Dissemination, antibiotic susceptibility, proteomic and genomic characterization of antibiotic-resistant staphylococci recovered from general public settings

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Collaborations and publications

Collaborations

Whole genome sequence	Sanger Institute	Gavin K Paterson
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BioNumerics analysis	Applied Math, Belgium	Katleen Vranckx, Bruno Pot
	Public Health England	Haroun Shah

Conferences

25th ECCMID	2016	Comparative genomics of an environmental multiple antibiotic-resistant *Staphylococcus epidermidis*	Poster Presentation
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18th PHE	2015	The spread of antibiotic resistant staphylococci in the public libraries	Poster presentation
6th FEMS	2015	Widespread distribution of multidrug resistance in community associated staphylococci	Poster presentation
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Publications

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Abstract

Staphylococci are opportunistic pathogens responsible for a range of infections. Many staphylococcal species are frequently found to be resistant to antibiotics. The environment is considered a potential reservoir of genes conferring antibiotic resistance, which known as the 'resistomes'. Monitoring the dissemination of antibiotic resistant staphylococci is instrumental to mitigating this global health risk. **The overall aim of this study was to generate informative data regarding dissemination of antibiotic resistance in environmental and public settings. This included looking into the distribution, epidemiology characteristic and transfer of oxacillin resistant determinant *mecA*; gaining an insight into genomic features that contribute to multiple antibiotic resistance and pathogenicity of one *S. epidermidis* isolate; and understanding the stress responses in mediating oxacillin resistance in *S. aureus*.**

The use of MALDI-TOF MS allowed identification of staphylococci to species level. MALDI-TOF MS data were used for taxonomic analysis of staphylococci, and taxonomic data were then combined with isolation sites and antimicrobial susceptibility profiles to aid the understanding of dissemination of environmental resistant staphylococci.

The widespread dissemination of antibiotic resistant staphylococci in the environment was demonstrated. 12% of staphylococci harboured *mecA* gene. Community associated SCC*mec* types IV and V were more prevalent than nosocomial associated SCC*mec* types I, II, and III in the environment. 52% of SCC*mec* were non-typable. In addition, 14 new environmental *S. epidermidis* MLST types were reported. 9 antibiotic resistant determinants that were responsible for the resistant to 7 antimicrobial classes have been

identified in environmental *S. epidermidis* 118 (G6_2). Proteomic analysis revealed that stress responses, including SOS response, stringent response and heat shock response, mediate oxacillin resistance in *S. aureus.* These results demonstrate widespread multiple drug resistance in different staphylococcal species isolated from non-healthcare environments. This uncontrolled dissemination of multidrug resistant bacteria poses a potential public health threats.

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List of abbreviations

3D	Three-dimensional
1D	One-dimensional
AAC	Acetyltransferase
ACME	Arginine catabolic mobile element
AN	Acetonitrile
ANT	Adenylyltransferase
APH	Phosphotransferase
ATCC	American Type Culture Collection
BCF	Baby care facilities
bp	Base pairs
BSAC	British society for antimicrobial chemotherapy
CA-MRSA	Community associated methicillin-resistant *Staphylococcus aureus*
CDC	Centers for Disease Control
CID	Collision-induced dissociation
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase-negative staphylococci
CSPD	Disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2y-(5odchloro) tricyclo [3.3.1.1^{3,7}]decan}-4-yl) phenyl phosphate
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
DSH	Different sites of hotels
DSL	Different sites of a library
DSR	Different sites of restaurants
DSS	Different sites of supermarkets
DST	Different sites of transportation facilities
DTT	Dithiothreitol
dUTP	Deoxyuridine Triphosphate
EDTA	Ethylenediaminetetraacetic acid
EFG	Elongation factor G
EUCAST	European Committee on Antimicrobial Susceptibility Testing

HAS	Hotel air samples
HB	Handbags
HCCA	α-Cyano-4-hydroxycinnamic acid
HH	Human hands
HPA	Health Protection Agency
HSPs	Heat shock proteins
kbp	Kilobase pairs
LC-MS/MS	Liquid chromatography technique coupled with tandem mass spectrometry
MALDI-TOF MS	Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry
Mbp	Megabase pairs
MES	2-(N-morpholino) ethanesulfonic acid
MIC	Minimum inhibitory concentration
min	Minutes
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol salt agar
m/z	Mass-to-charge ratio
NA	Nutrient agar
NCBI	National Center for Biotechnology Information
nl	Normal liters
ORSA	Oxacillin resistant *Staphylococcus aureus*
OS	Basic organic solvent
OS-MRSA	Oxacillin susceptible methicillin-resistant *Staphylococcus aureus*
PBP	Penicillin binding protein
PBPs	Penicillin-binding proteins
PBP2a	Penicillin binding protein 2a
PCR	Polymerase chain reaction
PAGE	Polyacrylamide gel electrophoresis
PFGE	Pulsed-field gel electrophoresis
QAC	Quaternary ammonium compounds
rRNA	Ribosomal RNA
s	Seconds
SCC*mec*	Staphylococcal cassette chromosome *mec*
SCV	Small colony variant
SD	Standard deviation
SDS	Sodium dodecyl sulfate
ST	Sequence type
TAE buffer	Tris base, acetic acid and EDTA buffer
TFA	Tri-fluor-acetic-acid
TGase	Transglycosylase
TPase	Transpeptidase
UTI	Brilliance™ UTI Agar
WGS	Whole genome sequencing

Chapter 1 Introduction

1.1 Microbiology of staphylococci

1.1.1 Morphology

Staphylococcus spp. are Gram positive cocci, which appear as non-motile, round clusters arranged in grape-like formations with diameters ranging from 0.5-1.5 μm (Mahon et al., 2014). The *Staphylococcus* genus is a member of the family *Staphylococcaceae,* which belongs to the order of *Bacillales* of the class *Bacilli*, which is a part of phylum *Firmicutes* (Vos et al., 2011). To date, forty-seven species and 23 sub-species of *Staphylococcus* spp. have been identified (Becker et al., 2014).

1.1.2 Biochemical properties

Based on their ability to produce coagulase, staphylococci species are generally divided into two groups:

1). Coagulase positive, which is almost exclusively represented by *S. aureus* that can be distinguished from other species by its ability to produce coagulase, an enzyme that clots the blood plasma. Six species (*S. aureus, S. simiae*, *S. intermedius*, *S. delphini*, *S. lutrae* and *S. pseudintermedius*) are currently defined as coagulase positive staphylococci. *S. aureus* is the only human–associated coagulase positive staphylococci (Becker et al., 2014).

2). Coagulase negative, which is represented by a large number of staphylococcal species that do not produce the enzyme coagulase. Forty-one species are regarded as coagulase negative staphylococci, and 3 species of this group are in fact coagulase variable (Taponen et al., 2012, Becker et al., 2014) (Table 1.1).

1.1.3 Taxonomy of *Staphylococcus* spp.

Recently, Lamers et al., (2012) proposed a refined classification for the *Staphylococcus* genus based on molecular data such as the noncoding 16S rRNA gene and three protein-encoding genes (*dnaJ*, *rpoB*, and *tuf*). In this four loci based classification species were classified into 15 cluster groups, which in turn were categorized into six species groups (Auricularis, Hyicus-Intermedius, Epidermidis-Aureus, Saprophyticus, Simulans, and Sciuri species groups) based on their phenotypic properties, such as oxidase, novobiocin susceptibility and coagulase properties by Becker et al., (2014) (Table 1.1).

Table 1. 1 Phenotypic and phylogenetic classification of staphylococci species (Becker et al., 2014)

Oxidase	Novobiocin	Coagulase	Species group	Cluster group	Species	Sub-species
Negative	Susceptible	Negative	Hyicus-intermedius	Muscae	*S. muscae* *S. microti* *S. rostri*	
		Positive[1] Variable[2] Negative[3]		Hyicus	*S. hyicus*[2] *S. agnetis*[2] *S. chromogenes*[3] *S. felis*[3]	
				Intermedius	*S. intermedius*[1] *S. delphini*[1] *S. lutrae*[1] *S. pseudintermedius*[1]	
					S. schleiferi	*ssp. schleiferi*[3] *ssp. coagulans*[1]
			Epidermidis-Aureus	Aureus	*S. aureus*	*ssp. aureus*[1] *ssp. anaerobius*[1]
					S. simiae[1]	
		Negative		Epidermidis	*S. epidermidis*	
					S. capitis	*ssp. capitis* *ssp. saccharolyticus*
				Warneri	*S. warneri* *S. pasteuri*	
				Haemolyticus	*S. haemolyticus* *S. devriesei* *S. hominis*	 *ssp. hominis*

Oxidase	Novobiocin	Coagulase	Species group	Cluster group	Species	Sub-species
Negative	Susceptible	Negative	Epidermidis-Aureus	Haemolyticus	*S. jettensis*	*ssp. novobioseticus*
					S. petrasii	*ssp. croceilyticus* *ssp. petrasii*
				Lugdunensis	*S. lugdunensis*	
			Auricularis	Auricularis	*S. auricularis*	
			Simulans	Simulans-Carnosus	*S. simulans*	
					S. carnosus	*ssp. carnosus* *ssp. utilis*
					S. condimenti	
					S. piscifermentans	
	Resistant		Saprophyticus	Pettenkoferi-Massiliensis	*S. pettenkoferi*	
					S. massiliensis	
				Saprophyticus	*S. saprothyticus*	*ssp . saprophyticus* *ssp. bovis*
					S. equorum	*ssp. equorum* *ssp. linens*
					S. gallinarum	
					S. succinus	*ssp. succinus* *ssp. casei*
					S. xylosus	
				Cohnii-Nepalensis	*S. cohnii*	*ssp. cohnii* *ssp. urealyticus*
					S. nepalensis	
				Arlettae-Kloosii	*S. arlettae*	
					S. kloosii	
Positive			Sciuri	Sciuri	*S. sciuri*	*ssp. sciuri* *ssp. carnaticus*

Oxidase	Novobiocin	Coagulase	Species group	Cluster group	Species	Sub-species
						ssp. rodentium
Positive	Resistant	Negative	Sciuri	Sciuri	*S. fleirettii* *S. lentus* *S. stepanovicii* *S. vitulinus*	

1.1.4 Lab identification

Staphylococci are Gram-positive cocci, and they occur singly, in pairs or tetrads (Baron, 1996). *S. aureus* is round, with a diameter of 0.8-1.0 μm and forms golden yellow colonies (Kearns, 2006, Mahon et al., 2014). Most coagulase negative staphylococci (CoNS) are 1.2-1.4 μm in diameter and mostly appear to be non-pigmented and smooth, forming unbroken, shiny, opaque colonies (Becker et al., 2014). Identification of Staphylococcal species is very important in recognition of an outbreak and in tracking resistance trends (Samb-Ba et al., 2014). Phenotypic, genotypic and proteomic approaches may all be applied to aid identification of the bacteria to species (Cherkaoui et al., 2010).

In the clinic, *S. aureus* is an often encountered pathogen in skin and soft-tissue infections (Stryjewski & Chambers, 2008) and adhering to medical devices and polymeric surfaces (Zmantar et al., 2010). Therefore, there is a need for rapid assays for the detection of *S. aureus* to aid disease diagnosis and clinical hygiene. In parallel with morphological and biochemical identification, molecular identification can be used as a relatively rapid, reliable and cost-effective assay. However, identification based on DNA and RNA detection and quantification is still time consuming and requires numerous consecutive steps (Cherkaoui et al., 2010). Alternatively, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) can be used as a sensitive molecular identification tool, which relies on bacterial proteome analysis to determine the species of isolates (Mellmann et al., 2008).

1.1.4.1 Conventional identification

For conventional identification, bacterial species can be determined by phenotypic profiles, including Gram-stain results, colony morphologies, growth requirement and metabolic activities (Samb-Ba et al., 2014).

Most of the staphylococci are non-fastidious organisms, and non-selective medium, such as nutrient agar, trypticase soy agar, will support their growth (Association et al., 1912). The culture of most staphylococcal species takes 18 to 24 hours at 35℃ to 37℃ to grow (Mahon et al., 2014). In contrast, the growth of small colony variant (SCV) takes between 48 and 72 hours at 35℃ to 37℃ (Becker et al., 2014). Growth of staphylococci appears as smooth, glistening, entire, opaque and yellow round colonies, with a diameter of colonies is 3-6 mm (Mahon et al., 2014). For SCV, the size of the colony is 10% of wild-type colonies, and normal growth can be restored under favourable conditions (Becker et al., 2014).

The morphology of staphylococci can be observed by direct microscopic examination, and preliminary identification may be achieved by culturing on selective medium. Mannitol salt agar (MSA) is a selective medium for staphylococcal species. It contains a high concentration of salt (7.5-10% NaCl, w/v), which supports the growth of Gram-positive bacteria and inhibit the growth of Gram-negative bacteria. The inclusion of mannitol and phenol red (pH indicator) in MSA is used to differentiate *S. aureus* from CoNS. Acidification caused by fermentation of mannitol by *S. aureus* produces yellow colonies with yellow zones. In comparison, other staphylococcal species cannot ferment mannitol giving small pink colonies with no colour change to the medium (Mahon et al., 2014). In addition to MSA, Brilliance™ UTI Agar (UTI) is a differential medium for the preliminary differentiation of all the main micro-organisms that cause urinary tract infections. UTI contains two chromogens, X-Gluc and Red-Gal. X-Gluc is used for

identification of enterococci with the presence of β-glucosidase, forming blue colonies. Red-Gal is cleaved by the enzyme β-galactosidase produced by *E. coli* to produce pink colonies, and both chromogens can be cleaved by coliform bacteria (*Enterobacter*, *Klebsiella*) to produce dark blue or purple colonies. In addition, the UTI also contains tryptophan, which detects deaminase activity of *Proteus, Morganella,* and *Providencia* spp., giving brown colonies. In contrast, *Staphylococcus* appears with normal pigmentation on UTI, and water-soluble pigments (pyocyanin and pyoverdin) produced by *Pseudomonas aeruginosa* give blue-green colour on solid agar (Mahon et al., 2014, Carricajo et al., 1999).

In addition to morphology and selective agar identification, the coagulase assay is a rapid method for identifying *S. aureus.* Cell-bound coagulase causes visible agglutination of antigen coated latex beads (Mahon et al., 2014). The API® STAPH test is a simplified commercial phenotypic identification approach combining a series of biochemical tests. After comparing the reads with reference chart, the unknown staphylococcal species can be identified (Mahon et al., 2014). Phenotypic tests are a significant component of a detailed identification profile; however limitations should be recognized. The phenotypic characterizations can be altered based on the culture conditions, environmental stresses or gene transfer. Non-accurate identification or failure can be caused by common species displaying atypical phenotypes, rare species presenting a non-characterized phenotype, and lags in updating the phenotypic database (Petti et al., 2005).

1.1.4.2 16S rRNA gene sequencing

The 16S rRNA gene is a component of the 30S small subunit of prokaryotic ribosomes (Woese & Fox, 1977). The 16S rRNA gene sequencing has been used to study bacterial taxonomy since 1970s (Janda & Abbott, 2007), and is a prevalent method in bacterial

identification (Clarridge, 2004). The 16S rRNA gene sequencing is used for identification as it is: evolutionarily stable; exists in all bacteria; and is informative (Janda & Abbott, 2007). The 16S rRNA gene sequencing procedure includes following steps: DNA extraction, mixture of PCR components (template DNA, primers, buffer, deoxynucleotides), PCR (denaturation, annealing and extension), and subsequent sequencing (Mahon et al., 2014). 16S rRNA gene sequencing provides reliable identification results, although the preceding steps are time consuming and costly (Cherkaoui et al., 2010).

1.1.4.3 MALDI-TOF MS

In 1996, a paper detailing the rapid identification of intact microorganisms using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was published in the Nature Biotechnology (Claydon et al., 1996). This revolutionary new technique allows automatic, reliable and fast identification without prior knowledge of the type of microorganism (Maier et al., 2006). The principle of MALDI-TOF MS identification is as followings: the whole cell protein biomarkers are analysed by using mass spectrometry (Maier et al., 2006, Ryzhov & Fenselau, 2001), and the procedure provides a unique mass spectral fingerprint of microorganism. Then the detected mass spectrum pattern is compared with reference patterns in the database for identification (Maier et al., 2006). 96 samples can be analysed within one run (Risch et al., 2010).

Sample preparation of MALDI-TOF MS is simple and spectra can be obtained within minutes (Maier et al., 2006), and different growth medium compositions have little effect in the peak pattern distribution (Maier et al., 2006). Low cost, rapid turnaround time and accuracy make MALDI-TOF MS more appealing than conventional and 16S rRNA gene sequencing identification (Cherkaoui et al., 2010).

1.1.4.3.1 Procedure of MALDI-TOF MS

MALDI-TOF MS identification is a six-step process. ① The cellular protein is extracted with formic acid and acetonitrile; ② The protein is mixed with a matrix (α-cyano-4-hydroxycinnamic acid; HCCA); ③ The matrix together with a sample are irradiated by UV light, and subsequently vaporized and ionized; ④ The velocity of ionized particles differ according to their mass-to-charge ratio (m/z) values, and lighter ions moves faster through the drift space until they reach the detector; ⑤ The detector detects the mass of the particles present in the peak; ⑥ Pattern matching of the unknown microorganism is accomplished through comparison of the peaks with the database. The database is generated with specific peak information by measurement of reference bacterial species (Maier et al., 2006, Karas & Krüger, 2003). Bruker biotyper 3.1 software (Bruker Daltonic, Coventry, UK) is used for analysing mass spectral (Lee et al., 2013). The score value generated by biotyper 3.1 (Bruker Daltonic, Coventry, UK) is determined by three components: ① the matches of the unknown spectrum with the reference spectrum, ② the matches of the reference spectrum against the unknown spectrum, ③ the intensities of the matched peaks. Score 0 (no match) to 1.000 (perfect match) is firstly generated, and then convert into a log score (0 to 3). The reliability of the identification is based on log score also known as score value: high confidence (log score $\geq$ 1.7), and incorrect (log score < 1.7) (Cherkaoui et al., 2010).

1.1.4.3.2 Reproducibility of MALDI-TOF MS

Reproducibility refers to the similarity of the replicate spectra of the same strain, and it is used to assess reliability and efficacy of the sample preparation process (Majcherczyk et al., 2006). Minor differences in MALDI-TOF MS profiles may result in misidentification of closely related strains; hence, it is of great important to assess

reproducibility of MALDI-TOF MS in identifying bacteria to strain level (Majcherczyk et al., 2006).

Automated data acquisition of MALDI-TOF MS present higher reproducibility than manual data acquisition as the automated method is more objective than a human operator (Schumaker et al., 2012). Even though few studies applied on the reproducibility of MALDI-TOF MS, it is necessary to measure and quantify the reproducibility to enable reproducibility located on a proper threshold to make sure the reliability of identification at species level (Schumaker et al., 2012).

1.1.4.4 Taxonomic classification

16S rRNA gene sequencing can be used for taxonomic classification and phylogenetic tree analysis (Takahashi et al., 1999, Grundmann et al., 2002, Zhang et al., 2006, Jørgensen et al., 2005). MALDI-TOF MS, as a new powerful tool for rapid and accurate identification of wide range microorganisms, has also been demonstrated to be a promising tool to taxonomically classify microbial species (Maier et al., 2006).

1.1.4.4.1 Phylogenetic relationship based on 16S rRNA gene

Comparison of 16S rRNA gene sequence variation is a classical method in determining phylogenetic relationship of bacteria, and it has been widely accepted and used in research and reference labs (Weisburg et al., 1991). Phylogenetic trees are important because they can be used to assess evolutionary distance and relationships between bacteria (Ludwig & Schleifer, 1994). Maximum likelihood methods and Pearson correlation methods are used to build phylogenetic tree (Ludwig & Schleifer, 1994).

1.1.4.4.2 Taxonomic relationship based upon MALDI-TOF MS profiles

A family tree, which is similar to 16S rRNA gene sequencing based phylogenetic tree, can be built based on MALDI-TOF MS profile. The family tree can be used to elucidate

the taxonomic relationships between staphylococcal species (Maier et al., 2006). Since the ribosomal protein has been proven to be stable and abundant in bacteria, the pattern of ribosomal protein observed by MALDI-TOF MS can reflect ribosomal DNA sequencing (Maier et al., 2006, Hotta et al., 2010).

1.1.4.4.3 Bionumeric

BioNumerics 7.5 (Applied Maths, Belgium) was released in 1996, is a platform for the management, storage and statistical analysis of all types of biological data, including sequences, Pulsed-field gel electrophoresis (PFGE) patterns, and spectra. In addition, BioNumerics 7.5 (Applied Maths, Belgium) can build dendrograms for any selected experiments， such as sequences and MALDI-TOF MS profile. With a majority of similarity and distance coefficients and clustering methods provided by BioNumerics 7.5 (Applied Maths, Belgium), the most appropriate clustering for all data types and clustering purposes can be achieved. BioNumerics 7.5 (Applied Maths, Belgium) can handle up to 20000 entries, and provide powerful and efficient interpreting tools, including two-way zoom-sliders, swapping and abridging of branches, rerooting of trees, displaying data in various modes, and assigning colours or symbols to groups. In addition, it can add entries or delete entries from large clustering without affecting other entries (http://www.applied-maths.com/).

1.1.5 Molecular characterization

Diverse genotypes of *S. aureus* have been characterized, however, coagulase negative staphylococci have not been studied to the same extent (Becker et al., 2014). Different molecular and genomic methods, including staphylococcal cassette chromosome *mec* (SCC*mec*), multi-locus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS) can be used for accurate identification of

clonal diversity of staphylococcal species (Leonard & Markey, 2008). However, single locus DNA-sequencing of the repeat region of the staphylococcal protein A gene (*spa*) analysis can be limited only to *S. aureus* (Leonard & Markey, 2008).

1.1.5.1 SCCmec

Staphylococcal cassette chromosome *mec* (SCC*mec*) is an important feature to define the clonal diversity in methicillin resistant staphylococci (Becker et al., 2014). The *mec* complex and the *ccr* complex are two essential components of SCC*mec* (Ito et al., 2003). The *mec* complex contains the *mecA* gene that encodes methicillin resistant penicillin binding protein 2a, and regulatory gene *mecI*, *mecR1* and *IS431* (Ito et al., 2003). The second essential region is the *ccr* complex, composed of two sites specific recombinase genes *ccrA* and *ccrB,* which are responsible for the mobility of SCC*mec* (Ito et al., 2003). SCC*mec* types are determined by varied combination of the *ccr* and the *mec* complex (Ito et al., 2003).

Until now, the *mec* complex has been assigned into 6 classes according to their structures (Table 1.2) (IWG-SCC, 2009), and 8 *ccr* types have been assigned (IWG-SCC, 2009) (Table 1.3). The rest component of SCC*mec* is a junkyard region, of which genes may involve in the non-β-lactam antibiotic resistance and heavy metal resistance (Ito et al., 2003).

Table 1. 2 Structure of *mec* complex (IWG-SCC, 2009)

mec complex	Structure
Class A	IS*431*–*mecA*–*mecR1*–*mecI*
Class B	IS*431*–*mecA*–*mecR1*–IS*1272*
Class C1	IS*431*–*mecA*–*mecR1*–IS*431* (two IS*431*s were in the same direction)
Class C2	IS*431*–*mecA*–*mecR1*–IS*431*(two IS*431*s were in the opposite direction)
Class D	IS*431*–*mecA*–*mecR1*
Class E	$mecA_{LGA251}$-$mecR1_{LGA251}$-$mecI_{LGA251}$

Table 1. 3 Structure of *ccr* complex (IWG-SCC, 2009)

ccr complex	*ccr* gene
Type 1	A1B1
Type 2	A2B2
Type 3	A3B3
Type4	A4B4
Type 5	C1
Type 6	A5B3
Type 7	A1B6
Type 8	A1B3

SCC*mec* has been classified into 11 allotypes based on the combination of *mec* complex and *ccr* complex, and can be further classified into subtypes according to the differences in junkyard region (IWG-SCC, 2009) (Table 1.4). In addition, the diversity of SCC*mec* types are also contributed by lack of *mec* complex, *ccr* complex or both *ccr* and *mec* complex (Katayama et al., 2003, Harrison et al., 2013, Shore & Coleman, 2013).

Table 1. 4 Currently identified SCC*mec* types in *S.aureus* strains

SCC*mec* types	*ccr* complex	*mec* complex
I	1(A1B1)	B
II	2(A2B2)	A
III	3(A3B3)	A
IV	2(A2B2)	B
V	5(C1)	C2
VI	4(A4B4)	B
VII	5(C1)	C1
VIII	4(A4B4)	A
IX	1(A1B1)	C2
X	7(A1B6)	C1
XI	8(A1B3)	E

Two SCC*mec* typing methods have been used: the first is introduced by Zhang et al., (2005), and the second one is reported by Kondo et al., (2007). According to Kondo et al., (2007), SCC*mec* types are determined by the combination of the type of *ccr* complex and class of *mec* complex. With this method, SCC*mec* type I, II, III, IV, V, VI, VIII and IX can be determined. For Zhang's method, SCC*mec* type I, II, III, V and IVa, IVb, IVc and IVd can be identified directly from PCR products (Zhang et al., 2005).

It is reported that SCC*mec* type I, II and III are healthcare associated types, and SCC*mec* type II and III are responsible for multiple non-β-lactam antibiotic resistance of *S. aureus* (Rybak & LaPlante, 2005). Meanwhile, SCC*mec* type IV and V is more associated with community isolated *S. aureus* (Rybak & LaPlante, 2005, Monecke et al., 2014).

1.1.5.2 MLST

Multi-locus sequence typing (MLST) is a highly discriminatory method for genotypic typing of staphylococcal species, and is excellent for exploring long-term epidemiologically unrelated isolates (Enright et al., 2000). Currently, MLST can be used for *S. aureus* and *S. epidermidis* typing. Seven housekeeping genes, chosen for MLST, are assigned as distinct alleles, and each allele is a partially conserved ribosomal gene. Seven allele sequences are transferred into allele numbers via the MLST database (Enright et al., 2000, Widerström et al., 2012) (http://www.mlst.net/). Afterwards, a sequence type (ST) can be assigned by combination of seven alleles. Identical ST is regarded as belonging to same lineage, and non-matching ST is considered to be of unknown type. For unknown ST type, a new ST type will be assigned (Strommenger et al., 2008).

1.1.5.3 PFGE

Pulse-field gel electrophoresis (PFGE) is a powerful epidemiological typing method, and is well known to differentiate genetic variation of related isolates (Strommenger et al., 2008). PFGE has been applied in clinical microbiology to determine the relatedness of bacteria from epidemic incidence (King, 2006). In United States, PFGE has been proven to be a discriminating way to monitoring the spread of ORSA (McDougal et al., 2003). PFGE can provide the fingerprint of the genome, and the fingerprint reflects the

differences in composition of genetic background (Sanches et al., 1995). Therefore PFGE can differentiate phenotypically closely related bacteria (On & Harrington, 2001).

PFGE is an umbrella term for the alternating of an electric field in more than one direction through a solid matrix to achieve the separation of fragments (Woodford & Johnson, 1998). PFGE involves embedding organisms in low gelling temperature agarose, lysing the cell in situ, digesting the DNA molecule with a restriction enzyme; transferring the prepared low gelling temperature agarose gel into the wells of agarose gel (Woodford & Johnson, 1998). Restriction enzyme digested fragments are then separated by CHEF Mapper® which changes the direction of current into predetermined pattern (Tenover et al., 1995). The restriction enzyme for digestion of *S. aureus* and coagulase negative staphylococci is *SmaI*, and the numbers of restriction fragments are approximately 15-20 pieces. However, the size of fragments is quite different, for *S. aureus* is 10-700 kb, and for coagulase negative staphylococci is 5-400 kb (Tenover et al., 1995). Because of varied fragments, *S. aureus* and coagulase negative staphylococci can be differentiated by PFGE patterns. PFGE has been proved to be an accurate tool in staphylococcal epidemiological studies; however, for comparison of epidemiologically unrelated staphylococci, MLST is more useful (Jørgensen et al., 2005). Therefore, it is recommended to combine MLST and PFGE when characterizing bacteria population (Jørgensen et al., 2005).

1.1.5.4 WGS

More precise epidemiology typing can be achieved by using the whole genome sequencing. This analysis for monitoring the outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) in clinical settings has been widely used (Harris et al., 2013). Comparative analysis of whole genome sequences can provide an insight into

evolution of virulence and antibiotic resistance, transmission and pathogenic diversity (Gill et al., 2005, Harris et al., 2013, Zhang et al., 2003).

1.2 Epidemiology of staphylococci

1.2.1 Ecological niches of staphylococci

Diverse microbial populations constitute the human microbiome. Among these, staphylococci are known to be associated with the skin and mucous membranes (Baron, 1996).

1.2.1.1 S. aureus

S. aureus is predominantly found in the nasal passage and axillae (Kloos & Bannerman, 1994). 20% of humans are persistent carriers of *S. aureus,* whereas 60% are intermediate carriers, and the remaining are non-carriers (Kluytmans et al., 1997). *S. aureus* is capable of long term survival outside of the host body, which is an important contributing factor to its dissemination in the environment (Spendlove & Fannin, 1983). Depending on the texture of the non-host surfaces and colony size, *S. aureus* can survive from days to months (Neely & Maley, 2000).

1.2.1.2 S. epidermidis

S. epidermidis is a normal resident of human skin making almost 90% of the total human skin microflora (Baron, 1996, Kloos & Bannerman, 1994, Pfaller & Herwaldt, 1988). In addition to human skin, *S. epidermidis* is known to be colonized on medical devices, and form biofilms. Moreover, *S. epidermidis* has been found in food and animals such as cats, cattle, dogs, goats, gorillas, horses, pigs, and sheep (Becker et al., 2014) (Table 1.5).

1.2.1.3 Other staphylococci

The main habitat of *S. auricularis* is external ear of human (Kloos & Schleifer, 1983) (Table 1.5). *S. capitis* is found on the forehead and scalp region of humans, and also found in animals such as cats, dogs, horses (Kloos & Schleifer, 1975) (Table 1.5). *S. caprae* is found on skin, anterior nares of human and animals such as goats (Vandenesch et al., 1995) (Table 1.5).

S. hominis and *S. haemolyticus* are widely distributed in the human body, including head, axillae, arms, legs, pubic, and inguinal regions. In addition, they can also be found in milk, fermented food and domestic animals such as cats, dogs, goats, and pigs (Palazzo et al., 2008) (Table 1.5).

S. pettenkoferi and *S. lugdunensis* mainly colonize the human skin. *S. lugdunensis* can also be found in animals such as cats, and dogs, whereas, the distribution of *S. pettenkoferi* in the environment has not been clarified (Becker et al., 2014; Trülzsch et al., 2007) (Table 1.5).

S. saprophyticus colonize rectum and genitourinary tract, and it is the second only to *E. coli* in its association with urinary tract infections (UTI) (Becker et al., 2014, Pfaller & Herwaldt, 1988). In addition, *S. saprophyticus* can be isolated from ferment food and animals such as cattle, cats, and sheep (Becker et al., 2014) (Table 1.5).

S. simulans is found on the human skin such as legs, arms heads and in the urethra of healthy woman (Otto, 2009). Moreover, it can also be found in animals such as cattle, horses and sheep (Becker et al., 2014) (Table 1.5).

S. warneri, *S. sciuri*, and *S. cohnii* are found on human skin and animals (Pfaller & Herwaldt, 1988, Grice & Segre, 2011); meanwhile, *S. warneri* is also known to colonize on fermented foods (Pfaller & Herwaldt, 1988) (Table 1.5).

S. equorum, *S. pasteuri* and *S. xylosus* are associated with animal and fermented food, such as milk, cheese, and sausage (Becker et al., 2014). *S. equorum* is frequently isolated from cattle, goats, horses, and sheep, while *S. pasteuri* is more associated with pigs, and *S. xylosus* is isolated from cats, clams, goats, horses, insectivores, lower primates, rodents, and sheep (Becker et al., 2014) (Table 1.5).

S. simiae has been isolated from South American squirrel monkeys over a decade ago (Pantucek, 2005), and *S. arlettae* is isolated from animals, such as cattle, goats, pigs, poultry, sheep, textile and industrial effluent (Wang et al., 2012) (Table 1.5).

Table 1. 5 Colonization site of each staphylococcal species

Species	Colonization sites
S. arlettae	Textile, tannery industrial effluents; Cattle, goats, pigs, poultry, sheep
S. aureus	Human nasal passage, axillae, anterior nares
S. auricularis	Human external ear
S. capitis	Human forehead, scalp; Cats, dogs, horses
S. caprae	Human skin, anterior nares; Goat
S. cohnii	Human skin; Dogs, goats, poultry
S. epidermidis	Human skin, mucous membranes of the nasopharynx; Fermented sausages; Cats, cattle, dogs, goats, gorillas, horses, pigs, sheep
S. equorum	Fermented food; Cattle, goats, horses, sheep
S. haemolyticus	Human skin; Milk, fermented food; Cats, cattle, dogs, horses, goats, pigs, sheep
S. hominis	Human skin; Goat milk, fermented food; Cats, dogs, goats, pigs, sheep
S. lugdunensis	Human skin; Cats, chinchillas, dogs, goats, guinea pigs
S. pasteuri	Fermented sausage; Pigs
S. pettenkoferi	Human skin
S. saprophyticus	Human skin; Fermented food; Horses, goats, sheep, cats,
S. sciuri	Human skin; Cattle, dolphins
S. simiae	Squirrel monkeys
S. simulans	Human skin; Cattle, horses, sheep
S. warneri	Human skin; Fermented food; Dogs, cats, goats, horses, insectivores, monkeys, pigs, prosimians, rodents, sheep
S. xylosus	Human skin; Fermented food; Cats, clams, goats, horses, insectivores, lower primates, rodents, sheep

1.2.2 Hospital associated staphylococci

S. aureus was defined as a pathogen in 1880, and its virulence was demonstrated in 1941 (Archer, 1998). The first clinical methicillin-resistant *S. aureus* (MRSA) infection was reported in United Kingdom in 1961. By 1968, MRSA infection cases have been described all over the world (Huang et al., 2006). Now, *S. aureus* is a virulent pathogen which is the most common causes of nosocomial infection (Archer, 1998).

S. aureus is an opportunistic pathogen, which can cause a range of pathologies from minor infection such as skin infections to life threatening diseases such as toxic shock syndrome (Parsonnet et al., 2005, Stevens et al., 2010). The toxic syndrome is caused by superantigenic toxic shock syndrome toxin-1 (TSST-1) and enterotoxin (Foster, 2005b).

In addition to this, *S. aureus* can cause bacteraemia, endocarditis, metastatic infections, and sepsis. It has been shown that bacteraemia and endocarditis are associated with use of catheters (Lowy, 1998), while in contrast; metastatic infection and sepsis develop more in minor infections (Lowy, 1998). *S. aureus* also produces enterotoxins (SEs), which can cause food poisoning (Le Loir et al., 2003) (Table 1.6).

Little is known about *S. simiae,* however, *S. simiae* and *S. aureus* may have evolved from a common ancestor, and they are then split by increased pathogenicity of *S. aureus* through horizontal gene transfer (Suzuki et al., 2012) No *S. simiae* associated infection has been reported (Table 1.6).

In microbiology labs, nosocomial CoNS have been recognized as culture contaminants for a long time, and their pathogenic role has only recently been recognized (Becker et al., 2014).

S. epidermidis is the most common causes of neonate infection, which often includes bacteraemia, foreign body-related sepsis (catheter infection, prothetic vascular grafts infection, cardiac devices infection), shunt-associated infection (cerebrospinal fluid infection), endocarditis, urinary infections, endophthalmitis and surgical site infection (Becker et al., 2014; Huebner & Goldmann, 1999). Most of infections occur in populations with either exposure to multiple risk factors (drug abuser, hospitalization) or with hypo immunity (Lowy, 1998; Vuong & Otto, 2002) (Table 1.6).

Other CoNS that can cause clinical infections are the followings: *S. auricularis, S. capitis, S. caprae, S. cohnii, S. equorum, S. haemolyticus, S. hominis, S. lugdunensis, S. pasteuri, S. pettenkoferi, S. saprophyticus, S. sciuri, S. simulans, S. warneri,* and *S. xylosus* (Becker et al., 2014). The infections caused by each CoNS species are shown below (Table 1.6).

S. hominis and *S. haemolyticus* are less frequently the causes of clinical infections than *S. aureus* and *S. epidermdis*, but relatively higher than other CoNS species (Becker et al., 2014). *S. hominis* is mainly associated with sepsis (Palazzo et al., 2008; Sorlozano et al., 2009), while *S. haemolyticus* is known to be a common cause of sepsis in hospital (Pereira et al., 2014) (Table 1.6).

Infections caused by *S. capitis* have been reported, but are rarer than those caused by *S. hominis* and *S. haemolyticus* (Becker et al., 2014). *S. capitis* is the main cause of infections in neonate intensive care unit (Gras-Le Guen et al., 2007) (Table 1.6).

S. saprophyticus is the common causes of urinary tract infection (Kuroda et al., 2005; Widerström et al., 2012). Additionally, it can also cause bacteraemia, endocarditis, sepsis and neonate infections (Table 1.6).

S. lugdunensis is associated with variety of human infections, such as endocarditis (Vandenesch et al., 1993), osteomyelitis (Murdoch et al., 1996), soft skin, corneal infection (Böcher et al., 2009), bacteraemia, and sepsis (Tee et al., 2003) (Table 1.6).

S. sciuri and *S. auricularis* are considered to be nosocomial staphylococcal species. *S. sciuri* is the common cause of endocarditis, wound and tissue infection (Stepanović et al., 2001). Whereas, *S. auricularis* has been reported to be implicated in skin, soft tissue and neonate infections (Kloos & Schleifer, 1983) (Table 1.6).

S. pettenkoferi has been firstly recovered from human clinical specimens as a pathogen in 2007 (Trülzsch et al., 2007), and more infections that are caused by *S. pettenkoferi* have been reported recently, such as sepsis, osteomyelitis, and bacteraemia (Hashi et al., 2015; Loiez et al., 2007; Mihaila et al., 2012; Song et al., 2009) (Table 1.6).

S. simulans, as an opportunistic pathogens, may cause bone, joint infection, sepsis and osteomyelitis (Kloos & Schleifer, 1975; Males et al., 1985; Widerström et al., 2012) and sometimes can be recovered from wounds, lesions and abscesses (Otto, 2009) (Table 1.6).

S. warneri is known to cause bacteremia, endocarditis and vertebral osteomyelitis, (Center et al., 2003), and *S. equorum* has been reported to be associated with sepsis and corneal infection (Pinna et al., 1999) Moreover, *S. caprae* may cause endocarditis, bone infection, urinary infection and sepsis (Ross et al., 2005; Vandenesch et al., 1995) (Table 1.6).

Few *S. cohnii*, *S. xylosus* and *S. pasteuri* associated infections are reported. *S. cohnii* has been first reported to cause bacteremia in a colon cancer patient in 2003 (Basaglia et al., 2003), and a more recent report of *S. cohnii* associated multiple brain abscesses is in 2005 (Yamashita et al., 2005). *S. xylosus* associated sepsis is reported in 2012 (Giordano et al., 2012) (Table 1.6). The pathogenic role of *S. pasteuri* has not been clarified; however, it is reported that *S. pasteuri* is responsible for causing sepsis in a patient (Savini et al., 2009) (Table 1.6).

S. arlettae is mainly associated with animals, and there is no clinical case reported for this strain (Table 1.6).

Table 1. 6 Infections caused by staphylococcal species

Species	Infections													
	Sepsis	Endocarditis	Bacteremia	Neonate	Soft tissue	Urinary	Bone	Skin	Osteomyelitis	Endovasculitis	Joint	Corneal	Respiratory	Endophthalmitis
S. aureus	√	√	√		√		√	√		√	√		√	
S. epidermidis	√	√	√	√		√								√
S. lugdunensis	√	√	√		√				√			√		
S. saprothyticus	√	√	√	√		√								
S. caprae	√	√				√	√							
S. simulans	√						√		√		√			
S. auricularis				√	√			√						
S. pettenkoferi	√		√						√					
S. sciuri		√			√			√						
S. warneri	√			√		√								
S. capitis		√		√										
S. cohnii	√		√											
S. equorum	√											√		
S. haemolyticus	√													
S. hominis	√													
S. pasteuri	√													
S. xylosus										√				
S. arlettae														
S. simiae														

1.2.3 Community associated staphylococci

The research of community associated staphylococci is focused on methicillin resistant *S. aureus*. Traditionally, MRSA has been considered to be a nosocomial acquired pathogen; however, it has emerged as community acquired pathogen. The first community-associated MRSA (CA-MRSA) infection in the United States was reported in 1980 (Becker et al., 2014). Prevalence of CA-MRSA infection in the United States began in the 1990s; followed by reports that patient with CA-MRSA associated infections lack risk factors such as hospitalization, nursing home, and immune-compromised conditions (Huang et al., 2006).

1.2.4 Environmental staphylococci

The environmental distribution of microorganisms is mainly in soil and water (natural water, drinking water, sewage, wastewater), and most of environmental microorganisms are non-pathogenic (Wright, 2010). Antibiotic resistant environmental microorganisms are widely distributed due to exposure of antibiotic-producing bacteria in soil, and therefore act as the biggest reservoir of antibiotic resistance genes (Cantas et al., 2013; Wright, 2010). In addition, human use of antibiotics has also reached the biosphere, and thus contributed to the antibiotic resistance of microorganisms (Cantas et al., 2013; Wright, 2010). Environmental antibiotic resistant staphylococci have been rarely reported, however, animal and food associated staphylococci have been studied, including species such as *S. arlettae*, *S. aureus*, *S. capitis*, *S. caprae*, *S. cohnii*, *S. epidermdis*, *S. equorum*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. pasteuri*, *S. saprophyticus*, *S. sciuri*, *S. simiae*, *S. simulans*, *S. warneri*, and *S. xylosus* (Becker et al., 2014) (Table 1.5).

1.2.5 Transmission of staphylococci

The transmission of antibiotic resistant pathogenic staphylococci is the main global cause of epidemic outbreaks in both health care facilities and the community (Widerström et al., 2012). In a clinical setting, person to person transmission of staphylococcal species occurs by exposure to the hands of staphylococci colonized health care workers and medical devices such as catheter, implanted ports (Becker et al., 2014; Lowy, 1998). In the community, the transmission of methicillin-resistant *S. aureus* (MRSA) occurs in crowded places, through personal items such as towels, cosmetics, lotion, bedding, nail clipper, toothpaste, headphones, and close human contact (Hudson et al., 2013; Johansson et al., 2007; Mollema et al., 2010). The transmission of MRSA is observed through household items, pets and public transport (Manian, 2003; Rankin et al., 2005; Scott et al., 2008; Simões et al., 2011; Turabelidze et al., 2006; Van Duijkeren et al., 2004).

Pets and livestock can be another reservoir of antibiotic resistance (Ho et al., 2011). Several studies demonstrated that MRSA in people is equal to or greater than its prevalence in pets (Gandolfi-Decristophoris et al., 2012). The transmission of MRSA from human to animals or from animals to human is not well studied; however, MRSA are more likely to be isolated from people who have direct contact with animals (Gandolfi-Decristophoris et al., 2012). Veterinary staff, pets owners, and farmers are high risk group for MRSA carriage although they do not have a direct contact with hospitals (Gandolfi-Decristophoris et al., 2012). The occupation risk for veterinary staff is relatively high because of close proximity to MRSA infected pets or livestock (Zhang et al., 2011). Without effective cleanliness and isolation practice, they may be under risk of MRSA colonization and transmission (Vincze et al., 2014). MRSA colonisation of animals and contaminated food products is a potential source of persistent infection of

those who handle animals and food, such as pet owners, famers, catering staff (Furuya & Lowy, 2006; Gandolfi-Decristophoris et al., 2012).

Public transportation associated transmission is quite different from household transmission. International travel has greatly accelerated the dissemination of antibiotic resistant isolates, including MRSA (Zhou et al., 2014). Individuals who travel from the developing world with poorer hygiene standards to economically developed countries are more likely to transfer antibiotic resistant isolates (Ostholm-Balkhed et al., 2013). Ostholm-Balkhed et al., (2013) reported that individuals in Scandinavian countries who have travelled to Asia at least once have 12.5-fold increase in propensity for colonization of antibiotic resistant isolates than individuals who have never visited Asia. Although well-designed studies are required to systematically evaluate the transmission risk in travellers, empirical studies and observations have confirmed that globalisation is playing an important role in the spread of antibiotic resistance (Zhou et al., 2014).

There are several factors that can affect the transmission in hospitals, public places and households. Community settings are characterized by transient contact of a diverse population, but households involve high-intensity contact between the same individuals, so the dynamic of transfer is different (Furuya & Lowy, 2006). Crowding in poor hygiene areas such as slums is one of the factors that increase the risk of transmission, and also duration of human colonization may increase the transmission (Hanssen et al., 2004). Hygiene measures and decolonization of a carrier can efficiently reduce the incidence of soft tissue infection caused by the antibiotic resistance bacteria (Furuya & Lowy, 2006).

1.3 Pathogenicity of staphylococci

The ability of an organism to cause diseases is defined as pathogenicity (COLOSS, 2016). The pathogenicity of staphylococci is reflected by human colonization and infection (Lowy, 1998).

The pathogenicity of *S. aureus* has been well characterized. The immune system protects humans from *S. aureus* infections in various ways, including physical barriers, innate response and acquired responses (Foster, 2005b). However, *S. aureus* can overwhelm immune defences by colonizing on catheters or medical devices, entering the host along with catheter, adhering to host, and obstructing the immune response (Foster, 2005b). Several proteins are involved in the abiotic surface attachment of *S. aureus*, including non-covalently linked surface-associated protein (*Atl*, *ClpP*) and covalently linked surface protein (*bap*). Proteins involved in the attachment to host also include non-covalently linked surface-associated protein (*efb*, *embp*) and covalently linked surface protein (*bap*, *sdrC*, *sdrD*, *sdrE*). After adherence to the host surface, *S. aureus* impede the immune response by secreting virulence factors (*pvl*, *hlg*), which inhibit or kill immune cells. Panton-Valentine leucocidin (*pvl*) and γ-haemolysin (*hlg*) are leukotoxin, which are toxic to leukocytes (Becker et al., 2014). In addition, *S. aureus* can be internalized by the host cells, and stay as small colony variant in these cells. Finally, *S. aureus* can cause infections by secretion of extracellular enzymes, such as proteinase, lipase, and nuclease. These extracellular enzymes will cause cytolytic effects of host cells, facilitate the destruction of host tissue and contribute to septic shock (Foster, 2005b).

In comparison with *S. aureus*, the pathogenicity of *S. epidermidis* is firstly contributed by their ability in colonize on medical devices such as catheters, implanted ports, and

form biofilms (Becker et al., 2014; Foster, 2005b). The genetic control of biofilm synthesis is intercellular adhesion gene (*ica*), which form biofilms by synthesising intercellular adhesion factor (Arciola et al., 2001) (Table 1.7). Biofilm formation is known to protect *S. epidermidis* against external adverse factors, and increase the pathogenicity (Cramton et al., 1999). Four steps are involved in the formation of biofilms, including attachment, formation of multi-layer cell aggregates, maturation and dissociation (Cramton et al., 1999). Like *S. aureus, S. epidermidis* can also penetrate the immune system of the host. The proteins that are involved in abiotic and biotic surface attachment of *S. epidermidis* includes: non-covalently linked surface-associated proteins (*AtlE*, *ClpP*) and covalently linked surface proteins (*bap, SdrF, SdrG, SdrH*). Moreover, covalently linked surface protein encoding gene *bap* in *S. epidermidis* is believed to be acquired from *S. aureus* via transfer of a pathogenic island (SaPIbov2) (Otto, 2013). Extracellular enzymes of *S. epidermidis,* such as *geh*, *lipA*, favour the bacteria to invade host tissue and defence system (Becker et al., 2014). Additionally, *S. epidermidis* produces a less toxic molecule - phenol soluble modulins that serve as immune evasion molecule (Foster, 2005b). The function of phenol soluble modulins involves in pathogenesis and initiate the host inflammatory response (Liles et al., 2001).

Many virulence factors facilitate the diseases and increasing pathogenicity of staphylococci, including extracellular toxins and surface proteins. The virulence factors in *S. aureus* have been well characterized; however, less is known about virulence factors in CoNS (Becker et al., 2014). Comparative genomic analysis showed numerous virulence factors of *S. epidermidis* are closely related to *S. aureus* (Gill et al., 2005).

1.3.1 Extracellular toxins

Many studies have been used to explore the extracellular toxins of staphylococci, and Table 1.7 shows a summary of extracellular toxins found in *S. aureus* (Gill et al., 2005). In addition to the *pvl* and *hlg* mentioned above, various virulence factors are identified in *S. aureus.* Enterotoxin and exotoxin are pyrogenic toxin superantigens, which are recognized by T-cell receptors, and cause non-specific activation of T cells. High level expression of cytokine by T-cells then leads to toxic shock syndrome (Dinges et al., 2000; Foster, 2005b). Serine proteinase (*htrA*), esterase (*lipA*), beta hemolysin (*hlb*), cell wall hydro-lase (*lytN*), and lytic transglycosylases (*isaA*) are involved in host tissue invasion (Frankel et al., 2011; O'Callaghan et al., 1997; Rigoulay et al., 2004; Stapleton et al., 2007; Su et al., 2004) (Table 1.7). Proteinase (*ssp*) and lipase (*lip*) are known to cause host tissue damage (Stehr et al., 2003; Zarfel et al., 2013) (Table 1.7), while Leukotoxin D (*lukD*) is an immunity cell damage factor (Malachowa et al., 2012) (Table 1.7). Thermonuclease (*nuc*) and staphylococcal protein A (*spa*) are responsible for immunity evasion (Berends et al., 2010) (Table 1.7). Clp protease (*Clp*) is recognized as a stress response protein, which can degrade misfolded protein and maintain the normal function of bacteria (Michel et al., 2006) (Table 1.7).

Table 1. 7 Extracellular toxins in *S. aureus* (Gill et al., 2005)

Extracellular toxins	Examples	Functions	Reference
Enterotoxin	*sea*	Pyrogenic toxin superantigen (Massive cytokine release triggered toxic shock syndrome)	Foster, 2005b
Exotoxin	*setC*	Pyrogenic toxin superantigen (Massive cytokine release triggered toxic shock syndrome)	Dinges et al., 2000
Serine protease	*htrA*	Host tissue invasion	Rigoulay et al., 2004
Protease	*ssp*	Host tissue damage (Cleave fibrinogen-binding protein)	Zarfel et al., 2013
Lipase	*lip*	Immunity cell damage (Damage surface structures of the immune cells)	Stehr et al., 2003
Leukotoxin D	*lukD*	Immunity cell damage (Killing of neutrophils)	Malachowa et al., 2012
Esterase	*lipA*	Host tissue invasion (Extracellular lipase, degrade phospholipids from lung surfactants)	Su et al., 2004
Beta hemolysin	*hlb*	Host tissue invasion (Lysing mammalian cells)	O'Callaghan et al., 1997
Thermonuclease	*nuc*	Immunity evasion (Encodes for a thermostable nuclease, promote neutrophil extracellular traps evasion)	Berends et al., 2010
Cell wall hydrolase	*lytN*	Host tissue invasion (Non-covalent surface assocation protein)	Frankel et al., 2011
Clp protease	*clp*	Stress adaptation (Degrade mis-fold protein) Biofilm formation	Michel et al., 2006
Lytic transglycosylases	*isaA*	Host tissue invasion (Required for normal growth and for successful host-pathogen interactions)	Stapleton et al., 2007
Staphylococcal protein A	*spa*	Immunity evasion (Exhibits broad binding specificity with other proteins, which favours evasion of the innate and adaptive immune systems)	Deis et al., 2014
Intercellular adhesion gene	*ica*	Biofilm synthesis (Synthesis polysaccharide intercellular adhesin)	Arciola et al., 2001

In addition to the virulence genes mentioned above, there are several genes that contribute to the increased pathogenicity of staphylococci. Quaternary ammonium compounds (QAC) are used in the food industry for low toxic detergent, however, the

QAC-resistant genes (*qac*) were emerged in staphylococci (Heir et al., 1998). *copZ_2*, *copA_2 and csoR_1* variants are associated with copper transport, whose function is known to manage cellular copper and adapt to copper stress (Harrison et al., 2000; Schelder et al., 2011). Zhu et al., (2013) reported that heavy metal may act as selection pressure of antibiotic resistance genes in bacteria. In addition, metal resistance is also associated with interspecies gene transfer (Méric et al., 2015).

The virulence gene, *geh*D, which mediate the binding of bacteria to human collagen, is known to be expressed exclusively by *S. epidermidis*. In addition, the homology *gehC* is involved in the colonization of *S. epidermidis* on skin (Vuong & Otto, 2002).

1.3.2 Surface proteins

The success of staphylococci as a pathogen is partially due to their various surface proteins, which facilitate host cell invasion and residency. As well as host cell invasion, surface proteins are involved in several other functions, including adhesion, evasion of immune system, and formation of biofilm (Becker et al., 2014). In addition, surface proteins are varied from strain to strain due to the acquisition and loss of certain virulence determinants, which further complicates medical treatment (Otto, 2010).

The surface protein genes found in *S. aureus* and *S. epdiermidis* are listed in the Table 1.8 and the function of most surface proteins are known to adherence to host tissues (Table 1.8).

Table 1. 8 Surface protein genes found in *S. aureus* and S. *epidermidis*

Gene	Functions	Sources	Reference
clf	a	*S. aureus*	Schaffer et al., 2006
fnb	a	*S. aureus*	Gill et al., 2005
sdrC	a	*S. aureus*	Gill et al., 2005
sdrD	a	*S. aureus*	McCrea et al., 2000
sdrE	a	*S. aureus*	Foster et al., 2014
sdrF	a	*S. epidermidis*	Foster et al., 2014
sdrG	a	*S. epidermidis*	Foster et al., 2014
sdrH	a	*S. epidermidis*	Foster et al., 2014
map	a	*S.aureus*	Gill et al., 2005
empbp	a	*S.aureus*	Gill et al., 2005
ebh	a	*S. aureus* *S. epidermidis*	Gill et al., 2005, Cheng et al., 2014
ebp	a	*S. aureus* *S. epidermidis*	Gill et al., 2005, Downer et al., 2002
atl	a	*S. aureus* *S. epidermidis*	Gill et al., 2005, Wang & Lin, 2008
sas	b	*S. aureus* *S. epidermidis*	Gill et al., 2005, Otto, 2013
efb	a	*S. aureus*	Gill et al., 2005
pls	c	*S. aureus*	Josefsson et al., 2005

Note: a: Adherence to host tissue (extracellular matrix, fibrinogen, fibronectin, collagen, elastin, endothelial and epithelial cells); b: Binding to heme-iron; c: Methicillin resistant surface protein

Autolysin (*atl*) is expressed in *S. aureus*, and for other CoNS, the homology of *atl* which mediates initial adhesion functions are found in *S. epidermidis* (*atlE*), *S. caprae* (*atlC*), *S. saprophyticus* (*aas*), *S. lugdunensis* (*atlL*), *S. warneri* (atl_{WM}) (Becker et al., 2014). In addition, the homologous of covalently linked surface protein (*bap*) is also found in *S. simulans*, *S. heamolyticus* and *S. cohnii* (Becker et al., 2014). Additionally, several surface proteins that are found both in *S. aureus* and *S. epidermidis*, including cell wall associated fibronectin binding protein (*ebh*), elastin binding protein (*ebp*), cell wall surface anchor protein (*sas*) and bifunctional autolysin (*atl*) (Gill et al., 2005) (Table 1.8). Moreover, cell wall surface anchor protein encoding gene *sas* is known to transfer from *S. epidermidis* to *S. aureus* via prophage (Otto, 2013). The serine-aspartate repeat (*sdr*) are characterized to be surface proteins (Foster et al., 2014). *sdrC*, *sdrD, sdrE* are found uniquely in *S. aureus*, while *sdrF, sdrG, sdrH* are exclusively expressed by *S. epidermidis* (Foster et al., 2014; McCrea et al., 2000). *pls* is believed to be the methicillin resistant surface protein (Gill et al., 2005).

1.3.3 Pathogenicity determination approaches

The pathogenesis of staphylococci is associated with cell surface proteins, adhesion factors and secreted toxins (Ythier et al., 2012). Different approaches have been used to study the pathogenicity of staphylococci, including genomics, transcriptomics, and proteomics (Ythier et al., 2012).

1.3.3.1 Whole genome sequencing

Whole genome sequencing (WGS) reveals genetic relatedness down to the level of the single nucleotide, and it offers a feasible method for local, national and international monitoring and infection control (Price et al., 2013). For important pathogens, whole genome sequencing can provide an insight into the genomic composition that facilitates to its increasing pathogenicity (Gill et al., 2005).

1.3.3.1.1 Whole genome sequencing technique

DNA fragments are sequenced by a WGS platform, and then resembled into contigs for further analysis (Price et al., 2013). Three generations of sequencing platforms have been launched since 1977, and each generation has become more rapid and cost effective. The third generation of sequencing allows real time observation of construction of DNA strands, whereas the others are looking at reconstructing DNA fragments (Price et al., 2013).

1.3.3.1.2 Genomic feature of staphylococci

The circular genome of staphylococci is composed of 2.5 to 2.8 million base pairs, including genetic background and genomic islands (Gill et al., 2005; Kuroda et al., 2005; Price et al., 2013; Takeuchi et al., 2005). The function of two parts DNA are as followings: (1) genetic background are inherited from ancestral bacteria, which contains housekeeping genes involved in basic synthetic functions that is essential for the survival of bacteria; (2) genetic islands (GI) are acquired by horizontal transfer and which encodes virulence or antibiotic resistance genes (Ito et al., 2003). The staphylococcal cassette chromosome *mec* (SCC*mec*) is one of the GI types which carry antibiotic resistance genes (Ito et al., 2003).

1.3.3.1.3 Whole genome variation

Genetic variation analysis can be determined by comparison of each genome within a population with their reference genome sequence of the species (Arber, 2000). Six types of genetic variation are found: (1) loss of one or more bases; (2) gain one or more bases; (3) base substitution; (4) rearrangement of multiple segments; (5) copy number variation; and (6) DNA segment inversion (Arber, 2000). Loss or gain of bases may cause two results. In the first case, deletion /insertion of one or two bases into a protein coding region can have profound influence, which can lead to complete malfunction of

the protein (frameshift) (Trun & Trempy, 2009). Deletion or insertion of three or multiples of three bases will lead to loss or gain one or several amino acids in the middle of encoding region (Trun & Trempy, 2009). In this case, the protein function may or may not be altered depending on the position and codon type (Trun & Trempy, 2009). Genomic variation may have huge impact on the organisms and result in different phenotypes, such as immune evasion ability changes (Richards et al., 2015), antimicrobial susceptibility changes (Chen et al., 2014; Dengler et al., 2013), and virulence changes (Sapp et al., 2014). In comparison, the plasmids are much smaller than the chromosomes, and encodes proteins that are not essential for survival of bacteria; however, many plasmids harbour genes confer adaptive advantages, such as antibiotic resistance genes, and virulence genes (Chen et al., 2014; Dengler et al., 2013; Sapp et al., 2014). The numbers of varied plasmids that confers different phenotype and pathogenicity of staphylococci can also be determined by whole genome sequencing (Gill et al., 2005).

1.3.3.2 Whole proteomic approach

Another approach towards understanding the pathogenicity of staphylococci uses proteomic analysis (Enany et al., 2014). The advantage of proteomic analysis is that it can determine the expression of virulence genes (Enany et al., 2014). Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is one of the techniques that are applied for staphylococci proteomic analysis (Bernardo et al., 2004).

1.3.3.2.1 LC-MS/MS

LC-MS/MS can be used for identification of bacterial proteins, and thus provide an insight into protein expression of bacteria (Murray, 1997). This approach has been widely applied for staphylococcal research by exploring the protein expression

differences of an isolate cultured under different conditions or between genetically closely related species (Murray, 1997).

LC-MS/MS can show protein expression differences of each individual with similar genome composition. Encoding of orthologous proteins, or similar proteins in different quantities can lead to different phenotypic characteristics and performance (Murray, 1997).

LC-MS/MS is a technique that combines liquid chromatography and mass spectrometry. Liquid chromatography (LC) is used to remove impurities from the sample, separate component mixtures and ionize samples (Niessen, 2006). One benefit of liquid chromatography is the separation of isomers. Isomers are known to have exactly the same mass and cannot be differentiated by mass spectra. Additionally, liquid chromatography removes the risk of ion suppression, where one compound affects the ionization of another compound (Jemal, 2000). The second part, MS/MS is the combination of two mass spectrum analysers in single instrument, and the advantages of MS/MS are to increase sensitivity. The precursor ions which are transferred by liquid chromatography are fragmented by the first MS filters, selected ions are then monitored by the second mass analyser (Jemal, 2000). The information produced by the mass spectrometer: in the form of a list of peak intensities and mass to charge (m/z) values, can be manipulated and compared with genome and protein databases to identify the proteins (Murray, 1997). Therefore, with the combination of liquid chromatography and MS/MS, all the compounds present in a peak can be identified and the purity of sample can be checked (Jemal, 2000).

1.3.3.2.2 Scaffold software

The reliability of protein identification is affected by the accuracy and reproducibility of LC-MS/MS studies. Searle, (2010) demonstrates that the confidence in protein

identification can be increased by using Scaffold, a software tool in bioinformatics, which converts the peptide scores produced by various database search engines, into probabilities of peptide identification. These results are corroborated and combined with the so-called *greedy algorithms*, a group calculation method developed by Scaffold to reduce falsely reported protein identification (Searle, 2010).

The staphylococcal genomic plasticity contributes to the development of virulence and multiple antibiotic resistance in staphylococci (Takeuchi et al., 2005), which in turn increase their pathogenicity and make antibiotic therapy less effective (Otto, 2010; Takeuchi et al., 2005). Complete genome sequencing and proteomic analysis can provide an insight into the genomic features and their expression which contribute to the increasing pathogenicity in staphylococci (Enany et al., 2014; Gill et al., 2005).

1.4 Antibiotic resistance

1.4.1 History of antibiotic resistance

When Fleming first discovered penicillin, he warned that the abuse of antibiotics may lead to the development of antibiotic resistance of microorganisms and contribute to their dissemination (Bartlett et al., 2013). Unfortunately, the public did not heed Fleming's warning and the continuing growth of antibiotic resistance in microorganisms has been driven by the massive overuse of antimicrobial agents (Bartlett et al., 2013). Penicillin was first introduced in World War II, and soon after introduction, penicillinase producing *S. aureus* has been widely found in hospital environments (Chambers, 2001). Currently, the development of new antibiotics has been hampered by economic and regulatory barriers, and few new antibiotics have been discovered in last decade (Bartlett et al., 2013); however, antibiotic resistance has been reported all over the world, including Africa, America, Asia, Eastern Mediterranean, European, and

Pacific region (WHO, 2014). In USA, the prevalence of MRSA increased from 5-10% to 50% in hospital isolates in the past two decades (Chambers, 2001). Meanwhile, it is shown that up to 40% MRSA infections were acquired in health individuals from the community (Chambers, 2001). Unfortunately, the phenomenon is of multiple, not single, antibiotic resistance. The first multiple antibiotics resistant microorganism were reported in the late 1950s (Levy & Marshall, 2004). In 2007, the number of multidrug-resistant bacteria infections was 400,000; 25,000 of these were lethal in Europe (Bush et al., 2011).

Bacterial antibiotic resistance has serious health and economic consequences. The infection caused by antibiotic resistant staphylococci makes treatment harder, and then result in worse clinical outcome, even death (Cosgrove, 2006; Palumbi, 2001). First, the side effects of second line (cefixime and colistin) and third line (rifabutin and levofloxacin) antibiotics are far more frequent and severe than first line antibiotics (penicillin), including dizziness, fever, diarrhoea, renal dysfunction and leukopenia (Cosgrove & Carmeli, 2003; Cunha, 2001; Iravani et al., 1988; Levin et al., 1999). Second, treatment may be delayed by less effective antimicrobials, and lead to surgical procedure to eradicate illness (Harris et al., 1999; Levine et al., 1991). Meanwhile, the cost of antibiotic resistance associated infection is significantly higher than non-antibiotic resistant related infection, as antibiotic resistance associated infection often leads to longer hospitalization, surgical treatment and use of other antibiotics. Thus the economic burden on patients, health care facilities and even the whole society is increased (Lautenbach et al., 2001; Silver, 2011).

1.4.2 Limitation in tackling with antibiotic resistance

Over 20 new classes of antibiotic agents have been marketed between 1929 and 1970s. This was the so-called ‘golden age of antibiotic discovery’ (Laxminarayan et al., 2013). Since 1987 just two new classes have been discovered and commercialised (Laxminarayan et al., 2013; Silver, 2011). Although 20 new antimicrobial agents have been launched since 2010, all of them are analogues of existing classes of antibiotics (Butler & Cooper, 2011). In 2015, a promising antibiotic, designated as teixobactin, was discovered, and has activity against Gram-positive bacteria only with no detectable resistance. However, the development of teixobactin is still in its early stages, its clinical efficacy remains to hope for (Ling et al., 2015). Despite the noble endeavours of academia and industry, the focus on low-risk synthetic approaches to develop analogues of existing classes instead of traditional screening of natural products, and over-reliance of genomic approaches has reduced our global antibiotic discovery infrastructure (Coates et al., 2011).

The development and administration of preventive vaccines against infectious diseases has reduced the use of antibiotics, however, this is only true in some cases, such as *Haemophilus influenza*, *Neisseria meningitides* and *Streptococcus pneumoniae* (Peltola, 2000; Frasch & Bash, 2003; Kyaw et al., 2006). Vaccine clinical development is a long, expensive and high-risk process involving extensive human evaluation of efficacy and safety before commercialisation (Curtiss, 2002).

Trends in the falling efficacy of several antibiotics and the consequential health and economic burdens require effective monitoring (Bartlett et al., 2013) The collection of antibiotic resistance data is crucial for informing public health authorities, governments, policy makers and industry stakeholders to make better decisions to mitigate this threat

and discourage the overuse of antibiotics (Bartlett et al., 2013). Natural microflora in humans will evolutionarily develop resistance to antibiotics after extended exposure to antimicrobial selection pressures, giving rise to the emergence of opportunistic bacterial 'superbugs' (Jernberg et al., 2010). These opportunistic bacteria are considered to be a major cause of pandemic infections worldwide, and threaten the lives of the most vulnerable individuals of society such as children, elderly and immune-compromised individuals (Bartlett et al., 2013). All the resistance genes that are present in humans, animals and the environment are defined as bacterial resistomes. Moreover, the transmissible characteristic of these resistant genetic elements contribute to the emergence of antimicrobial resistance in patient, clinical settings and the wider environment (Wright, 2007).

1.4.3 Antibiotic resistance mechanisms

Staphylococcal infection is a significant cause of morbidity all over the world (Diekema et al., 2001), and the presence of antibiotic resistance made staphylococcal infection even worse (Diekema et al., 2001). Antibiotics, also named as antimicrobial agents, are used for treatment of staphylococcal infections; however, staphylococci have evolved several mechanisms to reduce their susceptibility to antibiotics, such as mutations, and acquisition of resistance genes (Livermore, 2003).

1.4.3.1 Resistance to beta-lactam

After Fleming's serendipitous discovery of penicillin, during the 'penicillin era' between 1940 and 1960, penicillin was widely considered to be a "magic bullet" which can kill all Gram-positive bacteria without harming human hosts (Ehrlich & Himmelweit, 1956; Gensini et al., 2007). However penicillin resistance was observed in a hospital setting, as early as 1942, just two years after the introduction of penicillin for

clinical use. Within two decades, about 80% of both hospital- and community-acquired *S. aureus* isolates were observed to have developed resistance to penicillin (Appelbaum, 2007a). Resistant strains expressed penicillinase, a specific type of β-lactamase, shows specific activity against penicillin through hydrolysis of the β-lactam ring. Penicillin is inactivated and loses its ability to inhibit the synthesis of cell wall (Abraham & Chain, 1940). Four classes of β-lactamase have now been identified, and they are differentiated by nucleotide sequences and crystal structures. However, all of them share several highly conserved amino acid sequences, which is responsible for targeting β-lactam antibiotics (Appelbaum, 2007a).

1.4.3.2 Resistance to beta-lactam by expression additional penicillin binding protein

1960-1978 is the era of natural and synthetic penicillin development, which include methicillin, oxacillin, ampicillin and other semisynthetic penicillin, and they can inhibit the growth of Gram-negative and Gram-positive bacteria (Medeiros, 1997). The bactericidal mechanism of beta-lactam antibiotics are known to bind to penicillin binding protein (PBP) to disrupt the synthesis of the peptidoglycan which is essential for formation of the bacterial cell wall (Tomasz et al., 1989).

Methicillin resistant was reported in *S. aureus* in the United Kingdom in 1961, just a year after it was firstly introduced to clinical use in 1960. In 1968, MRSA was reported all over the world (Huang et al., 2006), Today, MRSA strains are found worldwide, and most are multidrug resistant (Appelbaum, 2006). There is a new mechanism in methicillin resistant staphylococci, which is markedly different from the penicillin resistance mechanism. Methicillin resistant in *S. aureus* involves an altered target site due to an acquired penicillin-binding protein 2a (PBP2a) with low affinity to β-lactam antibiotics. Even with the presence of methicillin, penicillin-binding protein 2a can still promote synthesis of the bacterial cell wall (Appelbaum, 2007b).

Cell wall is essential for survival of bacteria, as it protects microorganisms from intracellular and extracellular pressures, enabling normal cellular function and division (Typas et al., 2012). Penicillin-binding proteins (PBPs) catalyse the glycan strand polymerization and the cross-linking of glycan chains, which is necessary for synthesis of cell wall (Tulinski et al., 2012). There are 3 subdivisions of PBPs identified in staphylococci: Class A, Class B and Class C. PBP2 belongs to the class A division which is encoded by the *pbp2* gene, and PBP2a belongs to class B division encoded by the *pbp2a* gene (Tulinski et al., 2012). *pbp2* is located in all staphylococcal species, and TPase and TGase domain of PBP2 are known to catalyse the transpeptidation and transglycosylation in crosslinking of peptidoglycan (Pinho et al., 2001). In the presence of methicillin, the TPase domain of PBP2 will be blocked, and thus inhibits the transpeptidation. Methicillin resistant *S. aureus* additionally acquired a *mecA* gene, which encodes for penicillin binding protein 2a (PBP2a) with a low affinity to β-lactam antibiotics (Tulinski et al., 2012). TPase domain of PBP2a involves in transpeptidation, and TGase domain of PBP2 is collaborative for transglycosylation in presence of methicillin. Therefore, PBP2a and PBP2 maintain normal functions and thus resistant to β-lactam antibiotics (Pinho et al., 2001) (Fig 1.1). In addition to *mecA*, four categories of *mecA* gene homologs (*mecA1, mecA2, mecB, mecC*) have been reported based on their similarity to original *mecA* gene (Ito et al., 2012). The *mecC* gene shares less than 70% similarity with the original *mecA* gene, and is present in SCC*mec* type XI recovered from human, veterinary and wild sources (Becker et al., 2014). Although the mechanism by which the *mecC* gene mediated oxacillin resistance has not been elucidated, it is confirmed that the *mecC* gene encodes a different type of penicillin binding protein 2a ($PBP2a_{mecC}$). This $PBP2a_{mecC}$ mediate the high level oxacillin resistance of *S. aureus* (Paterson et al., 2014). The *mecA* and *mecC* gene are subject to

PCR and used to explore the methicillin resistant determinants (García-Álvarez et al., 2011; Murakami et al., 1991).

Methicillin-resistant *S. aureus* (MRSA) has been represented by *mecA* gene positive and methicillin (oxacillin) resistant; however, the presence of oxacillin susceptible MRSA (OS-MRSA) exhibit a new type of MRSA (Hososaka et al., 2007). Although the OS-MRSA is phenotypically susceptible to oxacillin, it is believed that most OS-MRSA is oxacillin hetero-resistance. Therefore the treatment with beta-lactam antibiotics may be ineffective (Ikonomidis et al., 2008).

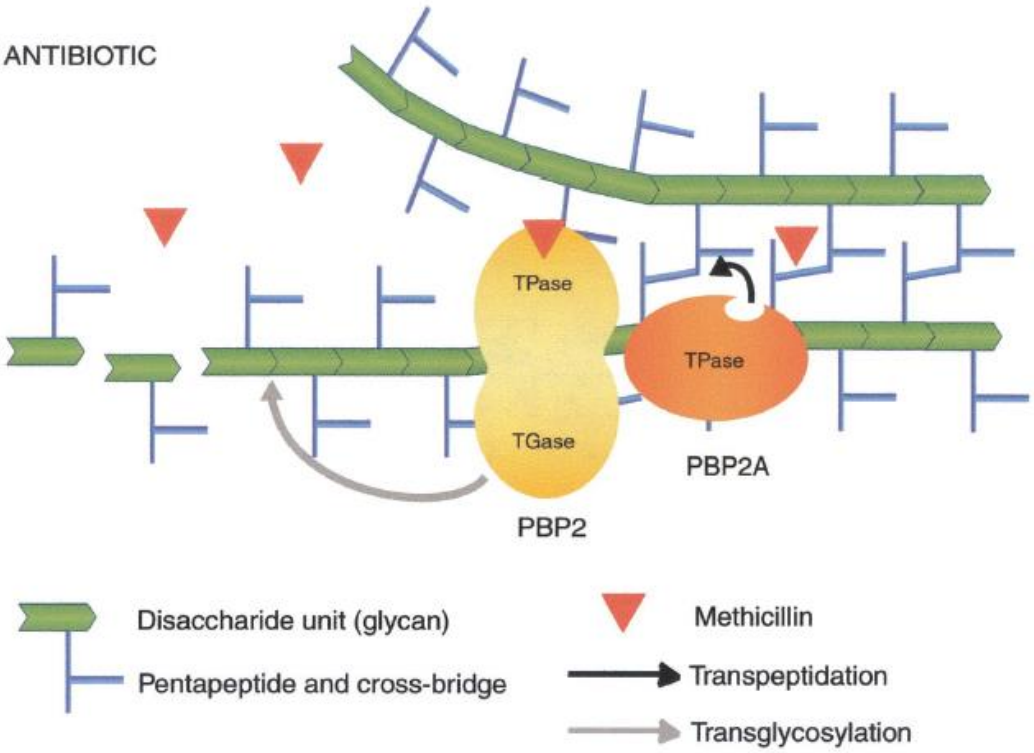


Figure 1. 1 The activity of PBP2 and PBP2a in the crosslinking of the peptidoglycan of methicillin-resistant *S. aureus.* The TPase domain of PBP2a is involved in transpeptidation, and the TGase domain of PBP2 is involved in transglycosylation in the presence of methicillin (Pinho et al., 2001).

1.4.3.3 Resistance to aminoglycoside

The anti-microbial activity of aminoglycoside antibiotics such as kanamycin, gentamicin, streptomycin, is based on their ability as protein synthesis inhibitors.

Aminoglycoside antibiotics are known to bind to the 30S ribosomal subunit and thus inhibit protein synthesis; however, the staphylococcal strains have evolved several mechanisms to inhibit aminoglycoside activity (Mingeot-Leclercq et al., 1999; Schmitz, 1999). Currently, two aminoglycoside resistance mechanisms have been widely accepted. The first mechanism involves reduced drug uptake, which is due to membrane impermeabilisation (Mingeot-Leclercq et al., 1999). The second is due to aminoglycoside-modifying enzymes produced by staphylococci that, inactivate the aminoglycosides by covalently attaching key functional groups of antibiotics, thus decreasing aminoglycosides ribosomal binding affinity, and resulting in high-level resistance (Mingeot-Leclercq et al., 1999; Schmitz, 1999). The aminoglycoside-modifying enzymes are encoded by acetyltransferase (AAC), adenylyltransferase (ANT) or phosphotransferase (APH), and resistant to gentamicin is mediated by a bifunctional enzyme displaying AAC and APH activity (Schmitz, 1999). Moreover, AAC-APH gene is located on a conjugative plasmid Tn4001, which is widely distributed in *S. aureus* and CoNS; whereas kanamycin and streptomycin resistance are determined by ANT and APH (Schmitz, 1999).

1.4.3.4 Resistance to macrolides

The antibacterial activity of macrolides is achieved by inhibiting protein synthesis (Vester & Douthwaite, 2001). Macrolides are characterized to be the polyketide group of compounds, which includes carbomycin and erythromycin (Vester & Douthwaite, 2001; Weisblum, 1995). Four macrolides resistance mechanisms have been identified. Firstly, the presence of macrolide efflux pumps (*msr*) in staphylococci has contributed to the macrolides resistances (Schmitz et al., 2000). In *S. aureus*, macrolide resistance is associated with *msrA* gene that encodes an ABC-transporter-mediated efflux (Matsuoka et al., 2003). Secondly, a *mph* gene located at downstream of *msrA* gene has been

known to inactivates macrolide antibiotics by encoding a phosphotransferase, and the expression of *mph* gene is associated with presence of *msrA* gene (Matsuoka et al., 2003). Thirdly, enzymes (*ere*) is known to inactivate macrolide by hydrolysing the lactone ring nucleus of macrolide (Schmitz et al., 2000). Finally, macrolide resistance in staphylococci can be achieved by alteration of macrolide target site – 23S rRNA (Weisblum, 1995). A gene named erythromycin ribosome methylation (*erm*) encodes 23S rRNA methylase, which is responsible for conformation change of 23S rRNA (Schmitz et al., 2000). *erm* mediated methylation of adenine residue of 23S rRNA domain V lead to the reduced affinity to macrolide, and thus confer to macrolide resistance (Vester & Douthwaite, 2001; Weisblum, 1995). The *erm* gene has been collected from diverse range of resources, and 30 different kinds of *erm* have now been identified (Weisblum, 1995).

1.4.3.5 Resistance to phenicols

Chloramphenicol is categorized to phenicols class, and is a bacteriostatic drug that stops bacterial growth by inhibiting protein synthesis. Chloramphenicol prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome (Jardetzky, 1963). Chloramphenicol resistance in staphylococci is due to an inducible enzyme: chloramphenicol acetyltransferase, which acetylates chloramphenicol, and thereby inactivates the chloramphenicol (Shaw et al., 1970).

1.4.3.6 Resistance to steroid and fosfomycin

Fusidic acid is a steroid antibiotic derived from the fungus *Fusidium coccineum* (Godtfredsen et al., 1962), which fight against severe Gram-positive infections by interfering with the function of the elongation factor G (EFG). EFG is known to transfer peptidyl-tRNA from the ribosomal A site to the P site, and then the messenger RNA is able to move one codon forward. Meanwhile, GTP is hydrolysed into GDP to provide

energy for this process, and EFG dissociates from the ribosome in a complex with GDP. EFG is encoded by *fusA* gene (Martemyanov et al., 2001). Fusidic acid prevents the dissociation of EFG from ribosome by binding to EFG on the ribosome, and thus blocks the next stage of protein synthesis (Martemyanov et al., 2001). Fusidic acid resistance in *S. aureus* is due to *fusA* gene mutation associated EFG structure alteration, and the altered EFG has low affinity to fusidic acid. Even in the presence of fusidic acid, EFG can be dissociated from the ribosome and is able to continue with next step in protein synthesis (Martemyanov et al., 2001). Brown & Thomas, (2002) has reported striking increase in fusidic acid resistance of clinical *S. aureus*, and warned about the decreased efficacy of fusidic acid in treatment of serious MSSA infections. Fosfomycin is an antibiotic that is known to inhibit cell wall synthesis, and fosfomycin resistance is mediated by *fosA* gene. The *fosA* gene encodes a glutathione S-transferase, which forms a covalent bond with fosfomycin, and then inactivates fosfomycin (Bernat et al., 1997).

1.4.3.7 Resistance to monoxycarbolic acid

Monoxycarbolic acid is a class of antibiotics, and mupirocin is one of the representative antibiotics (Cookson, 1998). Mupirocin was introduced into clinical practice in the UK in 1985, and it has been proved to be an extremely effective and successful topical antibiotic for treatment of nasal and skin MRSA infections (Cookson, 1998). Mupirocin is an analog of isoleucine which competitively binds to isoleucyl tRNA synthetase, and thus inhibit protein synthesis (Hodgson et al., 1994); however, resistant strains were reported shortly after introduction of the Mupirocin (Cookson, 1998). Different mechanisms are involved in low-level resistance and high-level resistance. *Ile* has been known to encode isoleucyl tRNA synthase, which specifically recognize isoleucine and transport to ribosome for protein synthesis (Lodish, 2008). Low-level resistance is due to the mutation in a chromosomally encoded *Ile*, and high-level resistance has been

shown to be due to the acquisition of an additional novel gene *IleS*. Mutation in *Ile* and acquisition of *IleS* are both lead to reduced affinity of isoleucyle tRNA synthase to mupirocin (Cookson, 1998; Hodgson et al., 1994).

1.4.3.8 Resistance to tetracycline

Tetracycline is a broad-spectrum polyketide antibiotic produced by the *Streptomycetes*, it is used for the treatment of bacterial infections as it inhibits protein synthesis (Ng et al., 2001). There are three mechanisms involved in tetracycline resistance, including: using energy-dependent efflux (encoded by *tet(K)* gene) of tetracycline (Gibbons & Udo, 2000); alteration of the ribosome to prevent the effective binding of tetracycline; enzymatic inactivation of tetracycline (Ng et al., 2001).

1.4.3.9 Resistance to glycopeptide

Glycopeptide antibiotics include vancomycin, teicoplanin, ramoplanin and decaplanin. Glycopeptides are known to inhibit the growth of bacteria by obstructing cell wall synthesis (Hiramatsu, 2001). For staphylococci, glycopeptide antitiobitcs can bind to acyl-D-alanyl-D-alanine in peptidoglycans, and therefore prevent the cross linking process of cell wall synthesis (Hiramatsu, 2001). Vancomycin resistance/intermediate *S. aureus* increase cell-wall thickness by producing more peptidoglycan, and thus vancomycin are trapped in peptidoglycan layers and cannot access to peptidoglycan synthesis sites. However, vancomycin resistance at the genetic level has not been clarified yet (Appelbaum, 2007a).

1.4.4 In vitro susceptibility testing overview

Antimicrobial susceptibility test is a standard clinical lab procedure, and rational selection of antibiotics for treatment is determined by assessing possible antibiotic resistance in bacteria (Jorgensen & Ferraro, 2009). In addition, susceptibility test can

provide information on decreased susceptibility of bacteria to antibiotics (Mahon et al., 2014). Nowadays, human, veterinary and agricultural use of antibiotics means that large quantities of antibiotics have been continuously released into the environment (Batt et al., 2006; Díaz-Cruz et al., 2003; Kummerer, 2003); however, little is known about the dissemination of antibiotic resistant staphylococci in environment.

1.4.4.1 Overview of commonly used susceptibility testing methods

Antibiotic susceptibility test is a routine procedure during phenotyping in clinical microbiology and microbiology research labs (Jorgensen & Ferraro, 2009). The most widely used testing methods in modern clinical lab are the antimicrobial gradient method and the disc diffusion method (Jorgensen & Ferraro, 2009).

The antimicrobial gradient method involves placing a commercial plastic antibiotic gradient strip on standardized bacterial suspension covered plates, and MIC is determined by inhibited growth point along the strip (Jorgensen & Ferraro, 2009). The gradient diffusion method is a simple and time efficient way to determine MIC of tested isolates. Moreover, the gradient diffusion method is consistent with the traditional broth dilution method (Jorgensen & Ferraro, 2009).

The disk diffusion method depends on the formation of a radial gradient around the antimicrobial agent. The antimicrobial agent is released from the disc and radially diffuses into the agar, giving a concentration gradient. At a specific distance from the centre, the concentration of antibiotic is too low to inhibit the growth of the test organism, and the inhibition zone is formed. After comparing the diameter of the inhibition zone with standard criteria, the susceptibility results are interpreted as `susceptible`,` intermediate` or `resistance`(Mahon et al., 2014).

1.4.4.2 Overview the standards for interpreting antimicrobial susceptibility results

With the global increase in microbial resistant to antibiotics, there is a need for universally recognized standards to interpret the susceptibility of microorganisms (Jorgensen & Ferraro, 2009). There are three globally recognized standards for the interpretation of antimicrobial susceptibility test results: British Society of Antimicrobial Chemotherapy (BSAC), the Clinical and Laboratory Standards Institute method (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The different standards of MIC and zone diameter breakpoints for staphylococci are showed in Table 1.9 (Creagh & Lucey, 2007; Howe & Andrews, 2012; Testing, 2014; Wikler, 2007).

Criteria for gentamicin, vancomycin, oxacillin and cefoxitin are different for interpreting *S. aureus* and CoNS, whereas, interpretive critertia for streptomycin and cefepime is specific for *S. aureus* (Creagh & Lucey, 2007; Howe & Andrews, 2012; Testing, 2014; Wikler, 2007; Wayne, 2014). Interpretive criteria for penicillin, amoxicillin, erythromycin, tetracycline, chloramphenicol, fusidic acid and mupirocin are generally for *Staphylococcus* spp. (Creagh & Lucey, 2007; Howe & Andrews, 2012; Testing, 2014;Wayne, 2014).

Table 1. 9 Zone Diameter and Minimum Inhibitory Concentration (MIC) Interpretive Standards for *Staphylococcus* spp. (Howe & Andrews, 2012; Testing, 2014; Wayne, 2014; Wikler, 2007)

	BSAC							CLSI							EUCAST						
	MIC breakpoint ($mg\ l^{-1}$)			Interpretation of zone Diameters (mm)			Comment	MIC breakpoint ($mg\ l^{-1}$)			Interpretation of zone diameters (mm)			Comment	MIC Breakpoint ($mg\ l^{-1}$)			Interpretation of zone diameters (mm)			Comment
Antibiotic (Disc content)	R>	I	S≤	R≤	I	S≥		R>	I	S≤	R≤	I	S≥		R>	I	S≤	R≤	I	S≥	
Aminoglycosides																					
Gentamicin (10 µg)	**1**	-	**1**	**19**	-	**20**		8	-	4	12	13-14	15		1	-	1	18	-	18	*S.aureus*
Gentamicin (10 µg)	-	-	-	-	-	-		-	-	-		-	-		1	-	1	22	-	22	CoNS
Streptomycin (10 µg)	-	-	-	-	-	-		-	-	-	**14**	-	**22**	*S. aureus*	-	-	-	-	-	-	
β-lactams																					
Oxacillin (1 µg)	2	-	1	14	-	15		**4**	-	**2**	**10**	-	**13**	*S. aureus*	2	-	0.25	-	-	-	
Oxacillin (1 µg)								**0.5**	-	**0.25**	**17**	-	**18**	CoNS	-	-	-	-	-	-	
Penicillin G (1U)	**0.12**	-	**0.12**	**24**	-	**25**		0.12	-	0.12	28	-	29	10 U	-	-	-	-	-	-	
Amoxicillin (10 µg)	-	-	-	-	-	-		**4**	-	**2**	**19**	-	**20**		-	-	-	-	-	-	

Note: The criteria in bold is used to interpret the antibiotic susceptibility test results in this study.
a: Andrews & Testing, 2001; b: Creagh & Lucey, 2007

Antibiotic (Disc content)	BSAC MIC breakpoint (mg l^{-1})			Interpretation Of zone Diameters (mm)			Comment	CLSI MIC breakpoint (mg l^{-1})			Interpretation of zone diameters (mm)			Comment	EUCAST MIC Breakpoint (mg l^{-1})			Interpretation of zone diameters (mm)			Comment
	R>	I	S≤	R≤	I	S≥		R>	I	S≤	R≤	I	S≥		R>	I	S≤	R≤	I	S≥	
Cefepime (30 µg)	-	-	-	-	-	-		**32**		**8**	**14**	**15-17**	**18**	*S.aureus*	-	-	-	-	-	-	
Cefoxitin (10 µg)	**4**			**21**	**-**	**22**	*S. aureus*	8		4	21		22	*S. aureus* 30 µg				22		22	*S. aureus* 30 µg
Cefoxitin (10 µg)	**4**			**21**	**22-26**	**27**	CoNS				24		25	CoNS 30 µg				25		25	CoNS 30 µg
Glycopeptides																					
Vancomycin (5 µg)	**2**	**-**	**2**		**-**		*S. aureus*	-	-	2	-	-	-	*S. aureus*	2		2	-		-	*S. aureus*
Vancomycin (5 µg)	**4**	**-**	**4**	**11[a]**	**-**	**12[a]**	CoNS			4				CoNS	4		4	-		-	CoNS
Macrolides																					
Erythromycin (5 µg)	**2**	**2**	**1**	**16**	**17-19**	**20**		8	-	0.5	13	14-22	23	15 µg	2		1	18		21	15 µg
Tetracyclines																					
Tetracycline (10 µg)	**2**	**2**	**1**	**19**	**-**	**20**		16	-	4	14	15-18	19	30 µg	2		1	19		22	30 µg
Phenicols																					
Chloramphenicol (30 µg)	8	-	8	14	-	15	10 µg	**32**	**-**	**8**	**12**	**13-17**	**18**		8		8	18		18	

Note: The criteria in bold is used to interpret the antibiotic susceptibility test results in this study.
a: Andrews & Testing, 2001; b: Creagh & Lucey, 2007

Antibiotic (Disc content)	BSAC MIC breakpoint (mg l^{-1})			Interpretation of zone Diameters (mm)			Comment	CLSI MIC breakpoint (mg l^{-1})			Interpretation of zone diameters (mm)			Comment	EUCAST MIC Breakpoint (mg l^{-1})			Interpretation of zone diameters (mm)			Comment
	R>	I	S≤	R≤	I	S≥		R>	I	S≤	R≤	I	S≥		R>	I	S≤	R≤	I	S≥	
Steroid																					
Fusidic acid (10 µg)	**1**	**-**	**1**	**29**	**-**	**30**		-	-	-	24	-	32		1		1	24		24	
Monoxycarbolic acid																					
Mupirocin (20 µg)	**256**	**2-256**	**1**	**6**	**7-26**	**27**		-	-	4	-	-	19[b]	5 µg	256	-	1	13	-	13	200 µg

Note: The criteria in bold is used to interpret the antibiotic susceptibility test results in this study.
a: Andrews & Testing, 2001; b: Creagh & Lucey, 2007

1.4.4.3 Quality control of susceptibility testing

S. aureus NCTC6571 is a β-lactamase negative isolate, and susceptible to all the routine antibiotics (Table 1.10). The MIC of *S. aureus* NCTC 6571 to oxacillin is 0.125 mg l^{-1} (Seaman et al., 2004). *S. aureus* NCTC 6571 is used as a control strain in all lab based susceptibility tests, including clinical diagnosis microbiology labs in the UK (Kearns, 2006). *S. aureus* NCTC 6571 has been included as a control in all the susceptibility tests in this study.

Table 1. 10 Antibiotic susceptibility profile of *S. aureus* NCTC6571

	Antibiotics													
	PEN	OX	VAN	MUP	CHL	TET	ERY	GEN	FD	AMP	CEC	CRO	RIF	TEC
S.aureus NCTC6571	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Note: R, resistant; S, susceptible; PEN, penicillin G; OX, oxacillin; VAN, vancomycin; MUP, mupirocin; CHL, chloramphenicol; TET, tetracycline; ERY,erythromycin; GEN, gentamicin; FD, fusidic acid; AMP, ampicillin; CEC, cefaclor; CRO, ceftriaxone; RIF, rifampicin; TEC, teicoplanin

1.5 Resistome

Much attention has been focused on clinical antibiotic resistant pathogens as they are the direct cause of illness; however, the antibiotic resistant microorganisms in the environment are also a cause for concern (Blair et al., 2014). Therefore, attentions should be focused not only on pathogenic, but also on non-pathogenic bacteria and potential antibiotic resistance genes (Wright, 2007). The development of antibiotic resistance in microorganism is a natural evolutionary phenomenon and exposure to antibiotic producing microorganisms may contribute to the selection of antibiotic resistance genes in environmental microorganisms (Blair et al., 2014). However, recent studies have shown that the mutation rates of microorganism increased when exposed to antibiotics, emphasising the role of antibiotics in driving the antibiotic resistance evolutionary process (Wright, 2007). In this case, antimicrobial agents from antimicrobial producing bacteria in soil habitats, humans, and animal therapeutics, sewage, agricultural and veterinary industries made environment a potential reservoir of antibiotic resistance genes (Cantas et al., 2013). Therefore, antibiotic resistance genes from environmental microorganism comprise a huge proportion of the resistome (Wright, 2007). It is necessary to include non-pathogenic microorganisms in antibiotic resistance research in order to impede the resistance before it appears in pathogens (Cantas et al., 2013).

1.6 Genomics related to antibiotic resistance

The presence of antibiotic resistance is determined by two factors: antibiotics and antibiotic resistant determinants (Levy & Marshall, 2004). The continuous flow of antibiotics from human treatment, veterinary and agricultural industries to the environment contribute to the selection of antibiotic resistant bacteria (Levy & Marshall,

2004); however, mechanistic details of the evolutionary development of antibiotic resistance still need to be clarified (Kemper, 2008; Zhang et al., 2009, Otto, 2013).

Acquisition of antibiotic-resistance significantly complicates the treatment of bacterial infections (Levy & Marshall, 2004), and it is belived that bacteria acquire antibiotic resistance via transfer of antibiotic resistant elements or genetic mutations (Otto, 2013). Horizontal transfer of antibiotic resistant determinants is considered to be reason for dissemination of resistance in bacteria (Bloemendaal et al., 2010). In addition, resistance traits caused by chromosome mutation can be transmitted vertically to offspring (Hastings et al., 2004).

1.6.1 Lateral transfer of antibiotic resistance

Recently, using different genome sequence approaches, Méric et al., (2015) has shown that *S. aureus* and *S. epidermidis* share half of the genome with 40% of the core genes in *S. epidermidis* and 24% of the core genes in *S. aureus* and considerable interspecies mobile genetic elements has been shared by both species, such as SCC*mec*, pathogenic islands, plasmids, and transposons. Staphylococcal species that share the same environmental niches are in close proximity for genetic exchange, such as conjugation, phage transduction, and uptake of naked DNA (Otto, 2013).

Methicillin resistant in staphylococcal species is due to the *mecA* gene, which is located on a mobile genetic island SCC*mec*. *mecA* encodes penicillin binding protein 2a, which has low affinity to beta-lactam antibiotics (Mkrtchyan et al., 2013). Methicillin susceptible isolates are considered to have acquired *mecA* genes via horizontal transfer of SCC*mec* elements, and then result in the dissemination of *mecA* gene between staphylococcal species (Bloemendaal et al., 2010). Clinical research showed that methicillin resistant *S. aureus* was formed *in vivo* by acquiring SCC*mec* from

methicillin resistant *S. epidermidis* (Bloemendaal et al., 2010). However, replication of *mecA* gene transfer via conjugation between the same two clinical staphylococci *in vitro* is not successful (Bloemendaal et al., 2010). To date, the transfer of SCC*mec* via conjugation and transformation has been seldom reported (Otto, 2013). Although the phage mediated SCC*mec* transfer was observed within *S. aureus*, the phage mediated transfer of SCC*mec* between different staphylococcal species has not been reported (Otto, 2013). For now, the mechanism of SCC*mec* transfer between staphylococcal species is yet to be clarified (Otto, 2013). To my knowledge, the replication of *mecA* gene transfers with environmental staphylococci *in vitro* has not yet been investigated.

1.6.2 Vertical transfer of antibiotic resistance

In addition to horizontal transfer of antibiotic resistance genes, bacteria inherit resistance vertically from their ancestors (Hastings et al., 2004). The resistance traits, which can be vertically transferred to their offspring, includes structural changes of the antibiotic target which reduces the antibiotic affinity; increasing efflux efficiency to remove the antibiotics from the bacteria, and increasing activity of the degradative system (Hastings et al., 2004). Efflux pump is one of the significant contributing factors for antibiotic resistance (Gupta et al., 2010). Efflux pump are membrane proteins that mediate energy dependent transportation of antimicrobial agents out of cell, including EmrB/QacA family drug resistance transporters and ATP binding cassette (ABC) transportor (Gupta et al., 2010; Solheim et al., 2007).

Genetic variation can be triggered by stress conditions, and confers adaptive mutations traits (Foster, 2005a). Stress environment, such as antibiotics, UV-light, pH, oxidative stress, temperature and heavy metals, increase the genetic variation and thus contribute selective advantages (Foster, 2005a). Bacterial stress responses can be categorized into

the SOS response, the general stress response, heat-shock response and stringent response (Foster, 2005a).

1.6.2.1 SOS response

The SOS response is triggered when the bacteria are subjected to DNA damage. LexA and RecA genes are involved in regulation of the SOS stress response in *E.coli*. LexA is a repressor of the SOS response, and RecA is promoter of SOS response (Miller et al., 2004). SOS response is known to be a defence mechanism for bacteria to resist β-lactam antibiotics (Miller et al., 2004). The SOS response is known to help bacterial propagation by inhibiting cell division during repair of DNA damage (Miller et al., 2004). In addition, The SOS system regulates a global response which upregulates genes involved in DNA repair and cell survival (Maiques et al., 2006).

1.6.2.2 General stress response

The *RpoS* and *MutS* genes are involved in the general stress response of *E. coli*, and *RpoS* is known to direct RNA polymerases to their promoters for transcribing proteins necessary for cell survival (Guisbert et al., 2008). Mismatch repair is crucial for maintaining the integrity of the chromosome, and *MutS* is the gene that is in charge of mismatch repair in eukaryotes. Mismatch repair has been known to have the following functions: (1) repair the errors of DNA replication process; (2) intermediate recombination process (Kolodner, 1996).

The upregulation of thioredoxin is a stress response of oxygen damage, and the upregulation of thioredoxin is essential to protect cells from oxygen damage (Bore et al., 2007), whereas, oxygen response has been reported in acid-shock response as indirect stress response (Bore et al., 2007).

1.6.2.3 Heat shock response

Heat shock response is triggered by subjecting a cell to a temperature increase, and involves upregulation of the transcription of heat shock proteins (HSPs) (Guisbert et al., 2008; Muthaiyan et al., 2012). HSPs are involved in part of the cell's internal repair mechanism and protein folding and stabilization (Guisbert et al., 2008; Muthaiyan et al., 2012). For example, GroE (Hsp 60) gene is crucial for maintaining the structure and formation of proteins at any temperature (Guisbert et al., 2008), and GroE can also protect RNA polymerase holoenzyme from heat inactivation (Ziemienowicz et al., 1993). DnaJ is Hsp40 chaperone, which has been known to control protein homeostasis in the cell (Cuéllar et al., 2013). Moreover, GrpE is another chaperone protein, which has been reported to assistant reactivation of heat-inactivated RNA polymerase (Ziemienowicz et al., 1993). The heat shock response can also be induced by DNA damage (Guisbert et al., 2008; Muthaiyan et al., 2012).

1.6.2.4 Stringent response

The stringent response is triggered by nutrient limitation. In *E. coli*, the main gene involved in stringent response is ppGpp gene. ppGpp gene regulates the expression of RNA (Guisbert et al., 2008). Stringent response generally reduces the capability of protein synthesis, however, it increases the synthesis of amino acid for protein that is lacked (Anderson et al., 2006).

1.7 Research Aims

Staphylococci are opportunistic pathogens responsible for the range of infections, and the presence of antibiotic resistance in staphylococci is a potential threat to public health. As a result of natural evolutionary process, the environment may act as a reservoir of antibiotic resistance genes. The uses of antibiotics are a major pressure for the mobilization of antibiotic resistance genes from environment to human pathogens. However, little is known about the antibiotic resistance in environmental staphylococci.

This study aims to:

1. Determine the dissemination of staphylococci in human related environment, and look to the taxonomic correlation of staphylococci isolated from different sites;
2. Assess antibiotic resistance of staphylococci in the environment, and investigate the antibiotic susceptibility profile variation of taxonomically closely related staphylococci;
3. Investigate molecular characterization of *mecA* gene positive staphylococci;
4. Investigate the genome features that contribute to the antibiotic resistance and virulence of one *S. epidermidis* isolate with high-level oxacillin resistance;
5. Assess *mecA* gene transfer with environmental staphylococci *in vitro*, and compare the protein expression differences of *S. aureus* cultured with and without oxacillin supplemented agar.

The study is composed of 11 chapters, including the chapter 1: the introduction, which discusses the research context of this project; chapter 2: outlines the material and methods used in experimental procedures; chapter 3, 4, 5, 6, 7, 8 are focused on results; chapter 9 includes discussion of the findings; Chapter 10 draws upon the entire thesis and gives a brief summary; and chapter 11 indicates the future work.

Chapter 2 Materials and Methods

The presence of antibiotic resistance genes is a natural evolutionary process, and it has been suggested that the environment may act as a reservoir of such antibiotic resistance genes (Wright, 2007). The development of new antibiotics does not seem assuring to solve the increasing threat of antibiotic resistance, as bacteria; in particular, staphylococci continue to adapt new strategies for their survival. Hence, it is necessary to generate data on the dissemination of antibiotic-resistant bacteria that would help the public health authorities to develop new strategies for infection control (Bartlett et al., 2013). In this study, different microbiological, molecular, genomic and proteomic techniques were used to determine the diversity, dissemination, resistance and virulence features of environmental staphylococci.

2.1 Sample collection

Bacterial cultures were recovered from multiple sources at various time points during 2012 to 2014. Sterile swabs were used to sample the surfaces of different general public settings and human hands. Human hands sampling was conducted with the general public without restriction of age, gender or race and not included healthcare residents. A high volume air sampler (Cherwell SAS Super 100) was used to collect air samples from one hotel. After collection, the samples were shipped to the lab within 1-3 hours.

All the samples (except air samples) in this study were collected using COPAN dry swabs (Copan Diagnostics Inc., USA), and all collection sites were in London, United Kingdom. The period and the sites of sample collection were as follows: ① Oct 2012 and Apr 2013 - different sites of hotels (DSH); ② Apr 2013 - hotel air samples (HAS); ③ Apr 2013, July 2013 and July 2014 - human hands (HH); ④ July 2013 - baby care facilities (BCF); ⑤ July 2013 - handbags (HB); ⑥ Sep 2013 - different sites of

supermarkets (DSS); ⑦ July 2014 - different sites of restaurants (DSR); ⑧ Aug 2014 and Sep 2014 - different sites of transportation facilities (DST); ⑨ Nov 2014 - different sites of a library (DSL). HAS were collected using a high volume air sampler (Cherwell SAS Super 100). These were collected using filter-based electret capture technology. Sterilized air sampler was filled with "contact plate", and the airflow of sample collection was between 200 and 1000 litres per min.

2.2 Isolation of staphylococci

In the laboratory, all swabs were suspended in 1 ml sterile 0.9 % saline, and then were inoculated onto Nutrient Agar (NA, Oxoid Basingstoke, UK), Mannitol Salt Agar plates (MSA, Oxoid Basingstoke, UK) and Brilliance UTI® agar (UTI, Oxoid Basingstoke, UK). These were incubated aerobically at 37℃ for 24-72 h. For air samples, the contact plates were transferred and incubated at 37℃ for 24-72 h.

The numbers of colonies on NA were recorded by counting colonies on the whole plate as such counting colonies as seen on the half of the plate and then multiplying it by 2 or counting the colonies on the quarter of plate and then multiplying by 4 to estimate the total number of colonies on each plate. MSA in this study was used for the preliminary discrimination of *S. aureus* (mannitol fermenting) and CoNS (mannitol non-fermenting), and also selectively isolate staphylococcal species. The acidic byproduct was produced by fermentation of mannitol will cause the phenol red in the MSA to turn yellow; otherwise, there is no colour change to the medium. UTI contains chromogenic substrates, which can provide preliminary colorimetric identification of the main microorganisms that cause urinary tract infection (Fig 2.1). The determination of species is in accordance to manufacturer's instructions. *E.coli*: pink/red colony; *Enterococcus* spp.: turquoise/blue-green colony; coliforms: dark blue/purple colony;

Proteus, *Morganella*, *Providencia*: brown halo colony; *Pseudomonas* spp.: brown/green colony; staphylococci, streptococci: non-pigmented white colony (http://www.oxoid.com/pdf/24021_oxoid_clarity_UTI.pdf) (Fig 2.1).

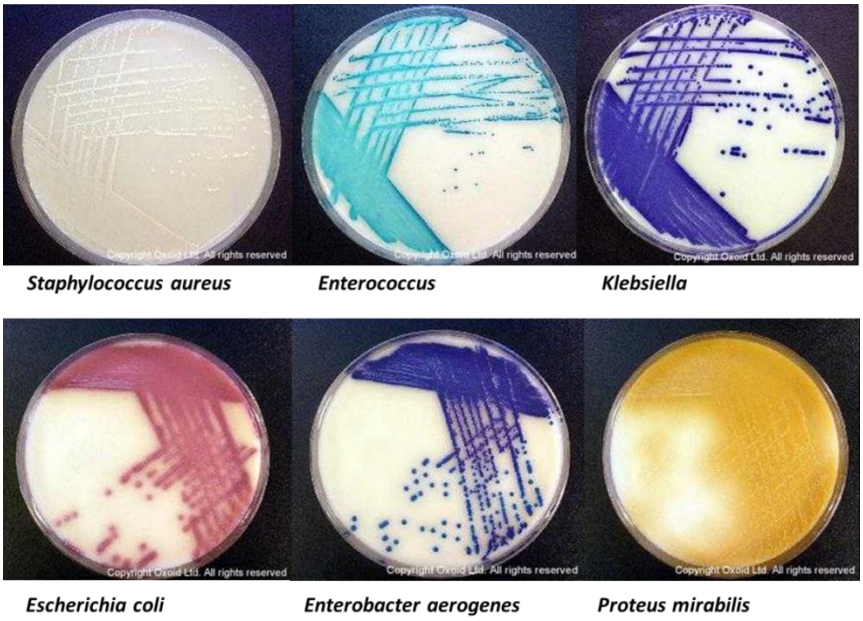


Figure 2. 1 Colour of bacteria on the dehydrate Brilliance^{TM} UTI agar. Image was adapted from:http://www.oxoid.com/UK/blue/prod_detail/prod_detail.asp? pr=CM0949.

Multiple morphological colonies were picked from NA, MSA accordingly, and resulting pure single colony transferred to a fresh NA plate, incubated 18-24 h. The culture was stored in the beads at – 80℃ (Microbank, Fisher Scientific, UK).

Partial sample collection and isolation was contributed by project students in 2014.

2.3 Identification

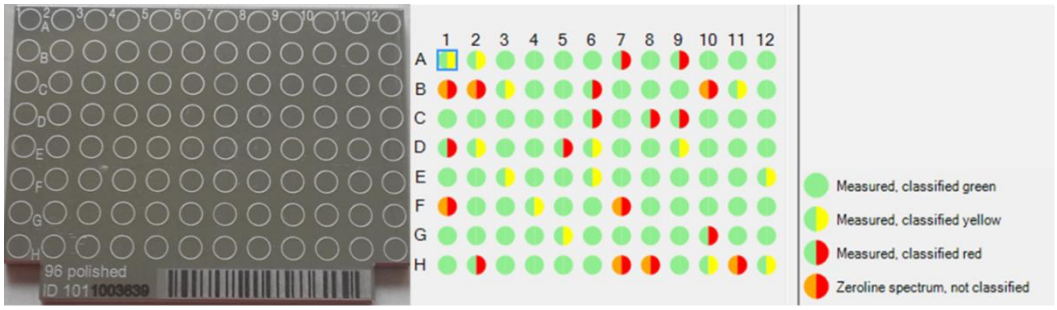
2.3.1 MALDI-TOF MS identification

Traditionally, identification of staphylococcal species has been assessed by testing phenotypic characteristics, or 16S rRNA gene sequencing methods (Janda & Abbott et al, 2007). A simple, rapid and reliable identification method – matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been employed for identification (Maier et al, 2006). This method has been widely available for identification of many microorganism species (Seng et al., 2009).

The preparation of matrix solution and sample preparation was carried out according the manufacturer's instructions (Bruker Daltonics, Coventry, UK).

Basic organic solvent (OS) was prepared with 50% (v/v) acetonitrile (Sigma-Aldrich, UK), and 2.5% (v/v) tri-fluor-acetic-acid (TFA) (Sigma-Aldrich, UK). To make the matrix solution, 250 µl OS was added to 2.5 mg α-Cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics, Coventry, UK) and vortexed until all the crystals were completely dissolved.

3-5 colonies of overnight culture were added into 300 µl distilled water, and mixed with 900 µl absolute ethanol. The suspension was centrifuged for 2 min at 13000 g, and the supernatant was then completely withdrawn by carefully pipetting. Dried pellets were mixed with 25 µl 70% (v/v) formic acid and then with 25 µl pure Acetonitrile (AN). The mixture was centrifuged for 2 min at 13000 g. 1 µl supernatant from the previous step was spotted on target plate (Bruker Daltonics, Coventry, UK) (Fig 2.2), and overlaid with 1 µl of α-Cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics, Coventry, UK).



2a 2b

Figure 2. 2 2a: Bruker 96 polished target plate; 2b: Biotyper 3.1 identification sectional drawing; Measured classified green (score value ≥ 2.0): highly probable species identification; Measured, classified yellow (2 ≥ score value ≥ 1.7): probable genus identification; Measured classified red (score value ≤ 1.7): not reliable identification; Zeroline spectrum, not classified: no spectrum was detected (Pictures were taken by me).

The spectra were detected by MALDI-TOF MS (Bruker Daltonics, Coventry, UK), and the resulting spectra for each isolate was analysed by Biotyper 3.1 software (Bruker Daltonic, Coventry, UK). *Escherichia coli* DH5α was used as a standard for calibration and quality control.

2.3.2 Reproducibility of MALDI-TOF MS

The definition of reproducibility is the capability of a technology to yield the same results when the same sample is tested repeatedly (Trindade et al., 2003). Isolates selected to assess the reproducibility of MALDI-TOF MS were prepared according to manufacturer's instructions (Bruker Daltonics, Coventry, UK) mentioned in 2.3.1, and two target plates were used at the same time in this study. The supernatant of each isolate was spotted twice on each target plate to make duplicates, and then covered with α-Cyano-4-hydroxycinnamic acid (HCCA) matrix (Bruker Daltonics, Coventry, UK). For reproducibility, one target plate was analysed by MALDI-TOF MS (Microflex LT), in the meantime, the other target plate was analysed by MALDI-TOF MS (Autoflex).

Spectra generated by both MALDI-TOF MS were analysed by Biotyper 3.1 for microbial identification.

2.3.3 16S rRNA gene sequencing identification

16S rRNA gene PCR and sequencing were also used to identify a small proportion of the environmental staphylococcal isolates to confirm the reliability of MALDI-TOF MS identification method.

2.3.3.1 Primers

The following primers were used for the amplification of the partial 16S rRNA gene sequence (Benagli et al., 2011):

UNI16S RNA-L (nucleotide sequence 5' -ATTCTTAGAGTTTGATCATGGCTCA- 3') and UNI16SRNA-R (nucleotide sequence 5' -ATGGTACCGTGTGACGGGCGGTGT GTA- 3'), which allows the amplification of a 1400 bp DNA fragment.

2.3.3.2 PCR reaction system

All PCR in this study was performed by $T100^{T}$ Thermal cycler (BIO-RAD, UK). Template DNA for PCR was prepared by resuspending one loop (10 µl, Thermo Scientific^{TM}, UK) of bacteria in 100 µl 1×TE buffer (10 mM Tris, 1 mM EDTA). The mixture was boiled for 10 min and then centrifuged at 3000 g for 5 min. The supernatant was used as DNA template (Hanssen et al., 2004). Alternatively, QIAamp DNA extraction kit (Qiagen, Crawley, UK) was used for DNA extraction.

PCR was prepared according to the protocol used by Benagli et al., (2011). The mixture was as followings: 20 units ml^{-1} Phusion® High-Fidelity DNA Polymerase (NEB, UK), 0.2 mM of each deoxynucleotide triphosphates, 1×Phusion® High-Fidelity Buffer,

approximately 10 ng template DNA, 1.5 mM $MgCl_2$ and 0.8 µM of each primer in a final volume of 25 µl.

PCR thermal cycling conditions were 5 min at 95°C for 1 cycle, followed by 35 cycles of 30 sec at 94°C, 30 sec at 52°C, 1 min at 72°C, and finalized by extension at 72°C for 10 min (Benagli et al., 2011).

2.3.3.3 Gel electrophoresis

Agarose gel electrophoresis was used to detect all PCR products in the size range of 100 bp to 10 kbp. Molecular grade agarose (Melford, UK) was dissolved in 1x TAE buffer (40 mM Tris base, 40 mM acetic acid, 1 mM EDTA) (Sigma-Aldrich, UK) to make 1.0% (w/v) agarose, and then mixed with ethidium bromide to a final concentration of 0.1 mg l^{-1} (Fisher Scientific). The solidified gel was then placed in gel electrophoresis buffer (1 ×TAE buffer), samples and 2-Log DNA ladder (0.1-10 kbp) (NEB, UK) were loaded into the wells, and 120 V was applied across the gel for 1 to 2 hours. Bromophenol blue/xylene cyanol dye front was monitored to see the migration of samples. The gel was visualized by a UV transilluminator (Syngene, Cambridge, UK) and saved as a jpg or TIFF files (Syngene, Cambridge, UK). The same gel electrophoresis system was used for *mecA* gene, *mecC* gene, SCC*mec* typing, and MLST detection.

2.3.3.4 Sequencing

The PCR products were purified by cycle pure kit (Qiagen, Crawley, UK) or gel extraction kit (Qiagen, Crawley, UK), and sequencing was performed commercially by Eurofins MWG operon (Eurofins Genomics, i54 Business Park, Valiant Way, Wolverhampton, UK). PCR purified products were sent out at concentrations of 50-100 ng $µl^{-1}$ along with the 10 pmol $µl^{-1}$ corresponding oligonucleotides. Results were

usually archived in the Eurofin account within 3 working days. The same sequencing system was used in MLST sequencing.

2.4 Antibiotic susceptibility test

In this study, the antibiotic susceptibility of staphylococci was measured by disc diffusion and antimicrobial gradient methods.

2.4.1 Disc diffusion test

Antibiotic susceptibility to 12 antibiotics were tested using standard disk diffusion methods as previously described (Mahon et al., 2014). Antibiotic susceptibilities to oxacillin (1 µg), vancomycin (5 µg), gentamicin (10 µg), mupirocin (20 µg), amoxicillin (10 µg), erythromycin (5 µg), tetracycline (10 µg), streptomycin (10 µg), cefepime (30 µg) , fusidic acid (10 µg) , penicillin G (1 unit) and chloramphenicol (30 µg) were tested (Mast Group, Merseyside, UK). The panel of 12 antibiotics used belonged to 8 different classes of antibiotics. These antibiotics were selected as these are the most common antibiotics used for profiling of antibiotic susceptibility in staphylococci. The amount of antibiotic on the discs is recommended by BSAC and CLSI standards (Howe & Andrew, 2012; Creagh & Lucey, 2007).

Antibiotic susceptibility tests of 120 staphylococcal isolates were carried out by the final year project students studying Biomedical Science program in 2014.

2.4.2 MIC test

The minimum inhibitory concentration (MIC) to oxacillin was additionally evaluated using ‘‘M.I.C. evaluators’’ (Oxoid Ltd., Basingstoke, UK). The antimicrobial gradient method towards oxacillin was applied to all *mecA* gene positive staphylococci. Test methods and interpretation were according to manufacturer’s instruction. The MIC strip (Oxoid Ltd, Basingstoke, UK) is a plastic strip with an antibiotic gradient from the

lowest concentration (0.015 mg l^{-1}) at the bottom to the highest concentration (256 mg l^{-1}) at the top. The MIC strips were placed on the surface of pre-inoculated iso-sensitest agar plates, and the scale was facing up. After 18-24 h incubation, the lids of petri dishes were removed, and the MICs were determined where the growth ellipse intersects the MIC strip (Fig 2.3) (Jorgensen & Ferraro, 2009).

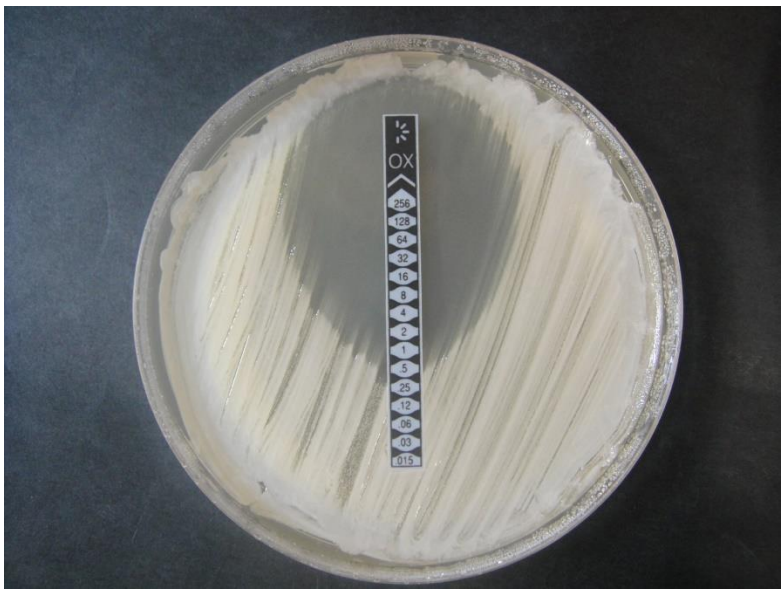


Figure 2. 3 Photograph of isosensitest agar plate with a MIC strip (oxacillin) (Picture was taken by me)

2.4.3 Interpretation

BSAC standards were used to interpret gentamicin, vancomycin, penicillin, erythromycin, tetracycline, fusidic acid and mupirocin (Howe & Andrews, 2012). The antibiotics (oxacillin, cefepime, streptomycin, chloramphenicol, amoxicillin) that cannot be interpreted by BSAC, were interpreted by CLSI standards (Creagh & Lucey, 2007; Wayne, 2014). In addition, MIC of oxacillin was interpreted with CLSI standards.

2.5 Molecular characterization of staphylococci

2.5.1 *mecA* gene detection

2.5.1.1 mecA gene determined by polymerase chain reaction

2.5.1.1.1 Primers

Two pairs of primers were used for *mecA* gene exploration, including met1, met2 (Hanssen et al., 2004) and mA1, mA2 (Kondo et al., 2007) (Table 2.1).

Table 2. 1 Two sets of primers for *mecA* gene PCR

mecA Primers	Primer sequence	Amplicon size (bp)	Reference
met1	GGGATCATAGCGTCATTATTC	527	Hanssen et al, 2004
met2	AACGATTGTGACACGATAGCC		
mA1	TGCTATCCACCCTCAAACAGG	286	Kondo et al, 2007
mA2	AACGTTGTAACCACCCCAAGA		

2.5.1.1.2 PCR reaction system

For met1 and met2 primers, the PCR was prepared according to Hanssen et al., (2004) with minor modification. The PCR were carried out with the standard PCR mixture with Phusion® High-Fidelity PCR Master Mix with HF Buffer (NEB, UK), which contains 20 units ml^{-1} Phusion® High-Fidelity DNA Polymerase, 0.2 mM each dNTP, 1× Phusion® High-Fidelity Buffer, approximately 10 ng template DNA and 1.5 mM $MgCl_2$. 0.8 µM of each primer in a final volume of 25 µl (Hanssen et al., 2004). For mA1 and mA2 primers, the PCR preparation was referred to Kondo et al., (2007). The reaction mixtures contained approximately 10 ng templates DNA, 0.1 µM oligo-nucleotide primers. 0.2 mM of each deoxynucleotide triphosphates, 3.2 mM $MgCl_2$, Ex Taq buffer, and 2.5 U Ex Taq polymerase (Takara Bio Inc., Tokyo. Japan) in a final volume of 25 µl (Kondo et al., 2007).

2.5.1.1.3 PCR cycle

The PCR program for met1 and met2 primers started with an initial denaturation step at 94℃ for 5 min followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 52℃ for 30 s, and extension at 72℃ for 1 min, ending with a final extension step at 72℃ for 10 min and followed by a hold at 4℃ (Hanssen et al., 2004). For mA primers, the PCR program began with an initial denaturation step at 94℃ for 2 min followed by 30 cycles of denaturation at 94℃ for 2 min, annealing at 57℃ for 1 min, and extension at 72℃ for 2 min; and a final elongation step at 72℃ for 2 min (Kondo et al., 2007).

2.5.1.2 mecA gene determination by Southern blotting

Southern blotting was performed for several isolates recovered from study to confirm the presence of *mecA* gene.

2.5.1.2.1 Genomic DNA extraction

Fresh culture was prepared on nutrient agar (Oxoid Basingstoke, UK) for 24 hours. DNA extraction was undertaken using the DNA extraction kit (Qiagen, Crawley, UK) according to manufacturer's instruction. The colonies of 2 to 4 plates were collected for one extraction column to obtain a high concentration of DNA.

2.5.1.2.2 DNA Probe

mecA gene primers (mA1 and mA2) were used to amplify the DNA probe for Southern blotting. The probe was amplified by PCR DIG probe synthesis kit with some minor modification (Roche, F. Hoffmann-La Roche Ltd). 50 µl PCR mixture tube contains: 0.25 µl of each primer, 5 µl 10×PCR buffer with $MgCl_2$, 5 µl 10×PCR DIG probe synthesis mix, 0.75 µl enzyme mix, approximately 10 ng template DNA , 3.4 µl $MgCl_2$ solution. The PCR program was as followings: denaturation at 94℃ for 5 min, 30

cycles of denaturation at 94℃ for 1 min, annealing at 50℃ for 1 min, extension at 72℃ for 2 min, and final extension was 72℃ for 10 min.

2.5.1.2.3 DNA digestion

The digestion of genomic DNA was carried out with restriction enzyme *Cla I* (5000U ml^{-1}) (Biolabs, New England) according to manufacturer's instruction with minor modification. The digestion system contained 2 µg genomic DNA, 5 µl buffer 4, 2 µl *Cla I* (5000 U ml^{-1}), BSA 0.5 µl and dH_2O 12.5 µl. The digestion was performed at 37℃ for 1 hour.

2.5.1.2.4 Gel electrophoresis

After running the gel at 100 V for 10 min, the voltage has been reduced to 36 V and left for 7 hours. An image of the gel was taken, and band size comparison was visualised by placing a ruler parallel to the length of the gel. Clear bands are indicative of complete digestion.

2.5.1.2.5 DNA Transfer

Firstly, the gel was bathed in depurination solution for 15 min, and then washed by dH_2O. Secondly, the gel was bathed in the denaturation buffer for 30 min, and washed by dH_2O. Finally, the gel was bathed in neutralisation buffer for 1 hour, and washed in dH_2O.

One layer of Whatman 3 mm paper was placed on the large plastic gel tray, with two sides in 20×SSC solution. The paper was soaked by 20×SSC, and a stripette was used to roll out any air bubbles. The paper was then topped by one layer of smaller Whatman 3 mm paper, and again the smaller Whatman 3mm paper was soaked in 20×SSC. Air bubbles in the smaller Whatman 3mm paper were rolled out. The agarose gel containing the genomic DNA digests was placed on top, soaked by 20×SSC, and then lined edges

by cling film. A waterproof seal was formed around it, so that all the 20×SSC was drawn up through only the gel and membrane. An Amersham Hybond-N Nylon membrane (GE Healthcare, UK) was lined up with the gel, and the right corner of the nylon membrane was cut for labelling. The nylon membrane was placed on the gel, soaked by 20×SSC and air bubbles were rolled out from nylon membrane. The nylon membrane was then topped with one gel sized Whatman 3 mm paper, again soaked by 20×SSC and air bubbles was rolled out from Whatman 3 mm paper. A packet of handtowels was piled up on the gel sized Whatman 3 mm paper, and topped by a plastic lid and weight. Two hours later, 20×SSC soaking through the gel and membrane into the tissue was checked and left overnight.

2.5.1.2.6 DNA hybridization

Pre-hybridization solution was warmed up to 68℃ and sonicated fish sperm DNA was thawed. The membrane was disassembled, and transferred into the UV crosslinker. Membrane DNA-side up was fixed by UV crosslinker, and the membrane was then soaked in 25 ml pre-hybridization solution mixed with 0.2 mg ml^{-1} sonicated fish sperm DNA, and incubated in a sealed box at 68℃ for 6 hours. The *mecA* gene probe was denatured at 80℃ for 15 min, and 30 µl of the probe was then added into the pre-hybridization solution to make hybridization solution. The membrane was soaked in hybridization solution, and incubated overnight in a sealed the box. The membrane was washed in 2×SSC, 0.2% (w/v) SDS solutions at room temperature for 15 min, and then washed in 0.2×SSC, 0.2% (w/v) SDS solutions at room temperature for 15 min.

After hybridization and stringency washes, the membrane was incubated in 100 ml blocking solution for 30 min at room temperature, and incubated in 20 ml antibody solution (2 µl Anti-Digoxigenin-AP, 20 ml Blocking solution) at room temperature for 30 min. The membrane was washed twice with 100 ml washing buffer, and then

equilibrated in 20 ml detection buffer at room temperature for 2-5 min. Membrane DNA-side up was placed on an opened-up plastic bag and 2 ml diluted CSPD (Appendix I.3) solution was applied on the top. The other side of the plastic bag was folded over the top of the membrane to make the CSPD solution distributed evenly over the membrane surface, and the edges around the membrane were sealed by the plastic sealer. The sealed membrane was kept in the cassette to avoid light damage, and incubated at 37℃ for 15 min. The membrane was exposed to X-ray film for 10 min, and the film was developed. First, the film was bathed in developer solution until the image appears, and then rinsed with water. Then, the film was bathed in fixer solution for 30 s, and rinsed with water again. Finally, the film was hung up to dry.

2.5.2 *mecC* gene detection

The presence of *mecC* gene was detected in oxacillin resistant staphylococcal isolates, and one pair of primers was used in this study.

2.5.2.1 Primers

*mecC*_Uni_F: GGATCTGGTACAGCATTACAACC, *mecC*_Uni_R: TGCTTTAAATC RATMTTGCCG was used to determine the *mecC* gene, which gives a 332 bp product (García-Álvarez et al., 2011).

2.5.2.2 PCR reaction system

The PCR was carried out by method used by García-Álvarez et al., (2011). A 25 µl PCR reaction was conducted containing 12.5 µl Phusion® High-Fidelity PCR Master Mix with HF Buffer (20 units ml^{-1} Phusion® High-Fidelity DNA Polymerase, 0.2 mM each dNTP, 1× Phusion® High-Fidelity Buffer) (NEB, UK), 4 mM $MgCl_2$, 0.15 mM KCl, 15 mM Tris, 4 µM of each primer, and approximately 10 ng of DNA template.

2.5.2.3 Cycling scheme for PCR

The cycling programme starts with a denaturation step at 94℃ for 4 min, and 32 cycles of denaturation for 45 s at 94℃, annealing for 45 s at 60℃, and extension for 45 s at 72℃, with a final extension step 72℃ for 5 min (García-Álvarez et al., 2011).

2.5.3 SCC*mec* typing

SCC*mec* is a mobile genetic island with two essential components, *mec* complex and *ccr* complex (IWG-SCC, 2009). Eleven SCC*mec* types have been identified to date. Typing was based on the combination of *mec* complex and *ccr* complex (IWG-SCC, 2009). SCC*mec* types I to IX (except VII) were tested in this study.

2.5.3.1 Primers

The SCC*mec* M-PCR typing assay contains 8 pairs of primers including the unique and specific primers for SCC*mec* types and subtypes I, II, III, IVa, IVb, IVc, IVd and V (Zhang et al., 2005). Primers for SCC*mec* types and subtypes were as followings: I (Type I-F, Type I-R), II (Type II-F, Type II-R), III (Type III-F, Type III-R), IVa (Type IVa-F, Type IVa-R), IVb (Type IVb-F, Type IVb-R), IVc (Type IVc-F, Type IVc-R), IVd (Type IVd-F, Type IVd-R) and V (Type V-F, Type V-R) (Table 2.2).

Another approach used for SCC*mec* typing was determined by the combination of *mec* complex and *ccr* complex types. Multiplex PCR was used to determine *mec* complex: class A *mec* (mI6, mA7), class B *mec* (IS7, mA7), and class C *mec* (IS2, mA7). Multiplex PCR was also used for *ccr* complex: type 1 *ccr* (α1, βc); type 2 *ccr* (α2, βc); and type 3 *ccr* (α3, βc). Single target PCR was applied to type 4 *ccr* (α4.2, β4.2) and type 5 *ccr* (γF, γR). These primers and their respective concentrations used in the PCR were listed in Table 2.2.

The specific concentration of each primer can be explained by the previously described protocols (Kondo's et al., 2007; Zhang et al., 2005), as they were designed for Multiplex PCR.

Table 2. 2 Primers for SCC*mec* typing

primer	Oligonucleotide sequence(5'-3')	Concentration (μM)	Amplicon Size (bp)	Specificity	Reference
Type I-F	GCTTTAAAGAGTGTCGTTACAGG	0.048	613	SCC*mec* I	Zhang et al., 2005
Type I-R	GTTCTCTCATAGTATGACGTCC	0.048			
Type II-F	CGTTGAAGATGATGAAGCG	0.032	398	SCC*mec* II	Zhang et al., 2005
Type II-R	CGAAATCAATGGTTAATGGACC	0.032			
Type III-F	CCATATTGTAGTACGATGCG	0.04	280	SCC*mec* III	Zhang et al., 2005
Type III-R	CCTTAGTTGTCGTAACAGATCG	0.04			
Type IVa-F	GCCTTATTCGAAGAAACCG	0.104	776	SCC*mec* IVa	Zhang et al., 2005
Type IVa-R	CTACTCTTCTGAAAAGCGTCG	0.104			
Type IVb-F	TCTGGAATTACTTCAGCTGC	0.092	493	SCC*mec* IVb	Zhang et al., 2005
Type IVb-R	AAACAATATTGCTCTCCCTC	0.092			
Type IVc-F	ACAATATTTGTATTATCGGGAGAGC	0.078	200	SCC*mec* IVc	Zhang et al., 2005
Type IVc-R	TTGGTATGAGGTATTGCTGG	0.078			
Type IVd-F	CTCAAAATACGGACCCCAATACA	0.28	881	SCC*mec* IVd	Zhang et al., 2005
Type IVd-R	TGCTCCAGTAATTGCTAAAG	0.28			
Type V-F	GAACATTGTTACTTAAATGAGCG	0.06	325	SCC*mec* V	Zhang et al., 2005
Type V-R	TGAAAGTTTGTACCCTTGACACC	0.06			
mI6	CATAACTTCCCATTCTGCAGATG	0.08	1963	Class A *mec*	Kondo et al., 2007
mA7	ATATACCAAACCCGACAACTACA	0.08			
IS7	ATGCTTAATGATAGCATCCGAATG	0.08	2827	Class B *mec*	Kondo et al., 2007
mA7	ATATACCAAACCCGACAACTACA	0.08			
IS2	TGAGGTTCAGATATTTCGATGT	0.08	804	Class C *mec*	Kondo et al., 2007
mA7	ATATACCAAACCCGACAACTACA	0.08			
βc	ATTGCCTTGATAATAGCCITCT	0.08	695	Type 1 *ccr*	Kondo et al., 2007

primer	Oligonucleotide sequence(5'-3')	Concentration (μM)	Amplicon Size (bp)	Specificity	Reference
α1	AACCTATATCATCAATCAGTACGT	0.08			
Bc	ATTGCCTTGATAATAGCCITCT	0.08	937	Type 2 *ccr*	Kondo et al., 2007
α2	TAAAGGCATCAATGCACAAACACT	0.08			
βc	ATTGCCTTGATAATAGCCITCT	0.08	1791	Type 3 *ccr*	Kondo et al., 2007
α3	AGCTCAAAAGCAAGCAATAGAAT	0.08			
α4.2	GTATCAATGCACCAGAACTT	0.08	1287	Type 4 *ccr*	Kondo et al., 2007
β4.2	TTGCGACTCTCTTGGCGTTT	0.08			
γR	CCTTTATAGACTGGATTATTCAAAATAT	0.08	518	Type 5 *ccr*	Kondo et al., 2007
γF	CGTCTATTACAAGATGTTAAGGATAAT	0.08			

2.5.3.2 PCR reaction system

For Zhang et al's method, PCR mixture contains 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate (dATP, dUTP, dGTP, and dCTP) (Fisher Scientific UK LTD), various concentration of the respective primers (Table 2.2), approximately 10 ng template DNA, 1× Phusion® High-Fidelity Buffer and 20 unit ml^{-1} of Phusion® High-Fidelity DNA Polymerase (NEB, UK) (Zhang et al., 2005).

For Kondo et al's method, the reaction mixture contain 10 ng chromosomal DNA, 0.1 µM primers, 0.2 mM each dNTP, 3.2 mM $MgCl_2$. 20 unit ml^{-1} of Phusion® High-Fidelity DNA Polymerase and 1× Phusion® High-Fidelity Buffer (Kondo et al., 2007).

2.5.3.3 Cycling scheme for PCR

The amplification for SCC*mec* types and SCC*mec* subtypes I, II, III, IVa, IVb, IVc, IVd and V was performed in a $T100^T$ Thermal cycler (Bio-rad, UK) beginning with an initial denaturation step at 94℃ for 5 min followed by 10 cycles of denaturation at 94℃ for 45 s, annealing at 65℃ for 45 s, and extension at 72℃ for 1.5 min. Another 25 cycles of denaturation at 94℃ for 45 s, annealing at 55℃ for 45 s, and extension at 72℃ for 1.5 min, ending with a final extension step at 72℃ for 10 min and followed by a hold at 4℃ (Zhang et al., 2005).

For *mec* complex and *ccr* complex, PCR was run with $T100^T$ Thermal cycler (Bio-rad, UK) beginning with an initial denaturation step at 94℃ for 2 min followed by 30 cycles of denaturation at 94℃ for 2 min, annealing at 57℃ for 1 min, and extension at 72℃ for 2 min; and a final elongation step at 72℃ for 2 min (Kondo et al., 2007).

2.5.4 Multi-locus sequence typing of *S. epidermidis*

Multi-locus sequence typing (MLST) determines the relationship of the isolates with international reported types using the DNA sequences of housekeeping genes (Thomas et al., 2007). In this study, MLST types were assigned to *S. epidermidis*.

2.5.4.1 primers

The primers used for Multi-locus sequence typing (MLST) of *S. epidermidis* are as followings: *arcC, aroE, gtr, mutS, pyr, tpi, yqil* (Table 2.3).

Table 2. 3 Primers for MLST of *S. epidermidis*

Gene	Primer	Sequence(5'-3')
Carbamate Kinase (*arcC*)	*arcC* F	TGTGATGAGCACGCTACCGTTAG
	arcC R	TCCAAGTAAACCCATCGGTCTG
Shikimate dehydrogenase (*aroE*)	*aroE* F	CATTGGATTACCTCTTTGTTCAGC
	aroE R	CAAGCGAAATCTGTTGGGG
ABC transporter (*gtr*)	*gtr* F	CAGCCAATTCTTTTATGACTTTT
	gtr R	GTGATTAAAGGTATTGATTTGAAT
DNA mismatch repair protein (*muts*)	*muts* F	GATATAAGAATAAGGGTTGTGAA
	m*uts* R	GTAATCGTCTCAGTTATCATGTT
Pyrimidine operon regulatory protein (*pyrR*)	*pyr* F	GTTACTAATACTTTTGCTGTGTTT
	pyr R	GTAGAATGTAAAGAGACTAAAATGAA
Triosephosphate isomerase (*tpiA*)	*tpi* F	ATCCAATTAGACGCTTTAGTAAC
	tpi R	TTAATGATGCGCCACCTACA
Acetyl coenzyme A acetyltransferase (*yqiL*)	*yqiL* F	CACGCATAGTATTAGCTGAAG
	yqiL R	CTAATGCCTTCATCTTGAGAAATAA

2.5.4.2 PCR reaction system

PCR was prepared according to the unified scheme of Thomas et al., (2007), Wisplinghoff et al., (2003) and Wang et al., (2003). PCR were carried out with the standard PCR mixture with Phusion® High-Fidelity PCR Master Mix with HF Buffer (NEB, UK), which contains 20 units ml^{-1} Phusion® High-Fidelity DNA Polymerase, 0.2 mM each dNTP, 1× Phusion® High-Fidelity Buffer, 1.5 mM $MgCl_2$. 0.8 µM of each primer and approximately 10 ng template DNA in a final volume of 25 µl.

2.5.4.3 Cycling scheme for PCR

PCR involved an initial denaturation of 95℃ for 3 min; 34 cycles of denaturation at 95℃ for 30 s, annealing at 50℃ for 1 min, and extension at 72℃ for 1 min; with a final extension of 72℃ for 10 min (Thomas et al., 2007; Wang et al., 2003; Wisplinghoff et al., 2003).

2.6 Whole genomic sequence

2.6.1 Identification of *S. epidermidis* 118 (G6_2)

The *S. epidermidis* 118 (G6_2) was recovered from hotel (DSH) in Oct 2012 in London, UK.

Preliminary identification was achieved by using Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (Microflex LT, MALDI-TOF MS, Bruker Daltonics, Coventry, UK) as described previously (Mkrtchyan et al., 2013).

Genomic DNA of *S. epidermidis* 118 (G6_2) was prepared using a QIAamp DNA extraction kit (Qiagen, Crawley, UK). 16S rRNA gene sequencing was performed as described previously (Okazaki et al., 2009), and amplified PCR products were

sequenced by Eurofins MWG operon (Eurofins Genomics, i54 Business Park, Valiant Way, Wolverhampton, UK).

2.6.2 General test of *S. epidermidis* 118 (G6_2)

Genomic DNA was extracted with the DNA extraction kit (Qiagen, Crawley, UK). *mecA* gene detection was determined by methods described by Hanssen et al., (2004). SCC*mec* type was carried out by *mec* and *ccr* complexes PCR accordingly (Kondo et al., 2007).

The antibiotic susceptibility of *S. epidermidis* 118 (G6_2) was tested against 13 antibiotics using disk diffusion methods (Mast Group, Merseyside, UK)， including oxacillin (1 µg), vancomycin (5 µg), gentamicin (10 µg), mupirocin (20 µg), amoxicillin (10 µg), erythromycin (5 µg), tetracycline (10 µg), streptomycin (10 µg), cefepime (30 µg) , fusidic acid (10 µg), penicillin G (1 unit), cefoxitin (10 µg), and chloramphenicol (30 µg). In addition, the minimum inhibitory concentration (MIC) of the isolate to oxacillin was determined using ‘‘M.I.C. evaluators’’ (Oxoid Ltd, Basingstoke, UK).

2.6.3 Whole genomic sequence assembly and comparative genomics

A draft genome sequence of *S. epidermidis* 118 (G6_2) was produced using HiSeq 2000 technology. Genomic DNA was extracted using the MasterPure™ Gram Positive DNA Purification Kit (Cambio, Dry Drayton, UK) from overnight cultures grown from single colonies in 5 ml of tryptic soy broth overnight at 37℃. Illumina library preparation was carried out as described previously (Quail et al., 2008), and genome sequencing using Hi-Seq 2000 performed following the manufacturer’s standard protocols (Illumina, Little Chesterfield, UK). The whole genome sequence was performed by Dr Gavin K Paterson (University of Hull).

The raw fastq data was retrieved and quality trimmed using Trimmomatic (version 0.35) with default settings, specifying a phred cutoff of Q20. Read quality was assessed using FastQC. The Kraken (version 0.10.5-beta) metagenomic pipeline, KronaTools Metagenomics App (version 2.5) was used to assess library purity, that is, it was not a mixed sample and validate that the species was *S. epidermidis*. *De novo* assemblies were performed using SPAdes Genomic Assembler (version 3.5.0), default PE settings, from which only contigs greater than 500 bp in length were taken for further analysis. Using the program, Andi (version 0.9.4-beta) the *de novo* assembled 118 (G6_2) genome along with 92 assembled staphylococcal genomes were aligned, clustered and visualized using PHYLIP and FigTree. Annotations were performed using the pipeline Prokka (version 1.11). The resultant annotated genome was used for all subsequent comparative genomic studies; including BLAST based genome comparisons visualized using the Blast Ring Image Generator (version 0.95) and Mauve (version snapshot 2015-02-13). The Presence/absence of genes was assessed using the Roary pipeline (version 3.4.2). BlastP was used to identify potential virulence factors, as defined by the VFDB (http://www.mgc.ac.cn/VFs/) and PHI-base (http://www.phi-base.org/about.php), whereby a cut off of ≥ 50% sequence identity over ≥ 50% of the total alignment percentage (= (alignment length/query length)*100) to help determine sequence homology.

2.6.4 Reference strains

Reference strains for comparative genomic analysis include methicillin resistant *S. aureus* N315 (ASM964v1), biofilm forming *S. epidermidis* RP62a (ASM1192v1), and non-biofilm forming *S. epidermidis* ATCC12228 (ASM764v1).

2.6.5 Nucleotide sequence accession numbers

Reads for *S. epidermidis* 118 (G6_2) was stored in European Bioinformatics Institute (EMBL-EBI).

2.6.6 Antibiotic resistance gene determination

The whole genomic sequences were uploaded to the ResFinder 2.1, which is one of the services provided by Center for Genomic Epidemiology (https://cge.cbs.dtu.dk /services/ResFinder/). The resistance genes were displayed on the website, with similarity to reference genes, and location sites in the genome.

2.7 *mecA* gene transfer via broth mating experiment

In order to find out the possibility of *mecA* gene transfer from environmental CoNS to *S. aureus in vitro,* mating experiment was performed.

2.7.1 Cell stock

The microorganisms used in mating experiment were listed in Table 2.4.

Table 2. 4 Organisms used in mating experiment

Organism name	Source
S. aureus NCTC 6571	Oxford University
S. hominis 399	Environmental culture collection

2.7.2 Mating experiment

Broth mating experiments were performed in triplicate using *mecA* gene positive *S. hominis* 399 as donor and *mecA* gene negative *S aureus* NCTC 6571 as a recipient. For conjugation, 1 ml of overnight culture of donor and 2 ml of the recipient were mixed and inoculated in 5 ml NB (Oxoid Ltd, Basingstoke, UK) and incubate for 18 h at 37℃ with gentle shaking. After incubation, 100 µl of the culture were spread on a MSA (Oxoid Ltd, Basingstoke, UK) supplemented with the selective agent oxacillin (4 mg l^{-1})

(Oxoid Ltd, Basingstoke, UK) and incubated at 37℃. The growth of colonies was detected after 24-48 h.

2.7.3 Prolex™ staph XTRA latex tests

The transconjugants were then identified by Prolex™ Staph XTRA Latex system (Prolab Diagnostics, Neston, South Wirral, UK) following the manufacturer's instruction.

One drop of the Prolex™ Staph XTRA Latex Reagent was dispensed on the test card, mixed with several colonies, and the agglutination/non-agglutination can be observed in 20 sec. A negative control was tested at the same time.

For positive results, a high level of agglutination can be observed within 20 sec; and no visible agglutination is indicative of negative results.

2.7.4 MIC oxacillin tests

The minimum inhibitory concentration (MIC) to oxacillin were evaluated using ''M.I.C. evaluators'' (Oxoid Ltd., Basingstoke, UK). Antimicrobial gradient method towards oxacillin was applied to transconjugant and recipient. Test methods and interpretation were undertaken according to manufacturer's instruction. Iso-sensitest agar overlaid with fresh culture was prepared for testing. MIC strips contain oxacillin was put on the surface of agar plates. After overnight incubation, the results were interpreted by the ellipse of inhibition area from the upper side of the plate.

2.7.5 Pulse-field gel electrophoresis

In addition to 16S rRNA gene sequencing of transconjugant, the pulse-field gel electrophoresis (PFGE) was applied to determine the genetic pattern of donor, recipient and transconjugant.

2.7.5.1 Preparation of genomic DNA digestion samples

The bacteria were pelleted and washed in PBS, before being re-suspended in 1% Low Melting Point Agarose (BioRad, UK) in buffer (10 mM Tris pH 8.0, 1 M NaCl) at a density of 10^8 cells per ml held at 42°C, prior to being dispensed into a mould (BioRad, UK) to form suitable DNA blocks. These were allowed to set on ice for 30 minutes before being transferred into a digestion buffer consisting of 3% (w/v) sarkosyl, 0.5 M EDTA and 100 μg ml^{-1} of proteinase K. Samples were incubated at 56°C for 24 hours before being stored at 4°C until use. DNA blocks were loaded onto a 1% (w/v) agarose gel and sealed using 1% (w/v) Low melting point agarose before being loaded onto the CHEF Mapper II system (BioRad, UK).

2.7.5.2 PFGE program

A phage λ ladder (48.5 kb-1,000 kb) was used as a marker. To separate chromosomal DNA from 200 kbp to 2.2 Mbp, a 1% (w/v) agarose gel was run in 0.5 × TBE at 12°C, at a gradient of 6 V cm^{-1} with the angle of 120 degree in a linear fashion for a total of 30 hours.

2.7.5.3 Gel screening

Following the electrophoresis, the gel was removed and stained a solution 1 mg ml^{-1} ethidium bromide in 1 × TAE electrophoresis buffer for 30 minutes before visualizing on a UV transilluminator (Syngene, Cambridge, UK).

The PFGE was performed by Dr Bruno Pichon (Public Health England, London, UK).

2.8 Proteomic analysis

Recepient and transconjugant were selected to do comparative proteomic analysis with LC-MS/MS, and the method follows the research procedure of Applied and Functional Genomics department, Public Health England.

2.8.1 Protein extraction

The cell lysate was produced by using the 'glass beads' method (Dekio et al., 2013). A full loop of fresh culture was collected and then transferred into a 2 ml vial with 150 µl lysis buffer (0.5 M sucrose, 20 mM maleic acid, pH 6.5, 20 mM $MgCl_2$, 6 mg ml^{-1} lysozyme, and 1 mM PMSF). After mixed thoroughly, 1 g glass beads (Sigma-Aldrich, UK) were added to the mixture. The highest setting of the vortex mixer was used, and the suspension was vortex mixed 3 - 5 times for 1 min. Each time the cells were kept on ice for 1 minute between vortex mixes.

After mixing, the cells were pelleted by centrifugation at 21000 g for 20 min. The supernatant were transferred into 1.5 ml eppendorf tubes, and kept at -20℃ until further use.

2.8.2 Protein concentration

Protein concentration was determined by Bradford assay (Bio-Rad, USA). BSA was used as standard, and the protein standard curve was obtained from seven concentration of BSA (0.05 mg ml^{-1}, 0.1 mg ml^{-1}, 0.2 mg ml^{-1}, 0.4 mg ml^{-1}, 0.6 mg ml^{-1}, 0.8 mg ml^{-1} and 1 mg ml^{-1}). Each sample was diluted 10 times, and 20 µl of cell lysate was mixed with 180 µl Bradford reagent (Sigma-Aldrich, UK). The absorbance at 595 nm of each protein sample was detected as duplicates in a 96 well plates using the FLUOstar Omega Microplate Reader (BMG LABTECH, Offenburg, Germany). The concentration was calculated using the FLUOstar Omega evaluation software (BMG LABTECH, Offenburg, Germany). Pre-set Bradford templates can be used to do the calculations.

2.8.3 One-dimensional SDS-PAGE

10 μg protein of each sample was loaded on a 10% (w/v) Bis–Tris gel (Invitrogen, UK), and protein was separated at a voltage of 180 V for 30 min using MES running buffer (Invitrogen, UK) in accordance with the manufacturer's instructions.

2.8.4 In-gel tryptic digestion of protein for LC-MS/MS

The gel was stained by colloidal Coomassie (Sigma-Aldrich, UK). Each gel lane was cut into 12 pieces and bands were placed in wells in a 96 well plate accordingly. The stain was washed by 50% (v/v) methanol (Fisher Scientific, UK) for 2 ×20 min, and then left in 50% (v/v) methanol overnight for thorough de-staining. Dehydration was achieved by soaking in 100% (v/v) acetonitrile (Fisher Scientific, UK) for 10 min and dried for 5 min, 10 mM DTT (GE Healthcare, UK) was used for reduction, and 55 mM iodoacetamide was added for alkylation. Dehydrated gels were incubated with10 ng μl^{-1} porcine trypsin (modified sequencing grade; Promega, USA) for 16 h at 37℃, and peptides were extracted by addition of 2% (v/v) acetonitrile (Fisher Scientific, UK) and 0.1% (v/v) trifluoroacetic acid (TFA) (Sigma-Aldrich, UK) for 1 h. After centrifugation, the supernatant were collected and stored at −80℃ for further use.

2.8.5 LC-MS/MS analysis of tryptic peptides

2.8.5.1 EASY-nLC

The proteolytic digests of the protein extracts were further separated by a split-free EASY-nLC 1000 liquid chromatograph system (Thermo Scientific, UK) and analysed by Thermo LTQ Orbitrap Classic mass spectrometers (Thermo Electron, Bremen, Germany). Peptide mixtures were initially trapped and desalted on a reversed phase trap column (C18, 300 μm i.d. × 3 mm, Thermo Scientific., UK) and further separated on an analytical reversed-phase (RP) nano column (C18, 3 μm particle size, 75 μm i.d. × 15 cm, Thermo Scientific., UK). Separation were achieved using a 38-minute linear

gradient of 4 to 45% solvent B (99.9% CH3CN/0.1% formic acid, v/v) versus solvent A (99.9% H_2O/0.1% formic acid, v/v), then to 90% B and held at 90% B for an additional 9 mins, at a flow rate of 300 nl min^{-1}.

2.8.5.2 LTQ Orbitrap

MS/MS experiments were performed on the Thermo Finnigan Orbitrap Classic mass spectrometer (Thermo Electron, Bremen, Germany) equipped with a nanospray ionization source and a Stainless Steel Emitters (length 105 mm, with sleeve O.D. 360 µm). The mass spectrometer was operated in positive mode. Helium was used as collision gas but no sheath and auxiliary gas were applied. Full MS scans were acquired in the Orbitrap mass analyzer over the m/z 350–2000 range with resolution 60,000 (m/z 400). The target value was 5.00E+05. The twenty most intense peaks with charge state ≥ 2 were selected for sequencing and fragmented in the ion trap with normalized collision energy of 35%, activation q = 0.25, activation time of 10 ms, and one microscan. The target value was 1.00E+04. The ion selection threshold was 500 counts, and the maximum allowed ion accumulation times were 500 ms for full scans and 100 ms for collision-induced dissociation (CID). Tandem MS (MS/MS) data was acquired in 'data-dependent' mode. The six most abundant peptide precursor ions detected in the preceding survey scan were dynamically selected and subjected for CID in the linear ion trap to generate MS/MS spectra. Samples were analysed as biological triplicates.

2.8.5.3 Searching database

Peptide identification was performed using Proteome Discoverer (version 1.4.1; Thermo Scientific) against staphylococcal database downloaded from Uniprot. The workflow consisting of the following nodes (and respective parameters): Spectrum Selector for spectra pre-processing (precursor mass range: 350–5000 Da; S/N Threshold: 1.5), Sequest-HT as search engine (Protein Database: see below; Enzyme: Trypsin; Max.

missed cleavage sites: 2; Peptide length range 6–144 amino acids; Max. Delta Cn: 0.05; Precursor mass tolerance of 10.0 ppm; Fragment mass tolerance of 0.60 Da; Static modification: cysteine carbamidomethylation; Dynamic modification: methionine oxidation), and percolator for peptide validation (FDR<1 % based on peptide q-value). EASY-nLC, LTQ Orbitrap, and searching database were performed by Dr Min Fang (Public Health England, London, UK).

2.9 Bioinformatic analysis

2.9.1 *mecA* and *mecC* gene

mecA and *mecC* gene PCR product in the gel were analysed by Syngene software (Syngene, Cambridge, UK), and band matching was performed by position to determine the size of PCR product.

2.9.2 SCC*mec* typing

PCR products of SCC*mec* typing were also analysed by band matching tool to confirm the size (Syngene, Cambridge, UK).

According to Zhang's SCC*mec* typing method, SCC*mec* type I to V was determined by the size of PCR products directly (Zhang et al., 2005).

SCC*mec* typing method introduced by Kondo et al., (2007) was based on the combination of *ccr* complex and *mec* complex. The types were determined using the guidelines proposed by the International Working Group on the Staphylococcal Cassette Chromosome elements (IWG-SCC, 2009) (http://www.sccmec.org/Pages/SCC_Classi-ficationEN.html).

2.9.3 BioNumerics analysis

In this study, BioNumerics 7.5 (Applied Maths, Belgium) was employed to analyse MALDI-TOF MS data of staphylococcal species. In order to improve the quality and

reliability of cluster analysis, isolates of each site with high quality spectra were chosen to do cluster analysis.

2.9.3.1 Taxonomic analysis of selected staphylococci

MALDI-TOF MS raw data were imported into BioNumerics 7.5 (Applied Maths, Belgium) software. The dendrogram tree was built by clicking 'Clustering>Calculate> Advance cluster analysis' in the comparison window. Topscore UPGMA was chosen to build a standard dendrogram tree, and the radial tree was generated by click 'Edit>Remove root'.

Three-dimensional (3D) scatter plots were built by clicking 'Clustering>Calculate> Cluster analysis (similarity matrix)', and comparison setting was based on 'pearson correlation>UPGMA method'. A standard dendrogram tree was built, and 3D image was generated by click 'Multi-dimensional scaling>Use metric algorithm'.

2.9.3.2 Taxonomic analysis of staphylococci based on isolation sites

MALDI-TOF mass spectrometry of isolates recovered from different sites were taxonomically analysed by BioNumerics 7.5 (Applied Maths, Belgium) software package. In order to differentiate each sample collection site, different colours were used, each of which indicates a specific site. The dendrogram tree and 3D images were built based on UPGMA method according to the BioNumerics 7.5 manual (Applied Math, Belgium).

2.9.3.3 Taxonomic analysis of staphylococci based on Antibiotic susceptibility

All selected isolates were additionally grouped based on their antibiotic susceptibility profiles. In these groups, red colour was selected to demonstrate the presence of multiple resistant (resistance to two or more antibiotics) staphylococci and green colour was selected for susceptible staphylococci isolates.

2.9.4 Multi-locus sequence typing

Each housekeeping gene locus sequence was uploaded into the MLST database (http://www.mlst.net/) to obtain a single locus type. All 7 single locus types were then combined to query for the match in the database (http://www.mlst.net/). New MLST types are required be sent to the *S. epidermidis* curator Dr Maria Miragaia via email (miragaia@itqb.unl.pt) for their assigning.

Chapter 3 Isolation, purification of environmental Staphylococci

Staphylococci on human skin, have been responsible for wide range of infections: from minor skin infection to life threatening toxic shock syndrome (Monecke et al., 2011). Various virulence factors and antibiotic resistance genes contribute to increased pathogenicity of staphylococci (Oliveira & Tomasz, 2002). Determination and clarification of the virulence factors and antibiotic resistance require collection of staphylococcal isolates from clinical environment (Oliveira & Tomasz, 2002). Since environmental staphylococci acts as a reservoir of antibiotic resistant determinants for clinical pathogens (Blair et al., 2014), it would be necessary to assess dissemination of antibiotic resistant environmental staphylococci. A large quantity of staphylococci recovered from hands and 8 inanimate sites were included in this study.

3.1 Sample collection

Permission was first granted to gain access and sample inanimate sites and hands of anonymous volunteers all over the London, United Kingdom. The findings for each site were given to each manager/owner for their permission. The sample collection detailed in Table 3.1, consists of the followings: ① 65 samples were collected from the baby care facilities (**BCF**); ② 188 swabs collected from different sites of four hotels (**DSH**); ③ 20 samples were collected from different sites of one public library (**DSL**); ④ 36 samples were collected from different sites of three restaurants (**DSR**); ⑤ a total of 37 samples were collected from different sites of five supermarkets (**DSS**); ⑥ 54 swabs were collected from different sites of transportation facilities (**DST**); ⑦ 12 hotel air samples (**HAS**) were collected from 12 hotel rooms; ⑧ 43 samples were isolated from

anonymous volunteers' handbags (**HB**); ⑨ 124 swabs were sampled from the hands of randomly selected anonymous volunteers (**HH**) (Table 3.1).

In addition, sampling sites of eight inanimate sites and human hands were as followings: ① **BCF** include dummies, soft play, mother's change bags, child car seats and nappy changing area; ② **DSH** includes TV remote controls, mattresses, pillows, duvets, tables, basin surface, lift buttons, hand dryers, water taps, paper dispensers, toilet rims, toilet floor, toilet handles, wardrobe handles, bedside lights, keyboards and room carpet floor; ③ **DSL** were sampled from books; ④ **DSR** include knife handles and fork handles; ⑤ **DSS** include shelves and trolley handles; ⑥ **DST** include seats, hand rail of buses, pelican crossing buttons on Mile End road, between Queen Mary University of London and Royal London hospital; ⑦ **HAS** were sampled from hotel room air; ⑧ **HB** includes anonymous volunteers' handbags; ⑨ **HH** include anonymous volunteers' hands in London area (Table 3.1). All specimens were transferred to the laboratory within 1-3 hrs, of the samples being taken.

Table 3. 1 Samples collected from hands and hands related inanimate sites

Sites	Specific sites	No of swabs	No of isolates
BCF	Dummies Soft play Mother's change bags Child car seats Nappy changing area	65	77
DSH	TV remote control Mattresses Pillows Duvets Tables Basin surface Lift buttons Hand dryers Water taps Paper dispensers Toilet rims Toilet floor Toilet handles Wardrobe handles Bedside lights Keyboards Room carpet floor	188	282
DSL	Books	20	50
DSR	Knife handles Fork handles	36	152
DSS	Shelves Trolley handles	37	176
DST	Pelican crossing buttons Seats Hand rails	54	113
HAS	Inside of hotel rooms	12	30
HB	Handbags	43	64
HH	Hands	124	287

Note: BCF- baby care facilities; DSH- different sites of hotels; DSL- different sites of a library; DSR- different sites of restaurants. DSS- different sites of supermarkets; DST- different sites of transportation facilities; HAS- hotel air samples; HB- handbags; HH- human hands.

3.2 Isolation

After sample collection, a total of 579 samples were plated onto NA (Oxoid Ltd, Basingstoke, UK), MSA (Oxoid Ltd, Basingstoke, UK) and UTI (Oxoid Ltd,

Basingstoke, UK) plates accordingly. The numbers of isolates recovered from each site were as follows: **BCF**: 77 isolates; **DSH**: 282 isolates; **DSL**: 50 isolates; **DSR**: 152 isolates; **DSS**: 176 isolates; **DST**: 113 isolates; **HAS**: 30 isolates; **HB**: 64 isolates; **HH**: 287 isolates (Table 3.1).

The numbers of colonies on NA ranged from none to uncountable. No uncountable results were found in following sites: DSL, DSR, DSS, DST, HAS, HB and HH. However, 40% of DSH samples had uncountable numbers of colonies, and followed by BCF samples (11%).

Mannitol fermented and not-fermented colonies were recovered from all nine sites: BCF, DSH, DSL, DSR, DSS, DST, HAS, HB and HH. The species found at each site differentiated by UTI (Oxoid Ltd, Basingstoke, UK) were as follows: **BCF**: *Enterococcus* spp., *E.coli*, *Proteus, Pseudomonas* spp. staphylococci; **DSH**: *Enterococcus* spp., *E.coli*, *Proteus*, *Pseudomonas* spp., staphylococci; **DSL**: *E.coli*, staphylococci; **DSR**: *Enterococcus* spp., *E.coli*, *Pseudomonas* spp., staphylococci; **DSS**: *Enterococcus* spp., *E.coli*, *Pseudomonas* spp., staphylococci; **DST**: *Enterococcus* spp., *E.coli*, staphylococci; **HAS**: *E.coli*, staphylococci; **HB**: *E.coli*, staphylococci; **HH**: *Enterococcus* spp., *E.coli*, *Pseudomonas* spp., staphylococci (Fig 3.1).

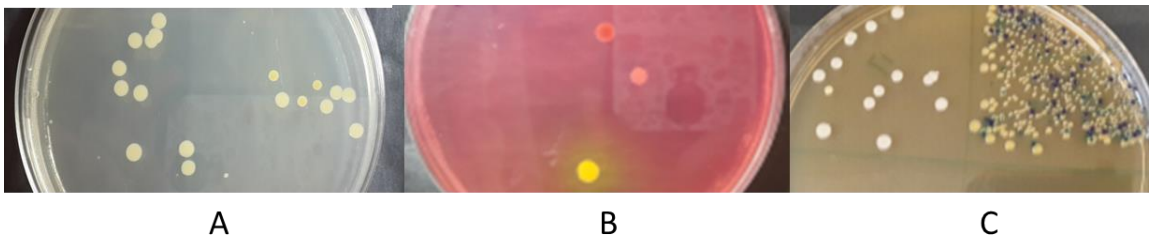


Figure 3. 1 Colonies on NA (A), MSA (B) and UTI (C) plates

3.3 Purification

Following the isolation process, a total of 1231 isolates were purified, including 77 isolates from **BCF**, 282 from **DSH**, 50 from **DSL**, 152 from **DSR**, 176 from **DSS**, 113 from **DST**, and 30 from **HAS**, 64 from **HB**, 287 from **HH** (Table 3.1). 79 out of 1231 isolates were eliminated from further investigation as their morphological characteristics were not consistent with staphylococci.

3.4 Chapter summary

The isolation of environmental bacteria included:

1. Nine sampling sites: BCF, DSH, DSL, DSR, DSS, DST, HAS, HB, HH were included in this study;
2. A total of 1231 isolates recovered from human hands and inanimate sites.

Determination the dissemination of antibiotic resistance requires the collection of staphylococcal isolates. This chapter introduced the collection and isolation of bacteria from human hands and 8 human-related inanimate sites using microbiology techniques. Baby care facilities and handbags are personal items, while hotels, hotel air, library, restaurants, supermarkets and transportation facilities are public settings. The importance of the predominant staphylococcal species of each site will be discussed in the discussion chapter. Evaluation of the sampling sites as reservoirs for antibiotic-resistant staphylococcal isolates will also be discussed in the discussion chapter.

Chapter 4 Identification of environmental staphylococci

The importance of rapid and accurate identification of microorganism has been well characterized (Valentine et al., 2005; Yao et al., 2002). MALDI-TOF MS has been proven to be a reliable and efficient tool for identification of clinical staphylococci (Van Veen et al., 2010). In our study, it was necessary to identify a large number of staphylococci, and MALDI-TOF MS was employed to determine these isolates. The reproducibility of MALDI-TOF MS was assessed in this study. In addition, classical 16S rRNA gene sequencing was employed to evaluate the efficacy of MALDI-TOF MS in identifying environmental staphylococci.

The spread of staphylococci from person to person are mainly via hands, and hands that frequently touch inanimate sites can be important bacterial reservoirs for transmission (Johansson et al., 2007; Mollema et al., 2010). However, little is known about the connection of staphylococci isolated from different sites. Here, I reported taxonomic analysis based on MALDI-TOF MS profile. A total of 411 staphylococci recovered from BCF, DSL, DSH, DSR, DSS, DST, HAS, HB, HH were selected to analyse the possible taxonomic relationship. In addition, the taxonomic relationship of each species was described separately.

4.1 Bacterial identification by MALDI-TOF MS

To determine the species of purified isolates, a total of 1152 isolates representing each of the collection sites were identified by MALDI-TOF MS. The control identification index of the MALDI-TOF MS, Biotyper 3.1 score values were 2.314 to 2.422 (with a mean of 2.371 ± 0.044 S.D) for *Escherichia coli* DH5α. Of the 1152 monomicrobial bacterial cultures, 991 (86%) produced acceptable identification scores of ≥ 1.7 using the MALDI-TOF MS - Biotyper 3.1 identification system. This included 844 (85%)

cultures with high confidence scores of ≥ 2.0 and 147 (15%) cultures with intermediate confidence scores of 1.70 to 1.99. Of 991 monomicrobial cultures, 971 (98%) contained Gram-positive organisms, 19 (2%) contained Gram-negative organisms, and one fungus. Within 991 monomicrobial cultures, 67 species were identified in this study, which, except for one fungus, consisted of 9 Gram-negative species, and 57 Gram-positive species (Fig 4.1).

Gram-positive bacteria: the distribution of confidence scores within the 971 Gram-positive cultures, 14% intermediate (1.7 to 1.99), and 86% high (2.0 to 3.0) (Table 4.1). 718 out of the 971 Gram-positive cultures were identified to be staphylococci, of which 618 produced high confidence scores of ≥ 2.0, at meantime, and 100 produced intermediate confidence scores (Table 4.1). Despite the diversity of Gram-positive species, most of these isolates were identified to be staphylococci. There were 19 staphylococcal species that were identified in this study: *S. arlettae, S. auricularis, S. aureus, S. capitis, S. caprae, S. cohnii, S. epidermidis, S. equorum, S. haemolyticus, S. hominis, S. lugdunensis, S. pasteuri, S. pettenkoferi, S. saprophyticus, S. sciuri, S. simiae, S. simulans, S. warneri,* and *S. xylosus,* which makes one third of all Gram-positive species. (Table 4.1)

Gram-negative bacteria: 11 of 19 (58%) Gram-negative cultures generated high confidence scores and 8 (42%) generated intermediate confidence scores (Table 4.1).

Table 4. 1 MALDI-TOF MS identification of environmental isolates

Organism	No of isolates	Division	MALDI-TOF MS score	
			1.7-1.99[b]	≥2.0[b]
Staphylococcus epidermidis	198	G+	28	170
Staphylococcus hominis	173	G+	19	154
Staphylococcus haemolyticus	79	G+	11	68
Staphylococcus capitis	79	G+	9	70
Staphylococcus warneri	68	G+	11	57
Staphylococcus pasteuri	34	G+	2	32
Staphylococcus saprophyticus	20	G+	5	15
Staphylococcus cohnii	14	G+	3	11
Staphylococcus aureus	12	G+	0	12
Staphylococcus simiae	10	G+	5	5
Staphylococcus sciuri	6	G+	1	5
Staphylococcus pettenkoferi	5	G+	3	2
Staphylococcus lugdunensis	5	G+	0	5
Staphylococcus equorum	3	G+	1	2
Staphylococcus caprae	2	G+	0	2
Staphylococcus xylosus	2	G+	1	1
Staphylococcus auricularis	2	G+	0	2
Staphylococcus simulans	1	G+	0	1
Staphylococcus arlettae	1	G+	0	1
Staphylococcus sp	4	G+	1	3
Acinetobacter lwoffii	4	G-	1	3
Acinetobacter sp	1	G-	1	0
Aerococcus viridans	5	G+	2	3
Alcaligenes faecalis	2	G-	1	1
Bacillus altitudinis	1	G+	1	0
Bacillus cereus	4	G+	3	1
Bacillus cohnii	1	G+	0	1
Bacillus flexus	1	G+	0	1
Bacillus licheniformis	5	G+	2	3
Bacillus megaterium	2	G+	1	1
Bacillus oshimensis	1	G+	1	0
Bacillus pumilus	2	G+	1	1
Bacillus subtilis	5	G+	3	2
Bacillus thuringiensis	1	G+	0	1
Bacillus weihenstephanensis	1	G+	1	0
Brevibacterium casei	5	G+	2	3
Candida parapsilosis	1	F[a]	0	1
Corynebacterium afermentans	3	G+	2	1
Corynebacterium amycolatum	1	G+	1	0
Corynebacterium aurimucosum	7	G+	0	7
Corynebacterium falsenii	1	G+	0	1
Corynebacterium minutissimum	2	G+	0	2
Corynebacterium pseudodiphtheriticum	1	G+	1	0
Corynebacterium striatum	1	G+	0	1
Dermacoccus nishinomiyaensis	3	G+	1	2
Dietzia cinnamea	1	G+	0	1
Enterobacter cloacae	1	G-	0	1

Note: a: F: fungus; b: The match of MALDI-TOF MS identification at the species level with a score value ⩾2.0 and at a genus level with a score value 1.7-1.99.

Organism	No of isolates	Division	MALDI-TOF MS score	
			1.7-1.99[b]	≥2.0[b]
Kocuria carniphila	7	G+	1	6
Kocuria kristinae	13	G+	1	12
Kocuria marina	3	G+	2	1
Kocuria palustris	13	G+	2	11
Kocuria rhizophila	9	G+	2	7
Kocuria rosea	2	G+	0	2
Kocuria sedentarius	2	G+	1	1
Kytococcus schroeteri	3	G+	0	3
Kytococcus sedentarius	3	G+	2	1
Micrococcus luteus	138	G+	4	134
Micrococcus lylae	1	G+	0	1
Micrococcus terreus	1	G+	1	0
Moraxella_sg_Moraxella osloensis	2	G-	1	1
Nesterenkonia lacusekhoensis	1	G+	0	1
Pantoea agglomerans	2	G-	2	0
Proteus mirabilis	5	G-	1	4
Pseudomonas luteola	1	G-	0	1
Pseudomonas oryzihabitans	1	G-	1	0
Rothia amarae	1	G+	0	1
Rothia dentocariosa	2	G+	1	1
Total no. of isolates	991		147	844
% genus			15	-
% species			-	85

Note: a: F: fungus;b: The match of MALDI-TOF MS identification at the species level with a score value ≥2.0 and at a genus level with a score value 1.7-1.99.

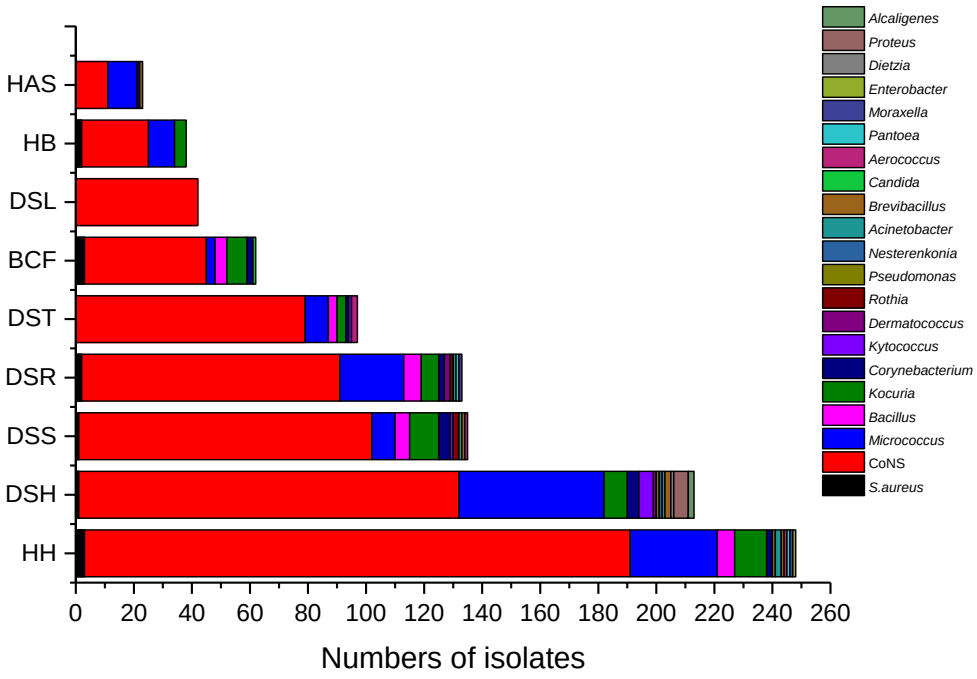


Figure 4. 1 MALDI-TOF MS identification of environmental isolates
BCF: baby care facilities; DSH: different sites of hotels; DSL: different sites of a library; DSR: different sites of restaurants; DSS: different sites of supermarkets; DST: different sites of transportation facilities; HAS: hotel air samples; HB: handbags; HH: human-hands.

4.2 Reproducibility of MALDI-TOF MS

MALDI-TOF MS reproducibility was assessed for environmental isolates. Reproducibility of MALDI-TOF MS identification was determined for 1-3 isolates of 18 species: *S. aureus*, *S. auricularis*, *S. capitis*, *S. caprae*, *S. cohnii*, *S. epidermidis*, *S. equorum*, *S. haemolyticus*, *S.hominis*, *S. lugdunensis*, *S. pasteuri*, *S. pettenkoferi*, *S. saprophyticus*, *S. sciuri*, *S. simiae*, *S. simulans*, *S. warneri*, and *S. xylosus*, and the results were shown in Table 4.2. Duplicates of thirty-four selected isolates were analysed by two different modes of MALDI-TOF MS.

Table 4. 2 Reproducibility of MALDI-TOF MS identification

ID	Bruker Microflex LT	Score value	Bruker Autoflex	Score value
12	*S. aureus*	2.37	*S. aureus*	2.264
12	*S. aureus*	2.442	*S. aureus*	2.28
13	*S. aureus*	2.402	*S. aureus*	2.315
13	*S. aureus*	2.429	*S. aureus*	2.335
15	*S. auricularis*	2.014	*S. auricularis*	1.831
15	*S. auricularis*	1.914	*S. auricularis*	1.857
52	*S. capitis*	2.235	*S. capitis*	2.275
52	*S. capitis*	2.011	*S. capitis*	2.177
53	*S. capitis*	1.926	*S. capitis*	1.994
53	*S. capitis*	1.888	*S. capitis*	1.985
95	*S. caprae*	2.015	*S. caprae*	1.84
95	*S. caprae*	2.149	*S. caprae*	1.983
96	*S. caprae*	1.986	*S. caprae*	1.821
96	*S. caprae*	1.898	*S. capitis*	1.8
107	*S. cohnii*	2.161	*S. cohnii*	2.049
107	*S. cohnii*	2.177	*S. cohnii*	2.165
122	*S. epidermidis*	2.215	*S. epidermidis*	2.216
122	*S. epidermidis*	2.15	*S. epidermidis*	2.154
134	*S. epidermidis*	2.299	*S. epidermidis*	2.135
134	*S. epidermidis*	2.166	*S. epidermidis*	2.098
135	*S. epidermidis*	2.11	*S. epidermidis*	2.246
135	*S. epidermidis*	2.125	*S. epidermidis*	2.235
310	*S. equorum*	1.8	*S. equorum*	1.998
310	*S. equorum*	1.76	*S. equorum*	1.886
311	*S. equorum*	2.041	*S. equorum*	1.86
311	*S. equorum*	2.165	*S. equorum*	1.959
377	*S. haemolyticus*	2.242	*S. haemolyticus*	2.016
377	*S. haemolyticus*	2.114	*S. haemolyticus*	2.04
384	*S. haemolyticus*	1.842	*S. haemolyticus*	1.746
384	*S. haemolyticus*	1.744	*S. haemolyticus*	1.784
385	*S. haemolyticus*	2.189	*S. haemolyticus*	2.102

Note: Isolate in bold suggests inconsistent identification.

ID	Bruker Microflex LT	Score value	Bruker Autoflex	Score value
385	*S. haemolyticus*	2.329	*S. haemolyticus*	2.043
400	*S. hominis*	2.504	*S. hominis*	2.529
400	*S. hominis*	2.137	*S. hominis*	2.22
402	*S. hominis*	2.325	*S. hominis*	2.12
402	*S. hominis*	2.251	*S. hominis*	2.045
564	*S. lugdunensis*	2.29	*S. lugdunensis*	2.183
564	*S. lugdunensis*	2.377	*S. lugdunensis*	2.332
567	*S. lugdunensis*	2.203	*S. lugdunensis*	2.061
567	*S. lugdunensis*	2.284	*S. lugdunensis*	2.282
579	*S. pasteuri*	2.055	*S. pasteuri*	2.054
579	*S. pasteuri*	1.981	*S. pasteuri*	2.049
597	*S. pasteuri*	2.238	*S. pasteuri*	2.069
597	*S. pasteuri*	2.127	*S. pasteuri*	2.087
607	*S. pettenkoferi*	2.01	*S. pettenkoferi*	1.935
607	*S. pettenkoferi*	1.929	*S. pettenkoferi*	2.009
608	*S. saprophyticus*	1.726	*S. saprophyticus*	1.763
608	***S. saprophyticus***	**1.735**	***S. cohnii***	**1.801**
			S. saprophyticus	**1.726**
609	*S. saprophyticus*	1.769	*S. saprophyticus*	1.984
609	*S. saprophyticus*	2.018	*S. saprophyticus*	1.94
632	*S. sciuri*	2.089	*S. sciuri*	2.028
632	*S. sciuri*	2.092	*S. sciuri*	2.03
632	*S. sciuri*	1.97	*S. sciuri*	1.957
632	*S. sciuri*	1.973	*S. sciuri*	2.005
636	*S. simiae*	1.845	*S. simiae*	1.825
636	*S. simiae*	1.996	*S. simiae*	1.922
638	*S. simiae*	2.021	*S. simiae*	1.844
638	*S. simiae*	1.93	*S. simiae*	1.942
644	*S. simulans*	1.837	*S. simulans*	1.826
644	*S. simulans*	1.949	*S. simulans*	1.84
654	*S. warneri*	2.391	*S. warneri*	2.209
654	*S. warneri*	2.48	*S. warneri*	2.112
686	*S. warneri*	2.249	*S. warneri*	2.26
686	*S. warneri*	2.455	*S. warneri*	2.134
704	*S. warneri*	1.977	*S. warneri*	2.175
704	*S. warneri*	1.996	*S. warneri*	2.163
713	*S. xylosus*	1.808	*S. xylosus*	1.997
713	*S. xylosus*	1.865	*S. xylosus*	2.1

Note: Isolate in bold suggests inconsistent identification.

A total of 68 targets were detected by two different modes of MALDI-TOF MS accordingly, and only one target (1.5%) showed an inconsistent result; however, the second match was found to be *S. saprothyticus*. This demonstrates the precision and relibility of the MALDI-TOF MS identification method. The scores generated by both MALDI-TOF MS were close to each other, although minor fluctuations were observed.

The fluctuation was between 0.001 and 0.368: thirty-six (53%) targets with score difference below 0.1, twenty three (34%) between 0.1 and 0.2, seven (10%) between 0.2 and 0.3, and 2 (3%) above 0.3.

Sixty-seven typing results could be confirmed, thus leading to an inter-laboratory reproducibility of 98.5%.

4.3 16S rRNA gene sequencing

To assess the reliability of MALDI-TOF MS identification, 60 isolates of 17 staphylococcal species, including *S. auricularis*, *S. capitis*, *S. caprae*, *S. cohnii*, *S. epidermidis*, *S. equorum*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. pasteuri*, *S. pettenkoferi*, *S. saprophyticus*, *S. sciuri*, *S. simiae*, *S. simulans*, *S. warneri*, and *S. xylosus,* were evaluated by 16S rRNA gene sequencing to compare with the results obtained by MALDI-TOF MS identification. Selected isolates were identified by MALDI-TOF MS, and 55 (92%) isolates were consistent with the results of 16S rRNA measurement. Five misidentified isolates were identified to be staphylococci, but different species.

Thirteen isolates were identified with score values ranging from 2.300 to 3.000 by MALDI-TOF MS, and 13 (100%) were consistent with 16S rRNA identification methods. Thirty-eight isolates were identified with score values ranging from 2.000 to 2.299, and 33 (87%) were concordant with 16S rRNA identification results. Nine isolates with score values ranging from 1.700 to 1.999 were 100% concordant to species with 16S rRNA method.

5 staphylococci strains, including 327, 331, 338, 409, 614, were determined different species by 16S rRNA gene sequencing, all of which produced confidence scores ranging from 2.000 to 2.299. Two of them were misidentified; the first (strain 338) was

identified as *S. aureus* using 16S rRNA and as *S. haemolyticus* using the MALDI-TOF MS. The other (strain 409) was identified as *S. equorum* according to 16S rRNA and as *S. hominis* using the MALDI-TOF MS. For the rest of 3 isolates (strain 327, 331, 614), 16S rRNA indicated possible identification as same as MALDI-TOF MS did, but not as the primary match. (Table 4.3) In general, 16S rRNA results support the MALDI-TOF MS identification. Discordant results were found in 5 isolates at the species level, and no discordant result was found at the genus level. In addition, the data obtained from MALDI–TOF MS method were consistent with the results of 16S rRNA measurement for 92% at the species level and 100% at genus level. Even for 9 isolates identified with score value range from 1.700 to 1.999, the 100% consistence was confirmed at the species level. With regard to turnaround time, it took only 2.5 min to obtain the results with MALDI-TOF MS in this study, which was more efficient than 16S rRNA PCR methods to yield the same results (Table 4.3).

Table 4. 3 Identification results obtained by MALDI–TOF MS in comparison to those obtained by partial 16S rRNA gene sequence-based species identification

ID	MALDI-TOF MS		16S rRNA
	Species	Score value	Species
15	*S. aurialuaris*	2.342	*S. auricularis* (T); ATCC 33753; D83358
52	*S. capitis*	1.821	*S. capitis* (T); L37599
95	*S. caprae*	2.318	*S. caprae* (T); ATCC 35538; AB009935
96	*S. caprae*	2.223	*S. caprae* (T); ATCC 35538; AB009935
99	*S. cohnii*	1.734	*S. cohnii* (T); ATCC 29974; D83361
106	*S. cohnii*	2.114	*S. cohnii* (T); ATCC 29974; D83361
107	*S. cohnii*	2.292	*S. cohnii* (T); ATCC 29974; D83361
120	*S. epidermidis*	2.094	*S. epidermidis* (T); ATCC 14990; D83363
122	*S. epidermidis*	2.293	*S. epidermidis* (T); ATCC 14990; D83363

ID	MALDI-TOF MS		16S rRNA
	Species	Score value	Species
310	*S. equorum*	1.846	*S. equorum* (T); RP29; AF527483
311	*S. equorum*	2.005	*S. equorum* (T); ATCC 43958; AB009939
317	*S. haemolyticus*	2.016	*S. haemolyticus* (T); CCM2737; X66100
318	*S. haemolyticus*	2.163	*S. haemolyticus* (T); CCM2737; X66100
321	*S. haemolyticus*	2.257	*S. haemolyticus* (T); CCM2737; X66100
322	*S. haemolyticus*	2.300	*S. haemolyticus* (T); CCM2737; X66100
323	*S. haemolyticus*	2.279	*S. haemolyticus* (T); CCM2737; X66100
325	*S. haemolyticus*	2.353	*S. haemolyticus* (T); CCM2737; X66100
327	*S. haemolyticus*	2.150	*S. saprophyticus* (T); ATCC 15305; AP008934 *S. haemolyticus* (T); CCM2737; X66100
328	*S. haemolyticus*	2.251	*S. haemolyticus* (T); CCM2737; X66100
329	*S. haemolyticus*	2.239	*S. haemolyticus* (T); CCM2737; X66100
330	*S. haemolyticus*	2.124	*S. haemolyticus* (T); CCM2737; X66100
331	*S. haemolyticus*	2.179	*S. aureus* (T); ATCC 12600; L36472 *S. haemolyticus* (T); CCM2737; X66100
334	*S. haemolyticus*	1.905	*S. haemolyticus* (T); CCM2737; X66100
335	*S. haemolyticus*	1.895	*S. haemolyticus* (T); CCM2737; X66100
336	*S. haemolyticus*	2.251	*S. haemolyticus* (T); CCM2737; X66100
337	*S. haemolyticus*	2.377	*S. haemolyticus* (T); CCM2737; X66100
338	*S. haemolyticus*	2.078	*S. aureus* (T); ATCC 12600; L36472
343	*S. haemolyticus*	1.949	*S. haemolyticus* (T); CCM2737; X66100
344	*S. haemolyticus*	2.257	*S. haemolyticus* (T); CCM2737; X66100
345	*S. haemolyticus*	2.141	*S. haemolyticus* (T); CCM2737; X66100
347	*S. haemolyticus*	2.209	*S. haemolyticus* (T); CCM2737; X66100

ID	MALDI-TOF MS		16S rRNA
	Species	Score value	Species
348	*S. haemolyticus*	2.344	*S. haemolyticus* (T); CCM2737; X66100
349	*S. haemolyticus*	2.030	*S. haemolyticus* (T); CCM2737; X66100
357	*S. haemolyticus*	2.320	*S. haemolyticus* (T); CCM2737; X66100
379	*S. haemolyticus*	2.191	*S. haemolyticus* (T); CCM2737; X66100
380	*S. haemolyticus*	2.362	*S. haemolyticus* (T); CCM2737; X66100
400	*S. hominis*	2.255	*S. hominis* (T); DSM 20328; X66101
401	*S. hominis*	2.433	*S. hominis* (T); DSM 20328; X66101
402	*S. hominis*	2.431	*S. hominis* (T); DSM 20328; X66101
403	*S. hominis*	2.352	*S. hominis (*T); DSM 20328; X66101
405	*S. hominis*	2.165	*S. hominis* (T); DSM 20328; X66101
406	*S. hominis*	2.333	*S. hominis* (T); DSM 20328; X66101
409	*S. hominis*	2.203	*S. equorum* (T); ATCC 43958; AB009939
567	*S. lugdunensis*	2.290	*S. lugdunensis* (T); ATCC 43809; AB009941
569	*S. pasteuri*	2.192	*S. pasteuri* (T); ATCC 51129; AB009944
579	*S. pasteuri*	2.294	*S. pasteuri* (T); ATCC 51129; AB009944
592	*S. pasteuri*	2.399	*S. pasteuri* (T);ATCC 51129; AB009944
604	*S. pettenkoferi*	1.772	*S. pettenkoferi* (T); B3117; AF322002
607	*S. pettenkoferi*	2.153	*S. pettenkoferi* (T); B3117; AF322002
609	*S. saprophyticus*	2.119	*S. saprophyticus* (T); ATCC 15305; AP008934
			S. capitis (T); ATCC 49326; AB009937
614	*S. saprophyticus*	2.235	*S. saprophyticus* (T); ATCC 15305; AP008934
630	*S. sciuri*	2.008	*S. sciuri* (T); DSM 20345T; AJ421446
632	*S. sciuri*	2.190	*S. sciuri* (T); DSM 20345T; AJ421446
636	*S. simiae*	2.049	*S. simiae* (T); CCM 7213; AY727530

ID	MALDI-TOF MS		16S rRNA
	Species	Score value	Species
639	*S. simiae*	1.897	*S. simiae* (T); CCM 7213; AY727530
644	*S. simulans*	1.879	*S. simulans* (T); ATCC 27848; D83373
653	*S. warneri*	2.236	*S. warneri* (T); L37603
654	*S. warneri*	2.254	*S. warneri* (T); L37603
656	*S. warneri*	2.069	*S. warneri* (T); L37603
714	*S. xylosus*	2.162	*S. xylosus* (T); ATCC 29971; D83374

4.4 MALDI-TOF MS data analysis

4.4.1 Cluster analysis of selected staphylococci

To find out the potential taxonomic relationships based on MALDI-TOF MS profiles of staphylococci, 411 isolates recovered from the general public and different environmental sites were selected for cluster analysis. This included isolates recovered from BCF; DSH; DSL; DSR; DSS; DST; HAS; HB; HH, and 13 out of 19 species identified were systematically analysed by BioNumerics 7.5 (Applied Math, Belgium). Eleven out of 411 isolates identified were *S. aureus,* and 12 other staphylococcal species include *S. epidermidis* (n=123), *S. hominis* (n=111), *S. warneri (*n=35), *S. capitis* (n=50), *S. haemolyticus* (n=42), *S. pasteuri* (n=21), *S. saprophyticus* (n=9), *S. simiae* (n=4); *S. cohnii* (n=2); *S. caprae* (n=1), *S. lugdunensis* (n=1), and *S. simulans* (n=1).

The unrooted dendrogram was built based on MALDI-TOF MS data. It was the reflection of the traditionally rooted dendrogram tree, but more compact. Each spot at the end of the branch represented a staphylococcal isolate. 9 major clusters, of staphylococci species were identified, including *S. hominis*, *S. haemolyticus*, *S.*

epidermidis, *S. pasteuri*, *S. warneri*, *S. aureus*, *S. saprophyticus*, *S. capitis*, and *S. simiae* (Fig 4.2).

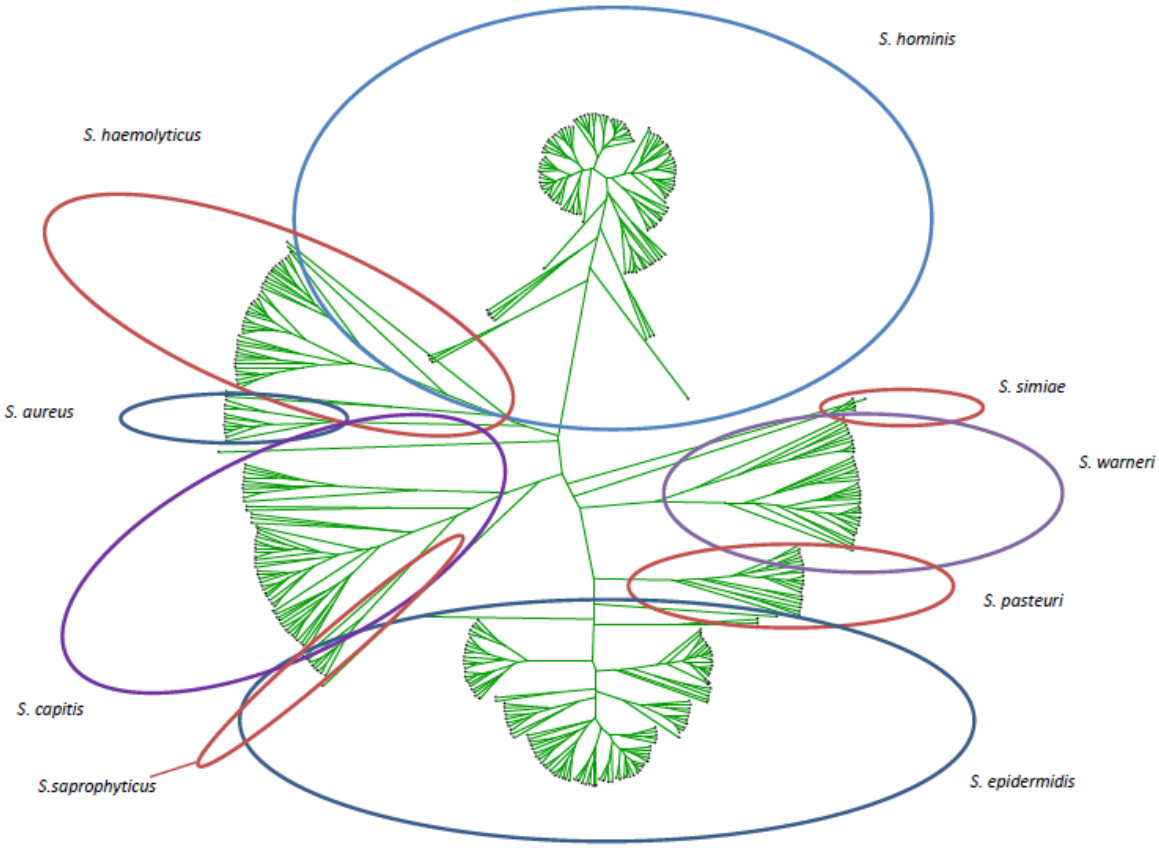


Figure 4. 2 Unrooted cluster analysis of staphylococci species using MALDI-TOF MS. Staphylococcal species were distributed in different clades and the circles were used to show the species forming these clades. The branches within each clade present the taxonomic relationship of staphylococci.

4.4.2 Cluster analysis of *Staphylococcus* spp. recovered from each site

4.4.2.1 Dominant species of each site

The presence of staphylococcal species differed between sites. The most common species isolated from DSL were *S. haemolyticus* and *S. epidermidis*; whereas *S. epidermidis* and *S. capitis* were predominant among the isolates recovered from DST

and HB. Moreover, *S. epidermidis* together with *S. hominis* were predominant among the isolates recovered from the HH, DSS and DSR. *S. haemolyticus* and *S. hominis* were predominant among the isolates recovered from the DSH. In addition, *S. haemolyticus* was predominant among the isolates recovered from HAS. The most common species isolated from BCF was *S. hominis* (Table 4.4).

Table 4. 4 Predominant and common staphylococcal species recovered from the human hands and different environmental sites

Sites	Predominant species	Commonly isolated species
BCF	*S. hominis*	*S. epidermidis*
DSH	*S. haemolyticus*	*S. hominis*
DSL	*S. haemolyticus*	*S. epidermidis*
DSR	*S. epidermidis*	*S. hominis*
DSS	*S. epidermidis*	*S. hominis*
DST	*S. epidermidis*	*S. capitis*
HAS	*S. haemolyticus*	-
HB	*S. epidermidis*	*S. capitis*
HH	*S. epidermidis*	*S. hominis*

Note: BCF- baby care facilities; DSH- different sites of hotels; DSL-different sites of a library; DSR: different sites of restaurants. DSS-different sites of supermarkets; DST-different sites of transportation facilities; HAS- hotel air samples; HB-handbags; HH-human hands.

4.4.2.2 Cluster analysis of isolates recovered from different sites

Three-dimensional scaling was performed to demonstrate the overall relationship of 411 staphylococcal isolates (Fig 4.3). Based on the data, all isolates were distributed into 4 groups. Groups 1, 2, 3 lacked the extensive diversity which was observed in the fourth group (Fig 4.3).

The stick is the visualization of the dendrogram, and the two spots that are connected by a stick are connected in the dendrogram. The X, Y, and Z axis are arbitrary units. It's the distance between the isolates that represent the similarity, and that distance exists of components in all three axis.

In the three-dimensional scaling, the similarity matrix is used to determine the 3D position. Isolates that have 100% similarity will be place on top of each other, with no distance between them. In contrast, the lower their similarity with each other, the higher their distance in the plot.

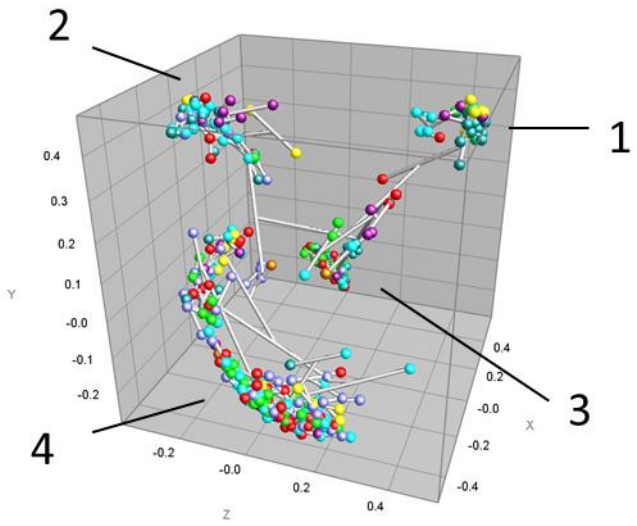


Figure 4. 3 Three-dimensional (3D) scatter plot of 411 staphylococci recovered from 9 sites. BCF ●; DSH ●; DSL ●; DSR ●; DSS ●; DST ●; HAS ●; HB ●; HH ●. X: -0.4 to 0.4; Y: -0.2 to 0.4; Z: -0.2 to 0.4.

4.4.2.3 Correlation of staphylococci isolated from different sites

Staphylococcal isolates recovered from different sites were taxonomically closely related. *Staphylococcus* spp. recovered from HH were taxonomically closely related to those isolated from BCF, DSH, DSL, DSR, DSS, DST, HAS, HB and HH (Fig 4.4a),

and staphylococcal isolates recovered from BCF were taxonomically closely related to those recovered from BCF, DSH, DSL, DSS, DST, HB and HH (Fig 4.4b). In addition, staphylococci isolated from DSS were taxonomically closely related to staphylococci isolated from BCF, DSH, DSR, DST, HH and DSS itself. Staphylococci recovered from DST were taxonomically closely related to staphylococci recovered from BCF, DSR, DSS, HH and DST (Fig 4.5a). Staphylococci recovered from DSH were taxonomically closely related to those recovered from BCF, DSS, HAS, HH and DSH (Fig 4.5b). Whilst staphylococci isolated from DSR were taxonomically closely related to isolates recovered from DST, HH and DSR, the staphylococci recovered from DSL were taxonomically closely related to those isolated from HH, BCF and DSL. In addition, staphylococci recovered from HB were taxonomically closely related to HH, BCF and HB.

Figure 4. 4 Isolates recovered from human hands were taxonomically closely related to staphylococci recovered from transportation facilities, restaurants, hotels, supermarkets, handbags, baby care facilities, library, hotel air and human hands itself. The blue line that was used to connect color spots and showed dissemination of taxonomically closely related staphylococci recovered from the indicated sites.

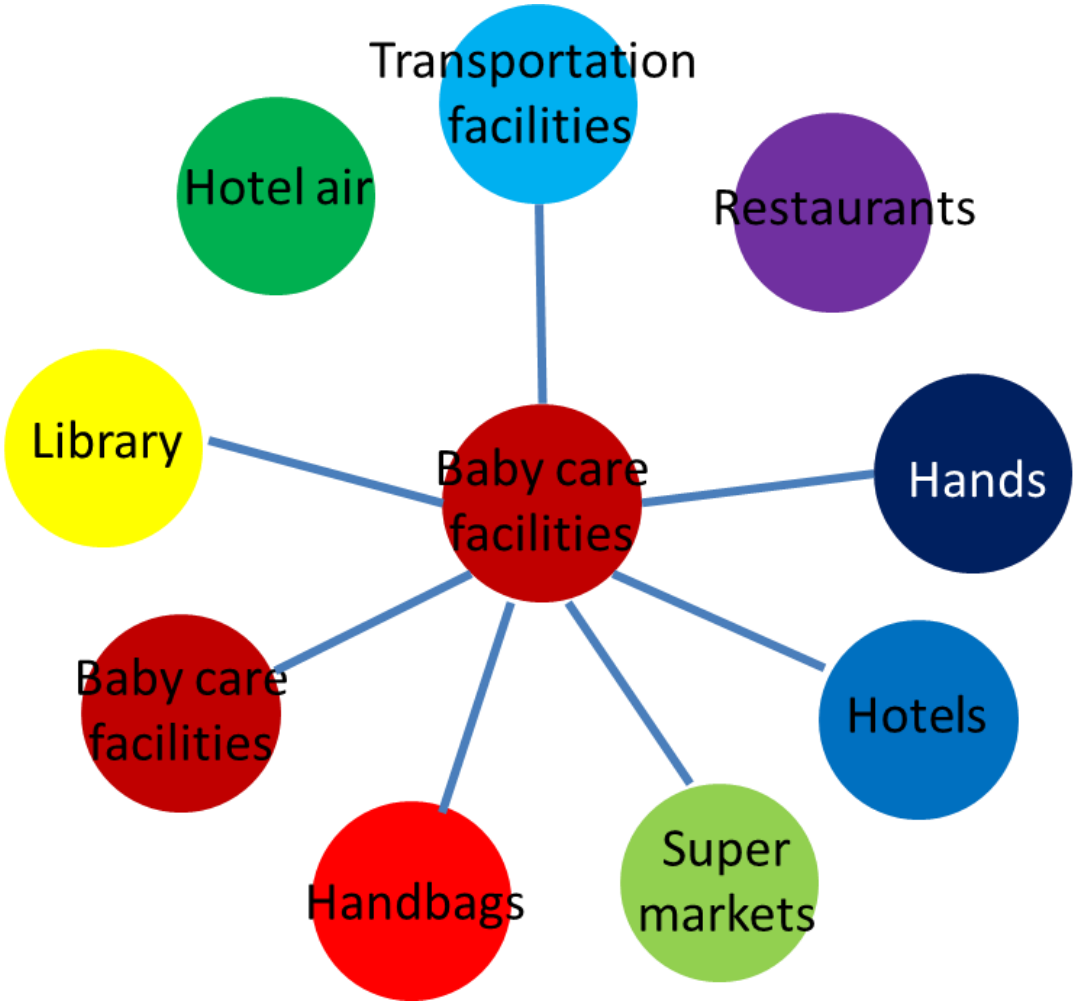


Figure 4. 5 Isolates recovered from baby care facilities were taxonomically closely related to staphylococci recovered from transportation facilities, hands, hotels, supermarkets, handbags, library and baby care facilities itself. The blue line that was used to connect color spots and showed dissemination of taxonomically closely related staphylococci recovered from the indicated sites.

Staphylococcal isolates recovered from HAS were taxonomically closely related to isolates from DSH, HH and HAS itself.

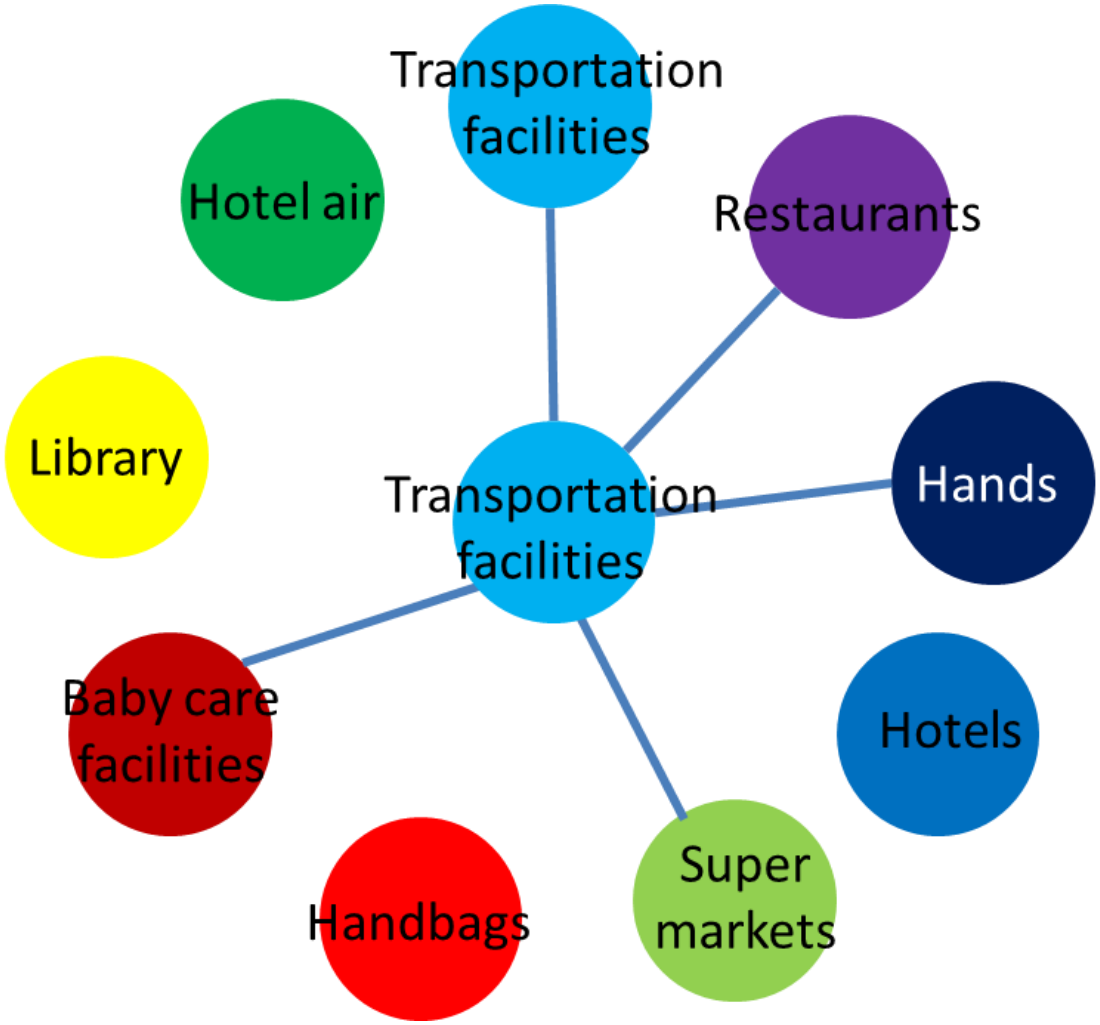


Figure 4. 6 Isolates recovered from transportation facilities were taxonomically closely related to staphylococci recovered from restaurants, hands, supermarkets, baby care facilities and transportation facilities itself. The blue line that was used to connect color spots and showed dissemination of taxonomically closely related staphylococci recovered from the indicated sites.

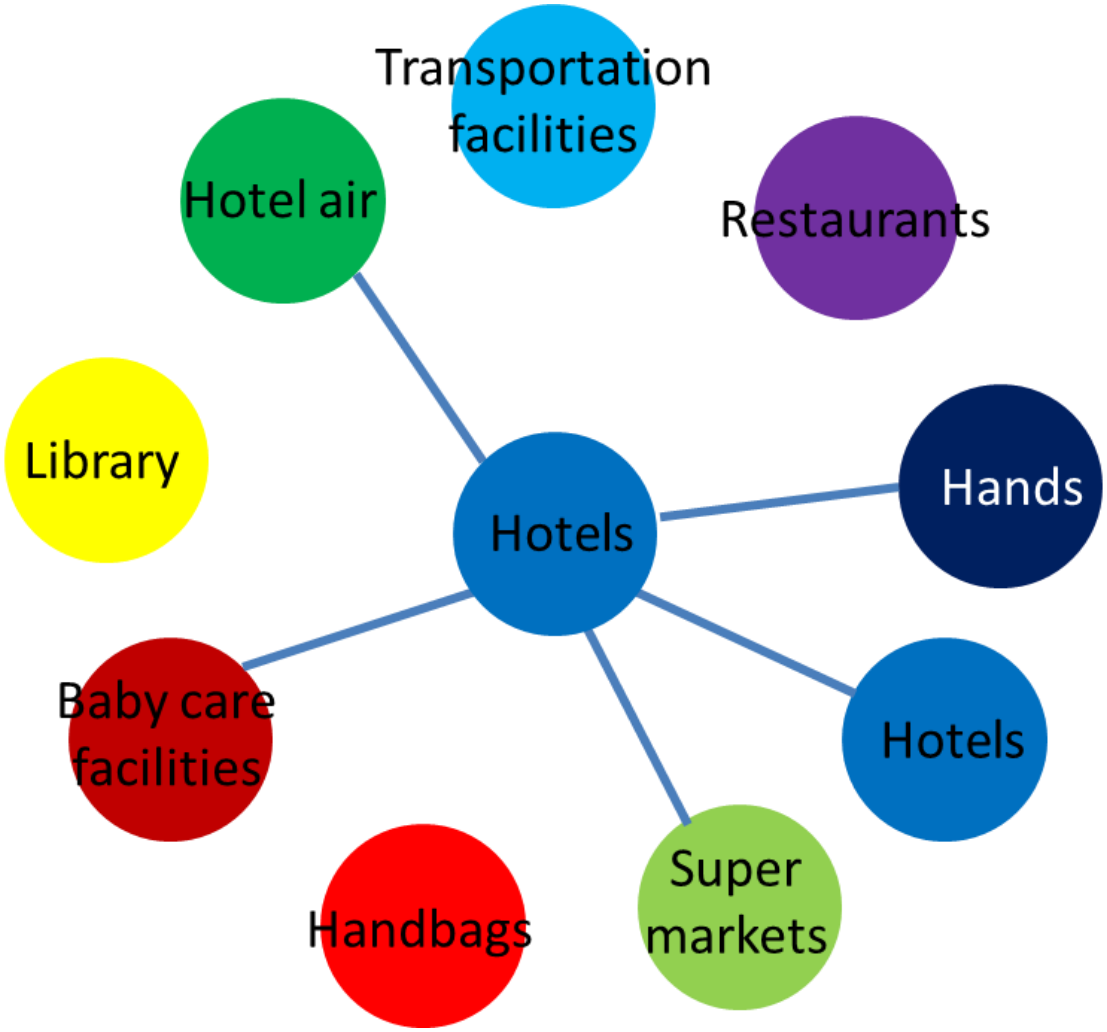


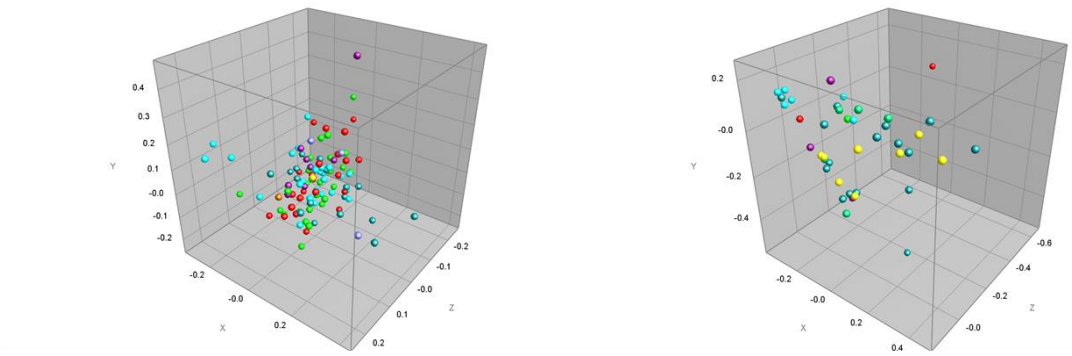
Figure 4. 7 Isolates recovered from hotels were taxonomically closely related to staphylococci recovered from hands, supermarkets, baby care facilities, hotel air and hotels itself. The blue line that was used to connect color spots and showed dissemination of taxonomically closely related staphylococci recovered from the indicated sites.

4.4.2.4 Cluster analysis of each staphylococcal species recovered from different sites

In addition, cluster analysis has been applied to each *Staphylococcus* spp. to determine the taxonomic relationships of each species isolated from different sites.

It was demonstrated that *S. hominis* isolates recovered from 8 different sites, including BCF, DSH, DSL, DSR, DSS, DST, HB and HH, were taxonomically related. The distribution of 111 *S. hominis* isolates in the unrooted cluster resulted in the formation of 13 clades. *S. hominis* isolates which were recovered from the same sites such as DSS,

HH, BCF, DSR were found to be taxonomically closely related as they appeared in the same cluster. However, it was also observed taxonomic relationship among these *S. hominis* isolates that were recovered from different sites such as DSS and DSR and those recovered from DSR and DSH. Moreover, *S. hominis* isolates recovered from DSS and HH, DSR and HH, DSH and HH, BCF and DSH, HB and BCF, BCF and DSL were also located in the same cluster, demonstrating their taxonomic close relationship (Fig 4.6a).



a b

Figure 4. 8 **8a and 8b** 8a Three-dimensional (3D) scatter plot of *S. hominis* isolates recovered from BCF, DSH, DSL, DSR, DSS, DST, HH, HB. BCF ●; DSH ●, DSL ●, DSR ●, DSS ●, DST ●, HH ●, HB ●.X: -0.2 to 0.2; Y: -0.2 to 0.4; Z: -0.2 to 0.2. 8b. Three-dimensional (3D) scatter plot of *S. haemolyticus* isolates recovered from BCF, DSH, DSL, DSR, DSS, HAS, HB, HH. BCF ●; DSH ●; DSL ●; DSR ●; DSS ●; HAS ●; HB ●;HH ●.X:-0.2 to 0.4; Y: -0.2 to 0.4; Z: -0.6 to 0.0.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

S. haemolyticus isolates recovered from 8 different sites, including BCF, DSH, DSL, DSR, DSS, HAS, HB, and HH were distributed in 3 large clades. One of these 3 clades was mainly formed by isolates recovered from HH. Similar to *S. hominis*, *S. haemolyticus* isolates recovered from DSH, DSL appeared in the same cluster,

indicating their taxonomically closely related. Interestingly, *S. haemolyticus* isolates recovered from different sites were also taxonomically closely related as they appeared in the same cluster. This included isolates recovered from HH and HAS, HH and DSR, HH and DSH, BCF and DSH. Moreover, *S. haemolyticus* recovered from DSH were located in the same cluster with *S. haemolyticus* recovered from HAS. Therefore, there is a possibility that isolates recovered from different sites in the hotels and air harbour the same populations of *Staphylococcus* spp. In addition, it was found that one of *S. haemolyticus* isolates recovered from DSR formed a distinctive branch (Fig 4.6b).

In relation to *S. epidermidis* isolates, it was found that isolates recovered from BCF, DSH, DSL, DSR, DSS, DST, HH, and HB were taxonomically related. In addition, these isolates were organised into nine large clusters. *S. epidermidis* recovered from HH, DSR, DSS, DSL and DST were in the same cluster with *S. epidermidis* recovered from HH, DSR, DSS, DSL, and DST. In addition, the results showed that *S. epidermidis* isolates recovered from DST were located in the same cluster as those isolated from BCF, indicating their taxonomically closely related. Additionally, *S. epidermidis* isolates recovered from HH and DSH, HH and DSR, HH and DST, HH and DSS, HH and BCF, HH and HB, DSS and DSL, DSS and BCF, DSS and DSR, DSS and DSH, HB and DSR, HB and BCF, DSR and DST were also located in same cluster (Fig 4.7a).

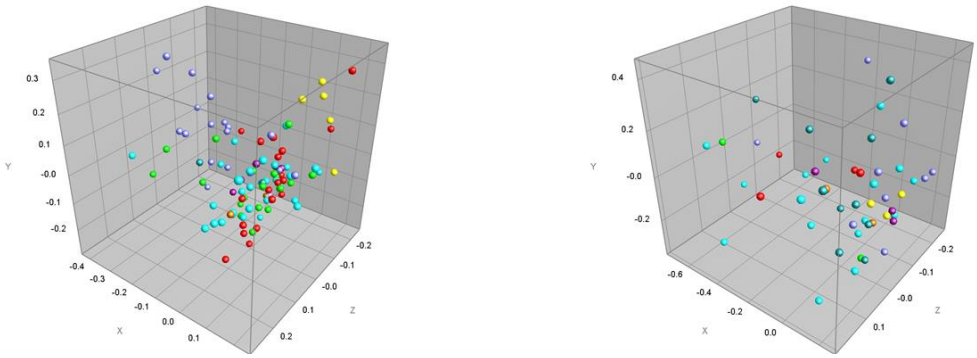


Figure 4. 9 **9a and 9b** 9a Three-dimensional (3D) scatter plot of *S. epidermidis* isolated from BCF, DSH, DSL, DSR, DSS, DST, HB, HH. BCF ●; DSH ●; DSL ●; DSR ●; DSS ●; DST ●; HB ●; HH ●. X: -0.4 to 0.1; Y: -0.2 to 0.3; Z: -0.2 to 0.2. 9b. Three-dimensional (3D) scatter plot of *S. capitis* isolated from BCF, DSH, DSL, DSR, DSS, DST, HB, HH. BCF ●; DSH ●; DSL ●; DSR ●; DSS ●; DST ●; HB ●; HH ●. X: -0.6 to 0.0; Y: -0.2 to 0.4; Z: -0.2 to 0.1.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

S. capitis isolates recovered from BCF, DSH, DSL, DSR, DSS, DST, HB and HH were arranged into three major clades. The majority of isolates in clade 1 included those recovered from DSL. The remaining isolates recovered from HH, DST, DSH, DSS, DSR, HB and BCF were evenly distributed within all 3 clades. *S. capitis* isolates recovered from DST, DSH, and HH were located in the same cluster as those recovered from DST, DSH, and HH. In addition, *S. capitis* isolates recovered from HH and DSH, HH and DSR, HH and DSS, BCF and DSL, BCF and DSH, DST and DSH, DST and DSR, DSH and DSS, DSH and DSL, were found to be located in the same cluster (Fig 4.7b).

Thirty-six *S. warneri* isolates recovered from BCF, DSH, DSL, DSR, DSS, DST and HH were used for cluster analyses and resulted in the formation of 3 major clades. The first clade was formed by *S. warneri* recovered from DST and HH, whereas the second clade was formed by those recovered from DSL and DSH and the third clade was formed by *S. warneri* recovered from BCF, HH and DSR. *S. warneri* recovered from DSR, HH, DST, and DSL were related to those isolates recovered from DSR, HH, DST, and DSL as they found to be located in the same clade. Moreover, *S. warneri* recovered from HH and DSR, HH and DST, DST and DSS, DST and DSH, DSS and DSH, DSL and BCF were originated from the same cluster (Fig 4.8a).

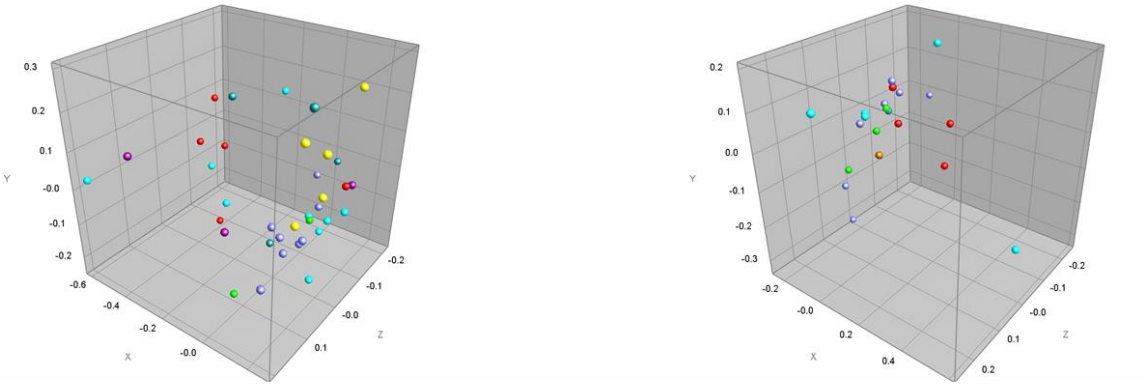


Figure 4. 10 **10a and 10b** 10a. Three-dimensional (3D) scatter plot of *S. warneri* isolated from BCF, DSH, DSL, DSR, DSS, DST, HH. BCF ●; DSH ●; DSL ●; DSR ●; DSS ●; DST ●; HH ●. X: -0.4 to 0.0; Y: -0.2 to 0.3; Z: -0.2 to 0.1. 10b. Three-dimensional (3D) scatter plot of *S. pasteuri* isolated from DSH, DSR, DSS, DST, HB, HH. DSH ●; DSR ●; DSS ●; DST ●; HB●; HH ●. X: -0.2 to 0.4; Y: -0.3 to 0.2; Z: -0.2 to 0.2.

The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

Nineteen *S. pasteuri* isolates recovered from DSH, DSR, DSS, DST, HB and HH formed a distinct clade. It was observed close taxonomic relationship between *S. pasteuri* isolates recovered from DST and DSS, HH and DSR as they were located in same cluster (Fig 4.8b).

Eleven *S. aureus* isolates have been analysed in this study. It was found that *S. aureus* isolates that were recovered from six different sites (BCF, DSH, DSR, DSS, HB, HH) formed 2 major clades. Two *S. aureus* isolates recovered from DSR were found to be located in the same clade. *S. aureus* isolates recovered from DSR and DSS were taxonomically closely related to those recovered from DSR and DSS. Apart from this, two *S. aureus* isolates, (one recovered from DSH and the other from HH) were found to be located in same cluster, demonstrating their close taxonomic relationship (Fig 4.9a).

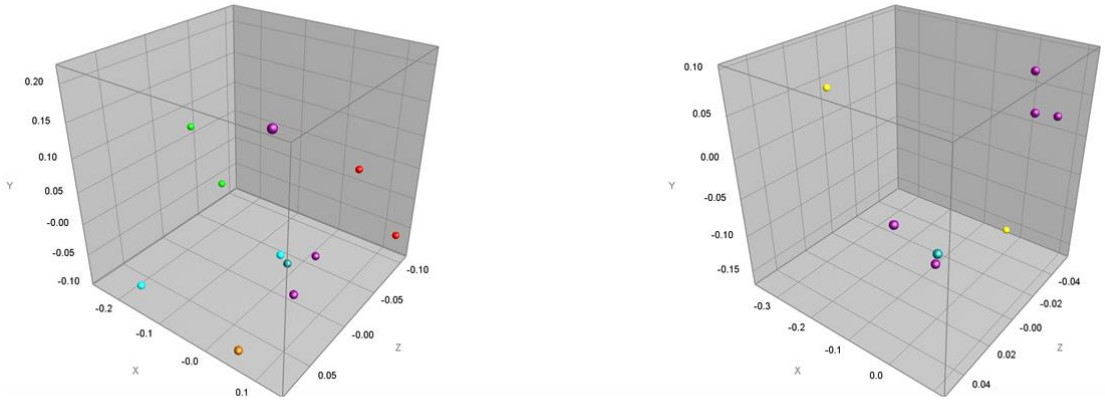


Figure 4. 11 **11a and 11b** 11a. Three dimensional (3D) scatter plot of *S. aureus* isolated from BCF, DSH, DSR, DSS, HB, HH. BCF ●; DSH ●; DSR ● ; DSS ●; HB ● ;HH ●. X: -0.2 to 0.1; Y: -0.1 to 0.2; Z: -0.1 to 0.05. 11b. Three-dimensional (3D) scatter plot of *S. saprothyticus* isolated from BCF, DSH, DSL. BCF ● ; DSH ● ; DSL ● .X: -0.3 to 0.0; Y: -0.15 to 0.1; Z: -0.04 to 0.04.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

Eight *S. saprophyticus* isolates were recovered from BCF, DSH, and DSL. *S. saprophyticus* isolated from DSH were located in same cluster as those isolated from BCF (Fig 4.9b).

Cluster analysis was applied to four *S. simiae* isolates, and they all were from DST. Interestingly, the clade formed by these isolates was located between *S. warneri* and *S. caprae* (Fig 4.10).

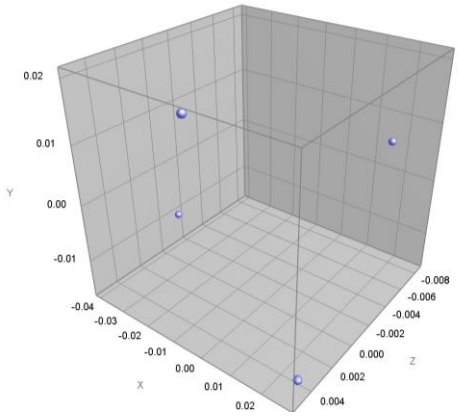


Figure 4. 12 Three-dimensional (3D) scatter plot of *S. simiae* isolates recovered from DST. DST ●. X: -0.04 to 0.02; Y: -0.01 to 0.02; Z: -0.008 to 0.004.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

4.5 Chapter summary

The identification of environmental bacteria showed that:

1. 991 out of 1152 (86%) were shown reliable identification;
2. 718 (62%) were identified to be *Staphylococcus* spp.;
3. Reproducibility of MALDI-TOF MS identification was confirmed by obtaining results from two modes of MALDI-TOF MS in fully automated fashion;

4. 16S rRNA gene sequencing results confirmed the reliability of MALDI-TOF MS identification;
5. Cluster analysis of staphylococci isolated from different sites was found to be taxonomically closely related.

It is important to do rapid and accurate identification of microorganisms in microbiology study. This chapter introduced the identification of staphylococcal isolates with MALDI-TOF MS, and tested the reproducibility and reliability of MALDI-TOF MS in identifying environmental staphylococci. Moreover, MALDI-TOF MS data were combined with isolation sites to do cluster analysis. Evaluation of the diversity of environmental staphylococcal species will be discussed in the discussion chapter. The importance of reliability and reproducibility in identifying environmental staphylococci will also be discussed later. Finally, the taxonomic relationship of staphylococci isolated from different sites will be assessed in the discussion chapter.

Chapter 5 Phenotypic analysis of environmental staphylococci

The emergency of antibiotic resistance in clinical staphylococci has been widely reported (Appelbaum, 2006; Brennan et al., 2011). The presence of antibiotic resistance put a great challenge in treatment (IWG-SCC, 2009), and the antibiotic susceptibility test is of great important for clinicians to select the right antimicrobial agents (Jorgensen & Ferraro, 2009). In addition, antimicrobial susceptibility tests can be applied to environmental microorganisms to survey the influence caused by overuse of antibiotics (Wang et al., 2008). Environmental staphylococci may act as a reservoir of antibiotic resistance genes, so it is necessary to include less frequently diseases associated microorganisms in antibiotic resistance research in order to prevent the resistance before it appears in pathogens (Blair et al., 2014). Antibiotic susceptibility tests were applied to 677 environmental recovered staphylococci. In addition, antibiotic susceptibility profiles were combined with MALDI-TOF MS identification data for systematic taxonomic analysis.

5.1 Antibiotic susceptibility test

The antibiotic susceptibility was determined by comparing the diameter of inhibition zone with BSAC or CLSI standards (Howe & Andrew., 2012; Creagh & Lucey., 2007). Antibiotic resistance patterns were determined for 677 strains of staphylococci from 9 sites, and 649 (96%) staphylococcal isolates were resistant to more than one antibiotic. Resistance to penicillin, and fusidic acid was recorded in more than 60% of all staphylococcal species. Resistance to the other compounds tested was as follows: erythromycin 33%, streptomycin 31%, amoxicillin 27%, vancomycin 24%, tetracycline 18%, mupirocin 16%, cefepime 10%, gentamicin 10%, oxacillin 7%, and chloramphenicol 5%. For the resistance to non-β-lactam antibiotics, 453 staphylococcal isolates (67%) were resistant to fusidic acid, 226 isolates (33%) were resistant to

erythromycin, and the varied antibiotic resistance ratio of other non-β-lactam antibiotics such as gentamicin, vancomycin and chloramphenicol (Table 5.1). In addition to these non-β-lactam antibiotic resistant strains, 448 (66%) of the staphylococcal strains were resistant to the traditional β-lactam antibiotic penicillin, and 50 (7%) of staphylococcal isolates were resistant to oxacillin which express additional penicillin-binding protein (Fig 5.1).

Apart from high ratio resistant to penicillin and fusidic acid, there were species patterns of resistance: *S. epidermidis, S. hominis* and *S. pasteuri* were predominantly resistant to erythromycin, whereas *S. capitis* has relatively low resistance ratio (1%) of gentamicin, and shows relatively high ratio of streptomycin (45%) resistance. Additionally, *S. warneri* presents higher resistant (47%) to streptomycin (Table 5.1). The data sets of the rest of the species were of less than 30 isolates, however, these staphylococcal species: *S. arlettae*, *S. aureus*, *S. auricularis*, *S. caprae*, *S. cohnii*, *S. equorum*, *S. lugdunensis*, *S. pettenkoferi*, *S. saprophyticus*, *S. sciuri*, *S. simiae*, *S. simulans*, and *S. xylosus*, had similar antibiograms with species which had more than 30 isolates. For example, there were high levels of penicillin and fusidic acid resistance in all of small data sets species except for *S. simiae* (n=10). In addition to penicillin and fusidic acid, *S. sciuri* has high resistance ratio of oxacillin (67%), mupirocin (67%), and streptomycin (67%).

Multi-resistance was seen in 677 tested staphylococcal species, including one isolate resistant to 11 antibiotics, one isolate resistant to 10 antibiotics, five to 9 antibiotics, seventeen isolates resistant to 8 antibiotics, thirteen to 7 antibiotics, forty-four to 6 antibiotics, fifty-eight to 5 antibiotics, one hundred and six to 4 antibiotics, one-hundred and thirty-eight to 3 antibiotics, one hundred and sixty-one to 2 antibiotics, and one hundred and five to 1 antibiotic. Twenty-eight staphylococcal isolates were susceptible to all the tested antibiotics. (Appendix II.1)

Table 5. 1 Antibiotic susceptibility profile of environmental staphylococci

Test result	No of resistant isolates/No of tested isolates (% resistance)											
Resistance to	oxacillin	penicillin	vancomycin	mupirocin	cefepime	gentamicin	fusidic acid	streptomycin	amoxicillin	erythromycin	tetracycline	chloram phenicol
S. epidermidis	15/181 (8)	**132/181 (73)**	53/181 (29)	30/181 (17)	18/181 (10)	13/181 (7)	**116/181 (64)**	39/181 (22)	48/181 (27)	**78/181 (43)**	31/181 (17)	6/181 (3)
S. hominis	4/164 (2)	**111/164 (68)**	22/164 (13)	17/164 (10)	9/164 (5)	10/164 (6)	**110/164 (67)**	36/164 (22)	30/164 (18)	**62/164 (38)**	34/164 (21)	5/164 (3)
S. capitis	3/75 (4)	**44//75 (59)**	13/75 (17)	12/75 (16)	3/75 (4)	**1/75 (1)**	**45/75 (60)**	34/75 (45)	17/75 (23)	10/75 (13)	7/75 (9)	4/75 (5)
S. haemolyticus	11/74 (14)	**41/74 (55)**	22/74 (30)	13/74 (18)	18/74 (24)	11/74 (15)	**51/74 (70)**	34/74 (46)	30/74 (41)	20/74 (27)	25/74 (32)	10/74 (14)
S. warneri	2/68 (3)	**38/68 (56)**	22/68 (32)	12/68 (18)	6/68 (9)	15/68 (22)	**41/68 (60)**	**32/68 (47)**	27/68 (40)	19/68 (28)	11/68 (16)	1/68 (1)
S. pasteuri	2/32 (6)	**22/32 (69)**	5/32 (16)	4/32 (12)	2/32 (6)	3/32 (9)	**22/32 (69)**	8/32 (25)	10/32 (31)	**14/32 (44)**	5/32 (16)	1/32 (3)
S.saprophyticus	3/20 (15)	**18/20 (90)**	4/20 (20)	5/20 (25)	1/20 (5)	2/20 (10)	**20/20 (100)**	2/20 (10)	5/20 (25)	7/20 (35)	3/20 (15)	2/20 (10)

Note: oxacillin (1μg); penicillin G (1 unit); vancomycin (5 μg); mupirocin (20 μg); cefepime (30 μg); gentamicin (10 μg); fusidic acid (10 μg); streptomycin (10 μg)； amoxicillin (10 μg); erythromycin (5 μg); tetracycline (10 μg); chloramphenicol (30 μg).

Test result	No of resistant isolates/No of tested isolates (% resistance)											
Resistance to	oxacillin	penicillin	vancomycin	mupirocin	cefepime	gentamicin	fusidic acid	streptomycin	amoxicillin	erythromycin	tetracycline	chloram phenicol
S. cohnii	4/14 (29)	**11/14 (79)**	3/14 (21)	3/14 (21)	5/14 (36)	0/14 (0)	**13/14 (93)**	8/14 (57)	4/14 (29)	9/14 (64)	2/14 (14)	2/14 (14)
S. aureus	0/12 (0)	**10/12 (83)**	6/12 (50)	2/12 (17)	0/12 (0)	7/12 (58)	**10/12 (83)**	4/12 (33)	6/12 (50)	3/12 (25)	0/12 (0)	1/12 (8)
S. simiae	0/10 (0)	**1/10 (10)**	3/10 (30)	0/10 (0)	0/10 (0)	0/10 (0)	**4/10 (40)**	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)
S. sciuri	**4/6 (67)**	**4/6 (67)**	2/6 (33)	**4/6 (67)**	1/6 (17)	1/6 (17)	**5/6 (83)**	**4/6 (67)**	2/6 (33)	0/6 (0)	0/6 (0)	1/6 (17)
S. pettenkoferi	1/5 (20)	**3/5 (60)**	1/5 (20)	0/5 (0)	1/5 (20)	1/5 (20)	**3/5 (60)**	3/5 (60)	2/5 (40)	1/5 (20)	1/5 (20)	1/5 (20)
S. lugdunensis	1/5 (20)	**4/5 (80)**	1/5 (20)	1/5 (20)	0/5 (0)	0/5 (0)	**3/5 (60)**	0/5 (0)	0/5 (20)	1/5 (20)	0/5 (0)	0/5 (0)
S. equorum	0/3 (0)	**2/3 (67)**	1/3 (33)	1/3 (67)	0/3 (0)	0/3 (0)	**1/3 (33)**	1/3 (33)	1/3 (33)	0/3 (0)	1/3 (33)	0/3 (0)
S. caprae	0/2 (0)	**2/2 (100)**	0/2 (0)	0/2 (0)	2/2 (100)	2/2 (100)	**2/2 (100)**	1/2 (50)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)

Note: oxacillin (1μg); penicillin G (1 unit); vancomycin (5 μg); mupirocin (20 μg); cefepime (30 μg); gentamicin (10 μg); fusidic acid (10 μg); streptomycin (10 μg); amoxicillin (10 μg); erythromycin (5 μg); tetracycline (10 μg); chloramphenicol (30 μg).

Test result	No of resistant isolates/No of tested isolates (% resistance)											
Resistance to	oxacillin	penicillin	vancomycin	mupirocin	cefepime	gentamicin	fusidic acid	streptomycin	amoxicillin	erythromycin	tetracycline	chloram phenicol
S. xylosus	0/2 (0)	**2/2 (100)**	1/2 (50)	1/2 (50)	1/2 (50)	1/2 (50)	**2/2(100)**	2/2(100)	0/2(0)	0/2(0)	1/2 (50)	0/2 (0)
S. auricularis	0/2 (0)	**1/2 (50)**	1/2 (50)	0/2 (0)	1/2 (50)	0/2 (0)	**1/2 (50)**	0/2 (0)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)
S. arlettae	0/1 (0)	**1/1 (100)**	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	**1/1 (100)**	1/1 (100)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
S. simulans	0/1 (0)	**1/1 (100)**	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	**1/1 (100)**	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
	50/677 (7)	**448/677 (66)**	160/677 (24)	105/677 (16)	68/677 (10)	67/677 (10)	**451/677 (67)**	209/677 (31)	185/677 (27)	225/677 (33)	121/677 (18)	34/677 (5)

Note: oxacillin (1µg); penicillin G (1 unit); vancomycin (5 µg); mupirocin (20 µg); cefepime (30 µg); gentamicin (10 µg); fusidic acid (10 µg); streptomycin (10 µg)； amoxicillin (10 µg); erythromycin (5 µg); tetracycline (10 µg); chloramphenicol (30 µg).

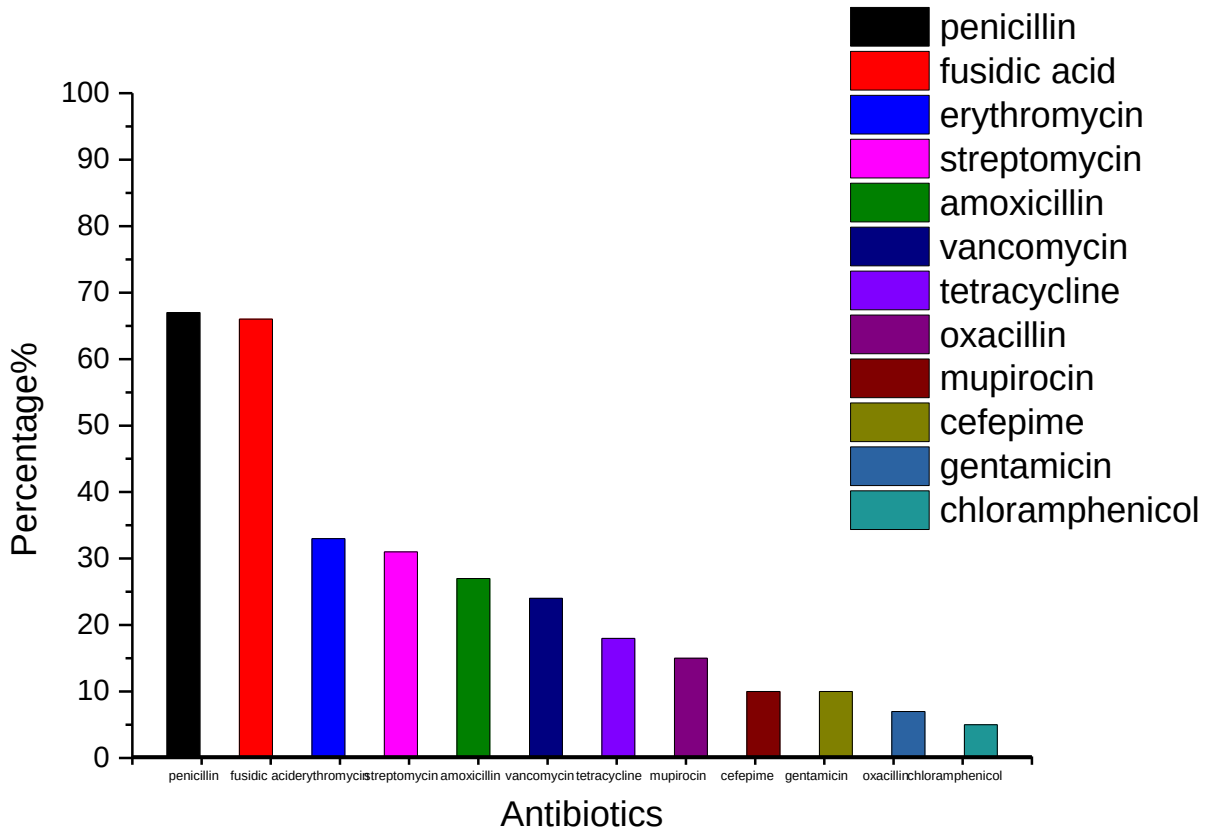


Figure 5. 1 Percent of environmental staphylococci resistant to penicillin G, fusidic acid, erythromycin, streptomycin, amoxicillin, vancomycin, tetracycline, oxacillin, mupirocin, cefepime, gentamicin and chloramphenicol

5.2 MIC (oxacillin) test

In addition to the antibiotic susceptibility tests, oxacillin MICs was determined for *mecA* gene positive coagulase negative staphylococci and all *S. aureus*. The oxacillin MICs for tested staphylococci were highly variable with MICs ranging from 0.015 mg l^{-1} to 256 mg l^{-1}.

5.3 Antibiotic susceptibility of taxonomically closely related staphylococci

As well as determinining the potential taxonomic relationships based upon MALDI-TOF MS profiles of staphylococci, antibiotic susceptibility profiles of these 411 staphylococcal isolates were combined to assess the antibiotic susceptibility variations of taxonomically closely related staphylococci.

5.3.1 Antibiotic susceptibility of staphylococci

Three-hundred and twenty-six (80%) out of 411 staphylococci were resistant against 2 or more antibiotics, including 1 staphylococci isolates resistant to 10 antibiotics, 4 to 9 antibiotics, 10 isolates were resistant to 8 antibiotics, 7 to 7 antibiotics, 20 to 6 antibiotics, 29 to 5 antibiotics, 59 to 4 antibiotics, 89 to 3 antibiotics, 107 to 2 antibiotics, and 63 to 1 antibiotic. Of all isolates tested only 22 were susceptible to all antibiotics tested.

5.3.2 Antibiotic resistance patterns of taxonomically closely related staphylococci

As noted above, MALDI-TOF MS data of 411 staphylococci were combined with antibiotic susceptibility profiles to determine the antibiotic resistant patterns of taxonomically closely related staphylococci. Staphylococci resistant to more than 2

antibiotics were considered as multiple antibiotic resistant, whereas susceptible isolates were those that demonstrated resistant to one antibiotic or none. The distribution of antibiotic resistance patterns in staphylococcal isolates was analysed with BioNumerics 7.5 (Applied Math, Belgium). It was demonstrated that susceptible and multiple resistant isolates were taxonomically closely related (Fig 5.2), and 30 multidrug resistant isolates were taxonomically closely related to 30 susceptible isolates respectively, indicating that these might belong to the same genotype of the founding strain.

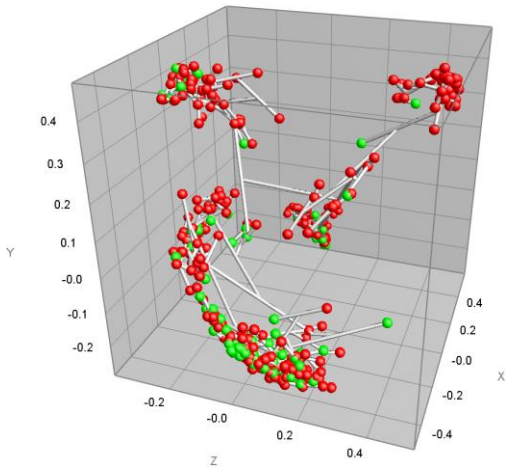


Figure 5. 2 Three-dimensional (3D) scatter plot of multiple antibiotic resistant ● and susceptible ● staphylococci. X: -0.4 to 0.4; Y: -0.2 to 0.4; Z: -0.2 to 0.4.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

5.3.3 Cluster analysis of each staphylococcal species combined with antibiotic susceptibility profile

Staphylococcal species selected for antibiotic susceptibility profile associated cluster analysis were *S. aureus*, *S. capitis*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. pasteuri*, *S. saprophyticus*, *S. simiae*, and *S. warneri*.

Ninety-four (85%) multiple resistant and 17 (15%) susceptible *S. hominis* were analysed. Eight multiple antibiotic resistant *S. hominis* were taxonomically closely related to 8 susceptible *S. hominis* (Fig 5.3a).

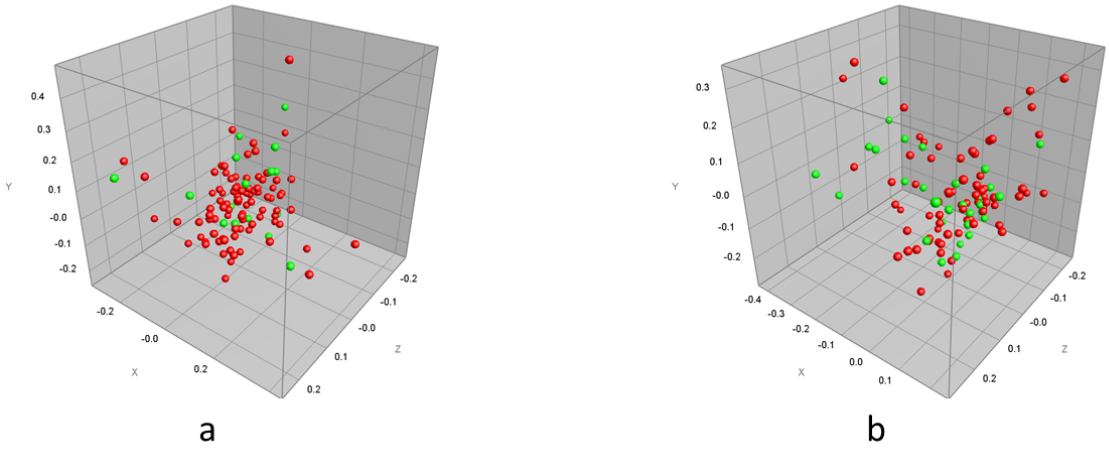


Figure 5. 3 **3a and 3b** 3a. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. hominis.* X:-0.2 to 0.2; Y: -0.2 to 0.4; Z: -0.2 to 0.2. 3b. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. epidermidis.* X: -0.4 to 0.1; Y: -0.2 to 0.3; Z: -0.2 to 0.2.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

Eighty-five (69%) multiple resistant and 38 (31%) susceptible *S. epidermidis* were analysed. It was found that 14 multiple antibiotic resistant *S. epidermidis* were taxonomically closely related to 14 susceptible *S. epidermidis* (Fig 5.3b).

In this study, thirty-nine (93%) multiple resistant and 3 (7%) susceptible *S. haemolyticus* were determined. Of those only one multiple resistant *S. haemolyticus* was found taxonomically closely related to one susceptible *S. haemolyticus* (Fig 5.4a).

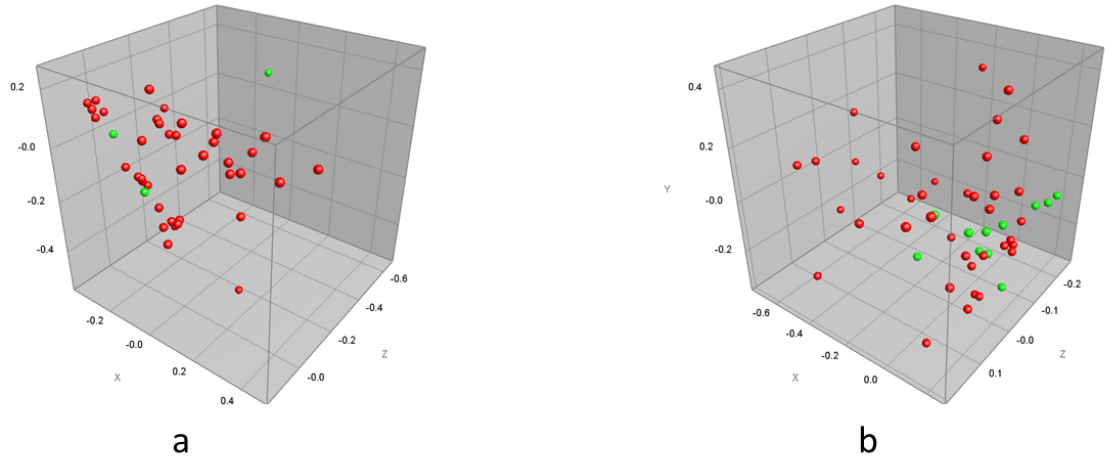


Figure 5. 4 **4a and 4b** 4a. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. haemolyticus.* X: -0.2 to 0.4; Y: -0.4 to 0.2; Z: -0.6 to 0.0. 4b. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. capitis.* X: -0.6 to 0.0; Y: -0.2 to 0.4; Z:-0.2 to 0.1.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

38 (76%) multiple resistant and 12 (24%) susceptible *S. capitis* were determined. It was found that 3 multiple resistant *S. capitis* were taxonomically closely related to 3 susceptible *S. capitis.* The distribution of the resistant (Red) *S. capitis* isolates on the left

side, and susceptible isolates on the right side of the cube indicated that these isolates were not related to one another (Fig 5.4b).

Thirty (86%) multiple resistant and 5 (14%) susceptible isolates of *S. warneri* were determined. From these isolates only 1 multiple resistant *S. warneri* was found to be taxonomically closely related to 1 susceptible *S. warneri* (Fig 5.5a).

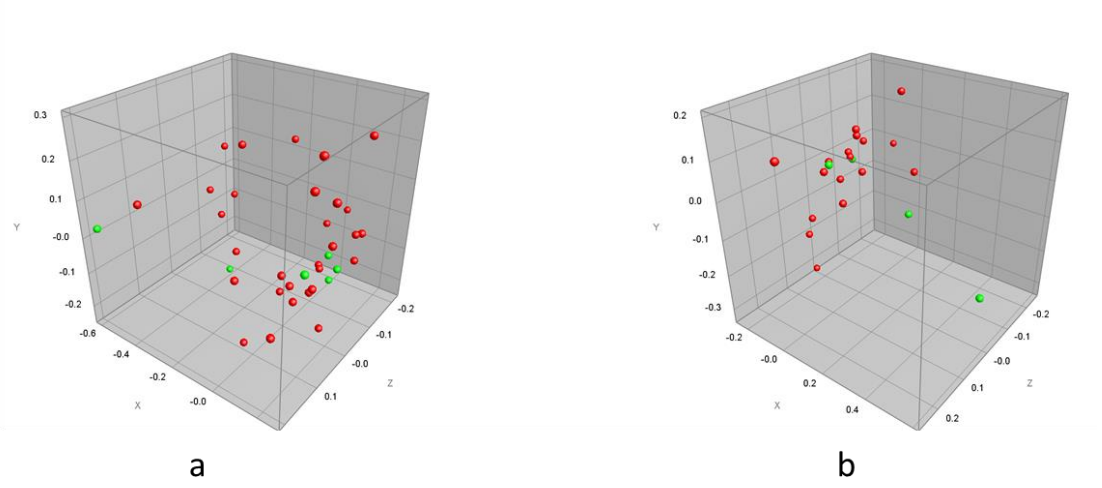


Figure 5. 5 **5a and 5b** 5a Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. warneri*. X: -0.6 to 0.0; Y: -0.2 to 0.3; Z: -0.2 to 0.1 5b. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. pasteuri.* X: -0.2 to 0.4; Y: -0.3 to 0.2; Z: -0.2 to 0.2.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

For *S. pasteuri*, 18 (86%) multiple resistance and 3 (14%) susceptible isolates were determined. It was found that two multiple resistant *S. pasteuri* were taxonomically closely related to two susceptible *S. pasteuri* (Fig 5.5b).

In this study, ten multiple resistant and 1 susceptible *S. aureus* were determined. It was showed that multiple resistant *S. aureus* were not related to this susceptible *S. aureus*

(Fig 5.6a). In addition, it was found that all *S. saprophyticus* isolates (n=9) were multiple resistant (Fig 5.6b). Moreover, 3 multiple resistant and 1 susceptible *S. simiae* were determined and it was found that one of multiple resistant *S. simiae* was taxonomically closely related to 1 susceptible *S. simiae* (Fig 5.7).

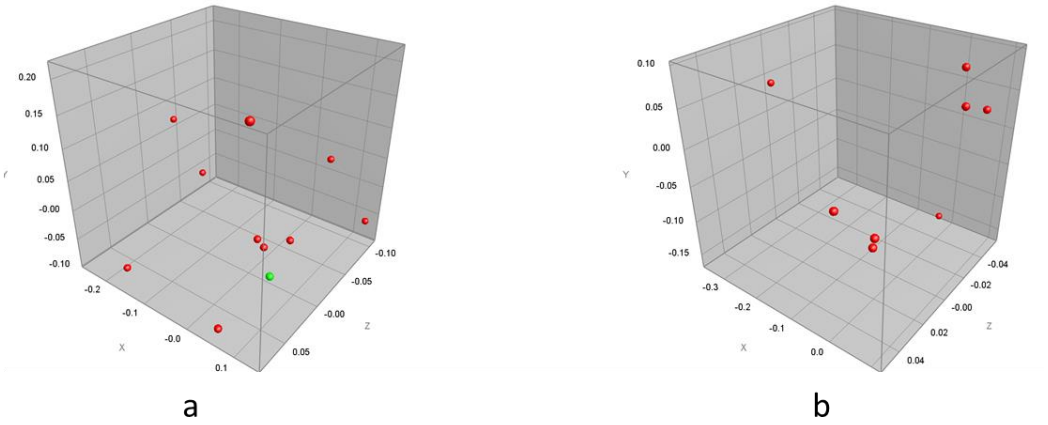


Figure 5. 6 **6a and 6b** 6a. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. aureus.* X: -0.2 to 0.1; Y: -0.1 to 0.2; Z:-0.1 to 0.05. 6b. Three-dimensional (3D) scatter plot of multiple resistant ● and susceptible ● isolates of *S. saprophyticus.* X: -0.3 to 0.0; Y: -0.15 to 0.1; Z:-0.04 to 0.04.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

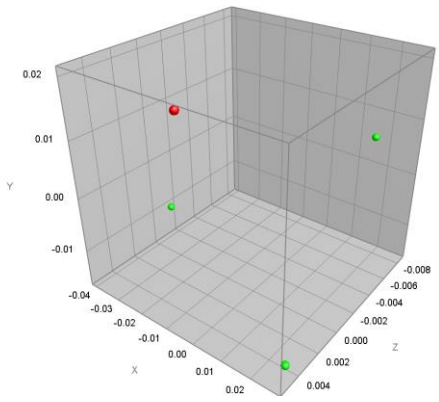


Figure 5. 7 Three-dimensional scatter plot of multiple resistant ● and susceptible ● isolates of *S. simiae*. X: -0.04 to 0.02; Y: -0.01 to 0.02; Z: -0.008 to 0.004.
The X, Y, and Z axises are arbitrary units. It represents the distance between the closely related isolates and that the distance exists in all three axises. Isolates that have 100% similarity are above each other, with no distance between them. As less their similarity is, the more the distance is between them in the plot.

5.3.4 Variations of antibiotic susceptibility profile of taxonomically closely related staphylococci

In order to identify antibiotic susceptibility variations of taxonomically closely related staphylococci, two representative isolates recovered from different sites as well as from the same sites were selected from each cluster. Staphylococcal species selected for these analyses were *S. hominis*, *S. epidermidis*, *S. haemolyticus*, *S. capitis* and *S. warneri* (Appendix II.2).

Thirty-five closely related clusters were determined for *S. hominis*. Twenty-five of these clusters were formed by the isolates recovered from different sites. In 2 clusters (clusters 1-2) were detected only one antibiotic susceptibility variation, in 3 clusters (clusters 3-5) two antibiotic susceptibility variations, in 10 clusters (clusters 6-15) 3, in 5 clusters (clusters 16-20) 4, in 2 clusters (clusters 21-22) 5, in 3 clusters (cluster 23- 25) 6. In total ten clusters were formed by the isolates recovered from the same site. There was no obvious antibiotic susceptibility variation observed in three clusters (cluster 26-

28). However, 1 antibiotic susceptibility variation was detected in 2 clusters (cluster 29-30), two in 2 clusters (cluster 31-32), three in 1 cluster (cluster 33), and four in 2 clusters (cluster 34-35) (Appendix II.2).

For *S. epidermidis*, thirty-four taxonomically closely related clusters were identified. Twenty-two of these clusters were formed by isolates recovered from different sites. No antibiotic sensitivity difference was detected in one cluster (cluster 1). One antibiotic susceptibility variation was detected in 3 clusters (cluster 2-4), two in 3 clusters (cluster 5-7), three in 7 clusters (cluster 8-14), four in 5 clusters (cluster 15-19), five in 1 cluster (cluster 20), six in 1 cluster (cluster 21), and seven in 1 cluster (cluster 22). *S. epidermidis* isolates recovered from the same sites formed twelve clusters. In two out of 12 clusters (cluster 23-24) there is no antibiotic susceptibility variation, however, one antibiotic susceptibility variation was detected in 4 clusters (cluster 25-28), two in 2 clusters (cluster 29-30), three in 1 cluster (cluster 31), six in 1 cluster (cluster 32), seven in 1 cluster (cluster 33), and eight in 1 cluster (cluster 34) (Appendix II.2).

Moreover, antibiotic susceptibility variations were determined for twelve taxonomically closely related clusters of *S. haemolyticus*. Six out of 12 clusters were formed by the isolates recovered from different sites. In 2 clusters (cluster 1-2) were detected 4 antibiotic variations, six in 1 cluster (cluster 3), seven in 1 cluster (cluster 4), and eight in 2 clusters (cluster 5-6). Six clusters were formed by isolates recovered from the same site and in 1 cluster (cluster 7) was found 1 antibiotic susceptibility variation, three in 1 cluster (cluster 8), five in 1 cluster (cluster 9), six in 2 clusters (cluster 10-11), and seven in 1 cluster (cluster 12) (Appendix II.2).

Fifteen taxonomically closely related clusters of *S. capitis* were analysed for antibiotic susceptibility variations. Eleven out of 15 clusters were formed by the isolates recovered from the different sites. In one such cluster (cluster 1) was found no antibiotic susceptibility variation, and further three variations in 3 clusters (cluster 2-4), four in 3 clusters (cluster 5-7), five in 3 clusters (cluster 8-10), and seven in 1 cluster (cluster 11). Among those clusters that were formed by isolates recovered from the same site two antibiotic susceptibility variations were detected in 2 clusters (cluster 12-13), three in 1 cluster (cluster 14), and nine antibiotic susceptibility variations in 1 cluster (cluster 15) (Appendix II.2).

10 taxonomically closely related clusters of *S. warneri*, were also examined for antibiotic susceptibility variations. Five out of 10 clusters were formed by isolates recovered from different sites, in one of which (cluster 1) was detected one antibiotic susceptibility variation. Three antibiotic variations were detected in 2 clusters (cluster 2-3), four in 1 cluster (cluster 4), and six in 1 cluster (cluster 5). Moreover, five clusters were formed by isolates from the same site. In 4 of five clusters (cluster 6-9) were observed 3 antibiotic variations and in 1 cluster (cluster 10) was detected 6 antibiotic variations (Appendix II.2).

5.3.5 Percentage of multiple resistant staphylococci recovered from each site

In addition to find out variation of antibiotic susceptibility profile of taxonomically closely related staphylococci, the number of susceptible and multiple resistant staphylococci in each site was summarized. A total of 325 (80%) of all staphylococci isolates were multiple resistant, and 86 were susceptible. Table 5.2 demonstrates the distribution of multiple resistant and susceptible staphylococci in each site. All five staphylococcal isolates recovered from HAS were multiple antibiotic resistant. Thirty-

one (94%) of all isolates recovered from BCF were multiple resistant staphylococci, and 2 (6%) were susceptible. More than 80% staphylococci isolates recovered from DSH (n=46), DSS (n=51), HH (n=86) and DSL (n=22) were also multiple resistant. Forty-eight (72%) of 67 isolates recovered from DSR were multiple resistant. In addition, 6 (60%) and 30 (58%) of staphylococci isolates recovered from HB and DST respectively were also multiple resistance (Table 5.2).

Table 5. 2 Distribution of multiple resistant and susceptible staphylococci within each site

Sites	Total isolates	Multiple resistant isolates (%)	Susceptible isolates (%)
Hotel air	5	5(100%)	0(0%)
Baby care facilities	33	31(94%)	2(6%)
Library	25	22(88%)	3(12%)
Hotels	53	46(87%)	7(13%)
Supermarkets	59	51(86%)	8(14%)
Human hands	107	86(80%)	21(20%)
Restaurants	67	48(72%)	19(28%)
Handbags	10	6(60%)	4(40%)
Transportation facilities	52	30(58%)	22(42%)

5.4 Chapter summary

The antibiotic susceptibility profile of staphylococci showed that:

1. Six hundred and forty-eight (96%) staphylococci were found to be resistant to at least one antibiotic;
2. Cluster analysis showed that 30 multidrug resistant staphylococci were taxonomically closely related to 30 susceptible staphylococci;
3. No obvious differences of antibiotic susceptibility profiles were observed between clusters that were formed by isolates recovered from different sites and clusters that were formed by isolates recovered from same site.

Antimicrobial susceptibility tests can be applied to environmental microorganisms to survey the dissemination of antibiotic-resistant environmental staphylococci. This chapter introduced the antibiotic susceptibility of staphylococci, and analysed the antibiotic susceptibility variation of taxonomically closely related staphylococci. The comparison of antibiotic susceptibility profiles and multiple antibiotic resistance ratios with clinical studies will be carried out in the discussion chapter. Evaluation of environment act as a reservoir of antibiotic-resistant staphylococci will be carried out in the discussion chapter. Finally, the antibiotic susceptibility profiles will be combined with MALDI-TOF MS data to assess antibiotic susceptibility variation of taxonomically closely related staphylococci.

Chapter 6 Genotypic analysis of selected staphylococci

Methicillin resistant *S. aureus* (MRSA) is a major public health problem, and infections caused by MRSA confer severe economic and healthy consequences (Köser et al., 2012). Methicillin resistant of MRSA is due to additionally acquired a *mecA* gene, which encodes for penicillin binding protein 2a (PBP2a) with a low affinity to β-lactam antibiotics (Tulinski et al., 2012). In this study, the *mecA* gene PCR was used to explore the dissemination of *mecA* gene positive environmental staphylococci.

Oxacillin susceptible *mecA* positive *S. aureus* (OS-MRSA) have been reported in clinical isolates, and precaution in avoiding selection of high oxacillin resistant MRSA during treatment processes has been advocated (Hososaka et al., 2007). However, little is known about oxacillin susceptible *mecA* positive coagulase negative staphylococci (OS-MRCoNS). In this study, the occurrence of OS-MRCoNS was assessed, and the expression of PBP2a of *mecA* gene positive staphylococci with varied MIC was determined.

The application the of molecular typing system has greatly promoted the pursuit of staphylococcal epidemiology studies, which in turn improved the efficiency of health care facilities to track the source and transmission of staphylococcal outbreak (Oliveira & Tomasz, 2002). In addition, molecular epidemiology has been employed to determine the population structure of environmental microbial pathogens (Oliveira & Tomasz, 2002). Here, SCC*mec* and MLST approaches were employed for the systematic typing of environmental staphylococci.

6.1 *mecA* gene

6.1.1 Detection of *mecA* gene for all staphylococci

The presence of *mecA* gene was determined for 714 staphylococcal isolates. PCR was undertaken with approapriate positive and negative controls. Eighty-nine (12%) *mecA* positive staphylococci were detected; however, among these MRSA was not found. *S. sciuri* had the highest *mecA* positive ratio (83%) among 19 staphylococcal species, followed by *S. cohnii* (36%), *S. haemolyticus* (24%), *S. pettenkoferi* (20%), and *S. lugdunensis* (20%). No *mecA* gene was found in 8 species, including *S. aureus*, *S. simiae*, *S. equorum*, *S. caprae*, *S. xylosus*, *S. auricularis*, *S. simulans*, and *S. arlettae*. Apart from the species mentioned above, the rest of the species' *mecA* positive ratio was between 6 to 13% (Table 6.1).

Table 6. 1 *mecA* gene positive isolates recovered from general public settings

Species	No of isolates	Number of *mecA* positive isolates (%)
S. epidermidis	198	24(12)
S. hominis	173	11(6)
S. haemolyticus	79	19(24)
S. capitis	79	6(8)
S. warneri	68	9(13)
S. pasteuri	34	4(12)
S. saprophyticus	20	4(20)
S. cohnii	14	5(36)
S. aureus	12	0(0)
S. simiae	10	0(0)
S. sciuri	6	5(83)
S. pettenkoferi	5	1(20)
S. lugdunensis	5	1(20)
S. equorum	3	0(0)
S. caprae	2	0(0)
S. xylosus	2	0(0)
S. auricularis	2	0(0)
S. simulans	1	0(0)
S. arlettae	1	0(0)
Total	714	89(12)

The antimicrobial resistances patterns of the 89 *mecA*-positive staphylococci are shown in Table 6.2. Eighty-four of *mecA* gene positive isolates were multidrug-resistant (resistant to more than 2 antibiotics); their resistances to antibiotics varied from 2 to 8 antibiotics.

Table 6. 2 Molecular characterisation and antibiotic resistance of *mecA* gene positive staphylococci isolated from environment

ID	Sites	Species	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C	*mecA*	*mec*	*ccr*	SCC*mec*	MIC/OX (mg l^{-1})
71	HH	*S. capitis*	S	S	S	S	S	S	R	S	S	S	S	+	-	-	I*	0.5
75	HH	*S. capitis*	R	S	S	S	S	R	R	S	S	S	S	+	Class A	NT	NT	0.5
81	HH	*S. capitis*	R	S	S	R	S	R	R	R	R	S	S	+	NT	5	NT	0.5
70	HH	*S. capitis*	R	S	S	S	S	S	R	S	S	R	S	+	NT	5	NT	**0.25**
83	HH	*S. capitis*	S	S	R	S	S	R	R	S	S	S	S	+	NT	5	NT	**0.12**
24	DSH	*S. capitis*	S	S	S	S	S	R	R	S	S	S	S	+	NT	1	NT	**0.12**
106	HAS	*S. cohnii*	R	S	R	R	S	R	I	S	S	S	S	+	Class C	5	V	2
108	HH	*S. cohnii*	S	S	S	I	S	R	R	S	R	R	S	+	Class A	NT	NT	1
107	HAS	*S. cohnii*	R	R	S	R	S	R	R	S	R	R	R	+	Class A	5	V	1
100	DSH	*S. cohnii*	R	S	R	S	S	S	S	R	R	S	S	+	Class A	5	5A	1
97	BCF	*S. cohnii*	R	S	S	R	S	R	R	S	R	S	S	+	Class B	1	I	**0.25**
118	**DSH**	***S. epidermidis***	**R**	**S**	**R**	**R**	**R**	**R**	**I**	**R**	**R**	**R**	**S**	**+**	**Class B**	**2**	**IV**	**256**
279	HH	*S. epidermidis*	R	R	S	S	S	R	S	S	R	S	S	+	Class B	2	IV	2
127	DSH	*S. epidermidis*	R	R	S	I	S	S	S	R	R	R	S	+	Class C	5	V	2
139	DSR	*S. epidermidis*	R	R	R	R	S	R	S	R	R	R	S	+	Class C	5	V	2
191	DSS	*S. epidermidis*	R	S	S	S	S	R	S	R	S	S	S	+	Class B	4	VI	2
308	HH	*S. epidermidis*	R	S	R	S	S	R	S	R	R	S	S	+	Class B	NT	NT	2
153	DSH	*S. epidermidis*	R	S	S	S	S	R	S	S	S	S	S	+	Class C	5	V	1
187	DSS	*S. epidermidis*	R	R	S	S	S	S	S	S	R	S	S	+	Class C	5	V	1
134	DSL	*S. epidermidis*	R	S	S	S	S	R	R	R	R	R	S	+	Class B	1	I	1

Note: * determined by Zhang's method. R: resistant, S sensitive. I: intermediate

BCF- baby care facility; DSH- different sites of hotels; DSL- different sites of a library; DSR- different sites of restaurants; DSS- different sites of supermarkets; DST- different sites of transportation facilities; HAS- hotel air samples; HB- handbags; HH- human hands.

A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Sites	Species	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C	*mecA*	*mec*	*ccr*	SCC*mec*	MIC/OX (mg l^{-1})
259	HH	*S. epidermidis*	R	S	S	R	S	R	R	R	R	S	S	+	Class C	5	V	1
234	HB	*S. epidermidis*	S	S	R	S	S	R	R	S	R	R	S	+	Class A	NT	NT	1
135	DSL	*S. epidermidis*	R	S	S	S	S	R	R	R	R	R	S	+	Class B	2	IV	0.5
124	DSH	*S. epidermidis*	R	R	S	R	S	R	R	R	S	R	S	+	Class B	2	IV	0.5
133	DSL	*S. epidermidis*	R	S	S	S	S	R	R	R	R	R	S	+	Class B	3	3B	0.5
126	HH	*S. epidermidis*	R	R	S	I	S	S	R	R	R	S	S	+	-	-	III*	0.5
257	HH	*S. epidermidis*	R	S	S	S	S	S	S	R	I	R	S	+	Class A	1	1A	**0.12**
249	DSH	*S. epidermidis*	R	R	S	S	S	R	R	S	S	R	S	+	NT	2	NT	**0.12**
119	DSH	*S. epidermidis*	R	S	S	S	S	S	S	R	I	R	S	+	Class C	5	V	**0.12**
111	BCF	*S. epidermidis*	R	S	S	S	S	S	S	S	S	S	S	+	Class A	2	II	**0.12**
202	DST	*S. epidermidis*	S	R	R	S	S	R	I	S	S	S	S	+	Class B	5	5B	**0.12**
264	HH	*S. epidermidis*	S	S	R	S	S	R	R	S	S	S	S	+	Class B	2	IV	**0.06**
125	DSH	*S. epidermidis*	S	R	S	I	S	S	R	S	S	S	S	+	NT	5	NT	**0.06**
185	DSS	*S. epidermidis*	R	R	S	S	S	S	S	S	S	S	S	+	Class C	NT	NT	**0.06**
129	DSL	*S. epidermidis*	R	S	S	S	S	R	R	R	R	R	S	+	Class B	1	I	**0.03**
379	HAS	*S. haemolyticus*	R	S	S	R	S	R	R	S	R	R	S	+	Class A	5	5A	256
316	DSH	*S. haemolyticus*	R	R	R	R	S	R	I	S	R	S	R	+	Class C	5	V	256
317	DSH	*S. haemolyticus*	R	R	S	R	R	R	R	R	R	R	R	+	Class A	2	II	256
318	DSH	*S. haemolyticus*	R	S	S	R	R	R	R	R	R	S	S	+	Class C	5	V	8
319	DSH	*S. haemolyticus*	R	S	S	R	S	R	I	R	R	S	S	+	Class C	5	V	8
362	DSL	*S. haemolyticus*	R	S	S	I	S	S	R	R	S	S	S	+	Class C	5	V	2
367	DSL	*S. haemolyticus*	R	S	S	R	S	S	R	R	S	S	S	+	Class C	5	V	2

Note: * determined by Zhang's method. R: resistant, S sensitive. I: intermediate

BCF- baby care facility; DSH- different sites of hotels; DSL- different sites of a library; DSR- different sites of restaurants; DSS- different sites of supermarkets; DST- different sites of transportation facilities; HAS- hotel air samples; HB- handbags; HH- human hands.

A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Sites	Species	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C	*mecA*	*mec*	*ccr*	SCC*mec*	MIC/OX (mg l^{-1})
355	DSH	*S. haemolyticus*	R	S	S	R	R	R	R	R	S	R	S	+	Class C	5	V	2
384	HH	*S. haemolyticus*	R	S	R	S	S	R	R	S	S	S	S	+	Class C	5	V	2
320	DSH	*S. haemolyticus*	R	R	R	R	S	R	I	S	S	R	R	+	Class A	1	1A	1
380	HAS	*S. haemolyticus*	R	R	R	S	S	R	R	S	R	R	R	+	Class C	5	V	0.5
321	DSH	*S. haemolyticus*	R	R	S	S	S	R	I	R	S	S	S	+	Class C	5	V	**0.25**
322	DSH	*S. haemolyticus*	R	S	S	S	S	S	I	R	S	R	S	+	Class A	1	1A	**0.25**
382	HH	*S. haemolyticus*	R	S	S	I	S	R	R	S	R	S	S	+	-	-	II*	**0.25**
324	DSH	*S. haemolyticus*	R	S	S	S	S	S	I	R	S	R	S	+	Class B	1	I	**0.12**
323	DSH	*S. haemolyticus*	S	S	S	S	R	S	R	R	I	R	S	+	Class A	2	II	**0.12**
381	HH	*S. haemolyticus*	S	S	S	I	S	S	R	S	S	S	S	+	Class B	5	5B	**0.12**
360	DSH	*S. haemolyticus*	S	S	S	S	S	R	S	S	S	S	R	+	Class B	5	5B	**0.06**
369	DSL	*S. haemolyticus*	R	S	S	S	S	R	R	S	R	S	R	+	Class B	1	I	**0.03**
399	DSH	*S. hominis*	R	R	S	S	S	R	S	S	S	S	R	+	Class A	1	1A	8
413	DSH	*S. hominis*	S	S	R	S	S	R	R	S	S	S	S	+	Class C	5	V	2
506	DSS	*S. hominis*	S	R	S	S	S	R	S	S	S	S	S	+	Class B	1	I	0.5
498	DSS	*S. hominis*	R	S	S	S	S	R	S	S	R	S	S	+	Class A	NT	NT	0.5
426	DSH	*S. hominis*	R	S	S	I	S	R	R	R	R	S	S	+	Class A	NT	NT	**0.25**
430	DSH	*S. hominis*	R	S	R	S	S	R	R	S	S	S	S	+	NT	5	NT	**0.12**
400	DSH	*S. hominis*	R	R	R	S	S	R	S	R	R	R	S	+	Class A	1	1A	**0.12**
326	DSH	*S. hominis*	S	S	S	S	S	S	S	R	I	S	S	+	Class A	1	1A	**0.06**
401	DSH	*S. hominis*	R	S	R	R	S	R	I	R	R	S	S	+	Class A	1	1A	**0.06**
412	DSH	*S. hominis*	R	S	S	S	S	R	S	R	R	S	S	+	NT	1	NT	**0.06**

Note: * determined by Zhang's method. R: resistant, S sensitive. I: intermediate

BCF- baby care facility; DSH- different sites of hotels; DSL- different sites of a library; DSR- different sites of restaurants; DSS- different sites of supermarkets; DST- different sites of transportation facilities; HAS- hotel air samples; HB- handbags; HH- human hands.

A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Sites	Species	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C	*mecA*	*mec*	*ccr*	SCC*mec*	MIC/OX (mg l^{-1})
391	BCF	*S. hominis*	R	S	S	S	S	R	S	S	S	S	S	+	NT	5	NT	**0.03**
564	DSH	*S. lugdunensis*	R	S	S	S	S	S	I	S	R	S	S	+	Class A	5	5A	0.5
593	HH	*S. pasteuri*	R	S	S	S	R	R	R	R	S	S	S	+	NT	5	NT	0.5
597	HH	*S. pasteuri*	R	S	R	I	S	S	R	S	S	S	S	+	NT	5	NT	0.5
589	HB	*S. pasteuri*	R	S	S	S	S	R	R	S	R	S	S	+	Class A	5	5A	**0.25**
592	HH	*S. pasteuri*	S	S	R	S	S	R	R	S	S	S	S	+	Class B	5	5B	**0.25**
603	DSH	*S. pettenkoferi*	R	R	S	R	R	R	R	R	R	R	R	+	Class A	5	5A	8
616	BCF	*S. saprophyticus*	R	R	R	S	S	R	I	R	R	R	S	+	NT	5	NT	256
612	BCF	*S. saprophyticus*	R	S	R	S	S	R	S	S	R	S	S	+	NT	NT	NT	1
627	HH	*S. saprophyticus*	R	S	I	S	S	R	S	S	S	R	S	+	Class B	5	5B	0.5
621	DSS	*S. saprophyticus*	R	S	R	R	S	R	S	R	S	R	S	+	Class B	2	IV	**0.25**
628	DSH	*S. sciuri*	R	R	S	R	S	R	I	S	S	S	R	+	Class A	5	5A	16
630	HH	*S. sciuri*	R	R	R	I	S	R	R	S	S	S	S	+	Class A	4	VIII	2
632	DSH	*S. sciuri*	R	S	S	I	S	R	R	R	S	S	S	+	Class A	5	5A	1
633	DSH	*S. sciuri*	R	S	R	I	R	R	R	R	S	S	S	+	Class B	5	5B	1
629	HH	*S. sciuri*	S	S	R	S	S	S	S	S	S	S	S	+	-	-	II*	0.25
659	DSH	*S. warneri*	R	R	R	S	S	R	R	R	S	S	S	+	NT	5	NT	0.5
704	HH	*S. warneri*	R	S	S	I	S	R	R	R	R	S	S	+	Class C	5	V	0.5
662	DSH	*S. warneri*	R	S	S	S	R	R	R	R	S	R	S	+	Class C	5	V	**0.25**
694	HH	*S. warneri*	R	S	S	S	S	S	S	S	S	R	S	+	-	-	I*	**0.25**
653	DSH	*S. warneri*	R	S	S	S	S	S	I	S	R	S	S	+	Class A	5	5A	**0.25**
654	DSH	*S. warneri*	R	R	S	S	S	R	I	S	S	S	S	+	Class C	5	V	**0.25**
655	BCF	*S. warneri*	S	S	R	S	R	R	R	S	I	S	S	+	Class B	1	I	**0.12**
648	BCF	*S. warneri*	R	S	S	S	R	R	S	R	S	S	S	+	NT	5	NT	**0.06**
645	BCF	*S. warneri*	R	S	S	S	S	R	S	S	S	S	S	+	NT	4	NT	**0.015**

Note: * determined by Zhang's method. R: resistant, S sensitive. I: intermediate

BCF- baby care facility; DSH- different sites of hotels; DSL- different sites of a library; DSR- different sites of restaurants; DSS- different sites of supermarkets; DST- different sites of transportation facilities; HAS- hotel air samples; HB- handbags; HH- human hands.

A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

No *mecA* gene was found in twelve *S. aureus* isolates, all of which were phenotypically susceptible to oxacillin. MICs of 12 *mecA*-negatives *S. aureus* isolates ranged from 0.06 to 0.12 mg l^{-1}.

The presence of the *mecA* gene was also demonstrated in 702 staphylococcal species, including: *S. epidermidis, S. haemolyticus, S. sciuri, S. cohnii, S. hominis, S. saprophyticus, S. lugdunensis, S. pasteuri, S. capitis, S. warneri,* and *S. pettenkoferi* (Table 6.2); however, the carriage of *mecA* did not always result in strains demonstrating significant levels of resistant to oxacillin. Thirty-nine of the 89 (44%) *mecA* positive staphylococci were found to have MICs below 0.5 mg l^{-1} to oxacillin (Table 6.2). Of 24 *mecA*-positive *S. epidermidis* isolates, 9 (38%) were classified as susceptible (oxacillin). Eight strains of 17 *S. haemolyticus* (47%) that tested positive for the *mecA* gene demonstrated susceptible to oxacillin. This inconsistency is also observed in other species, *S. hominis* (7/11), *S. sciuri* (1/5), *S. cohnii* (1/5), *S. saprophyticus* (1/4), *S. lugdunensis* (0/1), *S. pasteuri* (2/4), *S. capitis* (3/6), *S. warneri* (7/9), and *S. pettenkoferi* (0/1) (Table 6.2).

6.1.2 *mecA* gene expression in selected staphylococci

mecA gene is oxacillin resistance determinant (Tulinski et al., 2012), however, MICs of 44 % *mecA* gene positive isolates in this study were found to be below 0.5 mg l^{-1}. In order to clarify this inconsistence, LC-MS/MS were applied to 4 selected staphylococci with MICs ranged from 0.12 to 256 mg l^{-1}.

6.1.2.1 Protein extraction

To start *mecA* gene expression analysis, protein was extracted and the concentration was determined by Bradford assay (Sigma Aldrich, UK). The concentration of each sample is shown in the Table 6.3.

Table 6. 3 Protein concentration of 4 staphylococci

ID	Concentration (μg μl^{-1})
S. hominis 506	2.18081
S. haemolyticus 318	2.37212
S. epidermidis 118 (G6_2)	3.48394
S. epidermidis 111	3.10822

6.1.2.2 PBP 2a expression

Penicillin binding protein 2a was expressed in *S. haemolyticus* 318 and *S. epidermidis* 118 (G6_2), and the expression of PBP 2a in *S. epidermidis* 118 (G6_2) was 1.5 times more than that in *S. haemolyticus* 318; however, penicillin binding protein 2a was not found in *S. hominis* 506 and *S. epidermidis* 111 (Table 6.4).

Table 6. 4 Quantified expression of penicillin binding protein in 4 staphylococci by LC-MS/MS

ID	MIC (mg l^{-1})	*mecA* gene	Spectrum number count	
			PBP2a	PBP2
S.epidermidis 111	0.12	+	0	1
S.hominis 506	0.5	+	0	1
S.haemolyticus 318	8	+	1	7
S.epidermidis 118 (G6_2)	256	+	2	1

6.2 SCC*mec* typing

After determining the *mecA* gene positive staphylococcal isolates, the SCC*mec* types of 89 *mecA*-positive isolates were examined. 20 staphylococci (22%) carried SCC*mec* V, followed by SCC*mec* type I (9 isolates, 10%), type IV (6 isolates, 7%), type II (5 isolates, 6%), one type III (1%), one type VI (1%), and one type VIII (1%). In addition, seven isolates harboured a new SCC*mec* type 1A, which carried combination of class A *mec* complex and *ccr* type 1. Of the fifteen isolates that were non-typeable, eight carried a combination of class A *mec* complex and *ccrC*, six carried a combination of class B *mec* and *ccrC*, and one carried class B *mec* and *ccr* type 3. Additionally, 24 isolates

(29%) could not be typed due to lack of the *mec* complex or the *ccr* complex (Table 6.2 and 6.5).

Table 6. 5 Diversity of SCC*mec* types in coagulase negative staphylococci

	I	II	III	IV	V	VI	VIII	1A	3B	5A	5B	NT	Total No
S. capitis	1											5	6
S. cohnii	1				2					1		1	5
S. epidermidis	2	1	1	5	6	1		1	1		1	5	24
S. haemolyticus	2	3			9			2		1	2		19
S. hominis	1				1			4				5	11
S. lugdunensis										1			1
S. pasteuri										1	1	2	4
S. pettenkoferi										1			1
S. sciuri		1					1			2	1		5
S. saprophyticus				1							1	2	4
S. warneri	2				2					1		4	9
	9	5	1	6	20	1	1	7	1	8	6	24	89

NT: non-typable.

6.3 Multi-locus sequence typing

Following the determination of SCC*mec* types, MLST was performed to determine the housekeeping genes of 19 *S. epidermidis*. MLST is an accurate typing approach used for microorganisms, and MLST type is determined by combination of seven housekeeping gene locus (Thomas et al., 2007). Each housekeeping gene of 19 *S. epidermidis* were compared with the MLST database, and *arc C, aro E, gtr* of 120 were determined to be new locus as there was no match in the database. *gtr* of 119 was determined to be a new locus, as no homology in the database was found. MLST typing revealed that 17 *S. epidermidis* strains represent new MLST types. MLST type of *S. epidermidis* 120, 119，122，121, 279, 133, 134, 135, 126, 259, 124, 127, 234, 187, 308, 153 and 191 were assigned as ST515, ST516, ST517, ST518, ST599, ST600, ST600, ST600, ST601, ST602, ST602, ST603, ST604, ST605, ST606, ST607 and ST608. *S. epidermidis* 133, 134 and 135 were isolated from DSL, and they shared the same MLST type ST600. *S. epidermidis* 259 was isolated from HH, while *S. epidermidis* 124 was isolated from DSH. Both of them were ST602 (Table 6.6). In addition, *S. epidermidis* 139 were identified to be a known type ST360, and *S. epidermidis* 118 (G6_2) were determined to be ST59.

Table 6. 6 MLST types identified in *S. epidermidis*

ID	Species	Sites	*arcC*	*aroE*	*gtr*	*muts*	*pyr*	*Tpi*	*yqil*	ST
118	*S. epidermidis*	Hotels	2	1	1	1	2	1	1	ST59
119	*S. epidermidis*	Hotels	1	2	5	1	1	1	14	ST516
120	*S. epidermidis*	Hotels	28	25	5	5	7	5	11	ST515
121	*S. epidermidis*	Hotels	1	2	1	1	1	1	14	ST518
122	*S. epidermidis*	Hotels	1	2	2	6	2	16	1	ST517
124	*S. epidermidis*	Hotels	57	1	2	2	4	1	1	ST602
127	*S. epidermidis*	Hotels	57	10	5	5	10	16	21	ST603
133	*S. epidermidis*	Library	57	1	2	2	4	1	4	ST600
134	*S. epidermidis*	Library	57	1	2	2	4	1	4	ST600

ID	Species	Sites	*arcC*	*aroE*	*gtr*	*muts*	*pyr*	*Tpi*	*yqil*	ST
135	*S. epidermidis*	Library	57	1	2	2	4	1	4	ST600
139	*S. epidermidis*	Restaurants	3	3	5	5	7	4	4	ST360
153	*S. epidermidis*	Restaurants	57	1	22	2	2	16	1	ST607
187	*S. epidermidis*	Supermarkets	57	1	1	2	2	1	1	ST605
191	*S. epidermidis*	Supermarkets	57	3	5	5	7	14	11	ST608
234	*S. epidermidis*	Handbags	57	1	1	1	2	41	1	ST604
126	*S. epidermidis*	Hands	57	25	9	5	6	1	8	ST601
259	*S. epidermidis*	Hands	57	1	2	2	4	1	1	ST602
279	*S. epidermidis*	Hands	57	17	5	5	3	4	31	ST599
308	*S. epidermidis*	Hands	57	1	2	2	4	7	1	ST606

6.4 Chapter summary

Genotypic characterisation of staphylococci showed that:

1. *mecA* gene was found in eighty-nine (12%) staphylococcal isolates;
2. Thirty-nine of the 89 (44%) isolates that were *mecA* positive were found to have MICs below 0.5 mg l^{-1} to oxacillin;
3. Expression of PBP 2a was found in high oxacillin resistant *mecA* positive staphylococci, but not in oxacillin susceptible *mecA* positive staphylococci;
4. SCC*mec* type I, II, III, IV, V, VI, VIII were determined in environmental staphylococci;
5. Fourteen new MLST types were determined in 19 selected staphylococci.

MRSA is a potential public health threat, which has been recovered from both clinical and community associated settings all over the world. However, little is known about the dissemination of methicillin-resistant staphylococcal isolates recovered from the environment. This chapter introduced the dissemination of *mecA* gene positive staphylococci and molecular characterization of *mecA* gene positive staphylococci. The correlation of *mecA*-positive and phenotypic oxacillin susceptibility in environment staphylococci will be assessed by comparing with clinical staphylococci in the discussion chapter, and then the existence of the oxacillin susceptible *mecA*-positive environmental staphylococcal isolates will be assessed in the discussion chapter. The prevalent SCC*mec* types in the environment will be compared with community-associated SCC*mec* types and hospital-associated SCC*mec* types. Finally, the MLST types of environmental staphylococci will be compared with international reported MLST types to assess the dominant lineage of environmental staphylococci in the discussion chapter.

Chapter 7 Genomic analysis of *S. epidermidis* 118 (G6_2)

Complete genome sequencing of staphylococci can provide insights into important genomic features and assess the variation of plasmids that contribute to the pathogenicity of staphylococci (Gill et al., 2005). Whole genome sequencing has been used to analyse clinical significant staphylococci, transmission of *S. aureus* in health care facilities, and antibiotic resistance in clinical isolates (Harris et al., 2013; Peleg et al., 2012; Price et al., 2013); however, complete genomic features of environmental isolated staphylococci has been rarely reported. Since environmental staphylococci may act as a reservoir for antibiotic resistance genes (Wright, 2007), it would be necessary to gain insights into the genomic feature of environmental staphylococci. Whole genome sequencing was applied to *S. epidermidis* 118 (G6_2), which was recovered from a hotel room. MIC of *S. epidermidis* 118 (G6_2) against oxacillin was 256 mg l^{-1}, and phenotypically resistant to 11 out of 13 tested antibiotics.

7.1 Bacteria

S. epidermidis 118 (G6_2) was isolated and purified from a hotel room in London area in 2013, and it was resistant to all tested antibiotics, except vancomycin and chloramphenicol. Moreover, the MIC of *S. epidermidis* 118 (G6_2) was determined to be 256 mg l^{-1} against oxacillin. In addition, the *mecA* gene was identified and the SCC*mec* was determined to be type IV for this isolate.

The accession number for *S. epidermidis* 118 (G6_2) is ERR387168.

7.2 Phylogenetic relationship with clinical reference *S. epidermidis*

S. epidermidis 118 (G6_2) and publically available assembled 92 *S. epidermidis* were chosen to show the phylogenetic relationship (Fig 7.1). *S. epidermidis* 118 (G6_2) was highlighted in red. The *S. epidermidis* most similar to *S. epidermidis* 118 (G6_2) was boxed in red; however, the phylogenetic relationship was distinguishable as it formed a distinct branch (Fig 7.1).

The 92 reference isolates were referred to datasets of clinical *S. epidermidis* (Roach et al., 2015; Tewhey et al., 2014). Isolates SRR1656389, SRR1656376 and SE_BCM-HMP0060 (Roach et al., 2015) were in a distinct clade and shown to be most similar to *S. epidermidis* 118 (G6_2) (Fig 7.1). Whereby, *S. epidermidis* 118 (G6_2) is closest to the root of the clade.

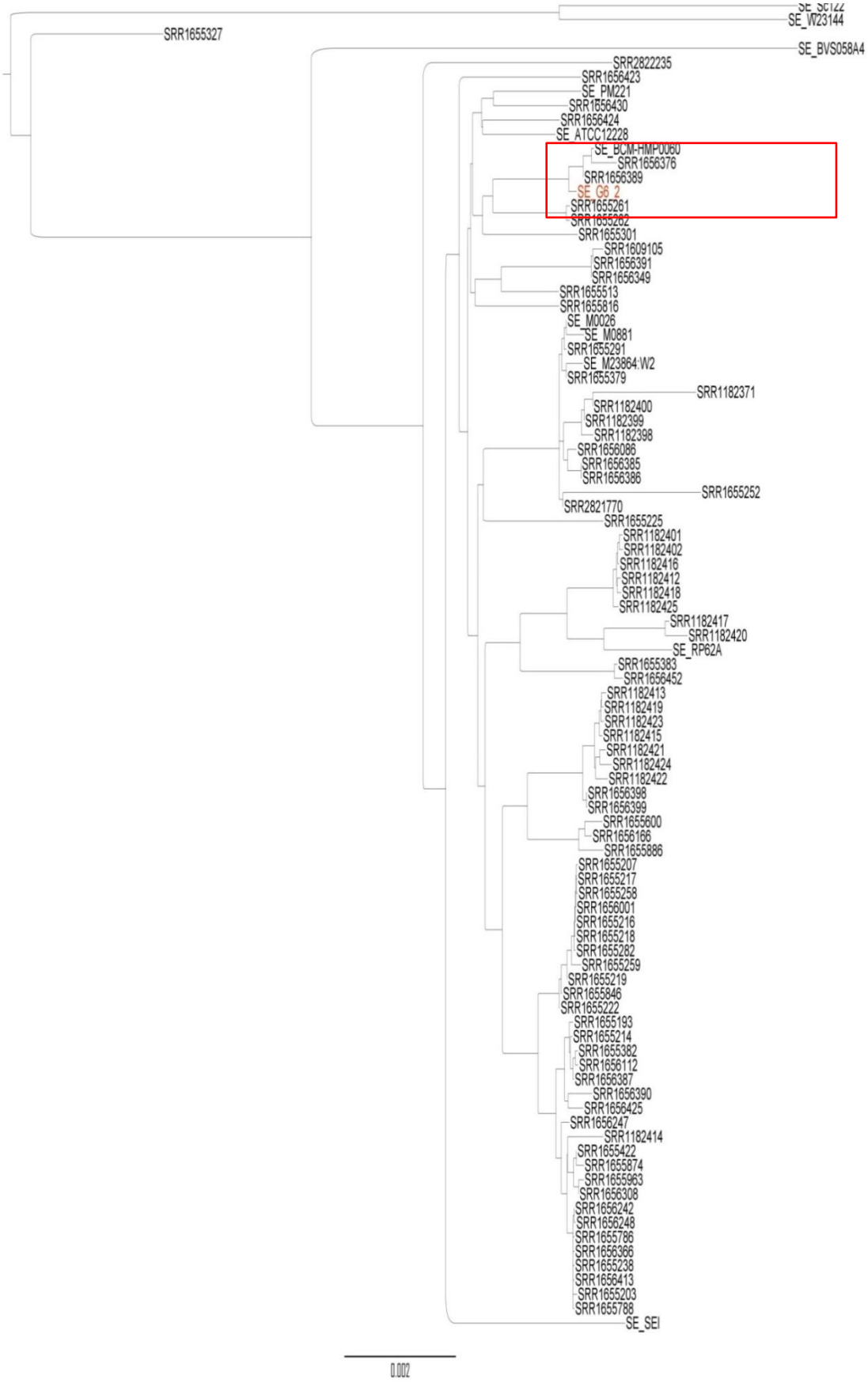


Figure 7. 1 Phylogenetic tree based on whole genome sequencing

7.3 General genomic features and plasmids

General genomic features of *S. epidermidis* 118 (G6_2), *S. epidermidis* PR62a, *S. epidermidis* ATCC12228 and *S. aureus* N315 were shown in Table 7.1. In general, the *S. epidermidis* 118 (G6_2) genome comprises of one chromosome (2408357 bp in length) and six plasmids, annotated as pG6_2_1 to pG6_2_6 (the largest, pG6_2_1, is 10570 and the smallest, pG6_2_6, is 3426 bp in length), with an average G+C content of 32.02%. It has a total (chromosome and plasmids) of 2213 predicted protein coding sequences, of which 21.5% were annotated as hypothetical and 14.3% were annotated as putative functions.

Table 7. 1 General features of *S. epidermidis* 118 (G6_2) and reference staphylococci

	S. aureus	*S. epidermidis*		
	N315	RP62a	ATCC 12228	118 (G6_2)
Chromosome				
Length of sequences	2814816	2616530	2499279	2408357
G+C content	32.80%	32.1%	32.10%	32.02%
Protein coding region	2595	2391	2419	2213
Ribosomal RNAs	16	19	16	4
16S	5	6	5	
23S	5	6	5	
5S	6	7	6	
Transfer RNAs	62	59	60	60
Plasmid				
Length of sequences	24653	27310	P1:4439	P1：10570
			P2:4679	P2：4909
			P3:8007	P3：4588
			P4:17261	P4：4576
			P5:24370	P5：4271
			P6:6585	P6：3426
Antibiotic resistance genes				
Beta-lactamase			+	+
tetracycline resistant protein			+	+
Bleomycin resistant protein				
Penicillin binding proteins				
Bifunctional AAC-APH		+		+
Aminoglycoside phosphotransferase	+	+		

	S. aureus	*S. epidermidis*		
	N315	RP62a	ATCC 12228	118 (G6_2)
Penicillin binding protein 2a	+	+		+
Fosfomycin resistance protein		+		+
Macrophage scavenger receptors				+
Inactivating enzymes				+
Isoleucyl RNA synthetase				+
Elongation factor G				+

7.4 Genotypic prediction of antibiotic resistance

Antibiotic resistance prediction, using the ResFinder (version 2.1) online tool (Center for Genomic Epidemiology), revealed a wide range of potential resistance features dispersed across the chromosome and plasmids (Table 7.2). A total of seven resistance phenotypes were predicted and included: aminoglycoside resistance (*aac(6') – aph(2")* genes, 100% identity); beta-lactam resistance (borne from the presence of both the *mecA* and *blaZ* genes, 100% identity); fosfomycin resistance (*fosA* gene, 100% identity); macrolide resistance (*mph(C*) gene, 100% identity); macrolide, lincosamide and streptogramin B resistance (*msr(A)* gene, 98.98% identity); and tetracycline resistance (*tet(K)* gene, 99.93% identity). Of these, only *mecA* and *fosA* were mapped to the chromosome, the remainder were localised to the plasmids, specifically, plasmid pG6_2_1 harboured *blaZ*, pG6_2_2 has the gene *tet(K)*, pG6_2_3 has both *mph(C)* and *msr(A)*, and pG6_2_4 possessed the *aac(6') – aph(2")* genes.

With the 13 tested antibiotics, *S. epidermidis* 118 (G6_2) was shown to be phenotypically resistant to streptomycin (aminoglycoside antibiotic), gentamicin (aminoglycoside antibiotic), penicillin (beta-lactam antibiotic), oxacillin (beta-lactam antibiotic), amoxicillin (beta-lactam antibiotic), cefepime (beta-lactam antibiotic), cefoxitin (beta-lactam antibiotic), erythromycin (macrolide antibiotic), tetracycline (tetracylines antibiotic), fusidic acid (steroid antibiotic), and mupirocin (monoxycarbolic acid antibiotic).

The antibiotic resistance genes determined by WGS correlated with the phenotypic data is as follows: *aac(6')-aph(2")* encodes for aminoglycoside-modifying enzymes, resulting in aminoglycoside resistance (streptomycin and gentamicin); *blaZ* encodes β-lactamase, responsible for beta-lactam resistance (penicillin); *mecA* encodes penicillin-

binding protein 2a, responsible for beta-lactam resistance (oxacillin, cefoxitin, cefepime and amoxicillin); *fosA* encodes fosfomycin resistance protein, responsible for fosfomycin resistance; *msr(A)* encodes macrophage scavenger receptors, responsible for macrolide, lincosamide and streptogramin B resistance; *mph(C)* encodes inactivating enzymes, responsible for macrolide resistance (erythromycin); and *tet(K)* encodes the tetracycline efflux pump, responsible for tetracycline resistance; The resistance determinants towards streptomycin, gentamicin, penicillin, oxacillin, cefoxitin, amoxicillin, cefepime, erythromycin, tetracycline were successfully predicted by ResFinder (version 2.1) online tool (Center for Genomic Epidemiology). These however did not predict genes responsible for fusidic acid and mupirocin resistance using ResFinder (version 2.1). The determinants responsible for mupirocin resistance have been found in Prokka annotated file. Mupirocin resistance in *S. aureus* can be caused by acquisition of an additional isoleucyl RNA synthetase (*ileS*) gene (Hodgson et al., 1994). In this study, *ileS* gene responsible for the resistance of mupirocin was present in *S. epidermidis* 118 (G6_2). Although it is expected to be captured by ResFinder tool, it does not make part of the database and hence it was overlooked. Fusidic acid resistance occurs due to the mutations in the *fusA* encoding elongation factor G (EF-G) or *rplF* (or *fusE*) (Howden & Grayson, 2006). These mutations cannot be captured by ResFinder as it only identifies acquired resistance genes.

Comparative analysis with reference strains revealed that *S. epidermidis* RP62a includes five antibiotic resistance genes, including aminoglycoside resistance *spc* genes (100% identity) and *aac(6') – aph(2")* genes (100% identity); beta-lactam resistance *mecA* (100% identity); fosfomycin resistance (*fosA* gene, 99.07% identity); macrolide resistance gene, *erm(A)* (100% identity). Only two resistance genes, including beta-lactam resistance *blaZ* genes (100% identity) and tetracycline resistance genes (*tet(K)*

gene, 99.93% identity) were found in *S. epidermidis* ATCC12228. Four antibiotic resistance genes were found in *S. aureus* N315: aminoglycoside resistance *spc* genes (100% identity) and *aadD* genes (99.74% identity); beta-lactam resistance *mecA* gene (100% identity); and macrolide resistance gene, *erm(A)* (100% identity).

Table 7. 2 Genotypic prediction of antibiotic resistance of *S. epidermidis* 118 (G6_2)

Product	Gene name	Accession number (Identity %)	Location	Position in contig	Function	Class of antibiotic	Antibiotics
Aminoglycoside -modifying enzymes	*aac(6')-aph(2'')*	M13771 (100)	pG6_2_4	2386323..2387762	Aminoglycoside resistance	Aminoglycoside	Gentamicin Streptomycin
β-lactamase	*blaZ*	AJ302698 (100)	pG6_2_1	1668561..1669406	Beta-lactam resistance		Penicillin Oxacillin
Penicillin-binding protein 2a	*mecA*	AB505628 (100)	Genome	2648..4657	Beta-lactam resistance	Beta-lactam	Amoxicillin Cefepime Cefoxitin
Fosfomycin resistance protein	*fosA*	ACHE01000077 (100)	Genome	104692..105120	Fosfomycin resistance	Phosphonic	Fosfomycin
Macrophage scavenger receptors	*msr(A)*	X52085 (98.98)	pG6_2_3	1472987..1474453	Macrolide, Lincosamide and Streptogramin B resistance	Microlide	Erythromycin
Inactivating enzymes	*mph(C)*	AF167161 (100)	pG6_2_3	1474552..1475451	Macrolide resistance		
Tetracycline efflux pump	*tet(K)*	U38428 (99.93)	pG6_2_2	430430..431809	Tetracycline resistance	Tetracycline	Tetracycline
Isoleucyl RNA synthetase	*ileS*	-	-	-	Fusidic acid resistance	Steroid	Fusidic acid
Elongation factor G	*fusA*	-	-	-	Monoxycarbolic resistance	Monoxycarbolic	Mupirocin

7.5 Functional genes uniquely found in *S. epidermidis* 118 (G6_2)

104 genes were uniquely found in the chromosome of *S. epidermidis* 118 (G6_2), when compared to the reference strains in this study. The majority were annotated as hypothetical, while the remainder comprised of a variety of functions, including heavy metal transport, regulatory, HTH domain, transpose and metabolic proteins. There were eight genes that were exclusively found in one of *S. epidermidis* 118 (G6_2) plasmids. The majority of these were annotated as hypothetical, of the remainder the gene *qacC*, *yheS* and Tn552 were identified.

7.6 Comparative virulence genes

Comparison of *S. epidermidis* 118 (G6_2) with *S. aureus* N315 showed that (1) 312 genes were in common; (2) 32 genes were uniquely found in *S. epidermidis* 118 (G6_2); (3) 522 genes were missing in *S. epidermidis* 118 (G6_2). Comparison of *S. epidermidis* 118 (G6_2) with *S. epidermidis* RP62a revealed that (1) 303 genes were in common; (2) 40 genes were uniquely found in *S. epidermidis* 118 (G6_2); (3) 75 genes were missing in *S. epidermidis* 118 (G6_2). Comparison of *S. epidermidis* 118 (G6_2) with *S. epidermidis* ATCC12228 showed that (1) 306 genes were common; (2) 38 genes were uniquely found in *S. epidermidis* 118 (G6_2); (3) 44 genes were missing in *S. epidermidis* 118 (G6_2) (Table 7.3).

Table 7. 3 Comparision of virulence genes between *S. epidermidis* 118 (G6_2) and reference staphylococci

S. epidermidis	Virulence genes	*S. aureus*	*S. epidermidis*	
		N315	RP62a	ATCC12228
	Unique	32	40	38
118 (G6_2)	Common	312	303	306
	Missing	522	75	44

There were 266 genes present in all four staphylococcal isolates in this study. The intracellular adhesion genes *icaA, icaB, icaC, icaD and icaR* responsible for biofilm formation (Cramton et al., 1999) were not present in *S. epidermidis* 118 (G6_2) and *S. epidermidis* ATCC12228. In contrast, *icaA* was present in *S. aureus* N315 (Zhang et al., 2003) and *S. epidermidis* RP62a (Kaplan et al., 2011). In addition, the genes *copZ_2, copA_2* and *csoR_1* variants associated with copper transport were determined in *S. epidermidis* 118 (G6_2) (Harrison et al., 2000; Schelder et al., 2011). A further 34 other heavy metal associated genes were shown to be homologous between *S. epidermidis* 118 (G6_2) and *S. epidermidis* RP62a and ATCC12228. Forty-eight genes were shown to be specific to *S. aureus* N315, primarily associated with iron transport and metabolism.

Virulence determinants in each staphylococcal species were shown in Table 7.4. A total of 29 virulence genes belonging to 14 virulence factors families were found in *S. epidermidis* 118 (G6_2), which is less than *S. aureus* N315 (n=61) but more than *S. epidermidis* RP62a (n=19) and ATCC 12228 (n=18). Thirteen virulence factors were found in common between the four staphylococcal strains, including *htrA, sspA, sspB, lip, lipA, hlb, nuc, clpP, clpB, clpX, clpC clpE* and *isaA.* Twelve virulence genes, *setC, sspP, lip_1, lip_2, lip2_1, lip2_2, lipR_2, lipM, lipL, lipR, sfpA,* and *sigD,* were uniquely found in *S. epidermidis* 118 (G6_2). *isaB, lytN* and *cptV* were found in both *S. epidermidis* 118 (G6_2) and *S. aureus* N315, but not in *S. epidermidis* RP62a and ATCC 12228. The gene *geh* was the only common gene found in *S. epidermidis* 118 (G6_2), RP62a and ATCC12228.

Table 7. 4 Virulence genes in *S. epidermidis* 118 (G6_2) and reference staphylococci

Virulence factor	*S.aureus*	*S.epidermidis*		
	N315 Gene	RP62a Gene	ATCC12228 Gene	118 (G6_2) Gene
Enterotoxin	*sea*			
	sec3			
	seg			
	sei			
	sel			
	sem			
	sen			
	seo			
	sep			
	yent1			
	yent2			
Exotoxin	*set1*			
	set2			
	set3			
	set4			
	Set6			
	set7			
	set8			
	set9			
	set10			
	set11			
	set12			
	set13			
	set14			
	set15			
	-			*setC*
Exfoliative toxin	*eta*			
Toxic shock syndrome toxin	*tsst*			
Serine protease	*splA*			
	splB			
	splC			
	splD			
	splF			
	htrA	*htrA*	*htrA*	*htrA*
Serine V8 protease	*sspA*	*sspA*	*sspA*	*sspA*
Cysteine protease	*sspB*	*sspB*	*sspB*	*sspB*
	sspC			
				sspP
Lipase	*lip*	*lip*	*lip*	*lip*
				lip_1
				lip_2
				lip2_1

Virulence factor	*S.aureus*	*S.epidermidis*		
	N315 Gene	RP62a Gene	ATCC12228 Gene	118 (G6_2) Gene
				lip2_2
				lipR_2
		geh	*geh*	*geh*
		geh1, gehC	*geh1,gehC*	
		geh2, gehD	*geh2, gehD*	
Lipase/esterase	*lipA*	*lipA*	*lipA*	*lipA*
				lipL
				lipM
				lipR
Leukotoxin D	*lukD*			
Leukotoxin E	*lukE*			
Leukocidin F	*lukF*			
Leukocidin M	*lukM*			
Alpha hemolysin	*hly*			
Beta hemolysin	*hlb*	*hlb*	*hlb*	*hlb*
Delta hemolysin	*hld*	*hld*	*hld*	
Gamma hemolysin, component A	*hlgA*			
Gamma hemolysin, component C	*hlgC*			
Gamma hemolysin, component B	*hlgB*			
Hyaluronate lyase	*hysA*			
Thermonuclease nuclease	*nuc*	*nuc*	*nuc*	*nuc*
Cell wall hydrolase	*lytN*			*lytN*
Clp protease, procolytic subunit	*clpP*	*clpP*	*clpP*	*clpP*
Clp protease, ATP binding subunit	*clpB*	*clpB*	*clpB*	*clpB*
Clp protease, ATP binding subunit	*clpX*	*clpX*	*clpX*	*clpX*
Clp protease, ATP binding subunit	*clpC*	*clpC*	*clpC*	*clpC*
Clp protease, ATP binding submit	*clpE*	*clpE*	*clpE*	*clpE*
Staphylococcal protein A Spa	*Spa*			
Phenol-soluble modulin	*hldc*			
Immunodominant staphylococcal antigen A	*isaA*	*isaA*	*isaA*	*isaA*
Immunodominant staphylococcal antigen B	*isaB*			*isaB*
cytoplasmic proteins	*isdl*			
Pilin Subunit Gene				*sfpA*
Sigma D factor				*sigD*

Virulence factor	*S.aureus*	*S.epidermidis*		
	N315 Gene	RP62a Gene	ATCC1228 Gene	118 (G6_2) Gene
Intercellular adhesion gene	*ica*	*ica*		
ATPase_copper_transporter	*ctpV*			*cptV*

Surface protein genes identified in each staphylococcal species were shown in Table 7.5. Eight surface protein genes were found in *S. epidermidis* 118 (G6_2), of which *sdrC*, *sdrD*, *sdrG*, *ebh*, *ebp*, and *atl* were involved in adherence to host tissue, *pls* involved in methicillin resistant, and *sasK* involved in binding to heme-iron (Gill et al., 2005). Surface protein genes, *sdrC* and *sdrD* were present in both *S. epidermidis* 118 (G6_2) and *S. aureus* N315, and *pls* were appeared in *S. epidermidis* 118 (G6_2) and RP62a. *sdrG* and *ebp* were present in three *S. epidermidis.* Furthermore, *sasK*, *ebh* and *atl* were found in all staphylococcal species. Moreover, surface protein genes found in *S. epidermidis* 118 (G6_2) (n=8) was less than *S. aureus* N315 (n=19) and *S. epidermidis* RP62a (n=9), and equal to *S. epidermidis* ATCC12228 (n=8).

Table 7. 5 Surface proteins of *S. epidermidis* 118 (G6_2) and reference staphylococci

Genes	Functional name	Function	*S.aureus*	*S.epidermidis*		
			N315	RP62a	ATCC12228	G6_2
clfA	Clumping factor A	a	+			
clfB	Clumping factor B	a	+			
fnbA	Fibronectin binding protein A	a	+			
fnbB	Fibronectin binding protein B	a	+			
sdrC	SdrC	a	+			+
sdrD	SdrD	a	+			+
sdrE	SdrE	a	+			
sdrF	SdrF	a		+	+	
sdrG	SdrG	a		+	+	+
sdrH	SdrH	a		+	+	
spa	ProteinA	a,b	+			
pls	Methicillin resistant surface protein	d		+		+
sasC	Cell wall surface anchor protein	d	+			
sasG	Cell wall surface anchor protein	d	+	+	+	
sasK	Cell wall surface anchor protein	c	+	+	+	+
isdC	Heme transporter	d	+			
isdI	Heme degrading moxooxygenase	a	+			
map	Extracellular adherence protein	a	+			
empbp	Extracellular matrix and plasma binding protein	a	+			
ebh	Cell wall-associated fibronectin binding protein	a	+	+	+	+
efb	Fibrinogen binding protein	a	+			
ebp	Elastin binding protein	a		+	+	+
atl	Biofunctional autolysin	a	+	+	+	+

Note: a, adherence to host tissue (extracellular matrix, fibrinogen, fibronectin, collagen, elastin, endothelial and epithelial cells); b, evasion of host defense; c, binding to heme-iron; d, unknown;

7.7 Chapter summary

Whole genome analysis of *S. epidermidis* 118 (G6_2) showed that:

1. Environmental *S. epidermidis* 118 (G6_2) formed distinctive branch with clinical reference *S. epidermidis*;

2. Nine antibiotic resistance genes were found in genome of *S. epidermidis* 118 (G6_2);

3. Multiple virulence and anti-detergent genes were determined in *S. epidermidis* 118 (G6_2).

Antibiotic resistance is a major global threat to public health and increasingly antibiotic resistant bacteria are emerging from different ecological niches, including environmental sources. Little is known of the genetic variations associated with strains isolated from environmental sources and/or general public settings.

S. epidermidis is an opportunistic pathogen primarily recovered from infections arising from healthcare associated medical devices. Genome sequencing of *S. epidermidis* strains have been reported, however, these have been limited to commensal and nosocomial strains. To understand the genetic background of environmental *S. epidermidis* strains, whole genome sequence analysis of multidrug-resistant *S. epidermidis* 118 (G6_2) was performed.

The comparative analysis of *S. epidermidis* 118 (G6_2) and clinical reference strains of *S. aureus* N315 and other *S. epidermidis* RP62a and ATCC12228 genomes will be discussed in the discussion chapter. Evaluation of the antibiotic resistance and virulence in environmental multidrug-resistant *S. epidermidis* 118 (G6_2) will be discussed in the discussion chapter.

Chapter 8 *mecA* gene transfer via mating experiment

Wielders et al., (2001) reported the evidence of *in vivo* transfer of *mecA* gene between clinical *S. aureus* isolates, and Bloemendaal et al., (2010) also showed the evidence of *in vivo* SCC*mec* transfer from *S. epidermidis* to *S. aureus* during antibiotic therapy, Bloemendaal et al have attempted to replicate the SCC*mec* transfer *in vitro*; however, the transfer of *mecA*/SCC*mec in vitro* has not been reported (Bloemendaal et al., 2010). After reviewing the present findings, mating experiment mediated transferring of *mecA*/SCC*mec* from environmental staphylococci to *S. aureus* NCTC6571 *in vitro* was applied. Additionally, comparative proteomic analysis provided functional genomics data for *S. aureus* to resistant to antibiotic.

8.1 *mecA* gene amplification in conjugants

The broth mating method was used to determine the *mecA* gene transfer, 10 trans-conjugants were isolated and purified with mannitol salt agar supplemented with 4 mg l^{-1} oxacillin, and *mecA* gene PCR were applied to these ten conjugants. *S aureus* NCTC6571 (recipient) showed a negative result, and *S. hominis* 399 (donor), conjugants No 3, 6, 7 and 10, showed positive results. These conjugants were then tested with another pair of *mecA* gene primers (286 bp), and conjugant No7 was showed positive results (Fig 8.1).

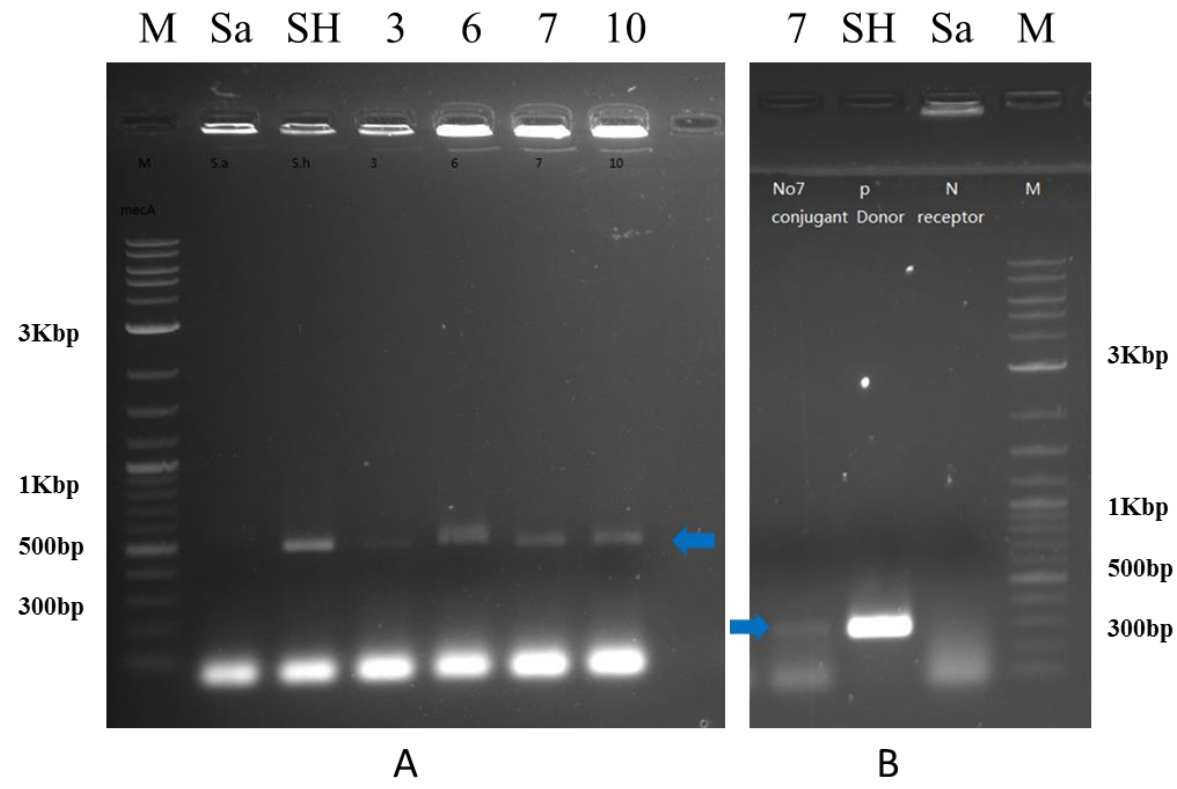


Figure 8. 1 Gel image of *mecA* gene PCR product. A 527 bp *mecA* primers (Hanssen et al., 2004), B 286 bp *mecA* primers (Kondo et al., 2007); Sa: *S. aureus*; SH: *S.hominis* 399; 3: conjugant No3; 6: conjugant No6; 7: conjugant No7; 10: conjugant No10; M: DNA ladder.

8.2 16S rRNA gene sequencing of conjugant

The species of conjugant was identified by partial 16S rRNA gene sequencing. The partial 16S rRNA gene of conjugant No7 was amplified, and the sequence was examined to be *S. aureus* in comparison with the nucleotide database at National Center for Biotechnology Information (NCBI) by using BLAST® (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

8.3 Prolex™ staph XTRA latex tests and MIC of conjugant

The conjugant was also tested by Staph latex test, which showed a positive result. The MICs of *S aureus* NCTC 6571 (recipient), *S. hominis* 399 (donor) and No7 (conjugant)

were determined. The recipient was fully susceptible to oxacillin, with MIC value of 0.125 mg l^{-1}, and the MIC of conjugant was 0.25 mg l^{-1}, which indicated fully susceptible to oxacillin. However, the conjugant could grow on mannitol salt agar supplemented with 4 mg l^{-1} oxacillin. The potential reason for this conflict phenomenon may be due to the mannitol salt agar, which contains high concentration of salt. In order to confirm the transfer of *mecA* gene between *S. hominis* and *S. aureus*, Southern blotting and PFGE were applied to donor, recipient and conjugant.

8.4 Southern blotting

After determining the MIC of conjugant, donor and recipient, Southern blotting was applied to determine the presence of the *mecA* gene in conjugant. According to Southern blotting results, the presence of *mecA* gene was confirmed in donor, but not in *S. aureus* NCTC6571 (recipient) and conjugant No7.

8.5 PFGE results

To avoid contamination of conjugant No7, pulse-field gel electrophoresis was used to examine the genetic patterns of donor, recipient and conjugant. PFGE showed that the conjugant and *S. aureus* NCTC 6571 (recipient) had the same pattern, but was different from the donor (Fig 8.2). The same PFGE pattern indicated the presence of one strain, and no contamination was found.

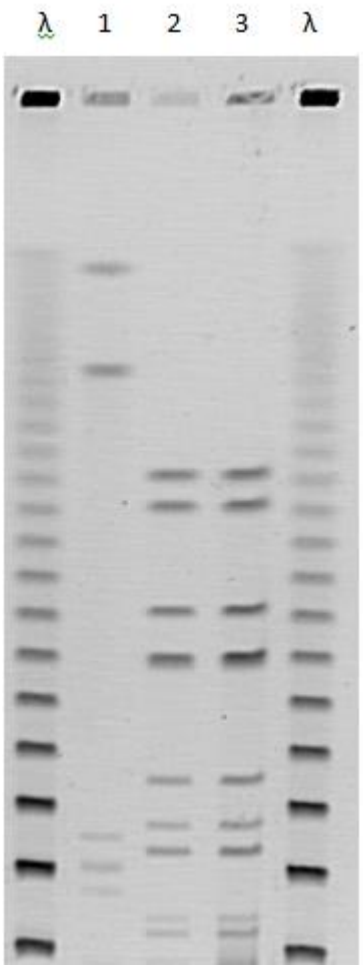


Figure 8. 2 PFGE patterns of donor, recipient and conjugant. λ = M Wt Ladder; Lane 1 = Donor (*S. hominis*, *mecA* positive); Lane 2 = Recipient (*S. aureus*, *mecA* negative); Lane 3 = Conjugant No7

8.6 Comparative proteomic analysis

As noted above, no *mecA* gene was detected in conjugant No7 based on Southern blotting, however, conjugant No7 could survive and propagate on 4 mg l^{-1} oxacillin supplemented MSA. The colonies of conjugant No7 on oxacillin supplemented MSA were small and sticky. Comparative proteomic analysis was then applied to clarify protein expression differences between *S. aureus* NCTC 6571 and conjugant No7.

8.6.1 Protein extraction

The first stage of comparative proteomic analysis was protein extraction. *S. aureus* NCTC 6571 was cultured on mannitol salt agar, and meanwhile conjugant No7 were cultured on mannitol salt agar supplemented with 4 mg l^{-1} oxacillin.

Protein was extracted and the concentration was determined by Bradford assay (Sigma Aldrich, UK). The concentration of each sample was shown in the Table 8.1.

Table 8. 1 Protein concentration of *S. aureus* NCTC6571 and conjugant No7

ID	Concentration 1 (µg $µl^{-1}$)	Concentration 2 (µg $µl^{-1}$)	Concentration 3 (µg $µl^{-1}$)
***S.aureus* NCTC 6571**	3.46308	1.66304	1.36051
Conjugant No 7	4.04837	7.67402	7.42345

8.6.2 In-gel trypsin digestion

Second, 10 µg of protein extract was loaded on to a gel and separated by 1D SDS-PAGE using MES running buffer (Invitrogen, UK) in accordance with the manufacturer's instructions. The 1D SDS-PAGE band patterns of *S. aureus* and conjugant No7 was showed in figure 8.3.

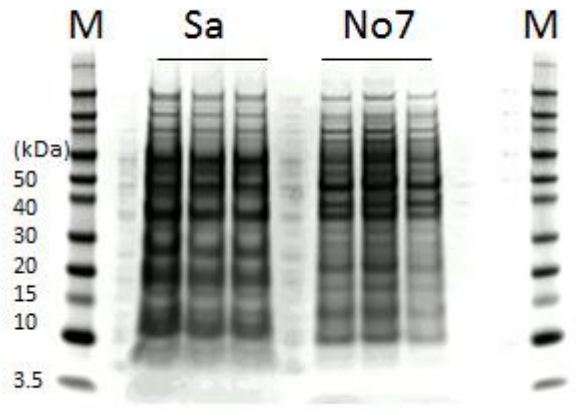


Figure 8. 3 1D SDS-PAGE (triplicates) band patterns of *S. aureus* and conjugant No7. M: protein ladder, Sa: *S. aureus* (cultured on MSA); No7: conjugant (cultured on oxacillin suppl MSA).

8.6.3 Comparative analysis of protein expression

The comparative analysis of protein expression demonstrated that various proteins were upregulated or exclusively expressed in conjugant No7 but not in *S. aureus* NCTC 6571. All of these proteins could be classified into 7 categories by function: including proteins involved in efflux, cell wall synthesis, virulence, reparation, stress response, degradation, and translation.

A total of 1353 proteins were detected by LC-MS/MS, and 757 proteins were both found in *S. aureus* (recipient) and conjugant No7. 300 proteins were uniquely expressed in *S. aureus* (recipient), and 296 proteins were uniquely expressed in conjugant No7 (Fig 8.4).

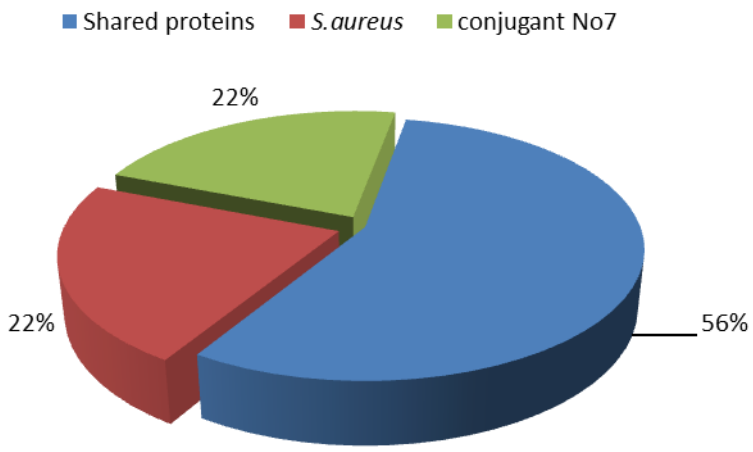


Figure 8. 4 Overview of protein expression differences in *S. aureus* NCTC 6571 and conjugant No7

No penicillin binding protein 2a was found in both *S. aureus* and conjugant. However, *EmrB/QacA* subfamily protein, drug resistance transporter (A6QJJ3, 71 KDa), and ATP-binding cassette transporter (A6QIF7, 33 KDa) were increasingly expressed in the conjugant. *EmrB/QacA* subfamily protein, drug resistance transporter and ATP binding

cassette transporter can be implicated in antibiotic resistance through facilitating the export of various cytotoxic drugs out of the membrane (Dawson & Locher, 2006; Lomovskaya & Lewis, 1992) (Table 8.2). In addition, the upregulation of these two transporters are also involved in the stringent response of bacteria (Anderson et al., 2006).

In addition to the protein involved in efflux, increased expression of proteins involved in cell wall synthesis were also observed in the conjugant, such as UDP-N-acetylmuramate-L-alanine ligase (A6QFM8, 45 kDa), penicillin binding protein 2 (A6QG81, 83 kDa); glycosyl transferase group 2 family protein (A6QDN2, 66 kDa) , and glutamate racemase (MURI, 30 kDa) (Fotheringham et al., 1998; Munshi et al., 2013; Rebets et al., 2014; Sauvage et al., 2008) (Table 8.2). However, the expression of penicillin binding protein 1 (A6QG81, 83 kDa) remains the same in both samples (Sauvage et al., 2008).

Moreover, in conjugant cultured with oxacillin, the virulence factors, including clumping factor A (CLFA, 97 kDa); zinc metalloprotease (A6QEG3, 78 kDa); *Clp* protease, procolytic subunit (CLPP, 22 kDa); *Clp* protease, chaperone protein (A6QFI5, 98 kDa); *Clp* protease, ATP- binding subunit (A6QEH7, 91 kDa), were specifically present or expressed at an increased level (Bloemendaal et al., 2010; Gill et al., 2005; Josefsson et al., 2001) (Table 8.2).

DNA ligase (DNLJ, 75 kDa) which is involved in DNA repair was found exclusively in conjugant (Cynthia Chen et al., 2002; Hanawalt & Cooper, 1979). Another repair protein is DNA mismatch repair protein *MutS* (MUTS, 100 kDa), whose function is known to correct DNA replication mismatch during the adverse influences on the genome (O'Neill, 2002). The expression of *MutS* was found to remain the same.

29 stress response proteins were identified, including 4 SOS response proteins, 13 stringent response proteins, and 12 heat-shock response proteins. The stress response proteins of bacteria are important for survival during imposition of environmental stresses (Anderson et al., 2006) (Table 8.2). All SOS response proteins were found to be upregulated in conjugant in comparison with *S. aureus*. 10 stringent response proteins (except aspartate-semialdehyde dehydrogenase, sulphite reductase and threonine synthase) were upregulated in the conjugant. The expression of 7 heat shock response proteins was increased, whereas the expression of 5 heat shock response proteins remains the same in conjugant. Notably, no cold shock protein was found in both isolates (Table 8.2).

Thioredoxin, which is essential to protect cells from oxygen damage, was upregulated in conjugant (Bore et al., 2007). Additionally, four proteins that belong to heat shock protein family were found to be upregulated in conjugant (Table 8.2). Chaperone protein DnaJ and DnaK is known to maintain normal function of cell and solubilize the protein aggregates (Cuéllar et al., 2013; Mogk et al., 2003), and small heat shock protein GrpE (Hsp20) and molecular chaperone Hsp31 are involved in protein folding and stabilization (Muthaiyan et al., 2012).

ATP-dependent protease subunit (HSLV, 20 kDa) and Dead-box RNA helicase (A6QIS5, 57 kDa) that are involved in degradation process in cells were found to be upregulated in the conjugant. HslU–HslV is a bacterial proteasome, the function of which is known to preserve cellular homeostasis by degrading substrate polypeptides (Shi & Kay, 2014). In addition, Dead box RNA helicase are involved in bacterial mRNA degradation (Py et al., 1996).

Expression differences were also found in 30S ribosomal proteins and 50S ribosomal protein. Increased expression of 30S ribosomal proteins S6, S9, S10, S11, S12, S13, S20, S21 and 50S ribosomal proteins L3, L4, L5, L6, L10, L13, L15, L17, L18, L19, L21, L22, L23 were observed in the conjugant. Apart from this, the 50S ribosomal proteins L24 and L32 were not expressed in conjugant but in *S. aureus* (recipient). However, 50S ribosomal protein L14 was exclusively expressed in conjugant (Table 8.2).

363 out of 475 (76 %) uncharacterized proteins were up-expressed in conjugant cells, and 92 uncharacterized proteins were uniquely expressed in the conjugant whereas 102 uncharacterized proteins were exclusively expressed in *S. aureus*. Unfortunately, the functions of these proteins are not currently known.

Table 8. 2 Comparative analysis of peptides identified in *S. aureus* NCTC 6571 and conjugant No7

Protein ID (Gene)	Size	Category	Spectrum count	
			S.aureus NCTC6571	Conjugant No7
Drug resistance transporter, EmrB/QacA subfamily protein (*opp*)	71 KDa	Efflux pumps	1	2
ABC transporter	33 KDa	Efflux pumps	1	4
Penicillin-binding protein 2 (*pbpB*)	83 KDa	Cell wall synthesis	3	9
Penicillin-binding protein 1 (*pbpA*)	83 KDa	Cell wall synthesis	1	1
Glycosyl transferase group 2 family protein	66 KDa	Cell wall synthesis	5	13
Glutamate racemase (*murl*)	30 KDa	Cell wall synthesis	0	5
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2 (*murA*)	45 KDa	Cell wall synthesis	2	8
Zinc metalloprotease (*ftsH*)	78 KDa	Virulence factor	9	15
Clp protease, procolytic subunit (*clpP*)	22 KDa	Virulence factor	12	18
Clp protease, Chaperone protein (*ClpB*)	98 KDa	Virulence factor	5	7

Protein ID (Gene)	Size	Category	Spectrum count	
			S. aureus NCTC6571	Conjugant No7
Clp protease, ATP binding subunit (*clpC*)	91 KDa	Virulence factor	15	24
Clumping factor A (*clfA*)	97 KDa	Virulence factor	0	3
DNA Ligase (*ligA*)	75 KDa	Reparation	0	1
DNA mismatch repair protein (*Muts*)	100 KDa	Reparation	1	1
Thioredoxin homolog	12 KDa	Oxygen response	4	11
Heat shock protein 20 (*GrpE*)	16 KDa	Heat shock protein	1	4
Chaperone protein DnaJ (*DnaJ*)	66 KDa	Heat shock protein	0	3
Chaperone protein DnaK (*DnaK*)	66 KDa	Heat shock protein	35	45
Molecular chaperone Hsp31 (*hchA*)	32 KDa	Heat shock protein	3	12
Protein recA (*recA*)	38 KDa	SOS response	2.6	4
Mechanosensitive channel protein (*mscL*)	14 KDa	SOS response	1	3
Excinuclease ABC, A subunit (*uvrA*)	105 KDa	SOS response	1	1.3
Excinuclease ABC, B subunit (*uvrB*)	77 KDa	SOS response	3	4
Accessory gene regulator protein A (*agrA*)	28 KDa	Stringent response	1	2
Glycine cleavage system T protein (*gcvT*)	40 KDa	Stringent response	11	16
Aspartate-semialdehyde dehydrogenase (*asd*)	36 KDa	Stringent response	1	1
Bifunctional autolysin (*atl*)	137 KDa	Stringent response	105	142
Fibrinogen-binding protein (*fbp*)	76 KDa	Stringent response	1	2
Histidinol-phosphate aminotransferase (*hisC*)	40 KDa	Stringent response	2	3
Oligopeptide ABC transporter (*oppC*)	40 KDa	Stringent response	2.3	2.6
Oligopeptide ABC transporter (*oppD*)	40 KDa	Stringent response	2	3
Oligopeptide ABC transporter (*oppF*)	36 KDa	Stringent response	5	7
Peptide methionine sulfoxide reductase (*msrA*)	21 KDa	Stringent response	4	7
Proline dehydrogenase (*putA*)	38 KDa	Stringent response	4	6
Sulfite reductase (*cysJ*)	72 KDa	Stringent response	2	2

Protein ID (Gene)	Size	Category	Spectrum count	
			S. aureus NCTC6571	Conjugant No7
Threonine synthase (*thrC*)	38 KDa	Stringent response	1	1
Riboflavin synthase, beta subunit (*ribH*)	16 KDa	Heat shock response	7	10
Glycine betaine/ carnitine/choline ABC transporter (*opuCA*)	46 KDa	Heat shock response	3	5
Orotidine 5-phosphate decarboxylase (*pyrF*)	26 KDa	Heat shock response	2	4
Purine nucleoside phosphorylase (*deoD*)	26 KDa	Heat shock response	13	18
Transcriptional regulator (*ctsR*)	18 KDa	Heat shock response	1	1
Urease, gamma subunit (*ureA*)	11 KDa	Heat shock response	10	10
Urease, beta subunit (*ureB*)	15 KDa	Heat shock response	6	7
Urease, alpha subunit (*ureC*)	62 KDa	Heat shock response	11	13
Urease accessory protein (*ureD*)	32 KDa	Heat shock response	1	1
Urease accessory protein (*ureE*)	17 KDa	Heat shock response	12	14
Urease accessory protein (*ureF*)	26 KDa	Heat shock response	1	1
Urease accessory protein (*ureG*)	22 KDa	Heat shock response	1	1
ATP-dependent protease subunit (*HslV*)	20 KDa	Degradation	1	7
Dead-box RNA helicase (*csh*)	57 KDa	Degradation	3	16
30S ribosomal protein S6	12 KDa	Translation	7	30
30S ribosomal protein S9	15 KDa	Translation	6	19
30S ribosomal protein S10	12 KDa	Translation	12	20
30S ribosomal protein S11	14 KDa	Translation	6	32
30S ribosomal protein S12	15 KDa	Translation	3	13
30S ribosomal protein S13	14 KDa	Translation	20	36
30S ribosomal protein S20	9 KDa	Translation	5	21
30S ribosomal protein S21	7 KDa	Translation	1	2
50S ribosomal protein L3	24 KDa	Translation	6	11

Protein ID (Gene)	Size	Category	Spectrum count	
			S. aureus NCTC6571	Conjugant No7
50S ribosomal protein L4	22 KDa	Translation	8	21
50S ribosomal protein L5	20 KDa	Translation	24	74
50S ribosomal protein L6	20 KDa	Translation	20	51
50S ribosomal protein L10	18 KDa	Translation	11	43
50S ribosomal protein L13	16 KDa	Translation	7	28
50S ribosomal protein L14	13 KDa	Translation	0	4
50S ribosomal protein L15	16 KDa	Translation	8	24
50S ribosomal protein L17	14 KDa	Translation	13	52
50S ribosomal protein L18	13 KDa	Translation	13	42
50S ribosomal protein L19	13 KDa	Translation	5	25
50S ribosomal protein L21	11 KDa	Translation	18	57
50S ribosomal protein L22	13 KDa	Translation	10	51
50S ribosomal protein L23	11 KDa	Translation	10	37
50S ribosomal protein L24	12 KDa	Translation	4	0
50S ribosomal protein L32	6 KDa	Translation	1	0

8.7 Chapter summary

The study of *mecA* gene transfer has confirmed that:

1. *mecA* gene transfer between staphylococcal species was not observed;
2. Proteins involved in efflux, virulence, stress response and gene expression regulation were exclusively or increasingly expressed in *S. aureus* cultured with 4 mg l^{-1} oxacillin.

Methicillin-resistant determinants *mecA* gene is known to be located on staphylococcal cassette chromosome *mec*. Although it has been defined as a mobile genetic element, the transfer mechanism of SCC*mec* has not been clarified. This chapter introduced the

mecA gene transfer via conjugation from environmental *S. hominis* to *S. aureus.* Although the transfer of *mecA* gene was not observed, the proteins involved in efflux, virulence, stress response, and cell wall synthesis were observed to be upregulated in conjugant. In the discussion chapter, the failure of *mecA* gene transfer and the proteins involved in oxacillin resistance in *mecA*-negative *S. aureus* will be discussed.

Chapter 9 Discussion

The introduction of antibiotics to clinical infection treatment has revolutionised medicine (Gensini et al., 2007), and thousands of lives have been saved since their first discovery (Fishman et al., 1998; Leibovici et al., 1998; Sykes, 2001; Wielders et al., 2001). However, the growing danger of antibiotic resistant is recognized internationally (Alanis, 2005). Currently, there is an alerting global threat of antibiotic resistance, and concerns have been raised all over the world (WHO, 2014). WHO Antimicrobial Resistance Global Report showed the β-lactam resistant proportion of *S. aureus* in 2014, including African Region (12-80%), Americas (21-90%), Eastern Mediterranean Region (10-53%), European Reigion (0.3-60%), South-East Asia (10-26%), Westen Pacific Region (4-84%). Lowest β-lactam resistance ratio was found in Aisa; however, only 3 countries were included. Together with publications of other Asia countries, the ratio was 2-81% (WHO, 2014).

Microorganisms are continuously evolving various antibiotic resistance mechanisms for their adaptation in environment, and thus the development of new antibiotics will not help to solve the problem of antibiotic resistance in the long term (Alanis, 2005). However, the collection of antibiotic resistance data is useful, since these data can inform decisions of public health organization to publicize the need for reduced antibiotic abuse and emphasis the optimal use of antibiotics (Bartlett et al., 2013). In this case, it is of great important to apply basic resistance-related research for antibiotic resistance screening (Bartlett et al., 2013).

The threat of antibiotic resistance in hospital and community associated staphylococci is a big concern for public health (Bradford, 2001; Hampton, 2013). The environment has been considered to be a potential reservoir of antibiotic resistance genes, which

immensely contribute to the resistome (Wright, 2007). Many studies addressed the issue of MRSA and MRCoNS in hospital settings but little is known about the situation in the environment and public settings (Brennan et al., 2011; Kinnevey et al., 2012; Zong et al., 2011).

This study has provided a systematic analysis of antibiotic resistance environmental staphylococci， includes identification of staphylococci with MALDI-TOF MS, statistical analysis of antibiotic resistance, the carriage of *mecA* gene, molecular characterision of *mecA* positive staphylococci, complete genomic analysis of one *S. epidermidis* isolate, assessment of the transfer of antibiotic resistance genes between staphylococcal species, and look into the stress response associated oxacillin resistance of MSSA.

Staphylococcus spp.

Staphylococci are classified into six species groups by Lamers et al., (2012) and Becker et al., (2014) according to their genotypic relationships and phenotypic properties. In this study, 19 staphylococcal species have been identified belonging to 5 species groups, except for Hyicus-Intermedius (Becker et al., 2014). *S. aureus* can cause numerous different kinds of infections, such as skin, soft-tissue, bone, joint, respiratory infections, endovasculitis, sepsis and endocarditis, followed by *S. epidermidis* (n=6), *S. lugdunensis* (n=6), *S. saprophyticus* (n=5), while infections caused by *S. simiae* and *S. arlettae* have not been reported (Lowy, 1998; Becker et al., 2014; Vandenesch et al., 1993; Murdoch et al., 1996; Bȍcher et al., 2009; Tee et al., 2003; Kuroda et al., 2005; Widerstrȍm et al., 2012; Suzuki et al., 2012; Wang et al., 2012).17 out of 19 staphylococcal species identified in this study have been previously reported to cause infections (Archer, 1998; Becker et al., 2014). The most common staphylococcal species were *S. epidermidis* (28%), followed by *S. hominis* (24%), and *S. haemolyticus*

(11%). *S. epidermidis*, *S. hominis*, and *S. haemolyticus* were also dominant in all isolation sites. *S. epidermidis* was predominantly found in hands, handbags, transportation facilities, supermarkets, restaurants, while *S. haemolyticus* was predominantly found in hotels, library, and hotel air samples, and *S. hominis* dominant in BCF. Soge et al., 2009 found that *S. epidermidis* was the most common CoNS species in US West Coast public marine beaches, followed by *S. saprophyticus* (Soge et al., 2009). *S. haemolyticus* was predominantly in both hotel and hotel air samples. Both samples were collected in the same hotel environment, which support the theory that the airborne bacteria are derived from the environment and building occupants (Fox et al., 2011). *S. hominis*, *S. haemolyticus* and *S. epidermidis* were previously recovered from the baby feces and breast milk (Albesharat et al., 2011). In this study, the sampling sites of baby care facilities include dummies and nappy changing area, and the predominant species were consistent with species that were found in the baby rectum. Many other species, including *S. capitis, S. warneri, S. pasteuri, S. saprophyticus, S. cohnii, S. aureus, S. simiae, S. sciuri, S. pettenkoferi, S. lugdunensis, S. equorum, S. caprae, S. xylosus, S. auricularis, S. simulans, S. arlettae,* were not prevalent. The findings of this study are consistent with those found in other reports studying CoNS isolated from patients, medical devices and the hospital environment (Minto et al., 1999; Sheikh & Mehdinejad, 2012). Sheikh & Mehdinejad, (2012) characterized 134 nosocomial associated CoNS belonging to 16 species, and the majority were identified to be *S. epidermidis* (19.4%) and *S. haemolyticus* (14.9%). Minto et al., (1999) found that *S. epidermidis* (68.2%), *S. haemolyticus* (11.1%), and *S. hominis* (3.2%) were predominant in 126 CoNS strains that were recovered from blood samples. Most of staphylococcal species were isolated from humans; and some staphylococci were recovered from sources such as soil, water and food (Kamal et al., 2013; Normanno et al., 2007). *S.*

simiae is reported to be isolated from squirrel monkeys of South American (Pantucek, 2005). In this study, 19 staphylococcal species were isolated from human-related environment, including baby care facilities, hotels, library, restaurants, supermarkets, transportation facilities, hands, handbags and air of hotels.

To our knowledge, this is the first systematic taxonomic analysis of staphylococci isolated from human hands and inanimate sites in the London region, UK. In this study, mass spectral patterns among strains were compared to discern intra- and inter-species taxonomic relationships. Staphylococci recovered from different sites were found to be taxonomically closely related, which aids the understanding of the transmission and dissemination of staphylococcal isolates. This has also been discussed by other authors (Simões et al., 2011) . Staphylococci isolates recovered from hands were taxonomically closely related to the isolates recovered from hotels, supermarkets, restaurants, library, transportation facilities, handbags, baby care facilities, air samples, and hands. Pratt et al., (2001) reported that poor hand hygiene is one of the major causes of cross contamination and antibiotic resistance transmission in health care facilities. The findings in this study support this hypothesis as the majority of the isolates were recovered from hand touched inanimate objects. In addition, de Neeling et al., (2007) reported the isolation of MRSA from slaughterhouse air samples, and demonstrated the transmission of MRSA via aerosols. Moreover, in this study air isolates were recovered from hotel environments, and the taxonomic relationship between isolates recovered from air and different sites in the same hotels was demonstrated.

MALDI-TOF MS

The importance of rapid and accurate identification of microorganisms have been demonstrated (Valentine et al., 2005; Yao et al., 2002). Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been

proved to be a high throughput technique for bacterial identification in clinical laboratories (Van Veen et al., 2010). MALDI-TOF MS identifies the species by comparing mass spectrum pattern with reference patterns in the database (Maier et al., 2006). MALDI-TOF MS has been employed to identify yeasts (Candida) and bacteria, such as *Actinomyces*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *S. aureus*, that were recovered from various clinical specimens, including blood, cerebrospinal fluid, pus, biopsies, respiratory tract, wounds, and stools (Dhiman et al., 2011; Seng et al., 2009). Dubois et al., (2012) tested 767 clinical isolates with MALDI-TOF MS, and 96.2% of isolates showed correct identification, including 86.7% of isolates were identified into species level and 9.5% of isolates were identified into genus level, while 1.3% misidentified, and 2.5% unidentified. In this study, 86% of 1152 environmental isolates provided reliable identification, including species level (73%), genus level (13%) and unidentified (14%). Generally, the percentage of reliable identification of environmental isolates is lower than clinical isolates. MALDI-TOF MS is a revolutionary new identification technique that was first introduced in 1996 (Claydon et al., 1996). The reproducibility and reliability of MALDI-TOF MS in identifying microorganisms has been extensively examined (Carbonnelle et al., 2007; Majcherczyk et al., 2006; Sandrin et al., 2013). 16S rRNA gene sequence is an older molecular technique for bacterial taxonomic study used since the 1980s (Janda & Abbott, 2007), and is a routine approach for identifying microorganisms in microbiology labs (Clarridge, 2004). In this study, MALDI-TOF MS was in good agreement (92%) with the 16S rRNA gene sequencing in identifying staphylococcal species. With 5 inconsistent isolates, 3 of them were consistent with the second match of 16S rRNA sequencing identification results, and 2 of them were inconsistent. The 16S rRNA sequencings were compared to NCBI

databases, various species with same score were displayed. Therefore, the identification accuracy of staphylococci may be affected (Loonen et al., 2012). Function stability, ubiquitous presence in all organisms and the fact that different positions in 16S rRNA sequences change at very different rates make 16S rRNA gene sequencing is an efficacious tool for phylogenetic analysis (Woese, 1987). Ribosomal protein, like 16S ribosomal DNA, is very stable (Maier et al., 2006). The ribosomal proteins patterns can be detected by MALDI-TOF MS, which correspond to the 16S ribosomal DNA sequence (Hotta et al., 2010; Maier et al., 2006). Therefore, profile analysis of MALDI-TOF MS result in a similar family tree in comparison with 16S rRNA gene sequences (Maier et al., 2006). It is reported that MALDI-TOF MS can be used for genotyping, phenotyping, and determining antibiotic resistance (DeMarco & Ford, 2013; Ghebranious et al., 2005). In this study, MALDI-TOF MS data have been used for taxonomic analysis of staphylococci.

In this study, a family tree was built based on the MALDI-TOF MS profile of environmental staphylococci, and taxonomic relationship of staphylococci recovered from different sites has been assessed. Taxonomic relationship can be analysed based on 16S rRNA gene sequences, though it is very difficult to handle a large quantity of data with regard to turnaround time and costs (Veen et al, 2010),

While MALDI-TOF MS has been certified to be a useful, rapid, reliable technique for identification microorganisms in medical and food safety industries, concerns have been raised on the reproducibility of this technique (Schumaker et al., 2012). Reproducibility can be referred to as the accuracy of identified strains (Sandrin et al., 2013). The reproducibility was assessed by testing fresh cultures from different days, or by different operators (Majcherczyk et al., 2006; Schumaker et al., 2012). However, no standardized approach has been used to report reproducibility (Sandrin et al., 2013).

Until now, reproducibility of MALDI-TOF MS has been assessed for clinical and food isolates, but assessment of environmental isolates has not been reported (Carbonnelle et al., 2007; Majcherczyk et al., 2006). In this study, 1-3 isolates of each staphylococcal species were selected to assess the reproducibility of MALDI-TOF MS in identifying environmental staphylococci. The findings from this study demonstrated that MALDI-TOF MS has an excellent reproducibility (98.5%) in identifiying environmental staphylococci, which is consistent with previous studies (Schumaker et al., 2012).

Antibiotic resistance

The presence of resistance towards most of the available antibiotics has been recognized in staphylococcal species (Becker et al., 2014). In comparation with clinical staphylococci, the antibiotic resistance in environmental staphylococcal isolates is less known. The susceptibility towards antibiotics can be determined using the antimicrobial susceptibility test (Jorgensen & Ferraro, 2009). Susceptibility patterns of staphylococcal species against antimicrobial agents in this study showed that the majority of staphylococci were resistant to penicillin (66%) and fusidic acid (67%). The antibiotic that the isolates displayed the least degree of resistance to was chloramphenicol (5%). Generally, it is widely accepted that clinical isolates demonstrate higher levels of antibiotic resistance due to consistent antibiotic exposure (Antoniadou et al., 2013). 80% of clinical CoNS were reported to be resistant to oxacillin in Europe (Hanberger et al., 2001); whereas 7% of isolates were resistant to oxacillin in this study, which is incompatible to their findings. Ferreira et al., (2002) reported 61.6% and 21.4% of clinical staphylococcal strains were resistant to oxacillin and mupirocin respectively, which is higher than the percentage of resistance in this study (oxacillin 7%, mupirocin 16%). Agvald-Ohman et al., (2004) reported 86%, 48%, and 54% of clinical staphylococci were resistant to oxacillin, erythromycin, and gentamicin accordingly,

which is higher than environmental staphylococci in this study (oxacillin 7%, erythromycin 33%, gentamicin 10%). In addition, Mohan et al., (2002) reported 40% of clinical staphylococci were resistant to chloramphenicol, whereas, 5% of staphylococci were resistant to chloramphenicol is in this study. Akinkunmi & Lamikanra, (2010) reported that 34.2% of clinical staphylococci were resistant to tetracycline in comparison with 18% of environmental staphylococci in this study. Fritsche et al., (2003) showed 21% of clinical staphylococci were resistant to cefepime, while 10% of staphylococci were resistant to cefepime in this study. Meanwhile, resistant to fusidic acid (32.2%), vancomycin (0%), amoxicillin (10%) and streptomycin (16%) in clinical staphylococcal strains is lower than the percentage of environmental staphylococci isolates (fusidic acid 67%, vancomycin 24%, amoxicillin 27%, streptomycin 31%) in this study (Akinkunmi & Lamikanra, 2010; Ferreira et al., 2002; Idriss et al., 2014).

Coagulase negative staphylococci are considered to be less virulent compared to *S. aureus* due to they rarely produce toxins or virulence factors (Otto, 2013). Neverthless, isolation of a wide range of multiple antibiotic resistant coagulase negative staphylococci in this study is a worrisome finding. Agvald-Ohman et al., (2004) reported multidrug resistant CoNS from clinical samples, which is compatible with environmental staphylococci. In this study, multi-resistance was commonly seen: including 0.3% of staphylococcal isolates resistant to more than 10 antibiotics; and 20.8% resistant to at least five tested antibiotics. It is reported that more than 80% of isolates recovered from swine and chicken manure were resistant to at least one antibiotic in China (Zhu et al., 2013). 96% staphylococcal species were resistant to at least 1 antibiotic in this study, which is higher than the percentage reported by Zhu et al., (2013). Only 4% staphylococcal isolates were susceptible to all the tested antibiotics in this study. The level of multiple antibiotic resistant isolates recovered from different

sites was varied. According to Soge et al., (2009), the multiple antibiotic resistant CoNS recovered from public beaches were 62%. In this study, the multiple antibiotic resistance ratios of CoNS recovered from baby care facilities, hotels, library, restaurants, supermarkets, hotel air samples, handbags, and hands were higher than the ratio determined in CoNS recovered from US West Coast public marine beaches. In general, all the isolates (100%) recovered from hotel air samples were multiple antibiotic resistance, while 58% of isolates recovered from transportation facilities showed lowest multiple antibiotic resistance ratio. The multiple antibiotic resistance ratios of hands were between baby care facilities and handbags (personal items) as well as hotels, hotel air, library, restaurants, supermarkets and transportation facilities (public settings). The variation of multiple antibiotic resistance ratio was also observed within personal items and public settings, and no pattern was determined.

Multiple drug resistant microorganisms were first reported in the late 1950s (Levy & Marshall, 2004), and later in 2007, the number of multidrug-resistant bacteria infections was 400,000, of which 25,000 were lethal in Europe only (Bush et al., 2011). In this study, multiple antibiotic resistant staphylococci were isolated from all 9 sites, and 544 (80%) staphylococcal isolates were resistant to two or more antibiotics. Staphylococci can acquire antibiotic resistance by horizontal gene transfer or genetic mutation (Otto, 2013). Different mechanisms contribute to the antibiotic resistance of microorganisms (Blair et al., 2014). The environment plays an important role in the development of antibiotic resistance in microorganisms (Cantas et al., 2013). Antimicrobial agents from antimicrobial producing bacteria in soil and the human therapeutics, animal therapeutics, sewage, agriculture and veterinary industries favour the selection of antibiotic resistance genes, and thus make the environment a reservoir of antibiotic resistance bacteria and antibiotic resistance genes (Cantas et al., 2013). The findings of high levels of multiple

resistant staphylococci in the environment support the theory that antibiotics in the natural environment contribute to the selection of antibiotic resistance microorganisms

Sexton et al., (2006) reported that the environment may play an important role in the dissemination of antibiotic resistance. In this study, MALDI-TOF MS data were combined with antibiotic resistance profiles for taxonomic analysis. 30 multiple antibiotic resistant staphylococci were taxonomically closely related to 30 susceptible staphylococci respectively, indicating that these might belong to the same genotype as the founding strain. Kraemer & Iandolo, (1990) reported the transfer of antibiotic resistance genes between species or interspecies, which may be a contributing factor in the development of different antibiotic resistance patterns in taxonomically closely related isolates. Additionally, Thouverez et al., (2003) has shown the correlation of antibiotic susceptibility and the MRSA genotype over a 4-year period. In this study, it was showed that taxonomically closely related antibiotic resistant staphylococci were recovered from different sites with varied antibiotic susceptibility profile. Up to 9 antibiotic susceptibility variations were observed in two taxonomically closely related staphylococci, which were recovered from same site, and up to 8 antibiotic susceptibility variations were found in other two taxonomically closely related staphylococci recovered from different sites. This finding also supports the theory that transfer of antibiotic resistant determinants contribute to the development of different antibiotic resistance patterns in taxonomically closely related isolates (Kraemer & Iandolo, 1990).

Community associated MRSA USA300, is an epidemic strain responsible for severe antibiotic resistance associated infections, which has reportedly been recovered from frequently touched surfaces in buses serving both hospital and community routes in potugal, which indicates the spillover of MRSA from hospital settings to the community

(Lutz et al., 2014). The taxonomic analysis of this study showed low diversity among each staphylococcal species. The dissemination of antimicrobial resistance in the environment is associated with ubiquitous bacteria. Staphylococci, as one of the ubiquitous bacteria, are known to be able to survive in the environment as well as colonize on humans (Cantas et al., 2013). In this study, staphylococci recovered from different sites were determined to be taxonomically closely related. In addition, no obvious differences of antibiotic susceptibility profile were observed between clusters that were formed by isolates recovered from different sites and clusters that were formed by isolates recovered from same site. This finding also supports the theory that the spread of antimicrobial resistance is associated with the dissemination of ubiquitous bacteria in the environment (Cantas et al., 2013).

Methicillin resistant staphylococci are a major public health problem, with severe economic and health consequences (Stefani & Varaldo, 2003). *mecA* gene encodes for penicillin binding protein 2a (PBP2a), which has a low affinity to β-lactam antibiotics and confers the methicillin resistant (Tulinski et al., 2012). Hussain et al., (2000) assessed the correlation of the presence of *mecA* gene and oxacillin susceptibility breakpoints (0.5 mg l^{-1}) in 493 clinical CoNS of 11 species, and classified these staphylococci into 4 categories. The *mecA* gene was determined in category I and II staphylococci, and the percentage of *mecA* positive isolates was as followings: *S. haemolyticus* (83.3%), *S. epidermidis* (61.9%), *S. hominis* (51.8%), *S. cohnii* (28.5%), *S. warneri* (27.3%), and *S. saprophyticus* (9.0%) (Hussain et al., 2000). Category II (*S. cohnii*, *S. warneri*, *S. saprophyticus*) differed from category I (*S. haemolyticus*, *S. epidermidis*, *S. hominis*) by their low *mecA*-positive ratio (Hussain et al., 2000). In this study, *S. haemolyticus* (24%), *S. epidermidis* (12%), *S. hominis* (6%) had *mecA*-positive strains but with lower *mecA*-positive ratios in comparision with clinical isolates. In

addition, *S. cohnii* (36%) and *S. saprophyticus* (20%) of category II recovered from this study showed higher *mecA*-positive ratio than clinical isolates, whereas, lower *mecA*-positive ratio was observed in S. *warneri* (13%) of this study. It is reported that no *mecA* gene was found in category III and IV staphylococci, including *S. xylosus, S. lugdunensis, S. capitis, S.simulans,* and *S. schleiferi* (Hussain et al., 2000). In this study, no *mecA* gene was determined in *S. xylosus* and *S. simulans*, which is consistent with clinical study report. In contrast, the presence of *mecA* gene was determined in *S. lugdunensis* (20%) and *S. capitis* (8%). It is also reported that category III (*S. lugdunensis* and *S. xylosus*) lack *mecA* gene, but phenotypically resistant to oxacillin. In their study, Hussain et al have not explained why this occurs (Hussain et al., 2000), however, proteomic data in this study suggested that expression of PBPs remain the same or upregulated, resulting the *mecA*-negative conjugant being phenotypically resistant to oxacillin. In contrast with Hussain et al's finding, *S. lugdunensis* recovered from this study was determined to be *mecA* positive and resistant to oxacillin. In this study, *S. simiae,* as sister species of *S. aureus,* was not identified to harbour *mecA* gene (Suzuki et al., 2012). The origin of *mecA* gene in *S. aureus* is considered to be from the common ancestor of *S. fleurettii, S. vitulinus* and *S. sciuri* (Tsubakishita et al., 2010). Moreover, *mecA1* gene of *S. sciuri* can mediate the high-level oxacillin resistance in *S. aureus* (Harrison et al., 2014). In this study, the high *mecA*-positive ratio of *S. sciuri* (83%) is a worrisome finding.

Oxacillin susceptible MRSA (OS-MRSA), that has been reported worldwide (Hososaka et al., 2007; Saeed et al., 2010), is defined as oxacillin susceptible *mecA* gene positive *S. aureus* (Hososaka et al., 2007). Hososaka et al (2007) also indicate the possibility of high level resistance induced by beta-lactam antibiotics. In this study, 12 *S. aureus* were neither oxacillin resistance nor *mecA* gene positive, and 89 *mecA* gene positive CoNS

were determined. The MICs (oxacillin) of *mecA* gene positive staphylococci varied from 0.015 to 256 mg l^{-1}, and 39 out of 89 *mecA*-positive CoNS were susceptible to oxacillin. The *mecA* gene positive, oxacillin susceptible CoNS were prevalent in environmental staphylococci. The *mecA* gene encodes a penicillin binding protein 2a, which has a low affinity to β-lactam antibiotics, and is thus phenotypically resistant to β-lactam antibiotics (Tomasz et al., 1989). In this study, the expression of *mecA* gene has been assessed in 4 CoNS with MIC range from 0.12 to 256 mg l^{-1}. The presence of PBP2a was determined in high oxacillin resistant isolates, but not in *S. epidermidis* 111 (MIC 0.12 mg l^{-1}) and *S. hominis* 506 (MIC 0.5 mg l^{-1}). The proteomic results of this study help to understand the oxacillin resistant variation of *mecA* gene positive staphylococci. Pinho et al., (2001) has reported that the optimal expression of methicillin resistant in MRSA requires collaboration of PBP2a and PBP2. TPase domain of PBP2a is involved in transpeptidation, and TGase domain of PBP2 is essential for transglycosylation in presence of methicillin (Pinho et al., 2001). Predictably, the presence of PBP2 has been determined in 2 PBP2a positive CoNS in this study.

The *mecC* gene shares less than 70% similarity with original *mecA* gene, and present in SCC*mec* type XI (Becker et al., 2014). *mecC* gene has been recovered from human and varied animal hosts (Loncaric et al., 2013); however, no *mecC* gene positive staphylococci was determined in this study. To date, there are 4 *mecA* gene homologues have been reported, including *mecA1* (80%), *mecA2* (90%), *mecB* (60%) and *mecC* (70%). It is believed that novel *mecA* homologues may be identified in the future (Ito et al, 2012).

SCC*mec* and MLST

SCC*mec* is a mobile genetic element, comprising of *mecA* gene, recombinase gene, regulatory elements and additional genes (Monecke et al., 2011). The variation of SCC*mec* confers the sources of MRSA (Monecke et al., 2011). Therefore, SCC*mec* is a molecular typing technique for epidemiological study of staphylococci (Oliveira & Tomasz, 2002). The structural diversity of SCC*mec* has been reported in hospital environments (Barbier et al., 2011; Zong et al., 2011). The variation in SCC*mec* is related to the high throughput of individuals in the hospital tested (Barbier et al., 2011). The first identification information of SCC*mec* type I, II, III elements are as followings: Type I (1961, UK), type II (1982, Japan) and type III (1985, New Zealand). SCC*mec* type I, II, III of MRSA has been reported to be associated with hospital (Monecke et al., 2011). Whilist, SCC*mec* type IV and V are present in community associated MRSA (Monecke et al., 2011). In addition, it is found that the presence of SCC*mec* elements is associated with slow growth rate (Monecke et al., 2011), and the slow growth rate has been considered to be a disadvantage of selection in the absence of an antibiotic selection pressure (Ender et al., 2004; Lee et al., 2007). In this case, isolates carrying SCC*mec* are not as competent as wild type strains in the absence of antibiotics (Monecke et al., 2011). Moreover, SCC*mec* type IV and I have similar structures (Oliveira & Tomasz, 2002). SCC*mec* type IV lacks a flanking region in comparison with SCC*mec* type I (Oliveira & Tomasz, 2002), and thus the type IV SCC*mec* element represents increased mobility by its smaller size (Oliveira & Tomasz, 2002). In this study, SCC*mec* types were identified in CoNS. 15 CoNS out of 89 (17%) *mecA* gene positive staphylococci were assigned to SCC*mec* type I, II or III, while SCC*mec* type IV and V took 29% (n=26). In the environment, community associated SCC*mec* type is more prevalent than nosocomial associated SCC*mec* types, which may support the

theory of the advantage in the spread of smaller SCC*mec* elements (Oliveira & Tomasz, 2002). SCC*mec* type VI was identified in clinical MRSA that were recovered from Portugal and has been redefined in 2006 (Oliveira & Tomasz, 2002). Moreover, type VIII SCC*mec* has been first identified in 2009 in Canada (Zhang et al., 2009). One of each type has been identified in this study, whereas SCC*mec* types IX were not detected in this study. It has been reported by others that the distribution of SCC*mec* types in MRCoNS varies and may depend on the human host and geographical locations of the isolates (IWG-SCC, 2009; Oliveira et al., 2006; Zhang et al., 2009; Zong et al., 2011). In previous reports, SCC*mec* types I, II, III and V were found to be the most common in environmental isolates, such isolates were taken from areas such as public beaches (Soge et al., 2009). In this study, SCC*mec* type I, IV, and V have been identified to be prevalent in environmental isolates. Becker et al., (2014) has summarized the community and livestock associated SCC*mec* types, including *S. capitis* (I, IA, II, III, IV, IVa, V, NT), *S. cohnii* (NT), *S. epidermidis* (I, IIa, IIb, III, III (variant), IV, IVa, IVb, IVc, IVd, IVe, IVg, V, VI, NT), *S. haemolyticus* (I, II, II.1, III, III (variant), IV, V, NT), *S. honomis* (I, III, IV, NT), *S. pasteuri* (IVc), *S. saprophyticus* (III, NT), *S. sciuri* (I, III, IIIA, V, VII, NT) and *S. warneri* (IV, IV.1, IVb, IVE, NT). In addition, NT, which stands for non-typeable and/or novel non-designated types, was identified in *S. capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. sciuri*, *S, warneri*. In this study, species associated SCC*mec* types differed from Becker et al. The SCC*mec* types found in *S. capitis* (I, NT), *S. haemolyticus* (I, II, V, NT) and *S. hominis* (I, V, NT) of this study is less than community associated SCC*mec* types. whereas, *S. cohnii* (I, V, NT), *S. pasteuri* (NT), *S. saprophyticus* (IV, NT), *S. sciuri* (II, VIII, NT), *S. warneri* (I, V, NT) harbors different SCC*mec* types in comparison with community associated staphylococci. SCC*mec* types of *S. epidermidis* in this study is consistent

with community associated *S. epidermidis*. In addition, associations were found between SCC*mec* carriage and certain species, for example SCC*mec* type V was preferentially associated with *S. haemolyticus*, *S. hominis*, *S. warneri*, and *S. epidermidis*. Previously, with clinical isolates, type V SCC*mec* was reported to be associated mainly with *S. haemolyticus* (Zong et al., 2011).

Apart from the variations in the classified SCC*mec* types isolated, 22 unclassified SCC*mec* types were also reported in this study. Eight of these had a combination of class A *mec* complex and *ccrC*, six carried a combination of class B *mec* and *ccrC*, one carried class B *mec* and *ccr3*, and seven had a combination of class A *mec* complex and *ccr* type 1. The 1A has been reported by other workers to be a new type 1A (Bouchami et al., 2011). SCC*mec* harbouring *mecA* but lacking *ccr* is known as pseudo (ψ)-SCC*mec*, while it is reported that SCC*mec*$_{12263}$ possess a *ccr* complex but lack *mecA* (Harrison et al., 2013; Katayama et al., 2003). 23 isolates (29%) of this study could not be typed as they lack either *mec* complex or *ccr* complex. It is known that SCC*mec* without *ccr* and *mec* genes have been classified as ψ SCC elements (Becker et al., 2014), and ψ SCC element was identified in one *S. saprophyticus* of this study.

S. epidermidis is considered to be clinical contaminant, and thus epidemiological studies of *S. epidermidis* are limited (Herwaldt et al., 1996; Wang et al., 2003). To date, studies on *S. epidermidis* have been focused on clinical isolates (Li et al., 2009), *S. epidermidis* ST2 has been found to be dominant in hospitals in China, Europe and USA, and is associated with the presence of *ica* operon and *IS256* (insertion sequences) positive (Li et al., 2009; Mendes et al., 2012; Miragaia et al., 2007). A wide range of genetic variation existing amongst *S. epidermidis* isolates has been demonstrated (Hussain et al., 2000) In this study, 14 new MLST types were assigned for 19 *S. epidermidis* isolates. MLST is a powerful tool for global epidemiological studies (Oliveira & Tomasz, 2002),

since it can identify important genetic background correlation of staphylococci recovered all over the world by comparing the MLST types (Diep et al., 2004), Internationally recognized clones *S. epidermidis* ST59 has been isolated from hospital in Taiwan, Denmark, Mexico, Cape Verde, Spain, Hungary, USA and China, and associated with SCC*mec* type V (Li et al., 2009; Mendes et al., 2012; Miragaia et al., 2007). In this study, *S. epidermidis* ST59 was associated with SCC*mec* type IV, and this is the first report of ST59 associated with SCC*mec* type IV, which is different to those already reported. Mendes et al., (2012) reported the isolation of *S. epidermidis* ST360 from clinical specimens in the USA, however, little information of *S. epidermdis* ST360 has been reported. In this study, *S. epidermidis* ST360 is combined with SCC*mec* type V. In addition, isolates recovered from human hands (259) and hotels (124) shared the same MLST type: ST602, which suggested the possible correlation of staphylococci recovered from different sites. However, *S. epidermidis* 259 harboured SCC*mec* type V, whereas *S. epidermidis* 124 was associated with SCC*mec* IV. 3 *S. epidermidis* isolates of this study recovered from library (133, 134, 135) were assigned the same MLST type; however, *S. epidermidis* 133, 134, 135 harboured SCC*mec* type 3B, I, IV accordingly. The same MLST type associated with different SCC*mec* types has been reported previously, such as *S. epidermidis* ST2 harbouring type II, III, IV and non-typable SCC*mec*, and ST22 is associated with SCC*mec* type III, IV and V (Miragaia et al., 2007). It is believed that different MRSA clones can appear in some clonal complexes (CC), and lead to the isolates with the same MLST type but different SCC*mec* type (Robinson & Enright, 2004). This theory is supported by the findings of same MLST type associated with different SCC*mec* types in this study.

Antibiotic resistant and virulence genes in *S. epidermidis* 118 (G6_2)

S. epidermidis is an important opportunistic pathogen, which is commonly related to infections due to indwelling medical devices (Becker et al., 2014). A pan-genome sequence analysis of 71 *S. epidermidis* that are recovered from healthy human bodies shows that formate dehydrogenase is exclusively present in the commensal lineage (Conlan et al., 2012). In this study, the formate dehydrogenase was present in *S. epidermidis* 118 (G6_2). In addition, the phylogenetic relationship indicates the close relationship of *S. epidermidis* 118 (G6_2) with SRR1656389, SRR1656376 and SE_BCM-HMP0060 strains that were recovered from intensive care units (Roach et al., 2015).

Antibiotic resistance genes detected in *S. epidermidis* 118 (G6_2)

Zankari et al., (2013) reported that WGS presented high antimicrobial susceptibility concordance with phenotypic tests in *E. coli*. Moreover, the promising feature of WGS for antimicrobial susceptibility prediction in a clinical isolate *S. aureus* was also demonstrated (Gordon et al., 2014). In this study, the correlation between antibiotic resistance genes and phenotypic antimicrobial susceptibility was determined in *S. epidermidis* 118 (G6_2). *aac(6')-aph(2'')*; *blaZ*; *mecA*; *fosA*; *msr(A)*; *mph(C)*; *tet(K)*; *ileS* and *fusA* in the genome of *S. epidermidis* 118 (G6_2); these are responsible for the streptomycin, gentamicin, penicillin, oxacillin, amoxicillin, cefepime, cefoxitin, fosfomycin, erythromycin, tetracycline, fusidic acid, and mupirocin resistance (Bernat et al., 1997; Bryan et al., 2004; Daigle et al., 1999; Fiebelkorn et al., 2003; Hodgson et al., 1994; Howden & Grayson, 2006; Olsen et al., 2006; Ubukata et al., 1989; Wang et al., 2008). *S. epidermidis* G6_2 harboured 9 antibiotic resistance genes, which is greater than those contained in clinical reference strains. *blaZ* is known to encode an enzyme inactivated β-lactam by hydrolysis of antibiotics. *aac(6')-aph(2''), fosA* and *mph(C)* are

known to inactivate aminoglycoside, fosfomycin and macrolide antibiotics by encoding transferases that form a covalent bond with antibiotics. *mecA* and *ileS* encodes penicillin binding protein 2a and additional isoleucyl tRNA synthetase which has low affinity to β-lactam antibiotics and mupirocin respectively. *msr(A)* and *tet(K)* encode efflux that confers to macrolide and tetracycline resistance. Finally, the *fusA* mutation has contributed to the fusidic acid resitance (Cookson, 1998; Hodgson et al., 1994; Howden & Grayson, 2006; Martemyanov et al., 2001; Matsuoka et al., 2003; Ng et al., 2001; Schmitz, 1999). Five different mechanisms are involved in multiple antibiotic resistances of *S. epidermidis* 118 (G6_2).

S. epidermidis has been considered to be a reservoir of antibiotic resistance genes, which facilitate the survival of *S. aureus* by horizontal transfer of antibiotic resistant determinants (Otto, 2013). There is evidence to support the theory that *S. epidermidis* is a reservoir of antibiotic resistance gene for *S. aureus*. Staphylococcal cassette chromosome *mec* (SCC*mec*) elements and arginine catabolic mobile elements (ACME) were found more frequently in *S. epidermidis* than *S. aureus*. Moreover, SCC*mec* type IV of *S. epidermidis* showed 98-98% similarity to SCC*mec* type IVa in *S. aureus*, and *ccr*AB gene in *S. epidermidis* are 100% identical to *S. aureus*. In addition, the methicillin resistant is more prevalent among *S. epidermidis* than *S. aureus* (Otto, 2013). In this study, *S. epidermidis* 118 (G6_2) was observed to harbour 9 antibiotic resistance determinants, thus supporting Otto's theory. In addition, Qin et al., (2012) reported a *Campylobacter coli* harbouring 6 aminoglycoside resistance genes isolated from chicken slaughterhouses. In contrast with *C. coli* harbouring 6 aminoglycoside resistance determinants, 9 antibiotic resistance determinants of *S. epidermidis* 118 (G6_2) are responsible for the resistance of 7 classes of antibiotics, including steroid, aminoglycoside, beta-lactam, fosfomycin, microlide, tetracycline, and monoxycarbolic

acid antibiotics. The development of antibiotic resistance is mostly due to the gene transfer (Otto, 2013). Mobile genetic elements, such as plasmids, transposons, pathogenic islands and chromosomal cassette, contribute to the dissemination of antibiotic resistance genes (Zhu et al., 2013). In this study, 5 out of 9 antibiotic resistance genes were located on plasmids of *S. epidermidis* 118 (G6_2), indicating the high mobility of these antibiotic resistance determinants. Recently, using different genome sequence approaches, Méric et al., (2015) has shown that *S. aureus* and *S. epidermidis* share considerable interspecies mobile genetic elements.

In addition to the multiple antibiotic resistant determinants, the copper responsive gene (*cptV*) and copper chaperone (*copZ*) were found in *S. epidermidis* 118 (G6_2), whose functions are known to encode proteins for copper efflux and adaption to copper stress (Schelder et al., 2011). Zhu et al., (2013) reported that the heavy metal is co-selective pressure, preserves the presence of antibiotic resistance genes in bacteria. Moreover, three functional genes: *qac*, *yehS* and Tn552, were found to be located in one of the *S. epidermidis* 118 (G6_2) plasmids. Quaternary ammonium compounds (QAC) are widely used in the food industry and clinical environment as low toxic detergents; however, QAC resistant staphylococci have been emerged in communities and the food industries (Heir et al., 1998). *qac* genes are known to encode QAC-resistance protein which are responsible for the efflux of QAC and dye from cells (Heir et al., 1998). In bacteria, ABC transporters are crucial for nutrient uptake and exportation of toxin and antibiotics (Davidson & Chen, 2004). *yehS* gene is known to be one of the genes that encode ABC transporter ATP-binding protein, which involves in nutrient uptake and secretion of toxins and antimicrobial agents from the cell (Davidson & Chen, 2004). Tn552 is a beta-lactamase related transposon, which is known for encoding beta-

lactamase and two regulatory proteins: *blaI, blaR1,* which control the expression of beta-lactamase (Rowland & Dyke, 1990).

Virulence genes determined in *S. epidermidis* 118 (G6_2)

Virulence genes found in *S. aureus* express a variety of virulence proteins, including enterotoxin, exotoxin, hemolysin (alpha, beta, gamma, and delta), nuclease, protease, and lipase (Dinges et al., 2000). Proteins encoded by virulence genes are generally involved in converting host tissue into nutrients for the growth of bacteria and invasion (Dinges et al., 2000). *setC* is a pyrogenic toxin superantigen, and can trigger toxic shock syndrome by causing massive cytokine release (Dinges et al., 2000). In this study, *setC* was exclusively determined in *S. epidermidis* 118 (G6_2). Alpha-hemolysin has neurotoxic and dermonecrotic effects on a variety of mammalian cells and delta-hemolysin is capable of lysing a wide range of mammalian cells (Dinges et al., 2000); however, the mechanism by which beta-hemolysin causes disease has not yet been clarified (Schwan et al., 2003). In this study, only beta-hemolysin encoding gene *hlb* was present in *S. epidermidis* 118 (G6_2). The nuclease can facilitate *S. aureus* escape from neutrophils and therefore undermine the immune system. *nuc* was present in *S. epidermidis* 118 (G6_2) and reference isolates (Berends et al., 2010). Extracellular protease, including serine proteinase (*sspA*), cysteine proteinase (*sspB*) and staphopain A (*sspP*), were known to have putative roles in virulence (Shaw et al., 2004; Zarfel et al., 2013). In addition to *sspA* and *sspB, S. epidermidis* G6_2 harbours *sspP* exclusively. *lip, geh and lipA* are members of the bacteria lipase family, and might be involved in pathogenicity by reducing the ability of immune cells to undertake phagocytosis ability (Stehr et al., 2003). *lip, geh* and *lipA* were all present in *S. epidermidis* 118 (G6_2). In addition, *clp* proteinase found in staphylococci is more like a stress response protein, and degrades misfolded proteins under stress conditions (Michel et al., 2006). Moreover,

lytN found in *S. aureus* N315 and *S. epidermidis* 118 (G6_2) encodes cell wall hydrolase/autolysin (Lindsay et al., 2005). Immunodominant staphylococcal antigen A (*isaA*) and immunodominant staphylococcal antigen B (*isaB*) were identified as a putative autolysin (Stapleton et al., 2007). Both *isaA* and *isaB* were determined in *S. epidermidis* 118 (G6_2). *sfpA* gene was reported to be located on a plasmid of *E. coli*, and encoding *sfp* fimbriae mediating the mannose-resistant hemagglutination (MRHA) (Müsken et al., 2008). The finding of this gene in *S. epidermidis* 118 (G6_2) strain is supportive evidence that the transfer of virulence factors may occur via plasmids (Zhu et al., 2013). In *C. difficile*, *sigD* is a regulon, which positively controls toxin expression via regulation of *tcdR* transcription (El Meouche et al., 2013). The *sigD* gene has been shown to regulate the expression of *tcdA, tcdB* toxins, which are involved in the early stages intestine tract colonization of *Clostridium difficile* and cause intestinal damage (El Meouche et al., 2013). *sigD* gene was detected in *S. epidermidis* 118 (G6_2) and to my knowledge this is the first report of the *sigD* gene found in staphylococci. The study also showed that *tcdA* was present in *S. epidermidis* 118 (G6_2). The polysaccharide intercellular adhesin gene *ica* is the gene that confers biofilm synthesis of staphylococcal species (Arciola et al., 2001; Zhang et al., 2003) No *ica* gene was detected in *S. epidermidis* 118 (G6_2) in this study, and thus *S. epidermidis* 118 (G6_2) is a non-biofilm forming isolate. Phenol soluble modulins responsible for immune evasion of *S. epidemidis* were determined in *S. epidermidis* 118 (G6_2), RP62a and ATCC12228 (Liles et al., 2001).

Various surface proteins contribute to the pathogenicity of staphylococci, which can enable them to invade hosts and remain there (Becker et al., 2014). *sdrC, sdrD, sdrE* are considered to be the surface protein of *S. aureus* (Foster et al., 2014), while *sdrF, sdrG, sdrH* are *S. epidermidis* associated surface proteins (McCrea et al., 2000). *sdrC, sdrD,*

and *sdrE* of *S. aureus* have crucial role in colonization and evasion of the host cells (Foster et al., 2014). In this study, *sdrC* and *sdrD* were first reported to be expressed in *S. epidermidis.* Autolysin (*atl*) is expressed in *S. aureus,* and for *S. epidermidis*, the homolog of *atl* which mediates initial adhesion functions is known as *atlE* (Becker et al., 2014). Moreover, cell wall associated fibronectin binding protein (*ebh*), elastin binding protein (*ebp*) and bifunctional autolysin (*atl*), are found in both *S. aureus* and *S. epidermidis* (Gill et al., 2005). Another surface protein, *sas* is known to transfer from *S. epidermidis* to *S. aureus* via prophage (Otto, 2013). Additionally, *pls* is found in *S. aureus* and *S. epidermidis*, and is known to regulate the adhesion process (Josefsson et al., 2005) and mediate methicillin resistant (Gill et al., 2005). In this study, *sasK, ebh, ebp* and *atl* were found in *S. epidermidis* 118 (G6_8) and all reference staphylococcal species, while *pls* was only found in *S. epidermidis* RP62a and 118 (G6_2). *S. epidermidis* 118 (G6_2) presented fewer virulence genes than *S. aureus* N315, however, it showed equivalent or even more virulence than two clinical *S. epidermidis* strains. Moreover, *S. epidermidis* 118 (G6_2) harbours 9 antibiotic resistance genes, more than were found in clinical reference staphylococci.

mecA* transfer *in vitro

Methicillin resistant is encoded on a mobile genomic island named SCC*mec* (Kondo et al., 2007). The origin of SCC*mec* in *S. aureus* is considered to be acquired from CoNS, which has been documented by Otto (2013). Theories supporting this hypothesis include the high level of SCC*mec* homologies in both *S. aureus* and CoNS as well as the occurrence of *mecA* gene in CoNS is more frequent than in *S. aureus* (Otto, 2013). Under laboratory conditions, *mecA* gene cannot be transferred by conjugation and transformation, whereas, phage mediated transduction of SCC*mec* within two *S. aureus* have been reported (Cohen & Sweeney, 1973). No phage mediated transduction of

SCC*mec* elements between different staphylococcal species has been reported (Katayama et al., 2000). The SCC*mec* transfer mechanism remains to be solved. In this study, the *mecA* gene was not transferred between staphylococcal species during the *in vitro* mating experiment, which was confirmed by Southern blotting, PFGE, LC-MS/MS and MIC assays. This finding is in concordance with previous report by Bloemendaal et al., (2010). Bloemendaal et al (2010) suggested that unsuccessful transfer of SCC*mec* is due to low frequencies or unfavourable *in vitro* conditions, since Bloemendaal et al (2010) indicated that one methicillin resistant *S. aureus* was derived from methicillin susceptible *S. aureus* by horizontal *mecA* gene transfer *in vivo*. 4 mg l^{-1} oxacillin supplemented mannitol salt agar is recommended to be used as a reliable screening medium for detection and identification of methicillin resistant *S. aureus* (Lally et al., 1985). However, it is reported that oxacillin supplemented MSA shows relatively low sensitivity in the selection of MRSA in comparison with conmercialized selective agar (Stoakes et al., 2006). PCR is a widely used genetic method for antibiotic resistance gene determination (Sakoulas et al., 2001; Zhang et al., 2008); However, false positive results have been reported previously (Tham et al., 1991). PFGE and Southern blotting are considered to be more reliable techniques in detecting the presence of specific genes (Trindade et al., 2003). In this study, the conjugant growing on 4 mg l^{-1} oxacillin supplemented mannitol salt agar (MSA, Oxoid Basingstoke UK) was determined to be *mecA* gene negative *S. aureus* (MSSA). In order to find out the reason of survival of MSSA on oxacillin supplemented MSA, it is essential to examine and compare, at a proteomic level, the successful transfer of *mecA*/SCC*mec*, as proteomics is a powerful tool to determine functional genomics (Ziebandt et al., 2010).

Stress response mediate the antibiotic resistance

It is well established that methicillin resistant is determined by penicillin binding protein 2a with low affinity to β-lactam antibiotics, encoded by the *mecA* gene (Wielders et al., 2002). No penicillin binding protein 2a was detected in either conjugant or *S. aureus* (recipient). However, upregulation of proteins involved in various stress responses was observed. Previous studies have revealed that bacterial can acquire antibiotic resistance through vertical transmission from ancestors, such as the target of an antibiotic being altered by mutation to reduce affinity, or adjust the efficiency of efflux pumps that are involved in clearance of antibiotic from cells or degradative systems (Hastings et al., 2004). Increased single nucleotide mutations were observed after exposure to antibiotics, including nucleotide mutation in *mprF* gene, point mutation in *spa* gene and *nos* gene mutant (Richards et al., 2015). Caspermeyer, (2015) has shown that adaptation and survival ability of *S. aureus* is increased after exposure to a single antibiotic, and emphasis on the role of mutation in antibiotic resistance evolution. Stress environment increase the mutation and thus contribute to selective advantages (Foster, 2005a). In this study, oxacillin most likely acted as a stress source, triggering stress responses of *S. aureus* and elevating the mutation rate, and thus contributing to the oxacillin resistant phenotype that allowed them to survive and proliferate on oxacillin supplemented mannitol salt agar. Finally, vertically transmission of these resistance traits to offspring enabled their survival in oxacillin supplemented mannitol salt agar. Proteomic level analysis is a unique approach to reveal the stress responses of bacteria (Xiao et al., 2003), and it has been successfully employed to look at the linezolid stress response of *S. aureus* (Bernardo et al., 2004).

The beta-lactam mediated SOS response has been previously reported in clinical *mecA* positive, oxacillin susceptible MRSA (Cuirolo et al., 2009), and the LexA/RecA protein

regulated SOS response increases the mutation rate which allows the selection of high oxacillin resistant populations from *mecA* positive, oxacillin susceptible MRSA (Cuirolo et al., 2009). Similar phenomena were found in this study, increased expression of RecA gene in *mecA* negative *S. aureus* was observed after exposure to beta-lactam antibiotic.

Mismatch repair is important for genomic integrity (Foster, 2005a); however, mismatch repair protein may remain at low levels under stress conditions so that mutations cannot be corrected (Foster, 2005a). It was found that the expression of *MutS* (key gene for mismatch repair) remained the same after exposure to beta-lactam antibiotic in this study. It is reported that bacteria can acquire resistant traits by mutation and altering the efficiency of degradation system. Additionally, these traits can be vertically transferred to offspring (Hastings et al., 2004). Upregulation of proteins that are involved in degradation were observed in this study.

The stringent response is known to be triggered by the stressful environment (Gao et al., 2010), and has been reported to tolerate antibiotic resistance in *Enterococcus faecalis* (Abranches et al., 2009). The proteins responsible for amino acid biosynthesis and transport processes are observed to be upregulated in the stringent responses (Anderson et al., 2006). ABC transporter is one such transport protein (Anderson et al., 2006), and is known to be one of the most significant contributing factors for antibiotic resistance (Gupta et al., 2010). In this study, ABC transporter and drug resistance transporter (EmrB/QacA) were found to be upregulated in presence of oxacillin.

The heat shock response is known to be induced by temperature or DNA damage (Guisbert et al., 2008; Muthaiyan et al., 2012). Heat-denatured proteins are known to be targeted by *ClpC* and then degraded by *ClpP* (Frees et al., 2004). As part of this system, *ClpB* is speculated to interact with heat shock proteins (*DnaK, DnaJ*) to solubilize the

protein aggregates (Mogk et al., 2003). Urease operon (*ureA-ureD*) are known to convert urease to ammonia and CO_2 (Anderson et al., 2006). The increased expression of urease operon is all known to help address the effects of temperature elevation (Anderson et al., 2006). GroE is important for transcription under heat elevation, as it protects the RNA polymerase holoenzyme from heat inactivation (Ziemienowicz et al., 1993). In this study, the isolates that were used for comparative proteomic analysis were both cultured at 37℃; however, these heat shock response associated proteins (*ClpB, ClpC, ClpP, urea-ureD, GroE*) were upregulated in presence of oxacillin. Foster, (2005a) has reported that other conditions responsible for unfolded proteins can trigger heat shock response. The finding of this study supports that the heat shock response can be triggered by antibiotic associated stress (Guisbert et al., 2008; Muthaiyan et al., 2012).

Heat shock protein is a family of proteins that are active during exposure to stress environments, such as excess heat, oxygen and UV light (Cao et al., 1999; Matz et al., 1995; Ritossa, 1962). Hsp40 (*DnaJ*) controls protein homeostasis in the cell (Cuéllar et al., 2013). Hsp20 (*GrpE*) has been reported to assist reactivation of heat-inactivated RNA polymerase (Ziemienowicz et al., 1993). Heat shock protein 20, 40, 70 were all found to be upregulated in conjugant of this study.

The upregulation of virulence genes is also induced under stress conditions (Anderson et al., 2006). In this study, virulence factors such as zinc metalloprotease (*ftsH*), protease, Clp protease (*clp*), and clumping factor A (*clfA*), were found to be expressed at increased levels in the presence of oxacillin. In addition, The PBPs are membrane-associated proteins that catalyse the synthesis of cell wall peptidoglycan in *S. aureus*, and peptidoglycan is an important component of the bacterial cell wall (Dmitriev et al., 2004; Memmi et al., 2008). Muthaiyan et al., (2012) has assessed the killing effect of

Valencia orange essential oil on *S. aureus*, and downregulation of PBPs and peptidoglycan was observed in the presence of the Valencia orange essential oil. In this study, expression of PBPs remained the same or upregulated, and upregulation of peptidoglycan biosynthesis associated proteins (*mur*) were observed in conjugant. This suggests that the conjugant was resistant to oxacillin in the absence of *mecA* gene.

Chapter 10 Concluding remarks

The introduction of antibiotic in clinical use is a revolutionary paradigm in morden medicine (Gensini et al., 2007). However, the misuse of antibiotics has led to the global crisis of antimicrobial resistance (Bartlett et al., 2013). The presence of antimicrobial resistance determinants is due to evolutionary selection by the environment, and thus the environment acts as a reservoir of antibiotic resistance determinants (Wright, 2007). Staphylococci, as an opportunistic pathogen, are the major cause of nosocomial infections, and the emergence of antibiotic resistance in staphylococci poses a major threat to public health (Becker et al., 2014). Meanwhile, development of new antimicrobial agents has been slowed down by the economic and regulatory barriers, and staphylococci continue adapting new antibiotic resistance feature for survival (Bartlett et al., 2013). Thus it is necessary to carry out antibiotic resistance screening. The data of resistance-related research can notify public health authorities to make the right strategies to control and limit the spread of antibiotic resistance in environment (Bartlett et al., 2013).

This study has assessed the distribution of antibiotic resistance of staphylococcal species in environment. The significant findings of my study are as follows:

1. 19 staphylococcal species were identified in this study, and 17 species were previously reported to be isolated from clinical specimens. Taxonomic correlations were determined in staphylococci recovered from human hands and 8 non-biological sites;
2. This is the first report of the employment of MALDI-TOF MS for identification of a large amount of environmental staphylococci. The reliability of MALDI-TOF MS in identifying environmental staphylococcal species was confirmed. In

addition, reproducibility of MALDI-TOF MS in identifying environmental staphylococci was assessed for the first time with two different modes of MALDI-TOF MS in automated fashion, and MALDI-TOF MS was confirmed to be a highly reproducible method for identifying environmental staphylococci.

3. Multiple antibiotic resistance staphylococci were widely distributed in the environment, and 80% staphylococci were resistant to two or more antibiotics. Varied antibiotic susceptibility profiles were observed within taxonomically closely related staphylococci, suggesting the acquisition of antibiotic resistance determinants by mutation and HGT. Cluster analysis also showed no significant difference between multidrug resistant and susceptible staphylococci. This demonstrates that antibiotic resistance genes are produced in both pathogenic and non-pathogenic bacteria as defined by 'resistomes'.
4. The ratio of *mecA*-positive environmental CoNS was generally lower than the ratios reported by clinical study; however, the species of *mecA*-positive environmental CoNS were more diverse than the species in clinical study reports. Oxacillin susceptible *mecA*-positive CoNS (OS-MRCoNS) was first determined in environmental CoNS. Unassigned or untypable SCC*mec* types were dominant in environmental staphylococci. For assigned SCC*mec* types, SCC*mec* type V was prevalent in the environment. 17 *S. epidermidis* harboured new MLST types, and ST59 was firstly reported to be associated with SCC*mec* type IV. Same ST type with varied SCC*mec* types was observed in this study.
5. Whole genome sequencing was applied to an environmental *S. epidermidis*, leading to the identification of 29 virulence genes, 8 surface proteins and 9 antibiotic resistance determinants. 5 out of 9 antibiotic resistance determinants were located on plasmids, which suggest the high mobility of these antibiotic

resistant determinants. Multiple virulence factors and antibiotic resistance genes suggest that *S. epidermidis* 118 (G6_2) could be more virulent and infections could be more difficult to treat. A highly pathogenic *S. epidermidis* 118 (G6_2), recovered from inanimate sites in hotel rooms has been reported.

6. *mecA* gene transfer in natural conditions via conjugation was not observed, and more sophisticated mechanisms may be required to trigger *mecA* gene transfer between staphylococcal species *in vitro*. Whole proteomic expression differences were detected and quantified in *S. aureus* cultured with and without oxacillin. Proteins involved in stress response, transporter mediated antibiotic resistance, virulence and gene expression regulation were exclusively or increasingly expressed in *S. aureus* when exposed to oxacillin. This finding indicates the new trait of antibiotic resistance of *Staphylococcus* spp., and may be a potential threat to public health.

In conclusion, the dissemination of multidrug resistance staphylococci in non-healthcare environments is evidence that these environments act as a reservoir for antibiotic resistant pathogens and determinants. Antibiotic resistance genes from environmental microorganisms comprise a huge proportion of the resistomes.

Chapter 11 Future work

This study provides a general overview of antibiotic resistance in environmental staphylococci, and it is a worrisome finding of wide-spread dissenmination of multiple antibiotic resistant staphylococci in non-healthcare related environments. New MLST types identified in environmental staphylococci displayed the distinctive lineage. In addition, a high virulence and antibiotic resistant *S. epidermidis* was recovered from hotel rooms at an establishment with a generally high standard of hygiene. Finally, *mecA* gene transfer was not observed *in vitro*; however, proteomic analysis has revealed that the stress responses of *S. aureus* were triggered to adapt to survive in the presence of oxacillin.

MALDI-TOF MS has been reported to be a useful, rapid, reliable tool in identifying microorganisms; however, concerns of reproducibility of this technique have been raised (Schumaker et al., 2012). The reproducibility of MALDI-TOF MS refers to the accuracy with which strains are identified (Sandrin et al., 2013). The reproducibility was obtained by testing fresh cultures from different days, and different operators (Majcherczyk et al., 2006; Schumaker et al., 2012). However, no standardized approach has been used to report reproducibility (Sandrin et al., 2013). In this study, the reproducibility of 34 environmental staphylococcal isolates belonging to 18 species was tested with two modes of MALDI-TOF MS. More environmental samples need to be tested for further validation of MALDI-TOF MS reproducibility in future.

In this study, the antibiotic resistance is determined by disc diffusion method, minimum inhibitory method or resistance gene PCR, however, disc diffusion assay and minimum inhibitory method require at least one working day to get the results, and PCR still needs several consecutive steps to get the results (Jorgensen & Ferraro., 2009;

Cherkaoui et al., 2010). As the development of MALDI-TOF MS, uses of MALDI-TOF MS for rapid identification of resistance against β-lactam antibiotics have been reported. The resistance of β-lactam can be detected by mass spectrometry by a molecular mass shift, which is caused by the hydrolysis of the β-lactam ring (Sparbier et al., 2012). In the future, it would be great to employ MALDI-TOF MS for rapid determination of the susceptibility towards β-lactam antibiotic instead of traditional disc diffusion methods.

The whole genomic sequence of *S. epidermidis* 118 (G6_2) provides an insight into the genomic composition of an environmental *S. epidermidis*. The presence of multiple virulence genes and antibiotic resistance genes make *S. epidermidis* 118 (G6_2) a potential threat to public health, as *S. epidermidis* 118 (G6_2) harbours more antibiotic resistance and virulence genes than other two well-known clinical reference *S. epidermidis* strains. In order to better characterize the pathogenicity of *S. epidermidis* 118 (G6_2), it is necessary to carry out animal research to assess the pathogenicity of the *S. epidermidis* 118 (G6_2) *in vivo*. In the future, the *S. epidermidis* 118 (G6_2) virulence can be assessed by in rabbit urinary tract infection, blood vessel infection, and endocarditis models. After clinical, histopathologic, bacteriological and serological examination of urinary tract infection, blood vessel infection and endocarditis, the pathogenicity of *S. epidermidis* 118 (G6_2) can be characterized.

Similar to *S. epidermidis* 118 (G6_2), 3 *S. haemolyticus* and 1 *S. saprophyticus* species were identified that displayed multiple antibiotic resistances and whose oxacillin MICs reach up to 256 mg l^{-1}. *S. saprophyticus* is the second only to *E. coli* as a major cause of urinary tract infection, and surface-associated protein of *S. saprophyticus* contributes to its ability to adhere to urothelial cells (Raz et al., 2005). *S. haemolyticus* and *S. epidermidis*, are the most prevalent staphylococcal species, and both are major causes of neonatal infections (Becker et al., 2014). The highly plastic genome of *S. haemolyticus*

confers frequent genomic rearrangement, insertion, and acquisition of antibiotic resistance (Takeuchi et al., 2005). There are a lot of genomic data on *S. aureus* and *S. epidermidis*, however, little is known on the other staphylococcal species. Therefore, these environmental *S. haemolyticus* and *S. saprophyticus* can be further assessed by whole genome sequencing to determine their genomic features that contribute to their pathogenicity. Moreover, further pan genome sequencing of environmental multidrug resistant *Staphylococcus* spp. would immensially contribute to the further findings of antibiotic resistance transmission between intra species. In the future, genomic work can broaden our knowledge to other staphylococci.

Finally, the transfer mechanism of SCC*mec* elements has not been elucidated. Bloemendaal et al., (2010) has reported that methicillin resistant *S. aureus* was derived from methicillin susceptible *S. aureus* by horizontal *mecA* gene transfer *in vivo*; however, this process can not be replicated with *in vitro* conjugation of the same *S. aureus* strains (Bloemendaal et al., 2010). Recently, Ray et al (2016) successfully observed the conjugative transfer of SCC*mec* from *S. epidermidis* to *S. aureus* by inserting the SCC*mec* elements into a staphylococcal plasmid, however, these manual modifications may not happen in nature. Previous studies have demonstrated enhanced horizontal transfer of mobile genetic elements in cells grown under biofilm formation conditions (Madsen et al., 2012), it will be worthwhile to try conjugative transfer of SCC*mec* using biofilm cultured cells. Additionally, the transformation of SCC*mec* elements faces difficulties by common laboratory method, and for now, transformation can not replicate this process (Otto, 2013). In the future, it may worth to try transformation of SCC*mec* elements via improved methods. In contrast, experimentally phage mediated transduction of SCC*mec* within two *S. aureus* has been reported (Cohen & Sweeney, 1973), however, no phage mediated transduction of SCC*mec* elements

between different staphylococcal species has been reported (Katayama et al., 2000). Recently, Chen et al., (2015) have described staphylococcal intra- and interspecies genetic elements transfer by *cos* phages. Phage mediated transduction is one of the important horizontal gene transfer mechanisms, and acts as one of the main evolutionary driving forces of bacteria (Chen et al., 2015). Therefore, it remains to find out if the SCC*mec* elements can be transferred by phage transduction. In the future, it is worth to try phage-mediated transduction to assess the SCC*mec* transfer between environmental staphylococcal species.

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Appendix I Buffers and Solutions

I.1 Culture mediums

Nutrient agar (Oxoid Ltd, Basingstoke, UK)
28 g nutrient agar powder
1 L ddH_2O

Nutrient broth (Oxoid Ltd, Basingstoke, UK)
13 g nutrient broth powder
1 L ddH_2O

Mannitol salt agar (Oxoid Ltd, Basingstoke, UK)
111 g mannitol salt agar powder
1 L ddH_2O

Brilliance^{TM} UTI clarity agar (Oxoid Ltd, Basingstoke, UK)
39 g Brilliance^{TM} UTI clarity agar powder
1 L ddH_2O

Iso-sensitest agar (Oxoid Ltd, Basingstoke, UK)
31.4 g iso-sensitest agar powder
1 L ddH_2O

Tryptic soy agar (Oxoid Ltd, Basingstoke, UK)
40 g tryptic soy agar powder
1 L ddH_2O

Tryptic soy broth (Oxoid Ltd, Basingstoke, UK)
30 g tryptic soy broth powder
1 L ddH_2O

I.2 General buffers

50×TAE buffer
0.04 M Tris-Acetate
0.001 M EDTA

6×DNA Loading dye
50 % (v/v) Glycerol
0.25 % (w/v) Bromophenol
0.25 % (w/v) Xylene cyanol

I.3 Southern hybridization buffer and solutions

Depurination solution
0.25 N HCl

Denaturation buffer
0.5 M NaOH
1.5 M NaCl
Dissolve in ddH_2O

Neutralization buffer
0.5 M Tris-HCl
3 M NaCl
Dissolve in ddH_2O
pH 7.0

20×SSC
3 M NaCl
0.3 M Trisodium citrate
Dissolve in ddH2O
pH 7.0

Maleic acid buffer
1 M Maleic acid buffer
1.5 M NaCl

Detection buffer
1 M Tris
1 M NaCl

Blocking solution
10 % (v/v) Blocking reagent (Roche)
90 % (w/v) Maleic acid buffer

I.4 PFGE buffers and solutions

SE Buffer
15 mM NaCl
5 mM EDTA

First lysis buffer
6 mM Tris,
100 mM EDTA,
1 M NaCl,
0.5 % (w/v) Brij 58,
0.2 % (w/v) Sodium deoxycholate,
0.5 % (w/v) N-Lauroyl sarcosine,
1 mM $MgCl_2$

Alkaline Lysis Buffer/proteinase K
1 % (w/v) N-Lauroyl sarcosine,
0.5 M EDTA

Dissolved in ddH_2O
pH 9.5

TE buffer
10 mM Tris
10 mM EDTA
Dissolved in ddH_2O
pH 7.5

0.5× TBE
45 mM Tris-borate
1 mM EDTA

Appendix II Antibiotic susceptibility data

II.1 Antibiotic susceptibility profile of all staphylococci

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
1	*Staphylococcus arlettae*	BCF	S	R	S	S	S	S	R	R	R	R	S	S
	1/1(tested/total)													
2	*Staphylococcus aureus*	BCF	S	S	S	S	S	S	R	S	R	I	S	I
3	*Staphylococcus aureus*	BCF	S	R	S	R	S	R	R	I	R	R	S	S
4	*Staphylococcus aureus*	BCF	S	R	S	R	S	S	R	S	S	S	S	S
5	*Staphylococcus aureus*	DSH	S	R	R	S	I	R	S	R	S	S	S	S
6	*Staphylococcus aureus*	DSH	S	R	R	S	I	R	S	S	R	S	S	S
7	*Staphylococcus aureus*	DSH	S	R	R	S	I	S	R	R	R	S	S	S
8	*Staphylococcus aureus*	DSR	S	R	S	S	S	R	R	R	S	R	S	S
9	*Staphylococcus aureus*	DSR	S	R	S	I	S	R	R	S	R	R	S	S
10	*Staphylococcus aureus*	DSS	S	R	R	I	S	S	R	S	S	I	S	S
11	*Staphylococcus aureus*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
12	*Staphylococcus aureus*	DSS	S	R	R	I	S	R	R	S	R	S	S	S
13	*Staphylococcus aureus*	HB	S	S	R	S	I	R	R	R	S	S	S	R
	12/12(tested/total)													
14	*Staphylococcus auricularis*	DST	S	S	S	S	S	S	S	S	S	S	S	S
15	*Staphylococcus auricularis*	DST	S	R	R	I	R	S	R	S	R	S	S	S
	2/2(tested/total)													

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
16	*Staphylococcus capitis*	BCF	S	S	S	R	S	S	R	S	S	S	S	S
17	*Staphylococcus capitis*	BCF	S	R	S	R	S	S	R	S	S	S	S	S
18	*Staphylococcus capitis*	BCF	S	R	S	S	S	S	R	S	R	S	S	S
19	*Staphylococcus capitis*	DSH	S	R	S	R	S	S	S	S	S	S	S	S
20	*Staphylococcus capitis*	DSH	S	R	R	S	S	S	R	I	S	I	S	S
21	*Staphylococcus capitis*	DSH	S	S	R	R	R	R	R	R	R	R	S	R
22	*Staphylococcus capitis*	DSH	S	R	S	S	S	S	S	R	S	S	S	S
23	*Staphylococcus capitis*	DSH	S	R	R	S	S	S	R	R	R	S	S	S
24	*Staphylococcus capitis*	DSH	S	S	S	S	S	S	R	R	S	S	S	S
25	*Staphylococcus capitis*	DSH	S	R	S	S	S	S	S	R	S	S	S	S
26	*Staphylococcus capitis*	DSH	S	S	R	S	I	S	S	S	S	S	S	S
27	*Staphylococcus capitis*	DSH	S	S	S	S	S	S	S	R	S	R	S	R
28	*Staphylococcus capitis*	DSH	S	R	S	S	S	S	S	R	S	S	S	S
29	*Staphylococcus capitis*	DSH	S	R	R	R	S	S	S	R	S	S	S	S
30	*Staphylococcus capitis*	DSL	S	S	S	S	S	S	R	R	S	S	R	S
31	*Staphylococcus capitis*	DSL	S	S	S	S	S	S	S	R	S	S	S	S
32	*Staphylococcus capitis*	DSL	S	R	S	S	S	S	S	R	R	S	S	S
33	*Staphylococcus capitis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
34	*Staphylococcus capitis*	DSR												
35	*Staphylococcus capitis*	DSR	S	R	S	S	S	S	S	S	S	R	S	S
36	*Staphylococcus capitis*	DSR	S	R	R	S	S	S	R	S	S	S	R	S
37	*Staphylococcus capitis*	DSR	S	S	S	S	S	S	R	S	S	S	S	S
38	*Staphylococcus capitis*	DSR	S	R	S	R	S	S	S	S	S	S	S	S
39	*Staphylococcus capitis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
40	*Staphylococcus capitis*	DSR												
41	*Staphylococcus capitis*	DSR												
42	*Staphylococcus capitis*	DSR												

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
43	*Staphylococcus capitis*	DSS	S	R	R	I	S	S	R	R	S	S	S	S
44	*Staphylococcus capitis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
45	*Staphylococcus capitis*	DSS	S	R	S	S	S	S	R	S	S	S	R	S
46	*Staphylococcus capitis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
47	*Staphylococcus capitis*	DSS	S	R	R	I	S	S	R	S	S	S	S	S
48	*Staphylococcus capitis*	DSS	S	R	S	S	S	S	R	S	S	S	R	S
49	*Staphylococcus capitis*	DSS	S	R	S	R	S	S	S	S	S	R	S	R
50	*Staphylococcus capitis*	DST	S	R	S	S	S	S	R	R	R	R	S	S
51	*Staphylococcus capitis*	DST	S	R	S	S	S	S	S	R	R	S	S	S
52	*Staphylococcus capitis*	DST	S	S	S	S	S	S	S	S	S	S	S	S
53	*Staphylococcus capitis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
54	*Staphylococcus capitis*	DST	S	R	S	S	S	S	R	S	S	S	S	S
55	*Staphylococcus capitis*	DST	S	S	S	R	S	S	S	S	S	S	S	S
56	*Staphylococcus capitis*	DST	S	S	S	S	S	S	S	S	S	S	S	S
57	*Staphylococcus capitis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
58	*Staphylococcus capitis*	DST	S	S	S	I	S	S	R	S	S	S	S	S
59	*Staphylococcus capitis*	DST	S	S	S	R	S	S	R	S	S	S	S	S
60	*Staphylococcus capitis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
61	*Staphylococcus capitis*	DST	S	R	S	S	S	S	R	S	S	S	S	S
62	*Staphylococcus capitis*	DST	S	R	S	S	S	S	R	S	S	S	S	S
63	*Staphylococcus capitis*	DST	S	S	S	S	I	S	S	S	S	S	S	S
64	*Staphylococcus capitis*	HB	S	S	S	S	S	S	S	R	S	R	S	S
65	*Staphylococcus capitis*	HB	S	R	S	S	S	S	S	S	S	S	S	S
66	*Staphylococcus capitis*	HB	S	S	S	S	S	S	S	R	S	S	S	S
67	*Staphylococcus capitis*	HB	S	R	S	S	S	S	R	R	R	S	S	S
68	*Staphylococcus capitis*	HH	S	R	R	R	S	S	S	R	R	S	S	S
69	*Staphylococcus capitis*	HH	S	R	S	S	S	S	R	R	R	S	R	S
70	*Staphylococcus capitis*	HH	S	R	S	S	S	S	S	R	S	S	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
71	*Staphylococcus capitis*	HH	R	S	S	S	S	S	S	R	S	S	S	S
72	*Staphylococcus capitis*	HH	S	S	S	S	S	S	S	R	S	S	S	S
73	*Staphylococcus capitis*	HH	S	S	S	S	S	S	R	R	S	S	S	S
74	*Staphylococcus capitis*	HH	S	R	S	S	S	S	R	R	R	S	S	S
75	*Staphylococcus capitis*	HH	R	R	S	S	S	S	R	R	S	S	S	S
76	*Staphylococcus capitis*	HH	S	S	S	S	I	S	S	R	S	S	S	S
77	*Staphylococcus capitis*	HH	S	R	S	S	S	S	R	R	S	S	S	S
78	*Staphylococcus capitis*	HH	S	R	S	S	I	S	R	R	R	S	S	S
79	*Staphylococcus capitis*	HH	S	R	S	S	S	S	R	R	R	S	S	S
80	*Staphylococcus capitis*	HH	S	S	S	S	S	S	S	S	R	S	S	S
81	*Staphylococcus capitis*	HH	R	R	S	S	R	S	R	R	R	R	S	S
82	*Staphylococcus capitis*	HH	S	R	S	S	S	S	S	R	R	S	S	S
83	*Staphylococcus capitis*	HH	S	S	S	R	S	S	R	R	S	S	S	S
84	*Staphylococcus capitis*	HH	S	S	S	S	S	S	R	R	S	S	S	S
85	*Staphylococcus capitis*	HH	S	S	R	I	S	S	R	S	S	R	S	S
86	*Staphylococcus capitis*	HH	S	S	S	I	S	S	R	S	S	I	S	S
87	*Staphylococcus capitis*	HH	S	R	S	I	S	S	R	S	R	S	R	S
88	*Staphylococcus capitis*	HH	S	S	R	S	S	S	S	S	S	R	S	S
89	*Staphylococcus capitis*	HH	S	R	S	I	S	S	R	S	S	S	S	S
90	*Staphylococcus capitis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
91	*Staphylococcus capitis*	HH	S	S	S	I	S	S	S	R	S	S	S	S
92	*Staphylococcus capitis*	HH	S	R	R	I	S	S	S	S	S	S	S	S
93	*Staphylococcus capitis*	HH	S	R	R	R	R	S	R	S	R	S	S	R
94	*Staphylococcus capitis*	HH	S	S	S	I	S	S	R	S	S	R	S	S
	75/79(tested/total)													
95	*Staphylococcus caprae*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
96	*Staphylococcus caprae*	HB	S	R	S	S	S	S	R	R	R	S	S	S
	2/2(tested/total)													

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
97	*Staphylococcus cohnii*	BCF	S	R	S	S	R	S	R	R	S	R	S	S
98	*Staphylococcus cohnii*	BCF	S	R	S	R	R	S	R	R	R	S	S	S
99	*Staphylococcus cohnii*	DSH	S	R	R	S	S	S	R	S	R	R	S	S
100	*Staphylococcus cohnii*	DSH	R	R	S	R	S	S	S	S	R	R	S	S
101	*Staphylococcus cohnii*	DSH	S	R	S	S	S	S	R	S	S	R	S	R
102	*Staphylococcus cohnii*	DSH	S	R	S	S	S	S	R	R	S	R	S	S
103	*Staphylococcus cohnii*	DSH	S	R	S	S	R	S	R	R	S	S	S	S
104	*Staphylococcus cohnii*	DSL	S	S	S	S	S	S	R	R	S	R	S	S
105	*Staphylococcus cohnii*	DSL	S	-	S	S	S	S	R	S	S	S	S	S
106	*Staphylococcus cohnii*	HAS	R	R	S	R	R	S	R	I	S	S	S	S
107	*Staphylococcus cohnii*	HAS	R	R	R	S	R	S	R	R	S	R	R	R
108	*Staphylococcus cohnii*	HH	R	S	S	S	I	S	R	R	S	R	R	S
109	*Staphylococcus cohnii*	HH	S	R	R	S	I	S	R	R	R	S	S	S
110	*Staphylococcus cohnii*	HH	S	R	S	S	S	S	R	S	S	R	S	S
	14/14(tested/total)													
111	*Staphylococcus epidermidis*	BCF	S	R	S	S	S	S	S	S	S	S	S	S
112	*Staphylococcus epidermidis*	BCF	S	R	S	S	S	S	R	S	R	S	S	S
113	*Staphylococcus epidermidis*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
114	*Staphylococcus epidermidis*	BCF	S	S	S	S	S	S	R	S	S	R	S	S
115	*Staphylococcus epidermidis*	BCF	S	R	S	S	S	S	S	S	S	R	S	S
116	*Staphylococcus epidermidis*	BCF	S	R	S	I	S	S	R	S	R	S	S	S
117	*Staphylococcus epidermidis*	BCF	S	R	S	S	S	S	R	S	R	I	R	S
118	*Staphylococcus epidermidis*	DSH	R	R	S	R	R	R	R	I	R	R	R	S
119	*Staphylococcus epidermidis*	DSH	S	R	S	S	S	S	S	S	R	I	R	S
120	*Staphylococcus epidermidis*	DSH	S	R	R	S	S	S	R	S	S	I	S	S
121	*Staphylococcus epidermidis*	DSH	S	R	S	S	S	S	R	R	R	I	R	R

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
122	*Staphylococcus epidermidis*	DSH	S	R	S	R	S	S	R	R	S	S	S	S
123	*Staphylococcus epidermidis*	DSH	S	R	S	S	I	S	R	R	S	S	S	S
124	*Staphylococcus epidermidis*	DSH	R	R	R	S	R	S	R	R	R	S	R	S
125	*Staphylococcus epidermidis*	DSH	S	S	R	S	I	S	S	R	S	S	S	S
126	*Staphylococcus epidermidis*	DSH	R	R	R	S	I	S	S	R	R	R	S	S
127	*Staphylococcus epidermidis*	DSH	R	R	R	S	I	S	S	S	R	R	R	S
128	*Staphylococcus epidermidis*	DSH	S	R	R	S	R	R	S	R	R	S	S	R
129	*Staphylococcus epidermidis*	DSL	S	R	S	S	S	S	R	R	R	R	R	S
130	*Staphylococcus epidermidis*	DSL	S	R	R	S	S	S	S	R	R	S	S	S
131	*Staphylococcus epidermidis*	DSL	S	R	R	R	R	S	R	S	R	S	S	S
132	*Staphylococcus epidermidis*	DSL	S	R	S	S	S	S	S	R	R	S	S	S
133	*Staphylococcus epidermidis*	DSL	R	R	S	S	S	S	R	R	R	R	R	S
134	*Staphylococcus epidermidis*	DSL	R	R	S	S	S	S	R	R	R	R	R	S
135	*Staphylococcus epidermidis*	DSL	R	R	S	S	S	S	R	R	R	R	R	S
136	*Staphylococcus epidermidis*	DSL	S	R	S	S	S	S	S	R	S	R	S	S
137	*Staphylococcus epidermidis*	DSL	S	R	S	S	S	S	S	R	R	R	S	S
138	*Staphylococcus epidermidis*	DSR	S	S	R	S	R	S	R	S	S	R	S	S
139	*Staphylococcus epidermidis*	DSR	R	R	R	R	R	S	R	S	R	R	R	S
140	*Staphylococcus epidermidis*	DSR	S	R	R	R	R	R	R	S	R	S	R	S
141	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	R	S	R	S
142	*Staphylococcus epidermidis*	DSR	S	R	R	I	S	S	R	S	S	R	S	S
143	*Staphylococcus epidermidis*	DSR	S	R	R	R	R	R	R	S	R	R	S	S
144	*Staphylococcus epidermidis*	DSR	S	S	S	S	S	S	R	S	S	S	S	S
145	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
146	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	R	S	S
147	*Staphylococcus epidermidis*	DSR	S	R	R	S	S	S	R	S	S	R	S	S
148	*Staphylococcus epidermidis*	DSR	S	S	S	S	S	S	R	S	S	R	S	S
149	*Staphylococcus epidermidis*	DSR	S	R	R	S	S	R	R	S	S	R	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
150	*Staphylococcus epidermidis*	DSR	S	R	R	S	S	R	S	S	S	R	S	S
151	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
152	*Staphylococcus epidermidis*	DSR	S	R	S	R	S	S	R	S	S	S	S	S
153	*Staphylococcus epidermidis*	DSR	R	R	S	S	S	S	R	S	S	S	S	S
154	*Staphylococcus epidermidis*	DSR	S	S	S	S	S	S	R	S	S	R	S	S
155	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
156	*Staphylococcus epidermidis*	DSR	S	R	S	S	R	R	S	S	S	S	S	S
157	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
158	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	R	S	S
159	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
160	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
161	*Staphylococcus epidermidis*	DSR	S	R	S	R	S	S	S	S	S	S	S	S
162	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
163	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	S	S	S	S	R	S
164	*Staphylococcus epidermidis*	DSR	S	R	S	I	S	S	S	S	S	S	S	S
165	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
166	*Staphylococcus epidermidis*	DSR												
167	*Staphylococcus epidermidis*	DSR												
168	*Staphylococcus epidermidis*	DSR	S	R	S	R	S	S	S	S	S	R	S	S
169	*Staphylococcus epidermidis*	DSR	S	S	S	S	S	S	R	S	S	R	S	S
170	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
171	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
172	*Staphylococcus epidermidis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
173	*Staphylococcus epidermidis*	DSR	S	S	S	S	S	S	R	S	S	S	S	S
174	*Staphylococcus epidermidis*	DSS												
175	*Staphylococcus epidermidis*	DSS	S	R	S	I	S	S	S	S	S	R	S	S
176	*Staphylococcus epidermidis*	DSS	S	S	S	S	S	S	R	S	S	I	S	S
177	*Staphylococcus epidermidis*	DSS	S	R	R	S	S	S	S	S	S	R	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
178	*Staphylococcus epidermidis*	DSS	S	R	R	S	S	S	R	S	S	R	R	S
179	*Staphylococcus epidermidis*	DSS	S	R	S	I	I	S	S	S	R	R	S	S
180	*Staphylococcus epidermidis*	DSS	S	R	R	I	S	S	R	S	S	S	S	S
181	*Staphylococcus epidermidis*	DSS	S	R	S	S	S	S	R	S	R	S	S	S
182	*Staphylococcus epidermidis*	DSS	S	S	S	I	S	S	S	S	S	S	S	S
183	*Staphylococcus epidermidis*	DSS	S	S	S	I	S	S	S	S	S	R	S	S
184	*Staphylococcus epidermidis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
185	*Staphylococcus epidermidis*	DSS	S	R	R	S	S	S	S	S	S	S	S	S
186	*Staphylococcus epidermidis*	DSS	S	R	S	S	S	S	S	S	R	S	S	S
187	*Staphylococcus epidermidis*	DSS	R	R	R	S	S	S	S	S	S	R	S	S
188	*Staphylococcus epidermidis*	DSS	S	R	S	R	R	R	R	S	R	S	S	S
189	*Staphylococcus epidermidis*	DSS	S	R	R	R	R	R	R	S	R	S	S	S
190	*Staphylococcus epidermidis*	DSS	S	S	S	S	S	S	S	S	S	S	S	S
191	*Staphylococcus epidermidis*	DSS	R	R	S	S	S	S	R	S	R	S	S	S
192	*Staphylococcus epidermidis*	DSS	S	R	R	S	S	S	R	S	S	S	S	S
193	*Staphylococcus epidermidis*	DSS	S	S	S	S	S	S	S	S	S	S	S	S
194	*Staphylococcus epidermidis*	DSS	S	S	S	S	S	S	S	S	S	S	S	S
195	*Staphylococcus epidermidis*	DSS	S	S	S	S	S	S	S	S	S	S	S	S
196	*Staphylococcus epidermidis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
197	*Staphylococcus epidermidis*	DSS	S	R	R	S	S	S	R	S	S	S	S	S
198	*Staphylococcus epidermidis*	DSS												
199	*Staphylococcus epidermidis*	DSS	S	R	S	I	S	S	R	S	S	S	S	S
200	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	R	S	R	R	S	S
201	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	S	R	R	R	R	S
202	*Staphylococcus epidermidis*	DST	S	S	R	R	S	S	R	I	S	S	S	S
203	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	R	S	S
204	*Staphylococcus epidermidis*	DST	S	S	R	R	S	S	S	S	S	R	S	S
205	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	S	R	R	R	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
206	*Staphylococcus epidermidis*	DST	S	R	R	S	S	S	R	S	R	R	S	S
207	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
208	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	S	S	S	R	S	S
209	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
210	*Staphylococcus epidermidis*	DST	S	S	R	R	S	S	R	S	S	R	S	S
211	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	R	S	R	R	S	S
212	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	R	S	S
213	*Staphylococcus epidermidis*	DST	S	S	R	S	S	S	R	S	S	S	S	S
214	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	S	S	R	R	S	S
215	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
216	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	R	S	R	R	S	S
217	*Staphylococcus epidermidis*	DST												
218	*Staphylococcus epidermidis*	DST												
219	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
220	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	R	S	R	R	S	S
221	*Staphylococcus epidermidis*	DST												
222	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	S	S	S	S	S	S
223	*Staphylococcus epidermidis*	DST	S	S	R	S	S	S	S	S	S	R	S	S
224	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
225	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	S	S	S	R	S	S
226	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	S	S	S	R	S	S
227	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
228	*Staphylococcus epidermidis*	DST	S	S	S	R	S	S	S	S	S	S	S	S
229	*Staphylococcus epidermidis*	DST	S	R	S	S	S	S	S	S	S	S	S	S
230	*Staphylococcus epidermidis*	DST	S	R	R	S	S	S	R	S	R	I	S	S
231	*Staphylococcus epidermidis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
232	*Staphylococcus epidermidis*	DST	S	R	S	I	S	S	S	S	S	R	S	S
233	*Staphylococcus epidermidis*	HB	S	R	S	S	I	S	S	S	S	R	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
234	*Staphylococcus epidermidis*	HB	R	S	S	R	S	S	R	R	S	R	R	S
235	*Staphylococcus epidermidis*	HB	S	S	S	S	S	S	S	S	S	S	R	S
236	*Staphylococcus epidermidis*	HB	S	S	R	S	R	R	R	R	S	S	R	S
237	*Staphylococcus epidermidis*	HB	S	S	S	S	S	S	R	S	S	S	S	S
238	*Staphylococcus epidermidis*	HB	S	R	S	S	S	S	R	R	S	S	S	S
239	*Staphylococcus epidermidis*	HH	S	S	S	R	S	S	S	S	S	S	S	R
240	*Staphylococcus epidermidis*	HH	S	S	R	S	S	R	R	S	S	S	S	S
241	*Staphylococcus epidermidis*	HH												
242	*Staphylococcus epidermidis*	HH												
243	*Staphylococcus epidermidis*	HH												
244	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	S	S	S	S	S	S
245	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	R	R	R	S	S
246	*Staphylococcus epidermidis*	HH	S	R	S	R	S	S	S	S	S	S	S	S
247	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	S	R	S	S	S	S
248	*Staphylococcus epidermidis*	HH	S	R	S	R	I	S	S	S	S	S	S	S
249	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	R	R	S	S	R	S
250	*Staphylococcus epidermidis*	HH	S	R	S	R	S	S	R	R	R	S	S	S
251	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	R	R	R	S	S
252	*Staphylococcus epidermidis*	HH	S	S	S	S	S	S	R	R	S	S	R	S
253	*Staphylococcus epidermidis*	HH	S	S	S	R	I	R	R	R	S	S	S	S
254	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	S	R	S	S	S	S
255	*Staphylococcus epidermidis*	HH	S	R	S	S	I	S	R	R	S	S	S	S
256	*Staphylococcus epidermidis*	HH	S	R	R	R	I	S	R	R	R	R	S	R
257	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	S	S	R	I	R	S
258	*Staphylococcus epidermidis*	HH	S	S	R	S	S	S	R	R	S	R	S	S
259	*Staphylococcus epidermidis*	HH	R	R	S	S	R	S	R	R	R	R	R	S
260	*Staphylococcus epidermidis*	HH	S	S	S	S	S	S	R	R	S	S	S	S
261	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	R	S	S	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
262	*Staphylococcus epidermidis*	HH	S	S	S	R	S	S	S	R	S	S	S	S
263	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	R	R	S	R	S
264	*Staphylococcus epidermidis*	HH	S	S	S	R	S	S	R	R	S	S	S	S
265	*Staphylococcus epidermidis*	HH	S	S	S	S	S	S	R	R	S	S	S	S
266	*Staphylococcus epidermidis*	HH	S	R	R	R	S	S	R	S	S	R	S	S
267	*Staphylococcus epidermidis*	HH	S	R	S	I	S	S	R	S	S	R	S	S
268	*Staphylococcus epidermidis*	HH	S	R	S	I	S	S	R	S	S	R	R	S
269	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	R	S	S	S	S	S
270	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	R	S	R	R	S	S
271	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	S	S	R	S	S
272	*Staphylococcus epidermidis*	HH	S	R	S	I	S	S	R	S	S	R	S	S
273	*Staphylococcus epidermidis*	HH	S	R	R	I	S	S	R	S	S	S	S	S
274	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	R	S	S	R	R	S
275	*Staphylococcus epidermidis*	HH	S	R	R	I	S	S	R	S	S	S	S	S
276	*Staphylococcus epidermidis*	HH	S	R	S	I	S	S	R	S	S	R	S	S
277	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	S	S	S	R	S	S
278	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	S	S	R	R	S	S
279	*Staphylococcus epidermidis*	HH	R	R	R	S	S	S	R	S	S	R	S	S
280	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	R	S	S	R	S	S
281	*Staphylococcus epidermidis*	HH	S	S	S	S	S	S	R	S	S	R	S	S
282	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	S	S	S	R	S	S
283	*Staphylococcus epidermidis*	HH	S	R	S	I	R	S	S	S	S	R	S	S
284	*Staphylococcus epidermidis*	HH	S	R	R	R	R	S	S	S	S	S	S	S
285	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	S	S	R	S	S
286	*Staphylococcus epidermidis*	HH	S	S	S	R	S	S	R	S	S	R	R	S
287	*Staphylococcus epidermidis*	HH	S	R	R	R	R	R	R	S	R	S	S	R
288	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
289	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	S	S	R	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
290	*Staphylococcus epidermidis*	HH	S	R	R	I	R	S	R	S	S	S	S	R
291	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	R	S	S	S	S
292	*Staphylococcus epidermidis*	HH	S	R	R	R	S	S	R	S	S	S	S	S
293	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	S	S	S	S	S	S
294	*Staphylococcus epidermidis*	HH												
295	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	S	S	S	S	S	S
296	*Staphylococcus epidermidis*	HH												
297	*Staphylococcus epidermidis*	HH	S	S	S	I	S	S	S	S	S	R	S	S
298	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	S	S	R	S	S
299	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	R	S	S	R	S	S
300	*Staphylococcus epidermidis*	HH												
301	*Staphylococcus epidermidis*	HH												
302	*Staphylococcus epidermidis*	HH												
303	*Staphylococcus epidermidis*	HH												
304	*Staphylococcus epidermidis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
305	*Staphylococcus epidermidis*	HH	S	R	R	S	S	S	S	S	S	R	R	S
306	*Staphylococcus epidermidis*	HH	S	R	S	R	S	S	R	S	S	R	S	S
307	*Staphylococcus epidermidis*	HH												
308	*Staphylococcus epidermidis*	HH	R	R	S	R	S	S	R	S	R	R	S	S
	181/198(tested/total)													
309	*Staphylococcus equorum*	DSH	S	R	S	S	S	S	S	S	S	I	S	S
310	*Staphylococcus equorum*	DST	S	R	R	R	S	S	R	R	R	S	R	S
311	*Staphylococcus equorum*	DST	S	S	S	S	S	S	S	S	S	I	S	S
	3/3(tested/total)													
312	*Staphylococcus haemolyticus*	BCF	S	S	S	S	S	S	S	S	S	S	S	S
313	*Staphylococcus haemolyticus*	BCF	S	S	R	S	S	S	R	R	S	S	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
314	*Staphylococcus haemolyticus*	BCF	S	R	S	R	R	R	R	R	R	I	S	R
315	*Staphylococcus haemolyticus*	BCF	S	R	S	I	S	S	R	S	R	R	S	S
316	*Staphylococcus haemolyticus*	DSH	R	R	R	R	R	S	R	I	S	R	S	R
317	*Staphylococcus haemolyticus*	DSH	R	R	R	S	R	R	R	R	R	S	R	R
318	*Staphylococcus haemolyticus*	DSH	R	R	S	S	R	R	R	R	R	R	S	S
319	*Staphylococcus haemolyticus*	DSH	R	R	S	S	R	S	R	I	R	R	S	S
320	*Staphylococcus haemolyticus*	DSH	R	R	R	R	R	S	R	I	S	S	R	R
321	*Staphylococcus haemolyticus*	DSH	S	R	R	S	S	S	R	I	R	S	S	S
322	*Staphylococcus haemolyticus*	DSH	S	R	S	S	S	S	S	I	R	S	R	S
323	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	R	S	R	R	I	R	S
324	*Staphylococcus haemolyticus*	DSH	S	R	S	S	S	S	S	I	R	S	R	S
325	*Staphylococcus haemolyticus*	DSH	S	R	R	R	S	R	R	S	R	I	S	S
326	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	S	S	S	R	I	S	S
327	*Staphylococcus haemolyticus*	DSH	S	R	S	R	S	S	R	S	R	S	S	S
328	*Staphylococcus haemolyticus*	DSH	S	R	S	R	S	S	R	S	R	I	S	S
329	*Staphylococcus haemolyticus*	DSH	S	R	S	S	S	S	R	R	R	I	S	S
330	*Staphylococcus haemolyticus*	DSH	S	S	R	S	R	S	R	S	R	S	S	S
331	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	S	R	S	R	I	S	S
332	*Staphylococcus haemolyticus*	DSH	S	S	S	S	R	S	S	S	R	I	R	S
333	*Staphylococcus haemolyticus*	DSH	S	R	R	S	S	S	R	S	S	I	S	S
334	*Staphylococcus haemolyticus*	DSH	S	R	S	S	S	S	R	S	S	I	S	S
335	*Staphylococcus haemolyticus*	DSH	S	S	S	S	R	S	S	S	R	I	R	R
336	*Staphylococcus haemolyticus*	DSH	S	S	R	S	S	S	S	S	R	I	R	S
337	*Staphylococcus haemolyticus*	DSH	S	R	S	S	R	S	R	R	S	I	R	S
338	*Staphylococcus haemolyticus*	DSH	S	R	S	S	S	S	S	R	R	I	S	S
339	*Staphylococcus haemolyticus*	DSH	S	R	R	S	S	S	R	R	R	I	S	S
340	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	S	S	S	S	I	R	S
341	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	S	S	S	R	I	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
342	*Staphylococcus haemolyticus*	DSH	S	R	S	S	S	S	R	S	S	I	R	S
343	*Staphylococcus haemolyticus*	DSH	S	S	S	R	S	S	R	S	S	S	S	S
344	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	R	R	S	S	I	R	S
345	*Staphylococcus haemolyticus*	DSH	S	S	S	S	R	S	R	S	S	I	S	S
346	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	S	S	S	S	I	S	S
347	*Staphylococcus haemolyticus*	DSH	S	R	S	R	R	S	R	R	S	R	R	R
348	*Staphylococcus haemolyticus*	DSH												
349	*Staphylococcus haemolyticus*	DSH												
350	*Staphylococcus haemolyticus*	DSH												
351	*Staphylococcus haemolyticus*	DSH												
352	*Staphylococcus haemolyticus*	DSH												
353	*Staphylococcus haemolyticus*	DSH	S	R	S	S	I	S	R	R	R	R	S	S
354	*Staphylococcus haemolyticus*	DSH	S	S	R	S	I	R	S	S	S	R	S	S
355	*Staphylococcus haemolyticus*	DSH	R	R	S	S	R	R	R	R	R	S	R	S
356	*Staphylococcus haemolyticus*	DSH	S	S	S	S	I	S	S	R	S	S	S	S
357	*Staphylococcus haemolyticus*	DSH	S	S	S	R	S	S	S	S	S	S	S	S
358	*Staphylococcus haemolyticus*	DSH	S	S	R	S	I	S	R	R	S	S	S	S
359	*Staphylococcus haemolyticus*	DSH	S	R	R	S	R	S	R	R	S	S	S	R
360	*Staphylococcus haemolyticus*	DSH	S	S	S	S	S	S	R	S	S	S	S	R
361	*Staphylococcus haemolyticus*	DSL	S	S	S	S	I	S	R	R	S	S	R	S
362	*Staphylococcus haemolyticus*	DSL	R	R	S	S	I	S	S	R	R	S	S	S
363	*Staphylococcus haemolyticus*	DSL	S	S	R	S	I	S	S	R	S	S	S	S
364	*Staphylococcus haemolyticus*	DSL	S	S	R	S	I	S	R	R	S	S	S	S
365	*Staphylococcus haemolyticus*	DSL	S	R	R	S	I	S	R	R	S	S	S	S
366	*Staphylococcus haemolyticus*	DSL	S	S	R	S	I	S	S	R	S	S	S	S
367	*Staphylococcus haemolyticus*	DSL	R	R	S	S	R	S	S	R	R	S	S	S
368	*Staphylococcus haemolyticus*	DSL	S	S	S	S	I	S	S	R	S	R	R	S
369	*Staphylococcus haemolyticus*	DSL	S	R	S	S	S	S	R	R	S	R	S	R

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
370	*Staphylococcus haemolyticus*	DSL	S	S	S	S	S	S	R	R	S	S	S	S
371	*Staphylococcus haemolyticus*	DSR	S	S	S	S	S	S	S	S	S	S	S	S
372	*Staphylococcus haemolyticus*	DSR	S	S	S	I	S	S	R	S	S	S	S	S
373	*Staphylococcus haemolyticus*	DSS	S	R	S	S	S	S	R	S	S	R	S	S
374	*Staphylococcus haemolyticus*	DSS	S	R	R	I	S	S	R	S	S	I	R	S
375	*Staphylococcus haemolyticus*	DSS	S	R	S	I	S	S	R	S	S	S	S	S
376	*Staphylococcus haemolyticus*	DSS	S	R	S	R	R	S	R	S	S	R	S	S
377	*Staphylococcus haemolyticus*	DST	S	R	S	S	S	R	R	R	R	R	S	S
378	*Staphylococcus haemolyticus*	DST	S	S	S	S	S	S	S	I	S	R	S	S
379	*Staphylococcus haemolyticus*	HAS	R	R	S	S	R	S	R	R	S	R	R	S
380	*Staphylococcus haemolyticus*	HAS	R	R	R	R	S	S	R	R	S	R	R	R
381	*Staphylococcus haemolyticus*	HH	S	S	S	S	I	S	S	R	S	S	S	S
382	*Staphylococcus haemolyticus*	HH	S	R	S	S	I	S	R	R	S	R	S	S
383	*Staphylococcus haemolyticus*	HH	S	R	R	R	I	R	R	R	R	S	R	S
384	*Staphylococcus haemolyticus*	HH	R	R	S	R	S	S	R	R	S	S	S	S
385	*Staphylococcus haemolyticus*	HH	S	S	S	S	R	S	R	R	R	R	R	S
386	*Staphylococcus haemolyticus*	HH	S	R	R	I	S	S	R	S	S	R	R	S
387	*Staphylococcus haemolyticus*	HH	S	S	S	S	S	S	R	S	S	R	S	S
388	*Staphylococcus haemolyticus*	HH	S	R	S	I	S	S	R	S	R	S	S	S
389	*Staphylococcus haemolyticus*	HH	S	R	S	I	S	S	R	S	S	S	S	S
390	*Staphylococcus haemolyticus*	HH	S	S	R	I	S	R	R	R	S	R	R	S
	74/79(tested/total)													
391	*Staphylococcus hominis*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
392	*Staphylococcus hominis*	BCF	S	R	S	S	S	S	R	S	R	S	S	S
393	*Staphylococcus hominis*	BCF	S	R	S	S	S	S	R	S	R	S	S	S
394	*Staphylococcus hominis*	BCF	S	S	S	S	S	S	R	S	S	S	S	S
395	*Staphylococcus hominis*	BCF	S	R	S	S	S	S	R	S	R	R	S	S

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
396	*Staphylococcus hominis*	BCF	S	S	S	R	S	S	R	S	S	S	S	S
397	*Staphylococcus hominis*	BCF	S	R	S	S	S	S	R	S	R	S	S	S
398	*Staphylococcus hominis*	BCF	S	R	S	S	R	S	R	S	R	R	R	S
399	*Staphylococcus hominis*	DSH	R	R	R	S	S	S	R	S	S	S	S	R
400	*Staphylococcus hominis*	DSH	S	R	R	R	S	S	R	S	R	R	R	S
401	*Staphylococcus hominis*	DSH	S	R	S	R	R	S	R	I	R	R	S	S
402	*Staphylococcus hominis*	DSH	S	S	S	S	S	S	R	S	R	S	S	S
403	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	R	S	R	S	S	S
404	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	R	R	S	I	S	S
405	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	S	R	S	S	S	S
406	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	S	S	S	S	S	S
407	*Staphylococcus hominis*	DSH	S	S	S	S	S	S	S	S	R	S	S	S
408	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	R	S	S	I	R	S
409	*Staphylococcus hominis*	DSH	S	S	S	S	S	S	R	S	S	S	S	S
410	*Staphylococcus hominis*	DSH	S	S	S	S	S	S	R	S	S	S	S	S
411	*Staphylococcus hominis*	DSH												
412	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	R	S	R	R	S	S
413	*Staphylococcus hominis*	DSH	R	S	S	R	S	S	R	R	S	S	S	S
414	*Staphylococcus hominis*	DSH	S	R	R	S	I	S	S	S	R	S	S	S
415	*Staphylococcus hominis*	DSH	S	S	S	S	I	S	S	S	S	S	S	S
416	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	S	R	R	S	R	S
417	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	S	R	S	S	S	S
418	*Staphylococcus hominis*	DSH	S	R	R	S	R	S	S	S	R	S	S	S
419	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	S	R	R	R	S	S
420	*Staphylococcus hominis*	DSH	S	R	S	S	R	S	R	R	R	R	S	S
421	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	R	R	S	S	S	S
422	*Staphylococcus hominis*	DSH	S	R	S	S	S	S	S	R	S	S	S	S
423	*Staphylococcus hominis*	DSH	S	R	S	S	I	R	S	R	R	S	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
424	*Staphylococcus hominis*	DSH	S	S	S	S	S	S	S	S	S	S	S	S
425	*Staphylococcus hominis*	DSH	S	S	S	R	S	S	S	R	S	S	S	S
426	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	R	R	R	R	S	S
427	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	S	S	S	S	S	S
428	*Staphylococcus hominis*	DSH	S	S	R	S	S	S	S	R	S	S	S	S
429	*Staphylococcus hominis*	DSH	S	S	S	R	S	S	S	S	S	S	S	S
430	*Staphylococcus hominis*	DSH	S	R	S	R	S	S	R	R	S	S	S	S
431	*Staphylococcus hominis*	DSH												
432	*Staphylococcus hominis*	DSH	S	R	S	S	I	S	R	R	S	S	S	S
433	*Staphylococcus hominis*	DSL	S	R	S	S	S	S	S	R	S	S	S	S
434	*Staphylococcus hominis*	DSR	S	S	S	I	S	S	R	S	S	S	S	S
435	*Staphylococcus hominis*	DSR	S	R	S	R	R	R	R	S	R	S	S	S
436	*Staphylococcus hominis*	DSR	S	S	S	S	S	R	R	S	S	S	S	S
437	*Staphylococcus hominis*	DSR	S	R	S	I	S	S	R	S	S	R	S	S
438	*Staphylococcus hominis*	DSR	S	R	S	R	S	S	R	S	S	S	R	S
439	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	R	S	S	R	S	S
440	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
441	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
442	*Staphylococcus hominis*	DSR	S	R	S	I	S	S	R	S	S	R	R	S
443	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
444	*Staphylococcus hominis*	DSR	S	R	S	R	S	S	R	S	S	S	R	S
445	*Staphylococcus hominis*	DSR	S	S	S	I	S	S	S	S	S	S	S	S
446	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	R	R	S
447	*Staphylococcus hominis*	DSR	S	R	R	I	S	S	R	S	R	S	R	S
448	*Staphylococcus hominis*	DSR	S	R	R	S	S	R	R	R	S	S	S	S
449	*Staphylococcus hominis*	DSR	S	R	S	I	S	S	R	S	S	R	S	S
450	*Staphylococcus hominis*	DSR	S	R	R	I	S	S	R	S	S	S	S	S
451	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	S	S	S	R	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
452	*Staphylococcus hominis*	DSR	S	S	S	I	S	R	R	S	S	R	S	S
453	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	S	S	S	S	S	S
454	*Staphylococcus hominis*	DSR	S	S	R	S	S	S	S	S	S	R	R	S
455	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
456	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
457	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	S	S	S	R	S	S
458	*Staphylococcus hominis*	DSR	S	R	S	I	S	S	S	S	S	R	S	S
459	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
460	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	S	S	S
461	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	R	S	S	R	S	S
462	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	R	S	S	S	S	S
463	*Staphylococcus hominis*	DSR	S	S	S	S	S	S	S	S	S	S	S	S
464	*Staphylococcus hominis*	DSR	S	S	S	I	S	S	R	S	S	S	S	S
465	*Staphylococcus hominis*	DSR												
466	*Staphylococcus hominis*	DSR	S	S	R	S	S	S	R	S	S	S	S	S
467	*Staphylococcus hominis*	DSR	S	R	S	S	S	S	R	S	S	R	S	S
468	*Staphylococcus hominis*	DSS	S	R	S	R	S	S	R	S	R	R	R	R
469	*Staphylococcus hominis*	DSS	S	S	S	R	S	S	S	S	S	S	S	S
470	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	R	S	S
471	*Staphylococcus hominis*	DSS	S	R	S	I	S	S	R	S	S	S	S	S
472	*Staphylococcus hominis*	DSS	S	R	R	I	S	S	R	S	S	S	R	S
473	*Staphylococcus hominis*	DSS	S	R	S	I	S	S	R	S	S	R	S	S
474	*Staphylococcus hominis*	DSS	S	R	S	R	S	S	S	S	S	R	R	S
475	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	R	S	S
476	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	S	S	S	S	S	S
477	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	R	S	S	R	S	S
478	*Staphylococcus hominis*	DSS	S	S	S	I	S	S	R	S	S	R	S	S
479	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	R	S	S	R	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
480	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	R	S	S	R	S	S
481	*Staphylococcus hominis*	DSS	S	R	S	I	S	S	R	S	S	R	S	S
482	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	S	S	S	S	R	S
483	*Staphylococcus hominis*	DSS	S	R	S	R	R	R	R	S	S	R	R	R
484	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
485	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	S	R	S	R	S	S
486	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
487	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
488	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	R	S
489	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
490	*Staphylococcus hominis*	DSS	S	R	S	S	S	R	R	S	S	S	S	S
491	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	R	S	R	S	R
492	*Staphylococcus hominis*	DSS	S	R	S	S	I	S	R	S	S	R	S	S
493	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	R	S
494	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	S	S	S	S	S	R
495	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	S	S	S	R	R	S
496	*Staphylococcus hominis*	DSS	S	S	R	S	S	S	S	S	S	S	S	S
497	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	S	S	S	R	R	S
498	*Staphylococcus hominis*	DSS	R	R	S	S	S	S	R	S	S	R	S	S
499	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
500	*Staphylococcus hominis*	DSS												
501	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	S	S	S	S	S	S
502	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	S	S	S	S	S	S
503	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	S	S	S	R	S	S
504	*Staphylococcus hominis*	DSS	S	S	S	S	S	S	R	S	S	S	S	S
505	*Staphylococcus hominis*	DSS	S	R	S	S	S	S	R	S	S	S	S	S
506	*Staphylococcus hominis*	DSS	R	S	R	S	S	S	R	S	S	S	S	S
507	*Staphylococcus hominis*	DST	S	R	S	S	S	S	R	R	R	S	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
508	*Staphylococcus hominis*	DST	S	R	S	S	S	S	R	R	R	S	S	S
509	*Staphylococcus hominis*	DST	S	R	S	S	S	S	S	S	S	S	S	S
510	*Staphylococcus hominis*	DST	S	R	S	S	S	S	R	R	R	R	R	S
511	*Staphylococcus hominis*	DST	S	R	S	S	S	S	S	R	R	R	S	S
512	*Staphylococcus hominis*	DST	S	S	S	S	S	S	R	S	S	R	S	S
513	*Staphylococcus hominis*	DST	S	S	S	S	S	S	R	S	S	S	S	S
514	*Staphylococcus hominis*	DST	S	R	S	S	S	S	S	S	S	S	S	S
515	*Staphylococcus hominis*	DST	S	R	S	S	S	S	S	S	S	S	S	S
516	*Staphylococcus hominis*	DST	S	S	S	S	S	S	S	S	S	S	S	S
517	*Staphylococcus hominis*	HB	S	R	S	S	S	S	R	R	S	R	S	S
518	*Staphylococcus hominis*	HB	S	R	R	S	S	S	S	S	S	S	R	S
519	*Staphylococcus hominis*	HH												
520	*Staphylococcus hominis*	HH	S	S	S	S	S	R	R	S	S	S	S	S
521	*Staphylococcus hominis*	HH	S	S	R	S	S	S	S	S	S	S	S	S
522	*Staphylococcus hominis*	HH	S	S	S	S	I	S	S	R	S	S	S	S
523	*Staphylococcus hominis*	HH												
524	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	R	S	S	S	S
525	*Staphylococcus hominis*	HH	S	S	S	R	S	S	R	R	S	S	S	S
526	*Staphylococcus hominis*	HH	S	S	S	S	R	S	R	R	S	S	S	S
527	*Staphylococcus hominis*	HH	S	R	S	S	I	S	R	R	S	R	S	S
528	*Staphylococcus hominis*	HH	S	R	R	S	R	S	S	S	R	S	S	S
529	*Staphylococcus hominis*	HH	S	S	S	S	I	S	R	R	S	S	R	S
530	*Staphylococcus hominis*	HH	S	R	R	S	S	S	R	S	S	S	S	S
531	*Staphylococcus hominis*	HH	S	R	S	I	S	S	R	S	S	S	S	S
532	*Staphylococcus hominis*	HH	S	S	S	I	S	S	R	S	S	R	S	S
533	*Staphylococcus hominis*	HH	S	R	S	S	S	S	S	S	S	R	S	S
534	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
535	*Staphylococcus hominis*	HH	S	R	S	R	I	S	R	S	R	R	R	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
536	*Staphylococcus hominis*	HH	S	S	S	S	S	S	S	S	S	R	R	S
537	*Staphylococcus hominis*	HH	S	R	R	S	S	S	S	S	S	R	S	S
538	*Staphylococcus hominis*	HH	S	R	R	S	S	S	R	R	S	R	R	S
539	*Staphylococcus hominis*	HH	S	S	S	I	S	S	S	S	S	S	S	S
540	*Staphylococcus hominis*	HH	S	R	S	S	S	R	S	S	S	R	R	S
541	*Staphylococcus hominis*	HH	S	R	S	S	S	S	S	R	S	R	R	S
542	*Staphylococcus hominis*	HH	S	R	S	S	S	S	S	S	R	R	S	S
543	*Staphylococcus hominis*	HH	S	S	S	S	S	S	S	S	S	S	S	S
544	*Staphylococcus hominis*	HH	S	R	S	S	S	S	S	S	S	R	R	S
545	*Staphylococcus hominis*	HH	S	R	S	I	S	S	R	S	S	R	R	S
546	*Staphylococcus hominis*	HH	S	S	S	S	S	S	R	R	S	R	S	S
547	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	R	S	S
548	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
549	*Staphylococcus hominis*	HH	S	R	S	I	S	S	R	S	S	R	R	S
550	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
551	*Staphylococcus hominis*	HH	S	S	S	I	S	R	R	R	S	R	S	S
552	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	S	S	S
553	*Staphylococcus hominis*	HH												
554	*Staphylococcus hominis*	HH	S	R	S	I	S	S	R	S	S	S	S	S
555	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	R	R	S
556	*Staphylococcus hominis*	HH	S	R	S	I	S	S	R	S	S	S	S	S
557	*Staphylococcus hominis*	HH	S	R	R	S	S	S	R	R	S	R	S	S
558	*Staphylococcus hominis*	HH	S	S	S	S	S	S	R	S	S	R	S	S
559	*Staphylococcus hominis*	HH	S	S	S	S	S	S	R	S	S	S	S	S
560	*Staphylococcus hominis*	HH	S	R	R	S	S	S	R	S	R	S	R	S
561	*Staphylococcus hominis*	HH	S	R	S	S	S	S	R	S	S	R	R	S
562	*Staphylococcus hominis*	HH												
563	*Staphylococcus hominis*	HH												

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

	164/173(tested/total)												
ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
564	*Staphylococcus lugdunensis*	DSH	R	R	S	S	S	S	S	I	S	R	S	S
565	*Staphylococcus lugdunensis*	DSH	S	R	S	R	S	S	R	S	S	I	S	S
566	*Staphylococcus lugdunensis*	DSH	S	R	S	S	S	S	R	S	S	I	S	S
567	*Staphylococcus lugdunensis*	DSL	S	R	R	S	S	S	R	I	S	S	S	S
568	*Staphylococcus lugdunensis*	DSL	S	S	S	S	S	S	S	S	S	S	S	S
	5/5(tested/total)												
569	*Staphylococcus pasteuri*	DSH	S	S	S	S	S	S	S	S	S	I	S	S
570	*Staphylococcus pasteuri*	DSR	S	R	S	I	S	S	R	S	S	S	S	S
571	*Staphylococcus pasteuri*	DSR	S	R	S	I	S	S	R	S	S	I	S	S
572	*Staphylococcus pasteuri*	DSR	S	R	S	I	S	S	R	S	S	R	R	S
573	*Staphylococcus pasteuri*	DSR	S	R	S	I	S	S	R	S	R	R	R	S
574	*Staphylococcus pasteuri*	DSR	S	S	S	S	S	S	R	S	S	S	S	S
575	*Staphylococcus pasteuri*	DSR	S	R	S	I	S	S	R	S	R	R	S	S
576	*Staphylococcus pasteuri*	DSS	S	R	R	I	S	S	R	S	S	R	S	S
577	*Staphylococcus pasteuri*	DSS	S	R	S	R	R	S	R	S	R	R	S	S
578	*Staphylococcus pasteuri*	DSS	S	R	R	S	S	S	S	S	S	R	S	S
579	*Staphylococcus pasteuri*	DST	S	R	S	S	S	R	R	R	S	S	S	S
580	*Staphylococcus pasteuri*	DST	S	R	S	S	S	S	R	S	R	R	S	S
581	*Staphylococcus pasteuri*	DST	S	R	S	R	R	S	R	S	R	R	S	S
582	*Staphylococcus pasteuri*	DST	S	R	S	S	S	S	R	S	S	S	S	S
583	*Staphylococcus pasteuri*	DST	S	R	S	S	S	S	R	S	S	R	S	S
584	*Staphylococcus pasteuri*	DST	S	S	S	S	S	S	S	S	S	S	R	S
585	*Staphylococcus pasteuri*	DST	S	S	S	S	S	S	S	S	S	S	S	S
586	*Staphylococcus pasteuri*	DST	S	R	S	S	S	S	S	S	R	S	S	S
587	*Staphylococcus pasteuri*	DST	S	R	R	S	S	S	S	S	R	S	S	S

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
588	*Staphylococcus pasteuri*	DST	S	R	R	S	S	S	S	S	R	S	S	S
589	*Staphylococcus pasteuri*	HB	S	R	S	S	S	S	R	R	S	R	S	S
590	*Staphylococcus pasteuri*	HH	S	S	S	S	I	S	S	R	S	S	S	R
591	*Staphylococcus pasteuri*	HH												
592	*Staphylococcus pasteuri*	HH	S	S	S	R	S	S	R	R	S	S	S	S
593	*Staphylococcus pasteuri*	HH	R	R	S	S	S	R	R	R	R	S	S	S
594	*Staphylococcus pasteuri*	HH												
595	*Staphylococcus pasteuri*	HH	S	S	S	S	S	R	R	R	S	S	S	S
596	*Staphylococcus pasteuri*	HH	S	R	S	S	I	S	R	R	R	R	R	S
597	*Staphylococcus pasteuri*	HH	R	R	S	R	I	S	S	R	S	S	S	S
598	*Staphylococcus pasteuri*	HH	S	R	R	S	S	S	S	S	S	R	R	S
599	*Staphylococcus pasteuri*	HH	S	R	S	S	S	S	R	S	S	R	S	S
600	*Staphylococcus pasteuri*	HH	S	S	S	S	S	S	R	S	S	R	S	S
601	*Staphylococcus pasteuri*	HH	S	S	S	I	S	S	R	S	S	S	S	S
602	*Staphylococcus pasteuri*	HH	S	S	S	I	S	S	R	S	S	S	S	S
	32/34(tested/total)													
603	*Staphylococcus pettenkoferi*	DSH	R	R	R	S	R	R	R	R	R	R	R	R
604	*Staphylococcus pettenkoferi*	DSH	S	R	S	S	S	S	S	S	S	I	S	S
605	*Staphylococcus pettenkoferi*	DSH	S	S	S	S	S	S	R	R	S	S	S	S
606	*Staphylococcus pettenkoferi*	DST	S	R	S	S	S	S	S	S	R	S	S	S
607	*Staphylococcus pettenkoferi*	HH	S	S	S	S	I	S	R	R	S	S	S	S
	5/5(tested/total)													
608	*Staphylococcus saprophyticus*	BCF	S	R	S	S	S	R	R	S	R	S	S	S
609	*Staphylococcus saprophyticus*	BCF	S	R	S	S	S	R	R	S	R	R	S	S
610	*Staphylococcus saprophyticus*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
611	*Staphylococcus saprophyticus*	BCF	S	R	S	R	S	S	R	S	S	R	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
612	*Staphylococcus saprophyticus*	BCF	R	R	S	R	S	S	R	S	S	R	S	S
613	*Staphylococcus saprophyticus*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
614	*Staphylococcus saprophyticus*	BCF	S	R	R	R	S	S	R	S	R	R	S	S
615	*Staphylococcus saprophyticus*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
616	*Staphylococcus saprophyticus*	BCF	R	R	R	R	S	S	R	I	R	R	R	S
617	*Staphylococcus saprophyticus*	DSH	S	R	R	S	I	S	R	R	S	R	S	S
618	*Staphylococcus saprophyticus*	DSL	S	R	S	S	S	S	R	R	S	S	S	S
619	*Staphylococcus saprophyticus*	DSL	S	R	S	S	S	S	R	S	S	S	S	S
620	*Staphylococcus saprophyticus*	DSS	S	R	R	I	S	S	R	S	S	R	S	S
621	*Staphylococcus saprophyticus*	DSS	S	R	S	R	R	S	R	S	R	S	R	S
622	*Staphylococcus saprophyticus*	DSS	S	R	S	S	S	S	R	S	S	S	S	R
623	*Staphylococcus saprophyticus*	DSS	S	R	S	S	S	S	R	S	S	S	S	R
624	*Staphylococcus saprophyticus*	HH	S	S	S	S	S	S	R	S	S	S	S	S
625	*Staphylococcus saprophyticus*	HH	S	S	S	S	S	S	R	S	S	S	S	S
626	*Staphylococcus saprophyticus*	HH	S	R	S	S	S	S	R	S	S	S	S	S
627	*Staphylococcus saprophyticus*	HH	R	R	S	I	S	S	R	S	S	S	R	S
	20/20(tested/total)													
628	*Staphylococcus sciuri*	DSH	R	R	R	S	R	S	R	I	S	S	S	R
629	*Staphylococcus sciuri*	DSH	S	S	S	R	S	S	S	S	S	S	S	S
630	*Staphylococcus sciuri*	DSH	R	R	R	R	I	S	R	R	S	S	S	S
631	*Staphylococcus sciuri*	DSH	S	S	S	R	I	S	R	R	S	S	S	S
632	*Staphylococcus sciuri*	DSH	R	R	S	S	I	S	R	R	R	S	S	S
633	*Staphylococcus sciuri*	DSH	R	R	S	R	I	R	R	R	R	S	S	S
	6/6(tested/total)													
634	*Staphylococcus simiae*	DST	S	S	S	S	S	S	R	S	S	S	S	S
635	*Staphylococcus simiae*	DST	S	3	R	S	S	S	S	S	S	S	S	S

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
636	*Staphylococcus simiae*	DST	S	S	R	S	S	S	R	S	S	S	S	S
637	*Staphylococcus simiae*	DST	S	S	S	I	S	S	S	S	S	S	S	S
638	*Staphylococcus simiae*	DST	S	S	S	S	S	S	R	S	S	S	S	S
639	*Staphylococcus simiae*	DST	S	S	S	S	S	S	R	S	S	S	S	S
640	*Staphylococcus simiae*	DST	S	S	S	S	S	S	S	S	S	S	S	S
641	*Staphylococcus simiae*	DST	S	S	S	S	S	S	S	S	S	S	S	S
642	*Staphylococcus simiae*	DST	S	S	S	S	S	S	S	S	S	S	S	S
643	*Staphylococcus simiae*	DST	S	R	R	S	S	S	S	S	S	S	S	S
	10/10(tested/total)													
644	*Staphylococcus simulans*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
	1/1(tested/total)													
645	*Staphylococcus warneri*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
646	*Staphylococcus warneri*	BCF	S	S	S	R	S	R	R	S	S	S	S	S
647	*Staphylococcus warneri*	BCF	S	R	S	S	S	R	R	S	R	S	S	S
648	*Staphylococcus warneri*	BCF	S	R	S	S	S	R	R	S	R	S	S	S
649	*Staphylococcus warneri*	BCF	S	S	S	I	S	S	R	S	S	R	S	S
650	*Staphylococcus warneri*	BCF	S	S	S	S	S	S	R	S	S	S	S	S
651	*Staphylococcus warneri*	BCF	S	S	S	S	S	S	R	S	S	S	S	S
652	*Staphylococcus warneri*	BCF	S	R	S	S	S	S	R	S	S	S	S	S
653	*Staphylococcus warneri*	DSH	S	R	S	S	S	S	S	I	S	R	S	S
654	*Staphylococcus warneri*	DSH	S	R	R	S	S	S	R	I	S	S	S	S
655	*Staphylococcus warneri*	DSH	S	S	S	R	S	R	R	R	S	I	S	S
656	*Staphylococcus warneri*	DSH	S	R	S	S	S	S	R	R	R	I	R	S
657	*Staphylococcus warneri*	DSH	S	R	R	S	S	S	R	S	S	I	S	S
658	*Staphylococcus warneri*	DSH	S	S	S	R	S	S	S	S	S	S	S	S
659	*Staphylococcus warneri*	DSH	R	R	R	R	S	S	R	R	R	S	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
660	*Staphylococcus warneri*	DSH	S	R	R	S	I	R	R	R	R	S	S	S
661	*Staphylococcus warneri*	DSH	S	R	S	S	R	S	R	R	R	R	S	S
662	*Staphylococcus warneri*	DSH	S	R	S	S	S	R	R	R	R	S	R	S
663	*Staphylococcus warneri*	DSH	S	R	S	S	I	S	S	S	R	S	R	S
664	*Staphylococcus warneri*	DSH	S	S	S	S	S	S	S	R	R	S	S	S
665	*Staphylococcus warneri*	DSH	S	S	S	S	I	R	R	R	S	R	S	S
666	*Staphylococcus warneri*	DSH	S	S	S	R	S	S	S	S	S	S	S	S
667	*Staphylococcus warneri*	DSH	S	S	R	S	S	S	S	S	S	S	S	S
668	*Staphylococcus warneri*	DSH	S	R	R	S	S	S	S	S	R	S	S	S
669	*Staphylococcus warneri*	DSH	S	S	R	S	S	S	S	S	S	S	S	S
670	*Staphylococcus warneri*	DSL	S	S	R	S	S	S	S	R	S	S	S	S
671	*Staphylococcus warneri*	DSL	S	S	S	S	S	S	S	R	S	S	S	S
672	*Staphylococcus warneri*	DSL	S	S	R	S	S	S	R	R	S	R	R	S
673	*Staphylococcus warneri*	DSL	S	S	R	S	S	S	R	R	R	S	S	S
674	*Staphylococcus warneri*	DSL	S	S	R	S	S	S	S	R	S	S	S	S
675	*Staphylococcus warneri*	DSL	S	R	R	S	S	S	R	R	R	S	S	S
676	*Staphylococcus warneri*	DSR	S	R	R	I	S	S	S	S	S	S	S	S
677	*Staphylococcus warneri*	DSR	S	R	R	S	S	R	S	S	S	R	S	S
678	*Staphylococcus warneri*	DSR	S	S	S	S	S	R	S	S	S	R	S	S
679	*Staphylococcus warneri*	DSR	S	R	S	S	S	R	R	S	S	R	R	S
680	*Staphylococcus warneri*	DSR	S	S	R	S	S	S	R	S	S	S	S	S
681	*Staphylococcus warneri*	DSR	S	R	S	S	S	S	S	S	S	S	S	S
682	*Staphylococcus warneri*	DSS	S	S	S	R	S	S	S	S	S	S	S	S
683	*Staphylococcus warneri*	DSS	S	R	R	R	S	S	R	S	S	S	S	S
684	*Staphylococcus warneri*	DST	S	R	R	R	S	R	S	R	R	I	S	S
685	*Staphylococcus warneri*	DST	S	R	R	R	S	R	S	R	R	S	S	S
686	*Staphylococcus warneri*	DST	S	R	R	R	S	R	S	R	R	S	S	S
687	*Staphylococcus warneri*	DST	S	S	S	S	S	R	R	S	S	S	S	S

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
688	*Staphylococcus warneri*	DST	S	R	S	S	S	S	R	S	R	R	S	S
689	*Staphylococcus warneri*	DST	S	R	S	S	S	S	R	S	R	S	S	S
690	*Staphylococcus warneri*	DST	S	R	S	S	S	S	R	S	R	S	S	S
691	*Staphylococcus warneri*	DST	S	S	S	S	S	S	R	S	R	S	S	S
692	*Staphylococcus warneri*	DST	S	S	S	S	S	S	S	S	S	S	S	S
693	*Staphylococcus warneri*	DST	S	S	S	S	S	S	R	S	S	S	S	S
694	*Staphylococcus warneri*	HB	S	R	S	S	S	S	S	S	S	S	R	S
695	*Staphylococcus warneri*	HB	S	S	S	S	I	S	R	R	S	R	S	R
696	*Staphylococcus warneri*	HH	S	S	S	R	S	R	S	R	S	R	S	S
697	*Staphylococcus warneri*	HH	S	S	S	S	I	S	R	R	S	S	S	S
698	*Staphylococcus warneri*	HH	S	R	S	S	S	S	R	R	R	R	S	S
699	*Staphylococcus warneri*	HH	S	R	S	S	S	S	R	R	R	R	S	S
700	*Staphylococcus warneri*	HH	S	R	R	S	I	R	R	R	S	R	R	S
701	*Staphylococcus warneri*	HH	S	S	S	R	S	S	R	R	S	I	S	S
702	*Staphylococcus warneri*	HH	S	R	S	S	S	R	R	R	R	S	R	S
703	*Staphylococcus warneri*	HH	S	S	R	S	R	R	R	R	R	R	R	S
704	*Staphylococcus warneri*	HH	R	R	S	S	I	S	R	R	R	R	S	S
705	*Staphylococcus warneri*	HH	S	R	R	S	R	S	R	R	R	R	S	S
706	*Staphylococcus warneri*	HH	S	S	S	S	R	S	R	R	S	S	S	S
707	*Staphylococcus warneri*	HH	S	R	S	S	S	S	R	S	S	S	S	S
708	*Staphylococcus warneri*	HH	S	S	S	S	R	S	R	R	R	R	R	S
709	*Staphylococcus warneri*	HH	S	R	S	S	S	S	S	R	S	S	S	S
710	*Staphylococcus warneri*	HH	S	R	S	S	R	S	R	R	R	R	S	S
711	*Staphylococcus warneri*	HH	S	R	S	S	S	S	R	S	S	S	S	S
712	*Staphylococcus warneri*	HH	S	R	S	S	S	S	S	S	S	S	R	S
	68/68(tested/total)													

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
713	*Staphylococcus xylosus*	HH	S	R	R	R	I	R	R	R	S	S	R	S
714	*Staphylococcus xylosus*	HH	S	R	S	S	R	S	R	R	S	S	S	S
	2/2(tested/total)													

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

BCF- baby care facility; DSH- different sites of hotels; DSL- different sites of a library; DSR- different sites of restaurants; DSS- different sites of supermarkets; DST- different sites of transportation facilities; HAS- hotel air samples; HB- handbags; HH- human hands.

II.2 Antibiotic susceptibility variation of closely related staphylococci

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
455	*Staphylococcus hominis*	1	Restaurants	S	R	S	S	S	S	R	S	S	S	S	S
554	*Staphylococcus hominis*		Hands				I								
471	*Staphylococcus hominis*	2	Supermarkets	S	R	S	I	S	S	R	S	S	S	S	S
440	*Staphylococcus hominis*		Restaurants				S								
458	*Staphylococcus hominis*	3	Restaurants	S	R	S	I	S	S	S	S	S	R	S	S
549	*Staphylococcus hominis*		Hands							R				R	
493	*Staphylococcus hominis*	4	Supermarkets	S	R	S	S	S	S	R	S	S	S	R	S
544	*Staphylococcus hominis*		Hands							S			R		
503	*Staphylococcus hominis*	5	Supermarkets	S	S	S	S	S	S	S	S	S	R	S	S
521	*Staphylococcus hominis*		Hands			R							S		
543	*Staphylococcus hominis*	6	Hands	S	S	S	S	S	S	S	S	S	S	S	S
403	*Staphylococcus hominis*		Hotels		R					R		R			
430	*Staphylococcus hominis*	7	Hotels	S	R	S	R	S	S	R	R	S	S	S	S
397	*Staphylococcus hominis*		Baby care facilities				S				S	R			
488	*Staphylococcus hominis*	8	Supermarkets	S	R	S	S	S	S	R	S	S	S	R	S
450	*Staphylococcus hominis*		Restaurants			R	I							S	
556	*Staphylococcus hominis*	9	Hands	S	R	S	I	S	S	R	S	S	S	S	S
395	*Staphylococcus hominis*		Baby care facilities				S					R	R		

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
517	*Staphylococcus hominis*	10	Handbages	S	R	S	S	S	S	R	R	S	R	S	S
394	*Staphylococcus hominis*		Baby care facilities		S						S		S		
442	*Staphylococcus hominis*	11	Restaurants	S	R	S	I	S	S	R	S	S	R	R	S
536	*Staphylococcus hominis*		Hands		S		S			S					
481	*Staphylococcus hominis*	12	Supermarkets	S	R	S	I	S	S	R	S	S	R	S	S
546	*Staphylococcus hominis*		Hands		S		S				R				
463	*Staphylococcus hominis*	13	Restaurants	S	S	S	S	S	S	S	S	S	S	S	S
526	*Staphylococcus hominis*		Hands					R		R	R				
482	*Staphylococcus hominis*	14	Supermarkets	S	R	S	S	S	S	S	S	S	S	R	S
445	*Staphylococcus hominis*		Restaurants		S		I							S	
508	*Staphylococcus hominis*	15	Supermarkets	S	R	S	S	S	S	R	R	R	S	S	S
502	*Staphylococcus hominis*		Hotels							S	S	S			
531	*Staphylococcus hominis*	16	Hands	S	R	S	I	S	S	R	S	S	S	S	S
425	*Staphylococcus hominis*		Hotels		S		R			S	R				
485	*Staphylococcus hominis*	17	Supermarkets	S	R	S	S	S	S	S	R	S	R	S	S
539	*Staphylococcus hominis*		Hands		S		I				S		S		
433	*Staphylococcus hominis*	18	Library	S	R	S	S	S	S	S	R	S	S	S	S
396	*Staphylococcus hominis*		Baby care facilities		S		R			R	S				

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
476	*Staphylococcus hominis*	19	Supermarkets	S	R	S	S	S	S	S	S	S	S	S	S
454	*Staphylococcus hominis*		Restaurants		S	R							R	R	
510	*Staphylococcus hominis*	20	Supermarkets	S	R	S	S	S	S	R	R	R	R	R	S
447	*Staphylococcus hominis*		Restaurants			R	I				S		S		
438	*Staphylococcus hominis*	21	Restaurants	S	R	S	R	S	S	R	S	S	S	R	S
401	*Staphylococcus hominis*		Hotels					R			I	R	R	S	
548	*Staphylococcus hominis*	22	Hands	S	R	S	S	S	S	R	S	S	S	S	S
400	*Staphylococcus hominis*		Hotels			R	R					R	R	R	
451	*Staphylococcus hominis*	23	Restaurants	S	S	S	S	S	S	S	S	S	R	S	S
399	*Staphylococcus hominis*		Hotels	R	R	R				R			S		R
483	*Staphylococcus hominis*	24	Supermarkets	S	R	S	R	R	R	R	S	S	R	R	R
534	*Staphylococcus hominis*		Hands				S	S	S				S	S	S
478	*Staphylococcus hominis*	25	Supermarkets	S	S	S	I	S	S	R	S	S	R	S	S
448	*Staphylococcus hominis*		Restaurants		R	R	S		R		R		S		
479	*Staphylococcus hominis*	26	Supermarkets	S	S	S	S	S	S	R	S	S	R	S	S
480	*Staphylococcus hominis*		Supermarkets												
499	*Staphylococcus hominis*	27	Supermarkets	S	R	S	S	S	S	R	S	S	S	S	S
505	*Staphylococcus hominis*		Supermarkets												

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
437	*Staphylococcus hominis*	28	Restaurants	S	R	S	I	S	S	R	S	S	R	S	S
449	*Staphylococcus hominis*		Restaurants												
459	*Staphylococcus hominis*	29	Restaurants	S	R	S	S	S	S	R	S	S	S	S	S
441	*Staphylococcus hominis*		Restaurants							S					
460	*Staphylococcus hominis*	30	Restaurants	S	R	S	S	S	S	R	S	S	S	S	S
462	*Staphylococcus hominis*		Restaurants		S										
464	*Staphylococcus hominis*	31	Restaurants	S	S	S	I	S	S	R	S	S	S	S	S
452	*Staphylococcus hominis*		Restaurants						R				R		
541	*Staphylococcus hominis*	32	Hands	S	R	S	S	S	S	S	R	S	R	R	S
540	*Staphylococcus hominis*		Hands						R		S				
393	*Staphylococcus hominis*	33	Baby care facilities	S	R	S	S	S	S	R	S	R	S	S	S
398	*Staphylococcus hominis*		Baby care facilities					R					R	R	
497	*Staphylococcus hominis*	34	Supermarkets	S	R	S	S	S	S	S	S	S	R	R	S
469	*Staphylococcus hominis*		Supermarkets		S		R						S	S	
538	*Staphylococcus hominis*	35	Hands	S	R	R	S	S	S	R	R	S	R	R	S
533	*Staphylococcus hominis*		Hands			S				S	S			S	
180	*Staphylococcus epidermidis*	1	Supermarkets	S	R	R	I	S	S	R	S	S	S	S	S
275	*Staphylococcus epidermidis*		Hands												

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
232	*Staphylococcus epidermidis*	2	Transportation facilities	S	R	S	I	S	S	S	S	S	R	S	S
115	*Staphylococcus epidermidis*		Baby care facilities				S						S		
175	*Staphylococcus epidermidis*	3	Supermarkets	S	R	S	I	S	S	S	S	S	R	S	S
164	*Staphylococcus epidermidis*		Restaurants										S		
194	*Staphylococcus epidermidis*	4	Supermarkets	S	S	S	S	S	S	S	S	S	S	S	S
293	*Staphylococcus epidermidis*		Hands		R										
186	*Staphylococcus epidermidis*	5	Supermarkets	S	R	S	S	S	S	S	S	R	S	S	S
160	*Staphylococcus epidermidis*		Restaurants							R		S			
150	*Staphylococcus epidermidis*	6	Restaurants	S	R	R	S	S	R	S	S	S	R	S	S
295	*Staphylococcus epidermidis*		Hands						S				S		
192	*Staphylococcus epidermidis*	7	Supermarkets	S	R	R	S	S	S	R	S	S	S	S	S
209	*Staphylococcus epidermidis*		Transportation facilities		S	S									
146	*Staphylococcus epidermidis*	8	Restaurants	S	R	S	S	S	S	R	S	S	R	S	S
223	*Staphylococcus epidermidis*		Transportation facilities		S	R				S					
196	*Staphylococcus epidermidis*	9	Supermarkets	S	R	S	S	S	S	R	S	S	S	S	S
136	*Staphylococcus epidermidis*		Library							S	R		R		
144	*Staphylococcus epidermidis*	10	Restaurants	S	S	S	S	S	S	R	S	S	S	S	S
306	*Staphylococcus epidermidis*		Hands		R		R						R		

Note: A: amoxicillin (10 μg); CEF: cefepime (30 μg); C: chloramphenicol (30 μg); E: erythromycin (5 μg); FC: fusidic acid (10 μg); GM: gentamicin (10 μg); MUP: mupirocin (20 μg); OX: Oxacillin (1 μg); PG: penicillin G (1 unit); S: streptomycin (10 μg); T: tetracycline (10 μg); VAN: vancomycin (5 μg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
176	*Staphylococcus epidermidis*	11	Supermarkets	S	S	S	S	S	S	R	S	S	I	S	S
117	*Staphylococcus epidermidis*		Baby care facilities		R							R		R	
203	*Staphylococcus epidermidis*	12	Transportation facilities	S	S	S	S	S	S	R	S	S	R	S	S
155	*Staphylococcus epidermidis*		Restaurants		R					S			S		
280	*Staphylococcus epidermidis*	13	Hands	S	R	R	S	S	S	R	S	S	R	S	S
111	*Staphylococcus epidermidis*		Baby care facilities			S				S			S		
216	*Staphylococcus epidermidis*	14	Transportation facilities	S	R	S	S	S	S	R	S	R	R	S	S
274	*Staphylococcus epidermidis*		Hands			R						S		R	
189	*Staphylococcus epidermidis*	15	Supermarkets	S	R	R	R	R	R	R	S	R	S	S	S
270	*Staphylococcus epidermidis*		Hands				S	S	S				R		
247	*Staphylococcus epidermidis*	16	Hands	S	R	S	S	S	S	S	R	S	S	S	S
233	*Staphylococcus epidermidis*		Handbags					I			S		R	R	
197	*Staphylococcus epidermidis*	17	Supermarkets	S	R	R	S	S	S	R	S	S	S	S	S
168	*Staphylococcus epidermidis*		Restaurants			S	R			S			R		
230	*Staphylococcus epidermidis*	18	Transportation facilities	S	R	R	S	S	S	R	S	R	I	S	S
114	*Staphylococcus epidermidis*		Baby care facilities		S	S						S	R		
228	*Staphylococcus epidermidis*	19	Transportation facilities	S	S	S	R	S	S	S	S	S	S	S	S
272	*Staphylococcus epidermidis*		Hands		R		I			R			R		

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
162	*Staphylococcus epidermidis*	20	Restaurants	S	R	S	S	S	S	S	S	S	S	S	S
290	*Staphylococcus epidermidis*		Hands			R	I	R		R					R
163	*Staphylococcus epidermidis*	21	Restaurants	S	R	S	S	S	S	S	S	S	S	R	S
236	*Staphylococcus epidermidis*		Handbags		S	R		R	R	R	R				
191	*Staphylococcus epidermidis*	22	Supermarkets	R	R	S	S	S	S	R	S	R	S	S	S
128	*Staphylococcus epidermidis*		Hotels	S		R		R	R	S	R				R
134	*Staphylococcus epidermidis*	23	Library	R	R	S	S	S	S	R	R	R	R	R	S
135	*Staphylococcus epidermidis*		Library												
215	*Staphylococcus epidermidis*	24	Transportation facilities	S	S	S	S	S	S	R	S	S	S	S	S
219	*Staphylococcus epidermidis*		Transportation facilities												
207	*Staphylococcus epidermidis*	25	Transportation facilities	S	S	S	S	S	S	R	S	S	S	S	S
213	*Staphylococcus epidermidis*		Transportation facilities			R									
208	*Staphylococcus epidermidis*	26	Transportation facilities	S	S	S	S	S	S	S	S	S	R	S	S
212	*Staphylococcus epidermidis*		Transportation facilities							R					
171	*Staphylococcus epidermidis*	27	Restaurants	S	R	S	S	S	S	S	S	S	S	S	S
151	*Staphylococcus epidermidis*		Restaurants							R					
304	*Staphylococcus epidermidis*	28	Hands	S	R	S	S	S	S	R	S	S	S	S	S
289	*Staphylococcus epidermidis*		Hands										R		

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
263	*Staphylococcus epidermidis*	29	Hands	S	R	S	S	S	S	R	R	R	S	R	S
251	*Staphylococcus epidermidis*		Hands										R	S	
227	*Staphylococcus epidermidis*	30	Transportation facilities	S	S	S	S	S	S	R	S	S	S	S	S
225	*Staphylococcus epidermidis*		Transportation facilities							S			R		
291	*Staphylococcus epidermidis*	31	Hands	S	R	S	S	S	S	R	R	S	S	S	S
292	*Staphylococcus epidermidis*		Hands			R	R				S				
249	*Staphylococcus epidermidis*	32	Hands	S	R	R	S	S	S	R	R	S	S	R	S
253	*Staphylococcus epidermidis*		Hands		S	S	R	I	R					S	
258	*Staphylococcus epidermidis*	33	Hands	S	S	R	S	S	S	R	R	S	R	S	S
248	*Staphylococcus epidermidis*		Hands		R	S	R	I		S	S		S		
156	*Staphylococcus epidermidis*	34	Restaurants	S	R	S	S	R	R	S	S	S	S	S	S
139	*Staphylococcus epidermidis*		Restaurants	R		R	R		S	R		R	R	R	
389	*Staphylococcus haemolyticus*	1	Hands	S	R	S	I	S	S	R	S	S	S	S	S
345	*Staphylococcus haemolyticus*		Hotels		S		S	R					I		
332	*Staphylococcus haemolyticus*	2	Hotels	S	S	S	S	R	S	S	S	R	I	R	S
312	*Staphylococcus haemolyticus*		Baby care facilities					S				S	S	S	
362	*Staphylococcus haemolyticus*	3	Library	R	R	S	S	I	S	S	R	R	S	S	S
337	*Staphylococcus haemolyticus*		Hotels	S				R		R		S	I	R	

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
371	*Staphylococcus haemolyticus*	4	Restaurants	S	S	S	S	S	S	S	S	S	S	S	S
390	*Staphylococcus haemolyticus*		Hands			R	I		R	R	R		R	R	
382	*Staphylococcus haemolyticus*	5	Hands	S	R	S	S	I	S	R	R	S	R	S	S
320	*Staphylococcus haemolyticus*		Hotels	R		R	R	R			I		S	R	R
326	*Staphylococcus haemolyticus*	6	Hotels	S	S	S	S	S	S	S	S	R	I	S	S
379	*Staphylococcus haemolyticus*		Hotel air samples	R	R			R		R	R	S	R	R	
364	*Staphylococcus haemolyticus*	7	Library	S	S	R	S	I	S	R	R	S	S	S	S
366	*Staphylococcus haemolyticus*		Library							S					
361	*Staphylococcus haemolyticus*	8	Library	S	S	S	S	I	S	R	R	S	S	R	S
363	*Staphylococcus haemolyticus*		Library			R				S				S	
368	*Staphylococcus haemolyticus*	9	Library	S	S	S	S	I	S	S	R	S	R	R	S
365	*Staphylococcus haemolyticus*		Library		R	R				R			S	S	
318	*Staphylococcus haemolyticus*	10	Hotels	R	R	S	S	R	R	R	R	R	R	S	S
347	*Staphylococcus haemolyticus*		Hotels	S			R		S			S		R	R
339	*Staphylococcus haemolyticus*	11	Hotels	S	R	R	S	S	S	R	R	R	I	S	S
340	*Staphylococcus haemolyticus*		Hotels		S	S				S	S	S		R	
322	*Staphylococcus haemolyticus*	12	Hotels	S	R	S	S	S	S	S	I	R	S	R	S
317	*Staphylococcus haemolyticus*		Hotels	R		R		R	R	R	R				R

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
39	*Staphylococcus capitis*	1	Restaurants	S	R	S	S	S	S	R	S	S	S	S	S
61	*Staphylococcus capitis*		Transportation facilities												
30	*Staphylococcus capitis*	2	Library	S	S	S	S	S	S	R	R	S	S	R	S
16	*Staphylococcus capitis*		Baby care facilities				R				S			S	
18	*Staphylococcus capitis*	3	Baby care facilities	S	R	S	S	S	S	R	S	R	S	S	S
19	*Staphylococcus capitis*		Hotels				R			S		S			
37	*Staphylococcus capitis*	4	Restaurants	S	S	S	S	S	S	R	S	S	S	S	S
88	*Staphylococcus capitis*		Hands			R				S			R		
48	*Staphylococcus capitis*	5	Supermarkets	S	R	S	S	S	S	R	S	S	S	R	S
92	*Staphylococcus capitis*		Hands			R	I			S				S	
54	*Staphylococcus capitis*	6	Transportation facilities	S	R	S	S	S	S	R	S	S	S	S	S
26	*Staphylococcus capitis*		Hotels		S	R		I		S					
60	*Staphylococcus capitis*	7	Transportation facilities	S	S	S	S	S	S	R	S	S	S	S	S
87	*Staphylococcus capitis*		Hands		R		I					R		R	
59	*Staphylococcus capitis*	8	Transportation facilities	S	S	S	R	S	S	R	S	S	S	S	S
69	*Staphylococcus capitis*		Hands		R		S				R	R		R	
75	*Staphylococcus capitis*	9	Hands	R	R	S	S	S	S	R	R	S	S	S	S
27	*Staphylococcus capitis*		Hotels	S	S					S			R		R

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
45	*Staphylococcus capitis*	10	Supermarkets	S	R	S	S	S	S	R	S	S	S	R	S
76	*Staphylococcus capitis*		Hands		S			I		S	R			S	
93	*Staphylococcus capitis*	11	Hands	S	R	R	R	R	S	R	S	R	S	S	R
28	*Staphylococcus capitis*		Hotels			S	S	S		S	R	S			S
90	*Staphylococcus capitis*	12	Hands	S	R	S	S	S	S	R	S	S	S	S	S
84	*Staphylococcus capitis*		Hands		S						R				
56	*Staphylococcus capitis*	13	Transportation facilities	S	S	S	S	S	S	S	S	S	S	S	S
58	*Staphylococcus capitis*		Transportation facilities				I			R					
71	*Staphylococcus capitis*	14	Hands	R	S	S	S	S	S	S	R	S	S	S	S
80	*Staphylococcus capitis*		Hands	S							S	R			
21	*Staphylococcus capitis*	15	Hotels	S	S	R	R	R	R	R	R	R	R	S	R
22	*Staphylococcus capitis*		Hotels		R	S	S	S	S	S		S	S		S
681	*Staphylococcus warneri*	1	Restaurants	S	R	S	S	S	S	S	S	S	S	S	S
712	*Staphylococcus warneri*		Hands											R	
711	*Staphylococcus warneri*	2	Hands	S	R	S	S	S	S	R	S	S	S	S	S
653	*Staphylococcus warneri*		Hotels							S	I		R		
690	*Staphylococcus warneri*	3	Transportation facilities	S	R	S	S	S	S	R	S	R	S	S	S
663	*Staphylococcus warneri*		Hotels					I		S				R	

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).

ID	Species	Cluster	Sites	OX	PG	VAN	MUP	CEF	GM	FC	S	A	E	T	C
671	*Staphylococcus warneri*	4	Library	S	S	S	S	S	S	S	R	S	S	S	S
649	*Staphylococcus warneri*		Baby care facilities				I			R	S		R		
684	*Staphylococcus warneri*	5	Transportation facilities	S	R	R	R	S	R	S	R	R	I	S	S
661	*Staphylococcus warneri*		Hotels			S	S	R	S	R			R		
679	*Staphylococcus warneri*	6	Restaurants	S	R	S	S	S	R	R	S	S	R	R	S
677	*Staphylococcus warneri*		Restaurants			R				S				S	
706	*Staphylococcus warneri*	7	Hands	S	S	S	S	R	S	R	R	S	S	S	S
708	*Staphylococcus warneri*		Hands									R	R	R	
688	*Staphylococcus warneri*	8	Transportation facilities	S	R	S	S	S	S	R	S	R	R	S	S
693	*Staphylococcus warneri*		Transportation facilities		S							S	S		
672	*Staphylococcus warneri*	9	Library	S	S	R	S	S	S	R	R	S	R	R	S
674	*Staphylococcus warneri*		Library							S			S	S	
685	*Staphylococcus warneri*	10	Transportation facilities	S	R	R	R	S	R	S	R	R	S	S	S
691	*Staphylococcus warneri*		Transportation facilities		S	S	S		S	R	S				

Note: A: amoxicillin (10 µg); CEF: cefepime (30 µg); C: chloramphenicol (30 µg); E: erythromycin (5 µg); FC: fusidic acid (10 µg); GM: gentamicin (10 µg); MUP: mupirocin (20 µg); OX: Oxacillin (1 µg); PG: penicillin G (1 unit); S: streptomycin (10 µg); T: tetracycline (10 µg); VAN: vancomycin (5 µg).