
blaI	β-lactamase repressor (regulatory protein)
blaR	β-lactamase regulatory protein

Continued overleaf

Appendix Table 1 continued. Antibiotic resistance, virulence, adhesion factors and biofilm associated genes detected by the Alere StaphyType microarray and the associated expected expression products or phenotype

Hybridisation (Ggene probe)	Expected expression product/phenotype
Resistance genotype	
erm(A)	Macrolide, lincosamide, streptogramin resistance
erm(B)	Macrolide, lincosamide, streptogramin resistance
erm(C)	Macrolide, lincosamide, streptogramin resistance
linA	Lincosamide resistance
msr(A)	Macrolide resistance
mef(A)	Macrolide resistance
mph(C) (formerly *mpbBM*)	Macrolide resistance
vatA	Streptogramin resistance
vatB	Streptogramin resistance
vga	Streptogramin resistance
vga(A)	Streptogramin resistance
vgb(A)	Streptogramin resistance
aacA-aphD	Aminoglycoside (gentamicin, tobramycin) resistance
aadD	Aminoglycoside (tobramycin, neomycin) resistance
aphA	Aminoglycoside (kanamycin, neomycin) resistance
sat	Streptothricin resistance
dfrA	Trimethoprim resistance
fusB (aka *far1*)	Fusidic acid resistance
Q6GD50 (aka *fusC*)	Putative fusidic acid resistance protein
ileS2 (aka *mupA*) (formerly *mupR*)	Mupirocin resistance
tetK	Tetracycline resistance
tetM	Tetracycline resistance
sdrm (formerly *tetEfflux*)	General antibiotic resistance efflux protein (formerly tetracycline resistance, putative transport protein)
cat	Chloramphenicol resistance
fexA	Chloramphenicol resistance
cfr	Resistance to phenicols, lincosamides, oxazolidinones (linezolid), pleuromutilins, streptogramin A Putative marker for fosfomycin, bleomycin resistance
fosB	Fosfomycin, bleomycin resistance
vanA	Vancomycin resistance
vanB	Vancomycin resistance
vanZ	Mupirocin resistance
Mercury resistance locus	Mercury resistance operon
qacA	Resistance to quaternary ammonium compounds and divalent cations (such as chlorhexidine)
qacC	Quaternary ammonium compound resistance
Hybridisation (gene probe)	**Expected expression product/phenotype**
Virulence genotype	
tst	Toxic shock syndrome toxin
sea (formerly *entA*)	Enterotoxin A
seb (formerly *entB*)	Enterotoxin B
sec (formerly *entC*)	Enterotoxin C
sed (formerly *entD*)	Enterotoxin D
see (formerly *entE*)	Enterotoxin E
seg (formerly *entG*)	Enterotoxin G
seh (formerly *entH*)	Enterotoxin H
sei (formerly *entI*)	Enterotoxin I
sej (formerly *entJ*)	Enterotoxin J
sek (formerly *entK*)	Enterotoxin K

Continued overleaf

Appendix Table 1 continued. Antibiotic resistance, virulence, adhesion factors and biofilm associated genes detected by the Alere StaphyType microarray and the associated expected expression products or phenotype

Hybridisation (gene probe)	Expected expression product/phenotype
Virulence genotype	
sel (formerly *entL*)	Enterotoxin L
sem (formerly *entM*)	Enterotoxin M
sen (formerly *entN*)	Enterotoxin N
seo (formerly *entO*)	Enterotoxin O
seq (formerly *entQ*)	Enterotoxin Q
ser (formerly *entR*)	Enterotoxin R
seu (formerly *entU*)	Enterotoxin U
egc-cluster	Enterotoxins *seg/sei/sem/sen/seo/seu*
PVL	Pantone-valentine leukocidin
lukM/lukF-P83	Bovine leukocidin
lukF	Haemolysin γ, component B
lukS	Haemolysin γ, component C
lukS-ST22+ST45	Haemolysin γ, component C, allele from ST22 and ST45
hlgA	Haemolysin γ, component A
lukD	Leukocidin D component
lukE	Leukocidin E component
lukX	Leukocidin/haemolysin toxin family protein
lukY	Leukocidin/haemolysin toxin family protein
hl	Hypothetical protein similar to Haemolysin
hla	Haemolysin α (α toxin)
hld	Haemolysin δ (amphiphylic membrane toxin)
hlIII	Putative haemolysin III
hlb	Haemolysine β (phospholipase C)
sak	Staphylokinase
chp	Chemotaxis inhibitory protein (CHIPS)
scn	Staphylococcal complement inhibitor (SCIN)
etA	Exfoliative toxin A
etB	Exfoliative toxin B
etD	Exfoliative toxin D
edinA	Epidermal cell differentiation inhibitor A
edinB	Epidermal cell differentiation inhibitor B
edinC	Epidermal cell differentiation inhibitor C
aur	Aureolysin
splA	Serine protease A
splB	Serine protease B
splE	Serine protease E
sspA	Glutamyl endopeptidase / V8-protease
sspB	Staphopain B
sspP	Staphopain A (Staphylopain A)
ACME-locus	Arginine catabolic mobile element
arcA-SCC	arginine deiminase
arcB-SCC	ornithine transcarbamoylase
arcC-SCC	carbamate kinase, locus 2
arcD-SCC	arginine/ornithine antiporter
Hybridisation (gene probe)	**Expected expression product/phenotype**
MSCRAMMs / adhaesion factors	
bbp	Bone sialoprotein-binding protein
clfA	Clumping factor A
clfB	Clumping factor B
cna	Collagen-binding adhesin
ebh	Cell wall associated fibronectin-binding protein

Continued overleaf

Appendix Table 1 continued. Antibiotic resistance, virulence, adhesion factors and biofilm associated genes detected by the Alere StaphyType microarray and the associated expected expression products or phenotype

Hybridisation (Gene probe)	Expected expression product/phenotype
MSCRAMMs / Adhaesion Factors	
eno	Enolase, phosphopyruvate hydratase
fib	Fibrinogen binding protein
ebpS	Cell wall associated fibronectin-binding protein
fnbA	Fibronectin-binding protein A
fnbB	Fibronectin-binding protein B
map	Major histocompatibility complex class II analog protein
sdrC	Ser-asp rich fibrinogen-binding, bone sialoprotein-binding protein C
sdrD	Ser-asp rich fibrinogen-binding, bone sialoprotein-binding protein D
vwb	Willebrand factor-binding protein
sasG	*S. aureus* surface protein G
Hybridisation (gene probe)	**Expected expression product/phenotype**
Biofilm associated genes	
icaA	Intercellular adhesion protein A (N-glycosyltransferase)
icaC	Intercellular adhesion protein C
icaD	Biofilm PIA synthesis protein D
bap	Surface protein involved in biofilm formation

Appendix Table 2. Microarray profile data for SCC*mec* genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

			SCC*mec* genes and specific probes																										
Patient	**Visit[b]**	**Species[c]**	*mecA*	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
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Appendix Table 2 continued. Microarray profile data for SCC*mec* genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	SCC*mec* genes and specific probes																																			
			mecA	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
30	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Samples taken from tooth or implant sites at visit 1 were pre treatment (mechanical debridement and oral hygiene advice), subsequent visits (2-6) are post treatment.

[c] Sa=*Staphylococcus aureus*, Se = *Staphylococcus epidermidis*

Appendix Table 3. Microarray profile data for antibiotic resistance genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

			Antibiotic resistance genes and specific probes																																											
Patient	**Visit[b]**	**Species[c]**	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50* : hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
7	1	Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+/-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	Sa	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
8	2	Sa	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1	Sa	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	-	-	+/-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	3	Sa	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 3 continued. Microarray profile data for antibiotic resistance genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

			Antibiotic resistance genes and specific probes																																												
Patient	**Visit**[b]	**Species**[c]	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50* : hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
10	1	Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	Sa	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
17	3	Sa	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Sa	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
21	1	Sa	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Sa	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	2	Se	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 3 continued. Microarray profile data for antibiotic resistance genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50* : hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
24	1	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	2	Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+/-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	Sa	+	+	+	+/-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	+/-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	1	Sa	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
32	1	Sa	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Sa	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Sa	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

Antibiotic resistance genes and specific probes (spanning header over gene columns)

Continued overleaf

Appendix Table 3 continued. Microarray profile data for antibiotic resistance genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

			Antibiotic resistance genes and specific probes																																													
Patient	**Visit[b]**	**Species[c]**	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50* : hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
32	3	Sa	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	Se	+	+	+	+	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
33	5	Sa	+	+	+/-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Sa	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
34	4	Sa	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1	Sa	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Samples taken from tooth or implant sites at visit 1 were pre treatment (mechanical debridement and oral hygiene advice), subsequent visits (2-6) are post treatment.

[c] Sa=*Staphylococcus aureus*, Se = *Staphylococcus epidermidis*

Appendix Table 4. Microarray profile data for virulence associated genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	Virulence associated genes and specific probes																																			
			tst: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
7	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	2	Sa	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1	Sa	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
9	3	Sa	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-

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Appendix Table 4 continued. Microarray profile data for virulence associated genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	Virulence associated genes and specific probes																																		
			tst: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
10	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
	5	Sa	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
17	3	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
		Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
21	1	Sa	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	+	+	+	+/-	+	-	-	-	-	-
		Sa	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	+	+	+	+/-	+	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-

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Appendix Table 4 continued. Microarray profile data for virulence associated genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	Virulence associated genes and specific probes																																			
			tst: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
24	1	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
30	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
	3	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	1	Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-

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Appendix Table 4 continued. Microarray profile data for virulence associated genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	Virulence associated genes and specific probes																																			
			tst: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
32	1	Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
	3	Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
	6	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
33	5	Sa	+	+/-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+/-	-	-	-	-	+/-	+/-	+	+/-	+/-	+	-	-	-	-	-	-
		Sa	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
34	4	Sa	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

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Appendix Table 4 continued. Microarray profile data for virulence associated genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

			Virulence associated genes and specific probes																																					
Patient	**Visit[b]**	**Species[c]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
35	1	Sa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Samples taken from tooth or implant sites at visit 1 were pre treatment (mechanical debridement and oral hygiene advice), subsequent visits (2-6) are post treatment.

[c] Sa=*Staphylococcus aureus*, Se = *Staphylococcus epidermidis*

Appendix Table 5. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	Biofilm, MSCRAMM and adhesion factor genes and specific probes																										
			icaA: hp_icaA_611	*icaC*: hp_icaC_611	*icaD*: hp_icaD_611	*bap*: hp_bap_611	*bbp*-all: hp_bbp_614	*bbp*-COL+MW2: hp_bbp_616	*bbp*-MRSA252: hp_bbp_613	*bbp*-Mu50: hp_bbp_617	*bbp*-RF122: hp_bbp_612	*bbp*-ST45: hp_bbp_611	*clfA*-all: hp_clfA_611	*clfA*-COL+RF122: hp_clfA_612	*clfA*-MRSA252: hp_clfA_613	*clfA*-Mu50+MW2: hp_clfA_614	*clfB*-all: hp_clfB_611	*clfB*-COL+Mu50: hp_clfB_612	*clfB*-MW2: hp_clfB_613	*clfB*-RF122: hp_clfB_614	*cna*: hp_cna_611	*ebh*-all: hp_ebh-3prime_611	*ebpS*: hp_ebpS_612	*ebpS*: hp_ebpS_614	*ebpS*-01-1111: hp_ebpS_611	*ebpS*-COL: hp_ebpS_613	*eno*: hp_eno_611
7	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	Sa	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+	-	-	-	-	+	+	+	-	+/-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	2	Sa	+	+	+	-	+	-	+	-	-	-	+	+/-	+	-	+	-	-	-	+	+	+	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	1	Sa	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	3	Sa	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+/-	+	-	+	+
10	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	Sa	+	+	+	-	+	-	+	-	-	-	+	+/-	+	-	+	-	+	+/-	-	+	+	+	-	-	+
17	3	Sa	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	+	+	-	+/-	+
		Sa	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+/-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	1	Sa	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+/-	+	-	+/-	+
		Sa	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+/-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	1	Sa	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+/-	-	-	-	-	+	+	+	-	-	+
		Sa	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+	-	-	-	-	+	+	+	-	-	+
		Sa	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+	-	-	-	-	+	+	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 5 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	Biofilm, MSCRAMM and adhesion factor genes and specific probes																								
			icaA: hp_icaA_611	*icaC*: hp_icaC_611	*icaD*: hp_icaD_611	*bap*: hp_bap_611	*bbp*-all: hp_bbp_614	*bbp*-COL+MW2: hp_bbp_616	*bbp*-MRSA252: hp_bbp_613	*bbp*-Mu50: hp_bbp_617	*bbp*-RF122: hp_bbp_612	*bbp*-ST45: hp_bbp_611	*clfA*-all: hp_clfA_611	*clfA*-COL+RF122: hp_clfA_612	*clfA*-MRSA252: hp_clfA_613	*clfA*-Mu50+MW2: hp_clfA_614	*clfB*-all: hp_clfB_611	*clfB*-COL+Mu50: hp_clfB_612	*clfB*-MW2: hp_clfB_613	*clfB*-RF122: hp_clfB_614	*cna*: hp_cna_611	*ebh*-all: hp_ebh-3prime_611	*ebpS*: hp_ebpS_612	*ebpS*: hp_ebpS_614	*ebpS*-01-1111: hp_ebpS_611	*ebpS*-COL: hp_ebpS_613	*eno*: hp_eno_611
30	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3	Sa	+	+/-	+/-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+/-	-	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	1	Sa	+	+	+	-	+	+	+/-	-	-	-	+	+	+/-	+/-	+	-	-	-	-	+	+	+	-	+	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32	1	Sa	+	+	+	-	+	+	-	-	-	-	+	+	+/-	+/-	+	+	-	-	-	+	+	+	-	+	+
		Sa	+	+	+	-	+	+	-	-	-	-	+	+	-	+/-	+	+	-	-	-	+	+	+	-	+	+
		Sa	+	+	+	-	+	+	-	-	-	-	+	+	-	+/-	+	+	-	-	-	+	+	+	-	+	+
	3	Sa	+	+	+	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+/-	+/-	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	6	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	5	Sa	+	+/-	+/-	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+/-	+	-	-	+
		Sa	+	+	+	-	+	-	+	-	-	-	+	-	+	-	+	-	-	-	+	+	+	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	4	Sa	+	+	+	-	+	+	-	-	-	-	+	+	-	+/-	+	+	-	-	-	+	+	+	-	+/-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1	Sa	+	+	+	+	+	+	-	-	-	-	+	+	-	+/-	+	-	+	+/-	-	+	+	+	-	+	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 5 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Species[c]	MSCRAMM and adhesion factor genes and specific probes																													
			fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
7	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	+/-	+
	3	Sa	+	-	+	-	-	-	-	-	+/-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	+/-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-
8	2	Sa	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	+	-	+	-	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-
9	1	Sa	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+/-	+	-	-	+	-	+	-	-	+	+	-	-	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 5 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Patient	MSCRAMM and adhesion factor genes and specific probes																																			
			fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
9	3	Sa	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+/-	+	-	-	+	-	+	-	-	+	-	-	+	+	-	-	+	+	+	-	+
10	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5	Sa	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+
17	3	Sa	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+/-	-	+	+	-	-	-	-	-	+	+	+	+/-
		Sa	+	-	+	+	-	-	-	-	+	+/-	-	+/-	-	-	-	+	+	-	+	-	-	+	-	+/-	-	+	+	-	-	-	-	-	+	+	+	+/-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	1	Sa	-	+	+	-	-	+	-	-	+/-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	+
		Sa	-	+	+	-	-	+	-	-	+/-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

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Appendix Table 5 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Patient	MSCRAMM and adhesion factor genes and specific probes																																			
			fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
21	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	1	Sa	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+/-	-	-	+	+	-	-	-	-	-	-	-	+	+/-
		Sa	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+/-	-	-	+	+	-	-	-	-	-	-	-	+	+/-
		Sa	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+	-	-	+	+	-	-	-	-	-	-	-	+	+/-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	2	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
	3	Sa	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+/-	-	-	-	-	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
31	1	Sa	+	-	+	-	-	-	-	-	+/-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	+	+/-

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Appendix Table 5 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Patient	MSCRAMM and adhesion factor genes and specific probes																													
			fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
31	1	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	+	-
32	1	Sa	+	-	+	+	-	-	-	+	+/-	-	-	-	-	+	-	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+/-
		Sa	+	-	+	+	-	-	-	+	+/-	-	-	-	-	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+/-
		Sa	+	-	+	+	-	-	-	+	+/-	-	-	-	-	+	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+/-
	3	Sa	-	-	+/-	+	-	-	-	+/-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	+	+	-	-	+	+	+	+/-
		Se	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	6	Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
33	5	Sa	-	+	+/-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+/-	-	-	-	+/-	+/-	+	-	+/-	-	-	+
		Sa	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	+	+	+	-	+	-	-	+
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 5 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* and *S. epidermidis* isolates recovered from the same patient[a]

Patient	Visit[b]	Patient	MSCRAMM and adhesion factor genes and specific probes																																			
			fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
34	4	Sa	+	-	+	+	-	-	-	+/-	+	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	-	+	-	+	+	+	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	1	Sa	+	-	+	+	-	-	-	+/-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	+/-	-	-	+	+	+	+	-	-	-	+	-	+	+/-
		Se	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Se	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Samples taken from tooth or implant sites at visit 1 were pre treatment (mechanical debridement and oral hygiene advice), subsequent visits (2-6) are post treatment.

[c] Sa=*Staphylococcus aureus*, Se = *Staphylococcus epidermidis*

Appendix Table 6. Microarray profile data for SCC*mec* genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	SCC*mec* genes and specific probes																													
		mecA	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
DDUH011b-1	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH011b-5	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH011b-7	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH042a-1	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH097a-2	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH122b-1	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH128b-2	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH130a-1	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH183b-2	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH405a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH479a-1	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH508b-1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 6 continued. Microarray profile data for SCC*mec* genes for *S. aureus* isolates[a]

		SCC*mec* genes and specific probes																													
Isolate ID	**Clonal complex (CC)[b]**	*mecA*	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
DDUH517b-3	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH559a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH616b-1	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
DDUH669a-2	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH703a-1	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH703a-2	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH705b-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH712a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH763a-3	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH764a-1	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH796a-1	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 6 continued. Microarray profile data for SCC*mec* genes for *S. aureus* isolates[a]

		SCC*mec* genes and specific probes																														
Isolate ID	**Clonal complex (CC)[b]**	*mecA*	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
DDUH837b-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH838a-2	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH858a-4	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH873a-1	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH914a-1	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH972a-2	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH974a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH975b-1	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Clonal complex as assigned by the Alere StaphyType DNA microarry.

Appendix Table 7. Microarray profile data for antibiotic resistance genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH011b-1	8	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH011b-5	8	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH011b-7	8	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH042a-1	9	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
DDUH097a-2	22	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH122b-1	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH128b-2	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH130a-1	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH183b-2	30	+	+	+	+/-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

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Appendix Table 7 continued. Microarray profile data for antibiotic resistance genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	Antibiotic resistance genes and specific probes																																													
		blaZ	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH405a-1	15	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-
DDUH479a-1	8	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH508b-1	5	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH517b-3	8	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH559a-1	15	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH616b-1	8	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH669a-2	7	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH703a-1	30	+	+	+/-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH703a-2	30	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

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Appendix Table 7 continued. Microarray profile data for antibiotic resistance genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	Antibiotic resistance genes and specific probes																																													
		blaZ	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH705b-1	15	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH712a-1	15	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH763a-3	22	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH764a-1	22	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH796a-1	9	+	+	+	+	+/-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH837b-1	15	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH838a-2	15	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH858a-4	30	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

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Appendix Table 7 continued. Microarray profile data for antibiotic resistance genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	Antibiotic resistance genes and specific probes																																												
		blaZ	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH873a-1	30	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH914a-1	22	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH972a-2	7	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH974a-1	15	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
DDUH975b-1	30	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Clonal complex as assigned by the Alere StaphyType DNA microarry.

Appendix Table 8. Microarray profile data for virulence associated genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	Virulence associated genes and specific probes																																	
		tst: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH011b-1	8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
DDUH011b-5	8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
DDUH011b-7	8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
DDUH042a-1	9	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	-	-	-	-	+/-	-	-	-	-	-
DDUH097a-2	22	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	+/-	-	-	-	+	+	+	+	+	-	-	-	-	-	-
DDUH122b-1	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-
DDUH128b-2	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-
DDUH130a-1	101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-
DDUH183b-2	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH405a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
DDUH479a-1	8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
DDUH508b-1	5	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	-	-
DDUH517b-3	8	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-

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Appendix Table 8 continued. Microarray profile data for virulence associated genes for *S. aureus* isolates[a]

		Virulence associated genes and specific probes																																				
Isolate ID	**Clonal complex (CC)[b]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH559a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
DDUH616b-1	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH669a-2	7	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
DDUH703a-1	30	+	+/-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	+/-	-	-	-	-	+/-	+/-	+	+/-	+/-	+	-	-	-	-	-
DDUH703a-2	30	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-
DDUH705b-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
DDUH712a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
DDUH763a-3	22	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	+	+	+	+/-	+	-	-	-	-	-
DDUH764a-1	22	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	+	+	+	+/-	+	-	-	-	-	-
DDUH796a-1	9	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-
DDUH837b-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
DDUH838a-2	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
DDUH858a-4	30	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-

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Appendix Table 8 continued. Microarray profile data for virulence associated genes for *S. aureus* isolates[a]

		Virulence associated genes and specific probes																																
Isolate ID	**Clonal complex (CC)[b]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH873a-1	30	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
DDUH838a-2	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
DDUH858a-4	30	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
DDUH873a-1	30	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
DDUH914a-1	22	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+/-	+	+/-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-
DDUH972a-2	7	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-
DDUH974a-1	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
DDUH975b-1	30	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] Clonal complex as assigned by the Alere StaphyType DNA microarry.

Appendix Table 9. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	Biofilm, MSCRAMM and adhesion factor genes and specific probes																								
		icaA: hp_icaA_611	*icaC*: hp_icaC_611	*icaD*: hp_icaD_611	*bap*: hp_bap_611	*bbp*-all: hp_bbp_614	*bbp*-COL+MW2: hp_bbp_616	*bbp*-MRSA252: hp_bbp_613	*bbp*-Mu50: hp_bbp_617	*bbp*-RF122: hp_bbp_612	*bbp*-ST45: hp_bbp_611	*clfA*-all: hp_clfA_611	*clfA*-COL+RF122: hp_clfA_612	*clfA*-MRSA252: hp_clfA_613	*clfA*-Mu50+MW2: hp_clfA_614	*clfB*-all: hp_clfB_611	*clfB*-COL+Mu50: hp_clfB_612	*clfB*-MW2: hp_clfB_613	*clfB*-RF122: hp_clfB_614	*cna*: hp_cna_611	*ebh*-all: hp_ebh-3prime_611	*ebpS*: hp_ebpS_612	*ebpS*: hp_ebpS_614	*ebpS*-01-1111: hp_ebpS_611	*ebpS*-COL: hp_ebpS_613	*eno*: hp_eno_611
DDUH011b-1	8	+	+	+	-	+	+	-	-	-	-	+	+	+/-	+/-	+	+	-	-	-	+	+	+	-	+	+
DDUH011b-5	8	+	+	+	-	+	+	-	-	-	-	+	+	-	+/-	+	+	-	-	-	+	+	+	-	+	+
DDUH011b-7	8	+	+	+	-	+	+	-	-	-	-	+	+	-	+/-	+	+	-	-	-	+	+	+	-	+	+
DDUH042a-1	9	+	+	+/-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	+/-	+	+	-	-	+
DDUH097a-2	22	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+	+
DDUH122b-1	101	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+	-	-	-	-	+	+	+	-	-	+
DDUH128b-2	101	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+/-	-	-	-	-	+	+	+	-	-	+
DDUH130a-1	101	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+	-	-	-	-	+	+	+	-	-	+
DDUH183b-2	30	+	+/-	+/-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+/-	-	+	-	-	+
DDUH405a-1	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+	+
DDUH479a-1	8	+	+	+	-	+	+	-	-	-	-	+	+	-	-	+	+	-	-	-	+/-	+/-	+	-	-	+
DDUH508b-1	5	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	+	-	-	-	+	+	+	-	-	+
DDUH517b-3	8	+	+	+	-	+	+	-	-	-	-	+	+	-	+/-	+	+	-	-	-	+	+	+	-	+/-	+
DDUH559a-1	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+/-	+
DDUH616b-1	8	+	+	+	+	+	+	-	-	-	-	+	+	-	+/-	+	-	+	+/-	-	+	+	+	-	+	+
DDUH669a-2	7	+	+	+	-	+	+	+/-	-	-	-	+	+	+/-	+/-	+	-	-	-	-	+	+	+	-	+	+
DDUH703a-1	30	+	+/-	+/-	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+/-	+	-	-	+
DDUH703a-2	30	+	+	+	-	+	-	+	-	-	-	+	-	+	-	+	-	-	-	+	+	+	+	-	-	+
DDUH705b-1	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+	+
DDUH712a-1	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+	+
DDUH763a-3	22	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+/-	+	-	+/-	+
DDUH764a-1	22	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+/-	+
DDUH796a-1	9	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	+	+	+	-	-	+

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Appendix Table 9 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* isolates[a]

		Biofilm, MSCRAMM and adhesion factor genes and specific probes																								
Isolate ID	**Clonal complex (CC)[b]**	*icaA*: hp_icaA_611	*icaC*: hp_icaC_611	*icaD*: hp_icaD_611	*bap*: hp_bap_611	*bbp*-all: hp_bbp_614	*bbp*-COL+MW2: hp_bbp_616	*bbp*-MRSA252: hp_bbp_613	*bbp*-Mu50: hp_bbp_617	*bbp*-RF122: hp_bbp_612	*bbp*-ST45: hp_bbp_611	*clfA*-all: hp_clfA_611	*clfA*-COL+RF122: hp_clfA_612	*clfA*-MRSA252: hp_clfA_613	*clfA*-Mu50+MW2: hp_clfA_614	*clfB*-all: hp_clfB_611	*clfB*-COL+Mu50: hp_clfB_612	*clfB*-MW2: hp_clfB_613	*clfB*-RF122: hp_clfB_614	*cna*: hp_cna_611	*ebh*-all: hp_ebh-3prime_611	*ebpS*: hp_ebpS_612	*ebpS*: hp_ebpS_614	*ebpS*-01-1111: hp_ebpS_611	*ebpS*-COL: hp_ebpS_613	*eno*: hp_eno_611
DDUH837b-1	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	+	+	-	+/-	+
DDUH838a-2	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+/-	+
DDUH858a-4	30	+	+	+	-	+	-	+	-	-	-	+	+/-	+	-	+	-	-	-	+	+	+	+	-	-	+
DDUH873a-1	30	+	+	+	-	+	-	+	-	-	-	+	+/-	+	-	+	-	+	+/-	-	+	+	+	-	-	+
DDUH914a-1	22	+	-	+	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	+/-	+	-	+	+
DDUH972a-2	7	+	+	+	-	+	+	+/-	-	-	-	+	+	-	+/-	+	-	-	-	-	+	+	+	-	+/-	+
DDUH974a-1	15	+	+	+	-	+	-	-	+	-	-	+	-	-	+	+	-	+	+/-	-	+	+	+	-	+	+
DDUH975b-1	30	+	+	+	-	-	-	-	-	-	-	+	+/-	+	-	+	-	-	-	+	+/-	+	+	-	-	+

[a] + = positive, - = negative, +/- = ambiguous.

[b] Clonal complex as assigned by the Alere StaphyType DNA microarry.

Appendix Table 9 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* isolates[a]

		MSCRAMM and adhesion factor genes and specific probes																																				
Isolate ID	**Clonal complex (CC)[b]**	*fib*: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH011b-1	8	+	-	+	+	-	-	-	+	+/-	-	-	-	-	+	-	-	+	-	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+/-
DDUH011b-5	8	+	-	+	+	-	-	-	+	+/-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+/-
DDUH011b-7	8	+	-	+	+	-	-	-	+	+/-	-	-	-	-	+	-	-	+	-	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+/-
DDUH042a-1	9	+	-	-	-	+	-	-	-	-	-	-	-	-	+/-	-	+	+	-	-	+	-	+	-	-	-	+/-	+	+	-	+	-	-	-	-	+	+/-
DDUH097a-2	22	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+/-	+	-	-	+	-	+	-	-	-	+/-	+	+	-	-	-	+	-	+	+	-	+
DDUH122b-1	101	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	+/-
DDUH128b-2	101	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+/-	-	-	-	+	+	-	-	-	-	-	-	-	+	+/-
DDUH130a-1	101	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	+/-	-	-	-	+	+	-	-	-	-	-	-	-	+	+/-
DDUH183b-2	30	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	+/-	-	-	-	-	-	-	+
DDUH405a-1	15	+	-	+	+	-	-	-	-	+	+/-	-	+/-	-	-	-	+	+	-	-	+	-	+	-	+	-	-	+	+	-	-	-	-	-	+	+	+	+/-
DDUH479a-1	8	-	-	+/-	+	-	-	-	+/-	+	-	-	-	-	+	-	-	+	-	-	+	-	+	-	-	-	-	+	+	+	-	-	-	+	-	+	+	+/-

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Appendix Table 9 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* isolates[a]

Isolate ID	Clonal complex (CC)[b]	MSCRAMM and adhesion factor genes and specific probes																																			
		fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH508b-1	5	+	-	+	-	-	+	-	-	+/-	+	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-	+	-	-	+	-	+	-	+	+	+/-
DDUH517b-3	8	+	-	+	+	-	-	-	+/-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	+/-	-	-	+	+	+	-	-	-	+	-	+	+	-
DDUH559a-1	15	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	+	+	+	+/-
DDUH616b-1	8	+	-	+	+	-	-	-	+/-	+	-	-	-	-	+	-	-	+	-	+	-	-	+	+/-	-	-	+	+	+	-	-	-	+	-	+	+	+/-
DDUH669a-2	7	+	-	+	-	-	-	-	-	+/-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	+	+/-
DDUH703a-1	30	-	+	+/-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+/-	-	-	-	-	+/-	+/-	+	-	+/-	-	-	-	-	-	-	+
DDUH703a-2	30	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+
DDUH705b-1	15	+	-	+	+	-	-	-	-	+	+/-	-	+/-	-	-	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	+	-	-	+	+	+	+/-
DDUH712a-1	15	+	-	+	+	-	-	-	-	+	+/-	-	+/-	-	-	-	+	+	-	+	-	-	+	-	+	-	+	+	-	-	+	-	-	+	+	+	+/-
DDUH763a-3	22	-	+	+	-	-	+	-	-	+/-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	+
DDUH764a-1	22	-	+	+	-	-	+	-	-	+/-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	+	+	-	+

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Appendix Table 9 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. aureus* isolates[a]

MSCRAMM and adhesion factor genes and specific probes

Isolate ID	Clonal complex (CC)[b]	*fib*: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612
DDUH796a-1	9	+	-	+	-	+	-	-	-	+	-	-	-	-	+	-	+	+	-
DDUH837b-1	15	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	+	-
DDUH838a-2	15	+	-	+	+	-	-	-	-	+	+/-	-	+/-	-	-	-	+	+	-
DDUH858a-4	30	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-
DDUH873a-1	30	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-
DDUH914a-1	22	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	+/-	+	-
DDUH972a-2	7	+	-	+	-	-	-	-	-	+/-	-	-	-	+	-	-	+	+	-
DDUH974a-1	15	+	-	+	+	-	-	-	-	+	+/-	-	+/-	-	-	-	+	+	-
DDUH975b-1	30	-	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-

Isolate ID	Clonal complex (CC)[b]	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH796a-1	9	-	+	+	+	-	-	+	+	+	-	+	-	-	-	-	-	+	+/-
DDUH837b-1	15	+	-	-	+	-	+/-	-	+	+	-	-	-	-	-	+	+	+	+/-
DDUH838a-2	15	+	-	-	+	-	+/-	-	+	+	-	-	-	-	-	+	+	+	+/-
DDUH858a-4	30	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+
DDUH873a-1	30	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+
DDUH914a-1	22	-	+	-	+	-	-	+	+	+	-	-	-	+	-	+	+	-	+
DDUH972a-2	7	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	+	+/-
DDUH974a-1	15	+	-	-	+	-	+	-	+	+	-	-	+	-	-	+	+	+	+/-
DDUH975b-1	30	-	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+

[a] + = positive, - = negative, +/- = ambiguous.
[b] Clonal complex as assigned by the Alere StaphyType DNA microarry.

Appendix Table 10. Microarray profile data for SCC*mec* genes for *S. epidermidis* isolates[a]

Isolate ID	MLST ST[b]	SCC*mec* genes and specific probes																													
		mecA	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
DDUH047a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH049b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH059b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH060a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH095a-1	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1000a-1	5	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1007a-1	U	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH116b-2	432	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH119a-1	14	-	-	-	-	-	-	-	-	-	-	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH124b-1	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH139a-1	284	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH141a-3	U	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH183a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH224a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH227a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH312a-1	153	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH315a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH318b-1	153	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH479a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Continued overleaf

Appendix Table 10 continued. Microarray profile data for SCC*mec* genes for *S. epidermidis* isolates[a]

		SCC*mec* genes and specific probes																												
Isolate ID	**MLST ST[b]**	*mecA*	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
DDUH513a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH515b-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH517a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH585a-1	U	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH590b-1	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH598a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH613b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH616b-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH669a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH703a-4	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH717a-2	U	+	+	+	-	-	-	-	+/-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH745a-1	32	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH761b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH764a-3	297	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH805b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH816a-1	U	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-
DDUH837a-1	U	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH838a-1	U	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH847a-1	U	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 10 continued. Microarray profile data for SCC*mec* genes for *S. epidermidis* isolates[a]

		SCC*mec* genes and specific probes																												
Isolate ID	**MLST ST[b]**	*mecA*	*delta_mecR*: hp_mecR_611	*ugpQ*: hp_ugpQ_611	*ccrA-1*: hp_ccrA-1_611	*ccrB-1*: hp_ccrB-1_612	*ccrB-1*: hp_ccrB-1_613	*plsSCC*-COL: hp_plsSCC_611	Q9XB68-*dcs*: hp_Q9XB68_611	*ccrA-2*: hp_ccrA-2_611	*ccrB-2*: hp_ccrB-2_611	*kdpA-SCC*: hp_kdpA-SCC_611	*kdpB-SCC*: hp_kdpB-SCC_611	*kdpC-SCC*: hp_kdpC-SCC_612	*kdpD-SCC*: hp_kdpD-SCC_611	*kdpE-SCC*: hp_kdpE-SCC_611	*mecI*: hp_mecI_611	*mecR*: hp_mecR_612	*xylR*: hp_xylR_611	*ccrA-3*: hp_ccrA-3_611	*ccrB-3*: hp_ccrB-3_611	*merA*: hp_merA_611	*merB*: hp_merB_611	*ccrAA*-MRSAZH47: hp_ccrAA_612	*ccrAA*-MRSAZH47: hp_ccrAA_613	*ccrC*-85-2082: hp_ccrC_611	*ccrA-4*: hp_ccrA-4_612	*ccrB-4*: hp_ccrB-4_611
DDUH858a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH894a-2	190	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+
DDUH901b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH963a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH972a-1	204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] U = sample has not been typed using MLST (untyped).

Appendix Table 11. Microarray profile data for antibiotic resistance genes for *S. epidermidis* isolates[a]

Isolate ID	MLST ST[b]	Antibiotic resistance genes and specific probes																																														
		blaZ	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB* (*far1*)	*Q6GD50*:hp_Q6GD50_611	*ileS2* (*mupR*)	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH047a-1	73	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH049b-1	U	+	+	+	+	+/-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH059b-1	U	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH060a-1	73	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH095a-1	17	+	+	+	+	-	-	+/-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1000a-1	5	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
DDUH1007a-1	U	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+/-	-	-	-	-
DDUH116b-2	432	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH119a-1	14	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH124b-1	200	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH139a-1	284	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH141a-3	U	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 11 continued. Microarray profile data for antibiotic resistance genes for *S. epidermidis* isolates[a]

		Antibiotic resistance genes and specific probes																																												
Isolate ID	**MLST ST[b]**	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB* (*far1*)	*Q6GD50*:hp_Q6GD50_611	*ileS2* (*mupR*)	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH183a-1	U	+	+	+	+	+/-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH224a-1	73	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH227a-1	U	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH312a-1	153	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH315a-1	U	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH318b-1	153	+	+	+	+	+/-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH479a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH513a-1	14	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH515b-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 11 continued. Microarray profile data for antibiotic resistance genes for *S. epidermidis* isolates[a]

Isolate ID	MLST ST[b]	Antibiotic resistance genes and specific probes																																												
		blaZ	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH517a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH585a-1	U	+	+	+	+	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH590b-1	193	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH598a-1	U	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
DDUH613b-1	73	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH616b-2	U	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH669a-1	U	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH703a-4	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH717a-2	U	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	+	+	+/-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

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Appendix Table 11 continued. Microarray profile data for antibiotic resistance genes for *S. epidermidis* isolates[a]

		Antibiotic resistance genes and specific probes																																														
Isolate ID	**MLST ST[b]**	*blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH745a-1	32	+	+	+	+	+	-	+	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH761b-1	73	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH764a-3	297	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH805b-1	U	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH816a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH837a-1	U	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH838a-1	U	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
DDUH847a-1	U	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH858a-1	73	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 11 continued. Microarray profile data for antibiotic resistance genes for *S. epidermidis* isolates[a]

Isolate ID	MLST ST[b]	Antibiotic resistance genes and specific probes: *blaZ*	*blaZ*: hp_blaZ_611	*blaI*: hp_blaI_611	*blaR*: hp_blaR_611	*blaR*: hp_blaR_612	*erm*(A)	*erm*(B)	*erm*(C)	*linA*	*msr*(A)	*mef*(A): hp_mefA_611	*mef*(A): hp_mefA_612	*mph*(C): hp_mpbBM_611	*mph*(C):: hp_mpbBM_612	*vatA*	*vatB*	*vga*	*vga*(A)	*vgb*(A)	*aacA_aphD*	*aadD*	*aphA_3*	*sat*	*dfrA*	*fusB (far1)*	*Q6GD50*:hp_Q6GD50_611	*ileS2 (mupR)*	*tetK*	*tetM*	*sdrm* : hp_tetEfflux_611	*cat*-pC221: hp_cat_613	*cat*-pC223: hp_cat_611	*cat*-pMC524: hp_cat_612	*cat*-pSBK203R: hp_cat_615	*cfr*: hp_cfr_611	*fexA*: hp_fexA_611	*fosB*: hp_fosB_611	*fosB*-plasmid: hp_fosB_612	*qacA*: hp_qacA_611	*qacC*: hp_qacC_611	*qacC*-equine: hp_qacC_614	*qacC*-SA5: hp_qacC_613	*qacC*-Ssap: hp_qacC_612	*qacC*-ST94: hp_qacC_615	*vanA*	*vanB*	*vanZ*
DDUH894a-2	190	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH901b-1	U	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH963a-1	73	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH972a-1	204	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.
[b] U = sample has not been typed using MLST (untyped).

Appendix Table 12. Microarray profile data for virulence associated genes for *S. epidermidis* isolates[a]

		Virulence associated genes and specific probes																																				
Isolate ID	**MLST ST[b]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH047a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
DDUH049b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH059b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH060a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH095a-1	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH1000a-1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH1007a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH116b-2	432	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH119a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH124b-1	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH139a-1	284	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH141a-3	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH183a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 12 continued. Microarray profile data for virulence associated genes for *S. epidermidis* isolates[a]

		Virulence associated genes and specific probes																																		
Isolate ID	**MLST ST[b]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH224a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH227a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH312a-1	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH315a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH318b-1	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH479a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH513a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH515b-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH517a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
DDUH585a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH590b-1	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH598a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
DDUH613b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 12 continued. Microarray profile data for virulence associated genes for *S. epidermidis* isolates[a]

		Virulence associated genes and specific probes																																		
Isolate ID	**MLST ST[b]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH616b-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
DDUH669a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH703a-4	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH717a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH745a-1	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH761b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH764a-3	297	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH805b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH816a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	+/-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH837a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH838a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH847a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-
DDUH858a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 12 continued. Microarray profile data for virulence associated genes for *S. epidermidis* isolates[a]

		Virulence associated genes and specific probes																																		
Isolate ID	**MLST ST[b]**	*tst*: hp_tst_611	*tst*1_other than RF122	*sea*	*sea*-320E	*sea*-N315 (aka entP)	*seb*	*sec*	*sec*M14: hp_entCM14_611	*sec*M14: hp_entCM14_612	*sed*	*see*	*seg*	*seh*	*sei*	*sej*	*sek*: hp_entK_611	*sek*: hp_entK_612	*sel*	*sem*	*sen*: hp_entN_611	*sen*_other than RF122	*seo*	*seo*: hp_entQ_611	*seo*: hp_entQ_612	*ser*	*seu*: hp_entU_611	*sak*	*sak*: hp_sak_611	*chp*: hp_chp_611	*chp*: hp_chp_612	*scn*: hp_scn_611	*etA*	*etB*	*etD*	*edinA*	*edinB*	*edinC*
DDUH894a-2	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH901b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH963a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
DDUH972a-1	204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.
[b] U = sample has not been typed using MLST (untyped).

Appendix Table 13. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. epidermidis* isolates[a]

		Biofilm, MSCRAMM and adhesion factor genes and specific probes																											
Isolate ID	**MLST ST[b]**	*icaA*: hp_icaA_611	*icaC*: hp_icaC_611	*icaD*: hp_icaD_611	*bap*: hp_bap_611	*bbp*-all: hp_bbp_614	*bbp*-COL+MW2: hp_bbp_616	*bbp*-MRSA252: hp_bbp_613	*bbp*-Mu50: hp_bbp_617	*bbp*-RF122: hp_bbp_612	*bbp*-ST45: hp_bbp_611	*clfA*-all: hp_clfA_611	*clfA*-COL+RF122: hp_clfA_612	*clfA*-MRSA252: hp_clfA_613	*clfA*-Mu50+MW2: hp_clfA_614	*clfB*-all: hp_clfB_611	*clfB*-COL+Mu50: hp_clfB_612	*clfB*-MW2: hp_clfB_613	*clfB*-RF122: hp_clfB_614	*cna*: hp_cna_611	*ebh*-all: hp_ebh-3prime_611	*ebpS*: hp_ebpS_612	*ebpS*: hp_ebpS_614	*ebpS*-01-1111: hp_ebpS_611	*ebpS*-COL: hp_ebpS_613	*eno*: hp_eno_611
DDUH047a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH049b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH059b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH060a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH095a-1	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1000a-1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1007a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH116b-2	432	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH119a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH124b-1	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH139a-1	284	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH141a-3	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH183a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH224a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH227a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH312a-1	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH315a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH318b-1	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH479a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH513a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH515b-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH517a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH585a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH590b-1	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Continued overleaf

Appendix Table 13 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. epidermidis* isolates[a]

		Biofilm, MSCRAMM and adhesion factor genes and specific probes																												
Isolate ID	**MLST ST[b]**	*icaA*: hp_icaA_611	*icaC*: hp_icaC_611	*icaD*: hp_icaD_611	*bap*: hp_bap_611	*bbp*-all: hp_bbp_614	*bbp*-COL+MW2: hp_bbp_616	*bbp*-MRSA252: hp_bbp_613	*bbp*-Mu50: hp_bbp_617	*bbp*-RF122: hp_bbp_612	*bbp*-ST45: hp_bbp_611	*clfA*-all: hp_clfA_611	*clfA*-COL+RF122: hp_clfA_612	*clfA*-MRSA252: hp_clfA_613	*clfA*-Mu50+MW2: hp_clfA_614	*clfB*-all: hp_clfB_611	*clfB*-COL+Mu50: hp_clfB_612	*clfB*-MW2: hp_clfB_613	*clfB*-RF122: hp_clfB_614	*cna*: hp_cna_611	*ebh*-all: hp_ebh-3prime_611	*ebpS*: hp_ebpS_612	*ebpS*: hp_ebpS_614	*ebpS*-01-1111: hp_ebpS_611	*ebpS*-COL: hp_ebpS_613	*eno*: hp_eno_611
DDUH598a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH613b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH616b-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH669a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH703a-4	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH717a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH745a-1	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH761b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH764a-3	297	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH805b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH816a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH837a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH838a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH847a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH858a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH894a-2	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH901b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH963a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH972a-1	204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.
[b] U = sample has not been typed using MLST (untyped).

Appendix Table 13 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. epidermidis* isolates[a]

		MSCRAMM and adhesion factor genes and specific probes																																			
Isolate ID	MLST ST[b]	*fib*: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH047a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH049b-1	U	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
DDUH059b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH060a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH095a-1	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1000a-1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH1007a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH116b-2	432	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH119a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH124b-1	200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH139a-1	284	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 13 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. epidermidis* isolates[a]

Isolate ID	MLST ST[b]	MSCRAMM and adhesion factor genes and specific probes																														
		fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH141a-3	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH183a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
DDUH224a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH227a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH312a-1	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	+
DDUH315a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	+/-	+
DDUH318b-1	153	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	+
DDUH479a-2	U	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
DDUH513a-1	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH515b-1	14	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH517a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 13 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. epidermidis* isolates[a]

Isolate ID	MLST ST[b]	MSCRAMM and adhesion factor genes and specific probes																																	
		fib: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH585a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH590b-1	193	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH598a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH613b-1	73	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH616b-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH669a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
DDUH703a-4	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH717a-2	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH745a-1	32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH761b-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
DDUH764a-3	297	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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Appendix Table 13 continued. Microarray profile data for biofilm, MSCRAMM and adhesion factor genes for *S. epidermidis* isolates[a]

		MSCRAMM and adhesion factor genes and specific probes																																				
Isolate ID	**MLST ST[b]**	*fib*: hp_fib_611	*fib*-MRSA252: hp_fib_612	*fnbA*-all: hp_fnbA_615	*fnbA*-COL: hp_fnbA_612	*fnbA*-MRSA252: hp_fnbA_613	*fnbA*-Mu50+MW2: hp_fnbA_611	*fnbA*-RF122: hp_fnbA_614	*fnbB*-COL: hp_fnbB_614	*fnbB*-COL+Mu50+MW2: hp_fnbB_616	*fnbB*-Mu50: hp_fnbB_611	*fnbB*-MW2: hp_fnbB_613	*fnbB*-ST15: hp_fnbB_612	*fnbB*-ST45-2: hp_fnbB_615	*map*-COL: hp_map_611	*map*-MRSA252: hp_map_613	*map*-Mu50+MW2: hp_map_612	*sdrC*-all: hp_sdrC_613	*sdrC*-B1: hp_sdrC_612	*sdrC*-COL: hp_sdrC_615	*sdrC*-Mu50: hp_sdrC_614	*sdrC*-MW2+MRSA252+RF122: hp_sdrC_616	*sdrC*-OtherThan252+122: hp_sdrC_611	*sdrD*-COL+MW2: hp_sdrD_612	*sdrD*-Mu50: hp_sdrD_613	*sdrD*-other1: hp_sdrD_611	*sdrD*-OtherThan252+122: hp_sdrD_614	*vwb*-all: hp_vwb_615	*vwb*-COL+MW2: hp_vwb_612	*vwb*-MRSA252: hp_vwb_613	*vwb*-Mu50: hp_vwb_614	*vwb*-RF122: hp_vwb_611	*sasG*-COL+Mu50: hp_sasG_613	*sasG*-MW2: hp_sasG_612	*sasG*-OtherThan252+122: hp_sasG_611	*isaB*: hp_isaB_611	*isaB*-MRSA252: hp_isaB_612
DDUH805b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH816a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH837a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH838a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH847a-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH858a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH894a-2	190	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH901b-1	U	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH963a-1	73	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DDUH972a-1	204	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] + = positive, - = negative, +/- = ambiguous.

[b] U = sample has not been typed using MLST (untyped).