Molecular studies of *Staphylococcus epidermidis*

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DECLARATION

This is to certify that:

i. This thesis comprises my original work towards the PhD except where indicated in the preface;

ii. Due acknowledgement has been made in the text to all other materials used;

iii. This thesis is fewer that 100,000 words in length, exclusive of tables, bibliographies and appendices.

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ABSTRACT

Staphylococcus epidermidis is a conspicuous member of the human microbiome, widely present on human skin. The shift in modern medicine towards invasive procedures has favoured its emergence as a significant nosocomial pathogen, especially in the setting of prosthetic devices. Despite being the most genetically diverse of the staphylococcal species, with 989 *S. epidermidis* multilocus sequence types characterised thus far, a single lineage, ST2, accounts for most clinical disease. Unfortunately, existing typing schemes lack the sensitivity to offer any epidemiological insights beyond this. Furthermore, the absence of dedicated molecular tools coupled with the genetically intractable nature of *S. epidermidis* has hindered the study of its molecular genetics, pathogenesis and treatment.

This thesis utilised new genome sequencing technologies to provide a modern perspective on the global molecular epidemiology of *S. epidermidis* with a focus on antimicrobial resistance. The project began with the functional analysis of the first complete genome of a multidrug resistant ST2 *S. epidermidis*. Then extended globally, to uncover the previously unrecognised international spread of three multidrug-resistant, hospital-adapted lineages of *S. epidermidis* (two ST2 and one ST23) that emerged in recent decades. Acquisition of a dual D471E plus I527M RpoB substitution was common to all three lineages and demonstrated to have become fixed in the populations. Analysis of isolates from 96 institutions in 24 countries identified the RpoB D471E plus I527M combination as the most common cause of rifampicin resistance in *S. epidermidis*, accounting for 86.6% of mutations. Furthermore, these substitutions were found to occur almost exclusively in isolates from the ST2 and ST23 lineages. By breaching lineage-specific DNA methylation restriction modification (RM) barriers, then performing site-specific mutagenesis, these *rpoB* mutations were shown to confer both rifampicin

resistance and reduced susceptibility to the glycopeptide antibiotics vancomycin and teicoplanin. Importantly, these findings suggest that current clinical practice has contributed to the generation of rifampicin and vancomycin resistance and spread of the three multidrug-resistant *S. epidermidis* lineages, warranting the review of staphylococcal treatment guidelines.

The final work in this thesis is the first comprehensive analyses of the barriers to the uptake of foreign DNA in *S. epidermidis*. Contrary to current assumptions, analyses revealed that the type I RM systems in *S. epidermidis* are not lineage specific like *Staphylococcus aureus*, instead demonstrating considerable diversity even within a single genetic lineage. This diversity can be attributed to marked differences in the gene arrangement, chromosomal location and movement of type I RM systems in *S. epidermidis* compared to *S. aureus*. Findings indicated that genetic manipulation of *S. epidermidis* must be tailored to each strain of interest. Using *Escherichia coli* plasmid artificial modification to express *S. epidermidis hsdMS*, the restriction barriers in *S. epidermidis* were readily overcome, with electroporation efficiencies equivalent to modification-deficient mutants. Through functional experiments, the use of genomic data to predict both the activity of type I RM systems and the potential for a strain to be electroporation proficient were demonstrated, and an efficient approach to the genetic manipulation of any *S. epidermidis* strain of interest, including those that have hitherto been intractable is presented.

Collectively, the works in this thesis offer new insights into the molecular epidemiology, resistance mechanisms, treatment recommendations and genetic manipulation of *S. epidermidis*. The method outlined for the genetic manipulation of any given *S. epidermidis* isolate should prove valuable in assisting future molecular studies and advancing knowledge about this significant nosocomial pathogen.

PREFACE

The work presented in this thesis was performed by the author except for the collaborator contributions outlined as follows:

Chapter 1

The content of this chapter includes excerpts from manuscripts that were prepared and published during this PhD candidature. The publications include those published in Chapters 2, 3 and 4 and the following manuscript: **Lee JYH**, Howden BP. 2015. Vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* – a clinician's guide to the science informing current practice. Expert Rev Anti Infect Ther 13:855-869. doi: 10.1586/14787210.2015.1041924

Chapter 2

Published manuscript: **Lee JYH**, Monk IR, Pidot SJ, Singh S, Chua KYL, Seemann T, Stinear TP, Howden BP. 2016. Functional analysis of the first complete genome sequence of a multidrug resistant sequence type 2 *Staphylococcus epidermidis*. Microb Genom 2:e000077. doi: 10.1099/mgen.0.000077

JYHL contributed 85% of the work in this manuscript as declared by the co-authors.

Author contributions: JYHL, KYLC and BPH were involved in the clinical care of the clinical case and collection of isolates; JYHL, BPH and TPS conceived the project; JYHL performed all experimental work, with assistance from IRM; JYHL performed all bioinformatic analysis with assistance from SJP, SS and TS. JYHL, IRM, BPH and TPS drafted the manuscript; all authors reviewed and contributed to the final manuscript.

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Author contributions: BPH and TPS conceived the project, which was supervised by BPH, TPS and IRM; JYHL performed all experimental work, with assistance from IRM; JYHL, AGDS, TS, TPS and BPH analysed data, including analysis of genome sequence data; KYLC, AK, RH, NW, MDB, BS, FL, MD, AD, RP, ARL and TMK established and analysed clinical and reference isolate datasets and performed susceptibility testing; JYHL, IRM, BPH and TPS drafted the manuscript; all authors reviewed and contributed to the final manuscript.

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LIST OF CONFERENCE PRESENTATIONS

Abstracts presented at national and international conferences:

JYH Lee, P Szczurek, KYL Chua, T Seemann, SL Baines, TP Stinear, BP Howden. Characterization of multidrug resistant *Staphylococcus epidermidis* isolated from clinical infections within a tertiary institution. *International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) 2014, Seoul, Korea.* (Poster).

JYH Lee, IR Monk, P Szczurek, KYL Chua, T Seemann, SL Baines, TP Stinear, BP Howden. Endemic presence of vancomycin heteroresistant *Staphylococcus epidermidis* within an Australian tertiary institution – the role of *rpoB* mutations in glycopeptide cross-resistance. *Australian Society for Antimicrobials (ASA) 2015, Brisbane, Australia.* (Oral presentation).

JYH Lee, IR Monk, SJ Pidot, S Singh, KYL Chua, T Seemann, TP Stinear, BP Howden. Functional analysis of the first complete genome and methylome of a multidrug resistant sequence type 2 *Staphylococcus epidermidis* and the use of plasmid artificial modification to overcome the restriction modification barrier. *International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) 2016, Chicago, USA.* (Poster).

JYH Lee, IR Monk, SJ Pidot, T Seemann, TP Stinear, BP Howden. The genomic diversity of the type I restriction modification systems of *Staphylococcus epidermidis* and use of plasmid artificial modification to bypass the restriction barrier. *BacPath 2017, Hahndorf, South Australia, Australia.* (Oral presentation).

JYH Lee, IR Monk, A Gonçalves da Silva, T Seemann, KYL Chua, A Kearns, R Hill, N Woodford, MD Bartels, B Strommenger, F Laurent, M Dodémont, A Deplano, R Patel, AR Larsen, TM Korman, TP Stinear, BP Howden. Genomics reveals global emergence of near pan-drug resistant *S. epidermidis*. *International Symposium on Staphylococci and Staphylococcal Infections (ISSSI) 2018, Copenhagen, Denmark.* (Oral presentation).

Invited speaker presentations:

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JYH Lee. Global spread and potential clinical impact of multidrug-resistant *S. epidermidis*. *American Society for Microbiology (ASM) Microbe 2019, San Francisco, USA.*

ABBREVIATIONS

ACME	Arginine catabolic mobile element
Amp	Ampicillin
ATCC	American Type Culture Collection
BAPS	Bayesian analysis of population structure
BARRGD	Bacterial antimicrobial resistance reference gene database
BHI	Brain heart infusion
BM	B media
BMD	Broth microdilution
CC	Clonal complex
ccr	Cassette chromosome recombinase gene
CFU	Colony forming units
CLABSI	Central line associated bloodstream infections
CLSI	Clinical and Laboratory Standards Institute
Cm	Chloramphenicol
CNS	Central nervous system
CoNS	Coagulase negative staphylococci
CR	Conserved region
CRISPR	Clustered regularly interspaced short palindromic repeat
CSF	Cerebrospinal fluid
eBURST	Evolution based upon related sequence types (clustering algorithm)
ENA	European Nucleotide Archive
EVD	External ventricular drain
FDA	Food and Drug Administration
GC	Genetic cluster
hierBAPS	Hierarchical Bayesian analysis of population structure
HPDI	Highest posterior density interval
hsd	Host specificity of DNA gene
hVISA	Heteroresistant vancomycin intermediate *Staphylococcus aureus*
ICU	Intensive care unit
IQR	Interquartile range
IS	Insertion sequence
Kan	Kanamycin
LB	Lysogeny broth
LSPN	List of Prokaryotic Names with Standing in Nomenclature (database)
MBC	Minimum bactericidal concentration
MCS	Multiple cloning site
MDRSE	Multidrug resistant *Staphylococcus epidermidis*
MET	Macromethod Etest
MIC	Minimum inhibitory concentration

MLST	Multilocus sequence type
MRSA	Methicillin resistant *Staphylococcus aureus*
NCBI	National Center for Biotechnology Information
NICU	Neonatal intensive care unit
OD_{600}	Optical density at 600 nm
PacBio	Pacific Biosciences
PAM	Plasmid artificial modification
PAP/AUC	Population analysis profile / Area under the curve
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PIA	Polysaccharide intercellular adhesin
PMN	Polymorphonuclear leucocytes
PVE	Prosthetic valve endocarditis
RM	Restriction modification
RRDR	Rifampicin resistance determining region
SCC	Staphylococcal cassette chromosome
SLiCE	Seamless ligation cloning extract
SMRT	Single molecule real-time
SNP	Single nucleotide polymorphism
SOE	Spliced overlap extension
SRA	Sequence Read Archive
ST	Sequence type (same as multilocus sequence type)
TEC	Teicoplanin
TRD	Target recognition domain
TRM	Target recognition motif
US	United States
VAN	Vancomycin
VFDB	Virulence factors of pathogenic bacteria database
VGA	Vancomycin gradient assay
VISA	Vancomycin intermediate *Staphylococcus aureus*
VRSA	Vancomycin resistant *Staphylococcus aureus*
VSSA	Vancomycin susceptible *Staphylococcus aureus*
WT	Wildtype

CHAPTER 1. Literature Review

1.1 Introduction

Staphylococcus epidermidis has long been recognised as the predominant bacterial coloniser of human skin [1]. Study of the healthy human microbiome has reinforced this, with *S. epidermidis* found to be universally present on the skin of study participants [2]. Due to this ubiquitous presence and fewer virulence factors compared to its more pathogenic relative, *Staphylococcus aureus*, historically *S. epidermidis* was perceived as non-pathogenic. However the shift in modern medicine towards interventional and invasive procedures has favoured the emergence of *S. epidermidis* as a significant nosocomial pathogen, particularly in the setting of prosthetic devices [3]. The ability of the organism to form biofilms over foreign bodies, in which bacteria embedded within a extracellular polymeric matrix are protected from both antibiotics and the host immune system, is key in the evolution of disease [4].

Although recognised as a significant nosocomial pathogen for over 30 years, knowledge about the epidemiology, transmission and molecular biology of *S. epidermidis* is limited compared to *S. aureus* [3,5,6]. Scientific review of *S. epidermidis* inevitably involves discussion of *S. aureus*. Historically, descriptions of *S. epidermidis* have been shaped by comparisons to *S. aureus* [7-9]. Furthermore, the genetically intractable nature of *S. epidermidis* and an absence of molecular methods specific for the manipulation of the species have hindered its study. In the absence of specific knowledge there has been a tendency to assume that findings about *S. aureus* can be extrapolated. However, as multidrug resistance proves increasingly common and the clinical impact and associated economic burden posed by *S. epidermidis* continues to increase [10-13], the time for dedicated study of this organism is long overdue.

1.2 Basic microbiology

S. epidermidis is a non-motile, nonspore-forming, gram-positive coccus, 0.8 – 1.0 μm in diameter, arranged in pairs and tetrads with occasional single cells [14,15]. Colonies are circular, 2.5 – 4.0 mm in diameter, smooth, raised, glistening and translucent to slightly opaque; typically, grey or greyish-white in colour, rare colonies possess a slight yellow or brownish pigment [15]; sticky consistency increases with age [14]. The cell wall is composed of peptidoglycan, teichoic acid and lipoteichoic acid. Certain strains can also produce polysaccharide intercellular adhesin (PIA) which can coat the cell and stimulate biofilm formation [10]. *S. epidermidis* can grow from 15 – 45°C, with optimal growth at 30 – 37°C [14]. Although a facultative anaerobe, it grows best under aerobic conditions. The G+C content of its DNA is 33.5 $\pm$ 0.2% [15].

1.3 Taxonomy

In 1964 *The Subcommittee on Taxonomy of Staphylococcus and Micrococcus* of *The International Committee on Nomenclature of Bacteria* proposed the recognition of two species of staphylococci, *Staphylococcus aureus* Rosenbach 1884 and *Staphylococcus epidermidis* (Winslow and Winslow 1908) Evans 1916 [9]. Prior to this, *Staphylococcus epidermidis* was assigned the following baysionyms: *Staphylococcus epidermidis albus* (Welch 1891) [8]; *Albococcus epidermidis* (Winslow and Winslow 1908) [16]; with transfer back to the genus *Staphylococcus* by Evans in 1916 [9]. A colonising isolate of *S. epidermidis* from a human nose, American Type Culture Collection (ATCC) strain 14990 was accessioned in 1963 [9,17]. In 1968, Hugh and Ellis proposed ATCC 14990 as the neotype strain for the species, as the original Winslow and Winslow strains of *Albococcus epidermidis* were no longer available for comparative studies [9].

The division of staphylococcal species on the basis of coagulase testing was proposed by Fairbrother in 1940 [18]. For a number of decades, medical microbiologists only focused on identifying the coagulase-positive species, *S. aureus*, based on the assumption that all coagulase negative staphylococci (CoNS) were non-pathogenic commensals [19]. In 1974, Kloos and Schleifer introduced the biochemical classification scheme for the differentiation of *Staphylococcus* species, on which current biotyping schemes are based [19]. Consequently, *S. epidermidis* became recognised as the major cause of all CoNS infections except those of the urinary tract [15,20-22], with *Staphylococcus saprophyticus* was found to account for 80% (12/15) of female urinary tract infections [20] . The original Kloos and Schleifer scheme described 11 different staphylococcal species [19]. Since then, 54 staphylococcal species have been catalogued by the *List of Prokaryotic Names with Standing in Nomenclature* (LPSN) database [23-25].

1.4 Clinical impact of *S. epidermidis*

Consistently recognised as a leading cause of nosocomial infections, *S. epidermidis* poses significant morbidity and mortality to individual patients and economic burden on healthcare systems. This impact is reflected in the summary data on *Antimicrobial-resistant pathogens associated with healthcare-associated infections* reported by the Centers for Disease Control and National Healthcare Safety Network, in which CoNS predominantly represented by *S. epidermidis*, were the identified as the: most common cause of central line associated bloodstream infections (CLABSI); second ranked cause of surgical site infections; and third most reported pathogen for hospital acquired infections [26].

Demonstrated to significantly prolong Intensive Care Unit (ICU) and general hospital length of stays, and result in higher mortality [3,13,27], CLABSI pose a significant cost, with a 2006 study estimating the attributable cost of each episode of ICU CLABSI at $11,971 [13]. *S. epidermidis* CLABSI have been estimated to cost $2 billion annually in the US alone [10].

With respect to the neonatal population, CoNS have been recognised as the leading cause of both early [28] and late (occurring after three days of age) neonatal sepsis [29,30], frequently associated with central venous access. Similar to the adult population, late neonatal sepsis has been associated with significantly prolonged hospital stay (79 vs 60 days, $p<0.001$) and increased mortality (18 vs 7%, $p<0.001$) compared to neonates without sepsis, especially in the very low birthweight cohort [30].

In terms of cardiac infections, *S. epidermidis* is recognised as a leading pathogen in prosthetic valve endocarditis (PVE), variably reported to accounting for 15 – 25% of cases [31-33]. Prospective longitudinal data from the *International Collaboration on Endocarditis* spanning 1984 to 1999 (n = 367) identified the complications of heart failure (54%, 29/54) and valvular abscesses (39%, 21/54) as significantly higher in CoNS PVE compared to PVE due to either *S. aureus* (heart failure: 33%, 19/58, $p=0.03$; valvular abscess: 22% 13/58, $p=0.06$) or viridans streptococci (heart failure: 32% 20/63, $p=0.02$; valvular abscess: 6%, 4/63, $p<0.001$) [32]. With an in-hospital mortality of 36% CoNS PVE was second only to *S. aureus* PVE, with a mortality of 47% ($p=0.22$) [32]. With minor variations in percentages, the observations regarding CoNS PVE complications with valvular abscesses held true in a follow-up study covering 2000 to 2005 (n = 537) [33]. Furthermore, it was observed that 48% (33/69) of CoNS PVE cases occurred between 60

– 365 days post valve implantation, with a high rate of methicillin resistance (68%) observed in the causative isolates [33].

Metanalysis performed as part of the British *Guidelines for the diagnosis, prevention and management of implantable cardiac electronic device infection* identified staphylococci as the causative organism in 68 – 93% of implantable cardiac electronic device infections, with an all-cause mortality of 0 – 35% [34]. CoNS specifically, were reported as the causative organism in 10 – 68% of infections [34].

CoNS have been identified as the predominant pathogen in various CNS infections including nosocomial drain-associated meningitis and ventriculitis (73%, 29/40) [35], as well as cerebrospinal fluid shunt associated infections, found in 37% (29/78) of specimens, followed by *S. aureus* (18%, 14/78) [36].

Although the rate of infective complications for prosthetic joints in the United States (US) is estimated as only 2 – 2.4% [12,37], the economic burden posed is immense, totalling $320 million in 2001, increasing to $566 million in 2009, with a projected annual cost over $1.62 billion by 2020 [11,12]. *S. epidermidis* and *S. aureus* account for more than half of all cases of prosthetic joint infections (PJIs) [37,38].

1.5 Epidemiology of *S. epidermidis*

S. epidermidis is characterised by marked genetic diversity, greater than that observed in other staphylococcal species [3]. To date, the majority of epidemiological studies of *S. epidermidis* have utilised pulsed-field gel electrophoresis (PFGE) as the typing method [39-44]. Despite pronounced genetic diversity within the species, as demonstrated in

community based colonisation studies [40,44], hospital based investigations suggest the predominance of a few hospital-associated, epidemic clonal lineages [39,45-49].

Individually, most nosocomial transmission studies have mostly focused on the presence of clones within specific high risk sites, such as the ICU [43], neonatal ICU (NICU) [49,50], cardiac care centres [5,39] and haemodialysis unit [41]. Typically, these studies have demonstrated the local prevalence of a few dominant clones. One NICU based study documented the emergence of a particular *S. epidermidis* clone responsible for 31% (20/65) of blood stream infections occurring within the unit over a year, as well as the longitudinal persistence of second clone over the 11 year period of the study [50]. While another prospective Italian study reported *S. epidermidis* to account for 30.4% (56/184) of all NICU-acquired infections over a three years period, with four main clones noted to cause most infections [49]. Compared to sporadic strains these clones were more antibiotic resistant, particularly to the agents used for empiric treatment within the unit. This observation of disseminated clones possessing more resistant antibiotic profiles is common to a number of studies, with methicillin resistant *S. epidermidis* reported to comprise up to 79% [39] to 92% [50] of hospital isolates.

The classification of *S. epidermidis* was revised in 2007, with introduction of an improved multilocus sequence type (MLST) scheme [51]. This saw the reclassification of ST27, previously identified as the major disease associated strain type, present in hospitals in both the US and Europe [52], as ST2. A study implementing the new scheme to analyse 217 nosocomial isolates from 17 different countries, divided the isolates into 74 MLST types, which using the eBURST algorithm formed nine endemic clonal lineages that were disseminated worldwide [46]. A single clonal lineage, clonal complex 2 (CC2), for which ST2 was the founder, accounted for 74% of isolates, with the remaining isolates distributed over eight minor and 13 singleton lineages. Furthermore, analysis of the data

suggested that recombination contributed to the generation of new alleles approximately twice as frequently as point mutations [46].

1.6 Mode of transmission

A limited number of studies have attempted to address the mode of transmission of *S. epidermidis* clones within the hospital environment. These investigations have yielded similar results, indicating replacement of less drug-resistant colonising strains with locally prevalent, drug-resistant *S. epidermidis* strains during the course of admission [5,53]. Both healthcare workers [5,41,53] and the hospital environment [53] have been implicated as potential reservoirs for hospital-adapted *S. epidermidis* clones.

A study conducted in 1982 by Archer *et al*. on patients undergoing cardiac surgery at the Medical College of Virginia found that only one in 26 patients who were not previously hospitalised were pre-operatively colonised with multidrug resistant *S. epidermidis* (MDRSE; defined as resistance to methicillin and gentamicin), however by day ten post-operatively 80% of patients were colonised [5]. Post-operative MDRSE isolates were resistant to at least three additional unrelated antibiotics (erythromycin, clindamycin, chloramphenicol, tetracycline or trimethoprim). Antibiotic usage was identified as a strong correlate for the nosocomial acquisition of MDRSE, with almost 70% of patients who were administered antibiotic prophylaxis prior to cardiac surgery subsequently colonised with drug-resistant CoNS (nearly exclusively *S. epidermidis*) [5]. Surveillance swabs of the surgical ICU personnel caring for these patients identified a 66% (number not specified) colonisation rate with an identical MDRSE molecular type (method unspecified) as their patients. Of note, screening of a comparator medical ward where

patients were infrequently administered antibiotics demonstrated only 11% colonisation of patients and personnel with drug-resistant CoNS [5].

Similarly, a recent prospective study of patients admitted from the community directly to ICU demonstrated that colonisation with hospital-associated genotypes of *S. epidermidis* (as determined by PFGE) typically occurred within three days of hospitalisation [53]. Both environmental surfaces within the ICU and healthcare workers, of whom 89% (33/37) had confirmed nasal carriage, were identified as potential reservoirs of these hospital-associated genotypes of *S. epidermidis* [53]. While another investigation for the reservoir of *S. epidermidis* within a haemodialysis unit, screened the palms and nasal mucosa of medical and nursing personnel and identified 38% (5/13) of staff to be colonised by the dominant clone using PFGE [41].

Collectively, these studies suggest that certain hospital locations create ideal circumstances for the maintenance and propagation of drug resistant *S. epidermidis* clones. Specifically, those in which the presence of prosthetic material *in situ* and administration of antibiotics constitute routine care for most patients. Within this setting, susceptible patients develop nosocomial MDRSE infections when conducive circumstances arise.

1.7 *S. epidermidis* genomics

At the time of commencing this work only four complete *S. epidermidis* genomes were published: reference strains ATCC 12228 [54], RP62a [55] and SEI [56]; and bovine strain PM221 [57]. More recently, the complete genome of 1457, an isolate frequently used in genetic studies of *S. epidermidis* was published [58]. A comparison of these genomes is

shown in Table 1.1, and a comparison of genome content relative to RP62a is shown in Figure 1.1. Of note, none of these strains represent ST2 the dominant lineage associated with clinical disease.

1.7.1 ATCC 12228 (FDA strain PCI 1200, WHO 12)

Catalogued in 1955, ATCC 12228 is a non-pathogenic, non-biofilm forming isolate that is highly drug-susceptible and used for the detection of residual antibiotics such as gentamicin, neomycin, and novobiocin in food products [54,59]. The first *S. epidermidis* genome to be closed, the complete ATCC 12228 genome was published in 2003 [54]. Only two virulence factors were identified in common with *S. aureus*, δ-haemolysin and β-haemolysin, with no other toxin genes identified [54]. In keeping with phenotypic data, the genome was found to lack methicillin resistance conferring *mec* genes and the intercellular adhesion locus *icaADBC*. Furthermore, no IS*256* or IS*257* elements, that are thought to be markers for invasive disease, were identified in the genome [54].

1.7.2 RP62a (ATCC 35984)

Biofilm producing isolate, RP62a, is multidrug resistant clinical *S. epidermidis* strain, isolated during an outbreak of intravascular catheter associated sepsis occurring at the City of Memphis Hospital, Memphis, USA between 1979 to 1980 [60]. The complete genome of RP62a was published in 2005 [55]. In addition to the presence of various antibiotic resistance genes such as *mecA* in the presence of IS*257*, compared to non-biofilm producing strain ATCC 12228, key differences in the genome of RP62a are the presence of the *ica* operon and *bhp* (homologue of *S. aureus* biofilm associated binding protein, Bap; that has been indicated in the pathogenesis and persistence of bovine mastitis) [55].

Table 1.1 Comparison of completed *S. epidermidis* genomes

Strain	*in silico* MLST	Isolated	Host	Clinical Presentation	Chromosome (Mb)	GC %	Plasmids	Reference
RP62a	10	1979-1980	Human	Bacteraemia	2.61	32.2	1	[55]
1457	86	1990	Human	Bacteraemia	2.45	32.3	1	[58]
ATCC 12228	8	1955	Non-clinical	Not applicable	2.50	32.0	6	[54]
SEI	*-	Unknown	Human	Unknown	2.50	32.0	1	[56]
PM221	184	1999-2002	Bovine	Mastitis	2.49	31.9	4	[57]

*Published reference strain SEI was not classifiable by existing multilocus sequence type (MLST) schema.

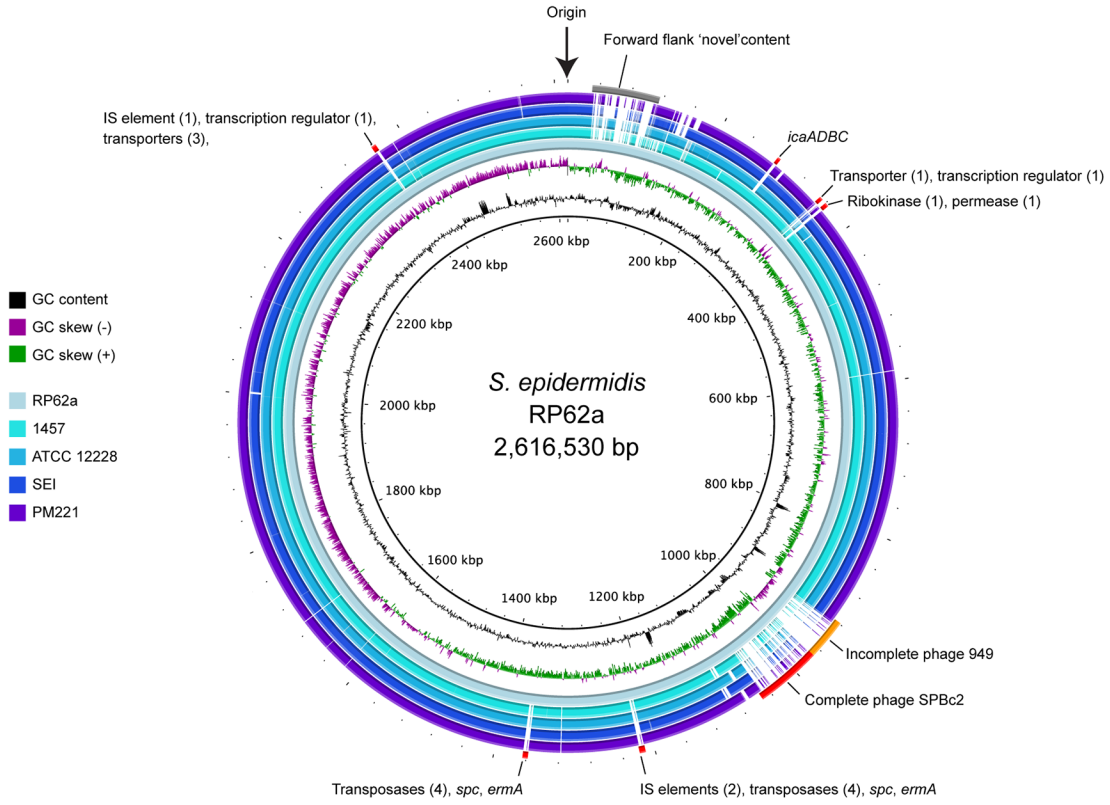


Figure 1.1 Gene content comparison for completed *S. epidermidis* genomes. A one-way comparison of gene content between the five published *S. epidermidis* genomes relative to RP62a, generated using BRIG [70]. Novel content in RP62a is annotated in the outermost ring.

1.7.3 SEI (ATCC 49134, AmMS 205, CDC 73-57501)

SEI is an isolate of clinical origin with an unknown date of isolation, that is used as a quality control strain for Microscan™ products [59]. Its complete genome was published in 2014 using a combination of Illumina short-read and long-insert 454 sequencing technology to close the genome, with preliminary annotation identifying at least seven virulence factors that were not specified [56]. Due to the proprietary nature of the isolate, there is a paucity of information regarding its phenotypic profile. Despite having all seven loci identifiable by the current *S. epidermidis* MLST database, isolate SEI is unclassified as the unique combination of allele variants (*arcC*[1], *aroE*[13], *gtr*[7] *mutS*[6], *pyrR*[2] *tpiA*[5] and *yqiL*[33]) are yet to be assigned a ST number.

1.7.4 PM221

PM221 was isolated from a spontaneous persistent bovine intramammary infection presenting to the Ambulatory Clinic of the Faculty of Veterinary Medicine, University of Helsinki between 1999 and 2002 [61]. *S. epidermidis* is rarely found in the normal bovine microbiota [62]. Epidemiological studies suggest that the *S. epidermidis* strains causing bovine mastitis originated from humans, with the same PGFE genotypes identified to colonise dairy farmers and in milk from affected cows [63]. The closed genome of PM221 was published in 2014 [57].

1.7.5 *S. epidermidis* 1457

S. epidermidis 1457 was isolated from an infected central venous catheter in 1990, at the Institute of Medical Microbiology and Immunology, University Hospital Eppendorf, Hamburg, Germany [64]. A biofilm producing isolate, pan-susceptible to commonly used staphylococcal antibiotic markers, it is frequently used in laboratory studies and amenable

to genetic manipulation by various methods including: phage transduction with *S. aureus* Φ187 [65,66] and *S. epidermidis* phage 48 [67]; transposon mutagenesis [67,68]; and allelic exchange [69]. The genome of 1457 was recently closed using PacBio RSII technology and demonstrated to lack a methylome [58], indicating no functional type I restriction modification system.

1.8 Determining clinically significant disease

As an ubiquitous coloniser of human skin [1], it is unsurprising that *S. epidermidis*, together with other CoNS, are the most frequent contaminants in blood cultures, representing 70 – 80% of all blood culture contaminants [71]. Typically introduced from the patient's (or specimen collector's) colonising flora due to poor aseptic technique during sampling. However, CoNS are also the most common cause of true nosocomial blood stream infections [13,26,72,73]. Therefore, distinguishing contaminants from clinical infections can be challenging, and poses significant cost [71]. In view of this, numerous studies have attempted to discriminate clinically significant from contaminating strains of *S. epidermidis* in a diagnostic context. Such studies have focused on the identification of markers for hospital dominant strains and those associated with invasive clinical disease.

Overall, the results of these studies demonstrate that the presence of *mecA* [52,74-79], *icaA* (or the full *icaADBC* operon) [52,74-78,80-82] and IS*256* [52,74-76,80,81,83] are most consistently associated with invasive isolates. When studies included phenotypic data, the presence of these markers usually correlated with the formation of biofilm in the case of *icaA* [75,76,80,81], and the presence of multidrug-resistance that included clindamycin, aminoglycosides and trimethoprim-sulfamethoxazole when IS*256* was present [77,80]. Two markers were found to be associated with colonising isolates, formate dehydrogenase

(*fdh*) [74,75], and the arginine catabolic mobile element (ACME) [75,80]. Studies including the intercellular protein adhesin *bhp* were conflicting. With two studies demonstrating a significant association with invasive *S. epidermidis* stains [75,80], but another inconclusive, with *bhp* present in equally small numbers of invasive and colonising isolates [76]. Markers investigated but determined to have no discriminatory value included: the biofilm associated genes - accumulation associated protein (*aap*) [76,80], autolysin *atlE* [76,78,84], fibronectin binding protein (*fbe*) [76], extracellular matrix binding protein (*embp*) [76]; and IS*257* [76,81].

1.9 Prosthetic device infections, *S. epidermidis* biofilms and immune evasion

Traditionally, infection of a prosthetic device can be classified as: 1. Early (occurring within three months of implantation); 2. Delayed (three to 24 months post implantation); or 3. Late (more than two years after implantation) [85,86]. Contamination during the time of device insertion typically results in early infections, unless the causative organism is of low virulence (such as *S. epidermidis* or *Cutibacterium acnes*) which may lead to delayed manifestation of infection [85-88]. Delayed and late infections can also occur due to haematogenous spread of bacteria seeding a prostheses [85,86,89].

In addition to a mechanical protective barrier, *S. epidermidis* biofilm has been demonstrated to impair the deposition of IgG and C3b resulting in increased resistance to phagocyte-mediated killing [90]. Other immune evasion mechanisms identified in *S. epidermidis* include the *aps* loci (homologous to the GraRSX three component system in *S. aureus*) that senses the presence of human antimicrobial peptides, and produces the protease SepA that destroys them [91]. While the quorum sensing system, accessory gene regulator (*agr*), that is known to regulate colonisation and virulence factors [92], is thought

to assist in resistance to killing by polymorphonuclear leucocytes (PMN) and their antimicrobial products [93]. In the case of *S. epidermidis* PJIs, studies have demonstrated that bacteria can invade and persist intracellularly within human fibroblasts and human and mouse osteoblasts, serving as a reservoir for chronic or delayed infection [37]. Primarily residing within acidic phagolysosomes that select for a small colony variant phenotype, when host cells die the *S. epidermidis* escapes into the extracellular environment potentially seeding the prosthesis resulting in a delayed implant-associated infection [37].

In animal models, the presence of a foreign body has been demonstrated to increase the risk of establishing a staphylococcal infection >100,000-fold, compared to the absence of foreign material [85,94]. The attributing reasons are multifactorial, including: defective host granulocyte response around implants [94,95]; inability of most antibiotics to penetrate the biofilm surrounding the foreign body [89,96,97]; and the lack of antibiotic activity against stationary phase bacteria within biofilms [96-98].

In a guinea pig subcutaneous foreign body model, PMNs localised around a foreign body have been demonstrated to contain decreased levels of granular enzymes and reduced ability to mount a respiratory burst [95]. It is hypothesised that prolonged stimulation of PMNs by the non-phagocytosable surface of an implanted device exhausts the host innate immune response, resulting in an impaired granulocyte response [95]. Due to limited oxygen and glucose, bacteria within biofilms are in a stationary phase of growth [85].

Infected devices can serve as a nidus for persistent and relapsing infection. While systemic antibiotics are effective against planktonic cells, most fail to kill bacteria

within biofilms, resulting in cycles of relapsing infection unless the foreign body is surgically removed [96]. Multiple animal studies [89,96,97] and decades of clinical observation [85-88] have demonstrated that once established, foreign body infections do not spontaneously clear. It is generally accepted that removal of a prosthesis with an established infection is required for cure, as sterilisation cannot be achieved [85-88,96,97].

1.10 Treatment of *S. epidermidis*

1.10.1 Glycopeptides - Vancomycin and teicoplanin

Vancomycin is glycopeptide antibiotic produced by the soil bacterium *Amycolatopsis orientalis* (originally classified *Streptomyces orientalis*) [99,100] (Figure 1.2A). Active against aerobic and anaerobic gram-positive bacteria, vancomycin inhibits bacterial cell wall peptidoglycan synthesis though rapid and irreversible binding to the D-Ala-D-Ala cell wall precursor at the external surface of the cytoplasmic membrane [100,101]. The result is bactericidal in most gram-positive organisms [101]. Discovered during a program aimed at the development of new anti-staphylococcal antimicrobial agents in response to the emergence of antibiotic resistant hospital strains of *S. aureus* in the early 1950s [99], Food and Drug Administration (FDA) approval of vancomycin in the US was expedited in 1958 in anticipation of clinical demand [100]. However, the adverse effects of infusion reactions, ototoxicity and nephrotoxicity reported in early vancomycin studies and the subsequent discovery of methicillin and anti-staphylococcal cephalosporins (both of which had favourable side-effect profiles), resulted in the preferential use of these ß-lactams until the increasing prevalence of methicillin resistant staphylococci in the 1980s necessitated the use of vancomycin [99,100]. Fortunately, it was determined that many of the adverse effects observed in early trials were due to impurities that were eliminated in preparations of vancomycin introduced in the 1970s [100]. Vancomycin is now the recommended first-

line agent for methicillin resistant staphylococci in numerous specialty specific guidelines [34,38,86,102-105].

Teicoplanin is a lipoglycopeptide chemically related to vancomycin, with a differing side-chain linkage pattern and carbohydrate groups attached, but the same mechanism of action [106,107] (Figure 1.2B). Comprised of six closely related teichomycin compounds (designated T-A_2-1 to 5 and T-A_3) [106] produced by *Actinoplanes teichomyceticus* [108], teicoplanin has a similar spectrum of activity to vancomycin [109].

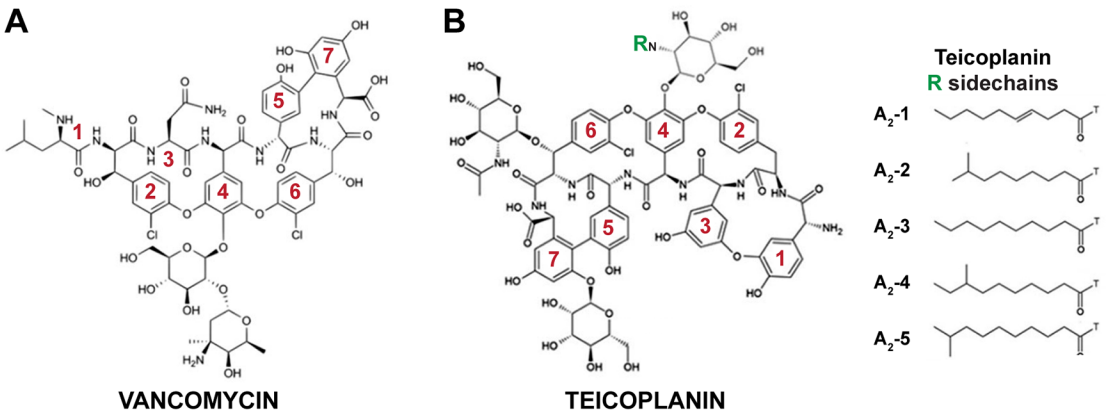


Figure 1.2 Chemical structures of glycopeptide antibiotics. Representation of chemical structures have been adapted from Kang and Park [110]. The seven core amino acid residues are numbered in red. **A.** Vancomycin is a group I glycopeptide (classification scheme based on type of residues at positions 1 and 3 [111]) that contains aliphatic residues at positions 1 and 3 and glucose-vancosamine disaccharide attached to amino acid 4. **B.** Teicoplanin is a group III glycopeptide with covalently joined aromatic residues at positions 1 and 3, mannose attached to position 7, *N*-acetylglucosamine attached to amino acid 6 and *N*-acylglucosamine attached to amino acid 4 with teicoplanin sidechain variants (green R) A_2-1 to 5 shown.

1.10.2 Mechanisms of glycopeptide resistance

1.10.2.1 Intrinsic resistance

Initial reports of clinically significant vancomycin resistance in CoNS began in the 1980s, raising concerns that the discovery may herald similar resistance in the more pathogenic, related species of *S. aureus* [112]. Since then, studies have demonstrated an upward creep in glycopeptide MICs for CoNS, particularly with teicoplanin, which has been attributed to increased use of such agents due to methicillin resistance [113-115]. Studies have suggested that *S. epidermidis* possesses low level intrinsic resistance to teicoplanin, that can be directly induced through exposure to teicoplanin, or cross induced with vancomycin exposure [112,116,117]. Furthermore, teicoplanin resistance is commonly associated with a modest increase in vancomycin resistance [113], it is therefore unsurprising that rates of vancomycin resistance are higher in *S. epidermidis* compared to *S. aureus* [3].

1.10.2.2 Heteroresistant vancomycin-intermediate and vancomycin-intermediate staphylococci

Knowledge regarding vancomycin resistance in staphylococci has predominantly been described and defined in terms of *S. aureus*. The first clinical case of *S. aureus* with reduced susceptibility to vancomycin (MIC 8 μg/ml) was reported by Hiramatsu *et al.* in 1997 [118]. Named Mu50, the strain was classified as vancomycin-intermediate *S. aureus* (VISA) [118]. Soon after, the same group described Mu3, a *S. aureus* strain identical to Mu50 on PFGE but with a vancomycin MIC of only 3 μg/ml (classified susceptible at the time) that possessed subpopulations capable of growth within the intermediate range [119]. Strains exhibiting this phenomenon came to be known as heteroresistant VISA (hVISA).

In 2006 the Clinical & Laboratory Standards Institute (CLSI) lowered vancomycin MIC breakpoints for *S. aureus*, thereby separating the species from CoNS, which had been classified together for over 20 years. This change was in recognition that heterogeneously resistant isolates of *S. aureus*, that is, those with MICs within the susceptible range for vancomycin when tested by routine methods, but which possess subpopulations capable of growth within the intermediate range, are precursors to VISA [120], and evidence that such isolates were associated with failure of vancomycin therapy [121]. Similarly, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) lowered their breakpoints for *S. aureus* in 2009, but opted to remove the ambiguity associated with interpretation of an intermediate MIC, defining only susceptible and resistant MICs [122]. Table 1.2 summarises the CLSI and EUCAST changes to vancomycin breakpoints in staphylococci. These alterations in defined breakpoints and international differences should be considered when interpreting the literature.

Table 1.2 Changes in vancomycin breakpoints for staphylococci.

Classification scheme	Broth microdilution MIC (μg/ml)			Reference
	Susceptible	Intermediate	Resistant	
S. aureus				
CLSI pre-2006	≤4	8-16	≥32	[121]
CLSI post-2006	≤2	4-8	≥16	[121]
EUCAST pre-2009	≤4	8	>8	[122]
EUCAST post-2009	≤2	-	>2	[122]
CoNS				
CLSI	≤4	8-16	≥32	[121]
EUCAST pre-2009	≤4	8	>8	[122]
EUCAST post-2009	≤4	-	>4	[122]

MIC: Minimum inhibitory concentration; CLSI: Clinical Standards Laboratory Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; CoNS: Coagulase negative staphylococci.

The single study that directly compared CoNS to *S. aureus* suggests that glycopeptide heteroresistance is significantly more prevalent in CoNS, with 13% (7/52) of CoNS but only 1.2% (1/81) of *S. aureus* isolates demonstrating heteroresistance to either, vancomycin, teicoplanin or both [123]. Although an accepted phenomenon with clinically significant consequences in *S. aureus*, heterogeneous vancomycin resistance in *S. epidermidis* and other CoNS is regarded with uncertainty and remains largely uncharacterised [124].

1.10.3 Vancomycin-resistant staphylococci

Demonstrated to arise from the cross-species, transposon mediated [125], transfer of the *vanA* operon from *Enterococcus faecalis* to *S. aureus* [126], the first clinical report of true high level (MIC 1024 μg/ml) vancomycin-resistance in staphylococci occurred 2002, in a patient co-colonised with vancomycin-resistant *E. faecalis* and vancomycin-resistant *S. aureus* (VRSA) [127]. Since then, around 50 clinical cases of VRSA have been reported [128], with 14 cases in the US [126,127,129] and additional cases reported in India [130,131], Iran [132], Pakistan [133], Brazil [134] and Portugal [135].

Studies suggest the presence of environmental reservoirs for vancomycin resistant staphylococci. A community prevalence study conducted on 1,000 healthy individuals residing in and around Bangalore, India, identified *S. aureus* or methicillin resistant *S. aureus* (MRSA) present on 22.5% and 16.6% of participants respectively; VRSA due to the presence of *vanA* was observed in 1.4% (n = 4) of those colonised with *S. aureus* [136]. A 2019 colonisation study conducted on meat samples (for human consumption) from 200 dromedary camel carcasses and 20 abattoir workers from three abattoirs in Egypt, detected *S. aureus* on 14.5% (29/200) of camel meat samples and 55% (11/20) abattoir

workers. Of which, 27% (8/29) camel and 54% (6/11) human isolates were identified as VRSA due to the presence of *vanA* or *vanB* [137]. Additionally, a Brazilian study in which health care workers (n = 102) were screened for the presence of vancomycin resistant staphylococci, identified five vancomycin resistant isolates (two *S. aureus*, three CoNS) [138]. The presence of *vanA* was only detected in a single *S. epidermidis* isolate colonising an ICU worker [138].

1.10.4 The pleiotropic nature of vancomycin heteroresistance

Within staphylococci, the prevalence of vancomycin heteroresistance has proven to be more common than high-level resistance. Best characterised in *S. aureus*, the mediators of vancomycin heteroresistance are complex and pleiotropic, interwoven with cross-resistance to other antimicrobial agents, rather than attributable to acquisition of a single resistance gene. Although not the first publication to explore the genetics behind non-VRSA reduced vancomycin susceptibility, the diversity of mutations associated with this phenotype was highlighted in a landmark study by Mwangi *et al*., that characterised sequential MRSA blood stream isolates over three months of vancomycin therapy that failed, from a patient with endocarditis [139]. Between the first (JH1) and last (JH9) isolate, progressive accumulation of 35 point mutations in 31 loci were shown to correlate with a stepwise increase in vancomycin MIC from 1 to 8 μg/ml. These 35 single nucleotide polymorphisms (SNPs), corresponded to 14 nonsynonymous substitutions, six frameshift mutations, one truncation, seven intergenic region mutations, one insertion in a tRNA, and six synonymous substitutions. Of these, seven SNPs affected six loci implicated in vancomycin resistance: missense mutations occurring in the *vraR* operon, *vraG* and *rpoB*; deletions causing frameshift mutations in *agrC* and *vraG*; a nonsense mutation in *yycH;* and a substitution in the intergenic region between divergently transcribed genes SA0526

and *nagB* [139]. Despite no exposure to daptomycin, increased resistance to the drug was also observed in progressive patient isolates, attributed to the acquisition of a missense mutation in *rpoC* and nonsense mutation in *yycH* [139]. Rifampicin resistance was acquired in the context of one dose of rifampicin single agent therapy, with four amino acid substitutions in RpoB (D471Y, A473S, A477S and E478D) [139].

Of note, a number of mutations occurred in genes that were essential (*rpoB*) or formed part of operons that encoded transcriptional regulators (*vraR, yycH* and *agr*) with far reaching downstream effects on gene expression. The global effects of mutations in such key loci was demonstrated in a related study that profiled the transcriptomes of JH1 and JH9, identifying 224 differentially expressed genes, the majority constitutively expressed and required for basic cellular functions including cell wall synthesis and cell division [140].

Reinforcing the pleiotropic nature of VISA genetics was a follow-on study by Vidaillac *et al*., which used two *in vitro* experimental systems (an endocarditis vegetation model with simulated clinical dosing of vancomycin; and passage under vancomycin selective pressure) to derive VISA mutants from clinical strain, JH1 [141]. Phenotypically, the two laboratory-derived mutants (JH R_1 and JH R_2) were indistinguishable from second clinical isolate (JH2), with comparable reductions in vancomycin and daptomycin susceptibility, and features typical of VISA. However, whole genome sequencing revealed that despite originating from the same parental isolate, the mutations possessed by each of the three strains differed [141]. JH2 acquired seven missense mutations in four loci: one in the *vraR* operon; four in *rpoB*, resulting in D471Y, A473S, A477S and E478D RpoB substitutions; one in *rpoC*; and another in SA1229 [139,141]. JH R_1 gained four SNPs causing one frameshift and three missense mutations [141], three of the affected genes had

unknown functions. However, a missense mutation in *yycG*, part of the *yycGF* (also known as *walRK*) essential two component regulatory system, was notable as mutations in this system are associated with a VISA phenotype. Experimental isolate JH R_2 acquired two missense mutations in *rpoB*, resulting in R484C and N641K RpoB substitutions [141]. Only one gene in common, *rpoB*, was mutated in both JH2 and the experimental isolates, however the acquired SNPs differed. Furthermore, none of the *in vitro* derived mutations were the same as the 35 SNPs in the final clinical strain JH9 [139,141]. Besides *rpoB*, the *yycGF* two component system was the only other locus at which both JH9 and the *in vitro* strains both acquired mutations, JH R_1 in *yycG* which encodes a histidine kinase [141], and JH9 in *yycH* which encodes an accessory protein [139]. It should be noted that while the cumulative list of putative mutations associated with an hVISA/VISA phenotype from this and other studies is extensive, causal effect has only been experimentally proven for a limited number of mutations [142].

Reduced vancomycin susceptibility associated with mutations within essential genes or operons is not unique to the JH1 strain, such mutations in other MRSA isolates have been identified in: the cell surface remodelling signalling pathway [143]; cell regulation operons [142]; genes involved in the expression of virulence factors [144] and other cell metabolism genes [145,146]. Irrespective of their genetic basis, hVISA/VISA mutants are phenotypically characterised by increased cell wall thickness, due to reduced cell wall turnover and decreased autolytic activity, thereby reducing access of vancomycin to its site of activity at the dividing cell septum [147]. Other mutation specific changes described include: excess peptidoglycan production; small colony variants; decreased growth rate; and vancomycin and daptomycin cross-resistance [147-149].

It is conceivable that the presence of mutations within key, regulatory loci under selective pressure from ongoing vancomycin therapy could create a cascade of altered signals pushing bacterial evolution towards increased resistance, and compensatory mutations that improve survival, consistent with reports of immune evasion and decreased virulence that favours a persistent disease state in some strains that have failed vancomycin therapy [149,150].

1.10.5 Vancomycin susceptibility testing

Studies evaluating methods for detecting reduced vancomycin susceptibility in staphylococci have all used the population analysis profile with area under the curve ratio (PAP/AUC) method described by Wootton *et al*. [151], as the reference method [152-154], this being the most reliable and reproducible method to confirm the presence of hVISA. A positive isolate defined by a ratio of ≥ 0.9 when compared to established hVISA strain, Mu3. A limited number of studies have attempted to apply the PAP method to CoNS species [113,117,123,155]. Given the cumbersome nature of the PAP/AUC method, it is only available at reference centres. Screening methods like the Macromethod Etest (MET) and Etest GRD can be (but generally are not routinely) performed in diagnostic laboratories [121,156].

1.10.6 Rifampicin

Rifampicin is a broad-spectrum, semi-synthetic antibiotic derived from the parent molecule rifamycin B, a bacterial polyketide secondary metabolite, extracted from the soil bacteria *Amycolatopsis mediterranei* (originally classified *Streptomyces mediterranei*) [157-159]. Although rifamycin B (Figure 1.3A) has only modest antibacterial activity, substitution of a carboxylic acid group with addition of a 4-methyl-

piperainaminyl side chain to form rifampicin (Figure 1.3B), produces a molecule with good bio-availability that is well tolerated with high antibiotic activity against *Mycobacteria*, Gram positive and Gram negative cocci, many gram negative bacilli and most anaerobes [157,159]. In susceptible organisms, the bactericidal effects of rifampicin are due to direct binding with the β subunit of DNA-dependent RNA polymerase (RpoB) to inhibit bacterial transcription [159] (Figure 1.4A). Rifampicin was first approved for clinical use in Italy, where it was developed, in 1968; approval in the US followed soon after in 1971 [157].

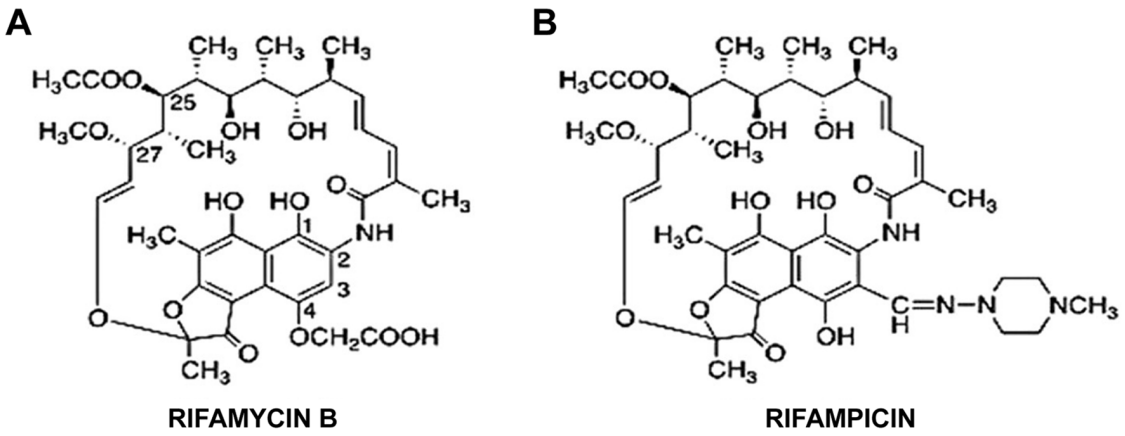


Figure 1.3 Chemical structures of rifamycin B and rifampicin. A. Rifamycin B **B.** Rifampicin. Adapted from Nigam *et al*. [160]

1.10.7 Mechanisms of rifampicin resistance

Despite low preservation in the amino acid identity across the complete protein alignments of RpoB between bacterial species, due to its essential function there is high conservation of the residues that comprise the active site at which RNA synthesis occurs (Figure 1.4B). The rifampicin-binding pocket of RpoB is located within 12 Å of the active site of RNA synthesis [161,162]. The mechanisms of rifampicin resistance have been

extensively studied in *Escherichia coli* and *Mycobacterium tuberculosis*, with identification of the amino acids integral to the conformation of the RpoB rifampicin-binding pocket. Mechanisms of rifampicin resistance are exclusively chromosomally mediated, the majority of mutations occur in a 27 amino acid sequence termed the rifampicin resistance determining region (RRDR), also known as Cluster I, within which three critical sites that form covalent bonds with rifampicin are most frequently affected [159,161] (Figure 1.4B). Substitution of amino acids at these three sites lowers the affinity for rifampicin within the binding pocket of RpoB. Experimental data in a number of species including *E. coli* [163], *M. tuberculosis* [159,164], and *S. aureus* [165,166] have demonstrated that such substitutions are capable of conferring high level rifampicin resistance at little or no fitness cost.

Outside of the RRDR, two other regions that contribute to the rifampicin-binding pocket through the formation of van der Waals bonds with rifampicin [159,167] have also been identified as sites at which rifampicin resistance mutations occur [159,161]. Known as the N-terminal cluster and Cluster II, the alignment of these regions for *E. coli*, *M. tuberculosis*, *S. aureus* and *S. epidermidis* are shown in Figure 1.4B, along with the commonly affected residues.

Based on alignment with the known sequence defined for other bacterial species: the RRDR in *S. epidermidis* was determined to span positions 462 to 488 (inclusive); the three critical positions were D471, H481 and S486 respectively; and key sites outside the RRDR corresponded to V135 and I527 (Figure 1.4B).

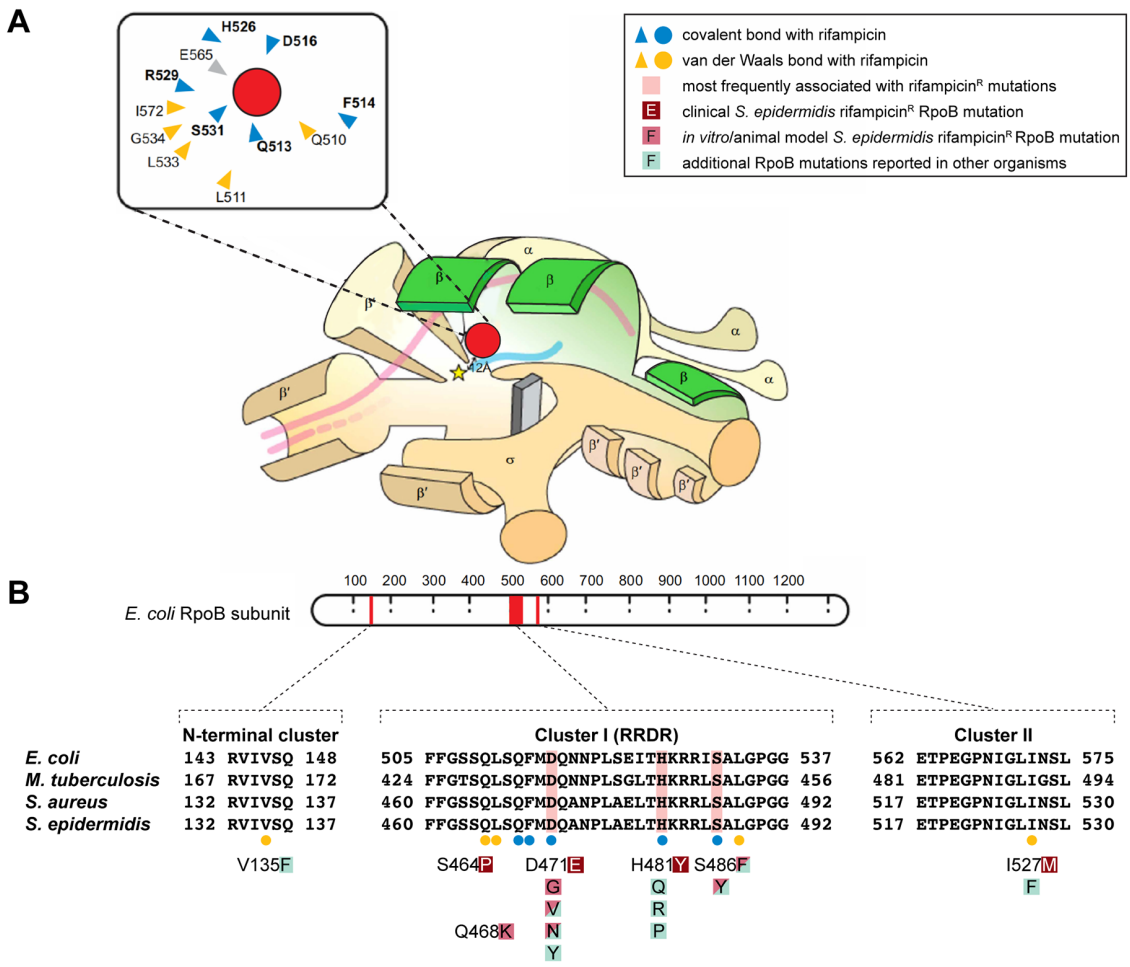


Figure 1.4 Schematic representation of the rifampicin resistance determining region (RRDR) of RpoB. A. Cartoon of rifampicin interacting with the structural elements of bacterial RNA polymerase adapted from Koch *et al.* [161]. A rifampicin molecule (red circle) is bound in the active site (yellow star) of the RpoB subunit (green) to inhibit transcription. DNA (pink lines) is shown as double-stranded entering the active site, then a single strand of template DNA once unwound. The growing RNA chain is represented by the blue line. Inset shows the rifampicin binding pocket of RpoB, highlighting the residues that interact with rifampicin, colour coded by the nature of the chemical bond (blue and yellow triangles). The grey triangle (*E. coli* RpoB E565) is the single interacting residue not known to be associated with a rifampicin resistance mutation. **B.** Alignment of the rifampicin resistance mutation hotspots in RpoB for various bacterial species, with reported mutations [159,161,168,169] overlaid.

1.10.8 Rifampicin resistance mutations in staphylococci

The translations of wildtype (WT) RpoB in *S. aureus* and *S. epidermidis* are both 1,184 amino acids in length, sharing 100% pairwise identity in the alignment of the RRDR, N-terminal cluster and Cluster II regions [169] (Figure 1.4B). Overall, the RpoB translations between the two species share 98.0% pairwise identity and 99.4% pairwise positive identity. Where pairwise identity refers to directly matching amino acid residues, whereas pairwise positive identity accounts for conservative substitutions in which the physio-chemical properties of amino acid resides are preserved [170]. Although this close consensus in RpoB translations between the species suggests that it is reasonable to assume that both interact with rifampicin in the same manner and generate the same resistance mutations, review of the literature suggests otherwise.

1.10.8.1 Rifampicin resistance mutations in S. epidermidis

A limited number of studies have investigated the diversity of rifampicin resistance mutations in *S. epidermidis* [168,169] (Figure 1.4B). Using two rifampicin susceptible (≤ 0.06 mg/L) clinical *S. epidermidis* isolates, reference strain RP62a (ST10) and IRDL-8883 (ST5; from a prosthetic joint infection), Wi *et al*., characterised the evolution of rifampicin resistance when the strains were subject to rifampicin selective pressure both *in vitro* and *in vivo* in a rat chronic foreign body model [169]. All mutations generated occurred in cluster I of RpoB with five *in vitro* (D471N, D471G, D471V, H481Y and S486Y) and three *in vivo* (Q468K, H481Y and S486F) substitutions generated. The D471N and D471G substitutions were found to confer only low-level rifampicin resistance, both with MICs of 0.25 mg/L. The D471V substitution was associated with an MIC of 32 mg/L and the remaining four mutants (H481Y, Q468K, S486Y and S486F) all conferred high level rifampicin resistance of >256 mg/L [169].

The largest study characterised 33 *S. epidermidis* isolates obtained during revision surgeries for prosthetic joint infections, performed at two Swedish hospitals [168]. Of the 33 isolates, 39% (n = 13) were rifampicin resistant, with the following amino acid substitutions identified: D471E + I527M (n = 9; MIC >32 mg/L), S464P + A534V (n = 2; MIC >32 mg/L), H481Y (n = 1; MIC >32 mg/L), and S464P (n = 1; MIC <6 mg/L).

1.10.8.2 Rifampicin resistance mutations in S. aureus

The rifampicin resistance mutations in *S. aureus* have been extensively catalogued [165,166]. A recent study of 7,099 *S. aureus* genomes demonstrated that eight RpoB mutations (H481N, L466S, A477D, D471G, H481Y, S464P, S529P, S529L and Q468K) accounted for 93% (469/505) of rifampicin resistance mutations identified [166]. No N-terminal regions mutations were detected, while a solitary D471N substitution identified in three isolates were thought to represent a new and rare rifampicin resistance mutation [166]. Unlike *S. epidermidis*, no D471E nor any I527 residue substitutions were observed, suggesting that the D471E plus I527M dual mutation prevalent in the clinical *S. epidermidis* isolates reported by Hellmark *et al*. [168], are not common in *S. aureus* despite the two species sharing near identical RpoB translations.

1.10.9 Rates of Rifampicin Resistance

Based on association with ecological and geological events, and inferred from eukaryotic molecular clock models, the long-term (over millions of years) genome-wide mutation rate for bacteria are estimated as 10^{-9} to 10^{-10} substitutions per year [171-173]. Depending on the organism studied and methodology, resistance mutation rates to rifamycins including rifampicin have been estimated at 10^{-10} to 10^{-7} [174], with a mutation rate of ~10^{-8} observed

in most organisms [157]. Using 0.016 to 32 ml/L of rifampicin, *S. epidermidis* strain RP62a was reported to have an *in vitro* rifampicin resistance frequency of 10^{-7} [175].

It has been suggested that mutation rates based on laboratory culture are elevated and do not reflect the actual, long-term rate of bacterial mutation [171]. *In vivo* bacteria are subject to environmental and genetic stresses such as those imposed by the host immune system, exposure to antibiotics and competing co-colonising pathogens which can influence mutation rates. Irrespective of laboratory derived estimates of mutation rate, clinically significant rifampicin resistance occurs in the hospital environment, with evidence suggesting a single dose of rifampicin can generate persistent colonisation with rifampicin resistant *S. epidermidis* [176]. A 1983 study by Archer *et al*. investigated the effect of antibiotic prophylaxis on the staphylococcal flora of cardiac surgery patients [176]. A single dose of rifampicin administered two hours preoperatively in conjunction with nafcillin was observed to induce newly detected rifampicin resistance in perianal CoNS (predominantly *S. epidermidis*) sampled in 72% of 61 patients whom underwent cardiac valve surgery by day seven postoperatively, one of whom developed prosthetic valve endocarditis with rifampicin resistant *S. epidermidis* [176]. Of the 61 patients isolates, over 80% were also found to be methicillin resistant. Since preoperative methicillin resistance was not screened for it is unclear if the prophylaxis administered was essentially rifampicin monotherapy. Of note, results markedly differed depending on the screening site. Newly developed rifampicin resistance was detected in only 5% of skin swabs from the nares and 3% of chest swabs, taken from the same 61 patients. The authors attributed this observation to the predominantly biliary excretion of rifampicin, which would lead to the ongoing presence of bioactive rifampicin metabolites in stool following administration [176]. Of the 14 patients for whom follow-up swabs were collected one to

two months postoperatively, 79% were found to be persistently colonised with rifampicin resistant CoNS on perianal screening. Furthermore, although chest swabs from these 14 patients demonstrated no rifampicin resistant CoNS at the time of discharge, repeat screening at one to two months found 57% had acquired rifampicin resistant CoNS on the skin of their chests [176], suggesting the spread of rifampicin resistant CoNS from the perianal region to other body sites. When screening was extended four to six months post-discharge, only 14% of perianal and 4% of chest swabs demonstrated the persistence of rifampicin resistant CoNS [176].

Without molecular epidemiology, it is unclear if the rifampicin resistant isolates identified in this study were the result of the *de novo* generation of new rifampicin resistant mutants in response to the selective pressure of rifampicin exposure, or colonisation and persistence of hospital dominant strains resulting from exposure during admission in the surgical ICU. However, despite no documented exposure to rifampicin, 19% (10/53) of patients administered single agent cephazolin as surgical prophylaxis became colonised with rifampicin resistant CoNS [176]. Of note, no rifampicin resistant strains were detected on preoperative screening of these patients. These rifampicin resistant isolates were typically also resistant to gentamicin and methicillin, suggesting the possible presence of a rifampicin resistant, multidrug resistant CoNS clone that could be selected for by use of unrelated β-lactam antibiotics to which the clone also exhibited resistance.

The rifampicin plus nafcillin surgical prophylaxis effectively eradicated *S. aureus* in 89% (16/18) of preoperatively colonised patients [176], a significantly higher eradication rate ($P < 0.001$) compared to single agent cephazolin, that eradicated *S. aureus* in only 48% (10/21) of patients [176]. While surgical prophylaxis is typically targeted towards *S.*

aureus, this study highlights the potential to generate increasing resistance in colonising CoNS species.

1.10.10 Biofilm activity of rifampicin

The only drug class demonstrated to be efficacious in the elimination of staphylococcal biofilms [89,97,177,178], rifampicin is frequently employed for both the treatment and prophylaxis of staphylococcal foreign body infections. *In vitro* studies using biofilm forming *S. epidermidis* strain, RP62a, identified rifampicin as the only antibiotic with activity against both replicating and non-replicating cells [178]. Furthermore, the bactericidal effect for rifampicin against non-growing cells was demonstrated to almost equal that of *S. epidermidis* growing in log phase. [178].

In vivo studies by Widmer *et al*. investigated the efficacy of a selection of antibiotics (vancomycin, daptomycin, teicoplanin, ciprofloxacin, rifampicin and netilmicin) against two clinical, methicillin-resistant, *S. epidermidis* strains: B3972, a known weak slime producer (presumably from the presence of PIA); and biofilm producing reference isolate RP62a [97]. Using a guinea pig tissue cage model infected with a 10^4 CFU local inoculum of *S. epidermidis*, rifampicin (25 mg/kg intraperitoneally 12 hourly for 4 days; 8 doses total) was the only drug that demonstrated sterilisation of 100% of implants (n = 12), typically within 48 hours of starting of treatment [97]. This effect was noted even when initiation of rifampicin was delayed until seven days post bacterial inoculation, with sterilisation of 12 of 12 implants when followed up to day 16 post-infection [97].

When lower dose rifampicin was used (7.5 mg/kg intraperitoneally 12 hourly for 4 days) bacterial counts decreased to below the limit of detection, but 25% of infections relapsed with the same rifampicin-sensitive strain. However, when low dose rifampicin

(7.5 mg/kg intraperitoneally 12 hourly) was combined with ciprofloxacin (10 mg/kg intraperitoneally 12 hourly) 100% of infections were cleared (n = 12) [97]. Of note, despite testing susceptible by standard MIC, single agent ciprofloxacin failed to eradicate any *S. epidermidis* foreign body infections in a guinea pig tissue-cage model (0/12). This is was attributed to a demonstrated lack of activity on stationary-phase and adherent *S. epidermidis* [97].

Like all animal models, it is debatable how well these studies reflect human disease and whether their findings can be meaningfully extrapolated to clinical management. For example, it is known that guinea pig models do not support certain antibiotics, such as β-lactams and clindamycin; and significant weight loss occurs if antibiotics are administered for more than four days [179]. Other animal models used to assess the efficacy of rifampicin for the treatment of staphylococcal foreign body infection or implant associated osteomyelitis include: rat chronic tissue cage [180]; rat wire-associated tibia osteomyelitis [11,181]; mouse knee joint infection with implanted wire [182,183]; and rabbit knee prosthesis [184]. Collectively, these studies suggested that rifampicin based combination treatments are most efficacious under the following experimental conditions: low bacterial inoculum; young biofilm; and prolonged treatment duration [179].

These findings in *S. epidermidis* in conjunction with that of their *S. aureus*-based studies [89,94,177], led the same group to trial rifampicin-based treatment combinations in staphylococcal orthopaedic implant related infections [98,185]. When combined with appropriate surgical intervention, rifampicin-based regimens, particularly those including a quinolone [97], enabled implant retention in early orthopaedic infections, with cure rates of up to 80 – 90% [85,98,185]. With similar success rates reported by other groups when implementing rifampicin [186,187]. These studies in early orthopaedic infections are the

only established exclusions to the surgical dogma that removal of an infected prosthesis is required for cure, as sterilisation cannot be achieved. Extrapolating from these studies, rifampicin-based regimens have become a cornerstone in the treatment of device related infections beyond orthopaedic manifestations and become incorporated into a number of specialty guidelines [34,38,102-105]. However, clinical concerns have been raised regarding lack of good quality evidence to support these guidelines and the potential to generate rifampicin resistance in colonising staphylococci and mycobacteria [188].

1.10.11 Rifampicin in combination therapy

Due to good tissue penetration and biofilm activity, rifampicin is frequently utilised in both the prevention (such as antibiotic impregnated venous [189] and CNS catheters [190], vascular grafts [191-193]), and treatment [34,38,97,98,102-105] of device associated infections. Since monotherapy is known to result in the rapid generation of rifampicin resistance [97,168,176,177,194] combination therapy is recommended.

1.10.11.1 Rifampicin and vancomycin

Despite a lack of robust supporting evidence, the adjunctive use of rifampicin with vancomycin for the treatment of staphylococci has pervaded a number of international specialty guidelines, particularly in the setting of prosthetic infections [34,38,102-105]. The combination a result of vancomycin being the first-line agent for the treatment of methicillin resistant staphylococci and the demonstrated ability of rifampicin to penetrate staphylococcal biofilms.

It is generally assumed that clinical outcomes for treatment approaches can be extrapolated interchangeably between staphylococcal species. Accordingly, a pair of

retrospective studies from 1983, in which adjunctive rifampicin was used for the treatment of methicillin-resistant *S. epidermidis* PVE [31,195] have come to inform current recommendations in the *Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus aureus Infections in Adults and Children* [103], and international cardiology guidelines for the treatment of staphylococcal infective endocarditis [104,105] and implantable cardiac electronic device infections [34]. Current guidelines for methicillin-resistant staphylococci advise the addition of rifampicin to: vancomycin plus gentamicin for the treatment of PVE [103-105]; vancomycin or daptomycin for the treatment of osteomyelitis/PJI [86,103]; vancomycin for CNS infections [103].

Vancomycin plus rifampicin therapy and the generation of rifampicin resistance

The literature regarding the combination of vancomycin and rifampicin for the treatment of staphylococci has been contradictory, with reports of both synergy [180,182] and antagonism [196]. Furthermore, concerns have been raised that poor penetration of vancomycin into certain tissues such as the brain and bone [197] relative to rifampicin, may result in rifampicin monotherapy and generate resistance to both agents. While it is generally assumed that unrelated drug classes should cross-protect against the development of resistance when combined, numerous animal trials [98,180,184], case series [198-200] and clinical studies [196,201,202] have reported treatment failure or the evolution of rifampicin resistance while patients were on the combination of vancomycin and rifampicin for the treatment of staphylococcal infections. Such that the validity of the combination was questioned as early as 1985, with the comment “There is neither microbiologic support for the use of these two agents in the hopes of obtaining synergy

nor any clinical evidence that vancomycin can inhibit the development of resistance to rifampicin" [199].

The most compelling evidence against the combination comes from three clinical studies. The first compared treatment regimens for *S. aureus* osteomyelitis and observed that patients administered vancomycin plus rifampicin did significantly worse with a 43% cure rate (3/7) compared to those whom received other rifampicin-based combinations, in which 84% (27/32) were cured ($p<0.02$) [196]. The second was a study that compared vancomycin plus rifampicin to vancomycin alone for the treatment of MRSA pneumonia in the ICU [201]. Fraught with clinical confounders and design issues, such as targeting a much lower than recommended vancomycin trough value, that prompt questions about its ability to address the planned purpose, the value of the study comes from unintended observations. Reported in the minor findings, rifampicin resistance developed in 34.1% (14/41) of patients who received dual therapy. Furthermore, microbiological eradication rate was significantly lower in patients that developed rifampicin resistance (38.5%) compared to those that did not (88.5%) ($p = 0.02$). Clinical cure rate at 14 days was no different between the subgroup that developed rifampicin resistance compared to the one that did not, both being poor at 50% and 55.6%, respectively. Which, as they performed better than the vancomycin alone cohort (clinical cure rate of 31%, 13/34), led the study to conclude that combination therapy seems more effective than vancomycin alone [201]. Although not reported, it would have been interesting to know the vancomycin MICs post therapy, rifampicin resistance rates beyond day 14, and clinical evolution of disease in the patients that were on dual therapy. The 60-day mortality of 26.8% (11/41) for the dual treatment group was significantly lower than 50% (21/42) for the vancomycin only group ($p = 0.042$), an observation which the authors were unable to account for [201]. Confounders aside, in context of current knowledge one could speculate that the

combination of rifampicin resistance and sub-therapeutic vancomycin within the lung environment (known to be particularly poorly penetrated at 5 – 41% of serum vancomycin levels [203]) may have selected for hVISA, with a shift towards a persistent phenotype with decreased virulence.

The final study was not any more convincing. A retrospective cohort analysis of the addition of rifampicin to standard therapy (vancomycin plus gentamicin 81%, nafcillin 17%, daptomycin 2%) for *S. aureus* native valve endocarditis reported 56% developed rifampicin resistance before clearance of bacteraemia. The study also found that patients who received rifampicin had a longer duration of bacteraemia (5.2 versus 2.1 days; $p < 0.001$) and higher mortality (21% versus 5%; $p = 0.048$) [202]. Hence guideline recommendations to not add rifampicin prior to clearance of bacteraemia [103].

The role for adjunct rifampicin for the clearance of *S. aureus* bacteraemia was clearly disproven in the recent multicentre, randomised, double-blind, placebo controlled, *Adjunctive rifampicin for S. aureus bacteraemia (ARREST)* trial [204]. Comparing adjunctive rifampicin (600 mg or 900 mg daily, according to weight; n = 370) to an identical placebo (n = 388), administered for two weeks together with standard antibiotic therapy, the study demonstrated that rifampicin had no significant effect on any of the composite primary or secondary measures of mortality, duration of bacteraemia or developing rifampicin resistance. Although a significant number of patients whom received rifampicin developed drug side-effects. It should be noted that the majority of isolates (82%, 619/758) were methicillin susceptible, with only 6% (47/758) MRSA [204], and it was not specified if the two patients whom received rifampicin and developed rifampicin-resistant *S. aureus* bacteraemias had received vancomycin as their standard therapy. While this study refutes previous animal studies that have suggested rifampicin

enhances blood killing of *S. aureus*, therefore reducing dissemination and death [89,94]; it did demonstrate a small but significant reduction in disease recurrence, suggesting that rifampicin does enhance the sterilisation of deep infective foci [204].

Rifampicin resistance mutations and vancomycin cross resistance

In *S. aureus* gene, a single point mutation within the rifampicin resistance determining region of *rpoB* is sufficient to produce both high-level rifampicin resistance and an hVISA phenotype [148,150,206,206]. Furthermore, *rpoB* mutations have been associated with dual heteroresistance to both daptomycin and vancomycin [207]. Although not universally present in VISA strains, the frequency at which RpoB mutations play a contributory role was highlighted in a genome wide association study by Alam *et al*. [208]. Attempting to identify genetic predictors of hVISA and VISA, 49 VSSA and 26 hVISA/VISA strains were analysed. Of 55,977 SNPs identified, only those causing substitutions at a sole locus, RpoB H481, were significantly associated ($p = 8.8 \times 10^{-8}$) with an hVISA/VISA phenotype [208]. Whether or not a similar association between rifampicin resistance mutations in RpoB and reduced glycopeptide susceptibility occurs in *S. epidermdis* or other CoNS is yet to be determined.

1.10.12 Consequences of rifampicin resistance mutations beyond rifampicin resistance

Since *rpoB* is essential for transcription, mutations within this gene are likely to have multiple physiological effects beyond just rifampicin resistance. In *E. coli* RpoB substitutions D516V and I572F have been demonstrated to have a high frequency of positive epistasis and are predicted to segregate in natural populations [209]. Furthermore, both these sites were associated with compensatory mutations in unrelated alleles that

conferred resistance to other antibiotics (such as quinolones and streptomycin), the fitness of isolates possessing mutations in both alleles was greater than that of isolates with only one, indicating that the effect of dual allele mutations were beneficial beyond the acquisition of drug resistance [209]. It was therefore hypothesised that even in the absence of persistent selective antibiotic pressure a multidrug resistant phenotype would be maintained, with potential acquisition of further compensatory mutations. Favouring this theory are the observations from experimental evolutionary studies demonstrating that compensatory mutations that abrogate the cost of acquired antibiotic resistance are more likely to occur than revertant mutations that restore drug susceptibility [165,210]. If the evolutionary benefit of the D516V or I572F RpoB substitutions demonstrated in *E. coli* hold true, then similar genetic interactions would be predicted to occur in other organisms [209]. How these observations extend to *S. epidermidis* at the equivalent RpoB positions of D471 and I527, which appear to be prevalent in the limited number of clinical isolates characterised so far, is yet to be investigated.

Should specific RpoB substitutions in *S. epidermidis* be demonstrated to cause co-resistance to vancomycin, as they do in *S. aureus* [148,150,206,206], then exposure to either vancomycin or rifampicin would select for isolates possessing these substitutions. In view of the prevalent use of vancomycin in the hospital environment, and trends towards addition of rifampicin for its biofilm penetrating properties in device associated infections, determining if the same detrimental relationship exists between *rpoB* mutations and the generation of a heterogeneous vancomycin intermediate phenotype in *S. epidermidis* is of particular importance to guide future treatment recommendations.

1.11 Genetic manipulation of *S. epidermidis*

Historically, *S. epidermidis* has proven to be genetically intractable with the exception of a few isolates with limited clinical relevance, such as ST86 strain, 1457 [58] and ST10 strain, RP62a [55,211]. Notably, no ST2 isolates have been successfully manipulated. Thereby leaving a void in the scientific knowledge regarding the molecular biology of the dominant hospital *S. epidermidis* strains.

1.11.1 Restriction modification systems

Restriction modification (RM) systems are proposed to have evolved as a form of bacterial immunity that targets and degrades incoming DNA from viruses and other foreign donors [212]. Of the four different types of RM systems, only three are found in staphylococci [213]. Type I and IV pose significant barriers to the genetic manipulation of staphylococci [211], the former determining the presence of adenine methylation of host DNA, the latter cytosine methylation [213]. Unless methylated at appropriate sites, DNA is recognised as foreign and degraded [214]. Type I RM systems are comprised of three host specificity of DNA (*hsd*) genes that encode a specificity protein (HsdS), modification protein (HsdM) and restriction endonuclease (HsdR). Together these function as a single protein complex in which: HsdS determines the DNA target recognition motif (TRM) that is methylated by HsdM; while HsdR cleaves unmodified and non-self-modified DNA [215]. Type IV RM systems consist of a single restriction endonuclease named *sepiRPI* in *S. epidermidis* and *sauUSI* in *S. aureus* [211]. Plasmid artificial modification (PAM) is a method to overcome the barrier imposed by RM systems, whereby plasmid DNA is passaged through a cytosine methylation deficient *E. coli* host (DC10B) constructed to heterologously express the *hsdMS* of a staphylococcal strain of interest, mimicking the

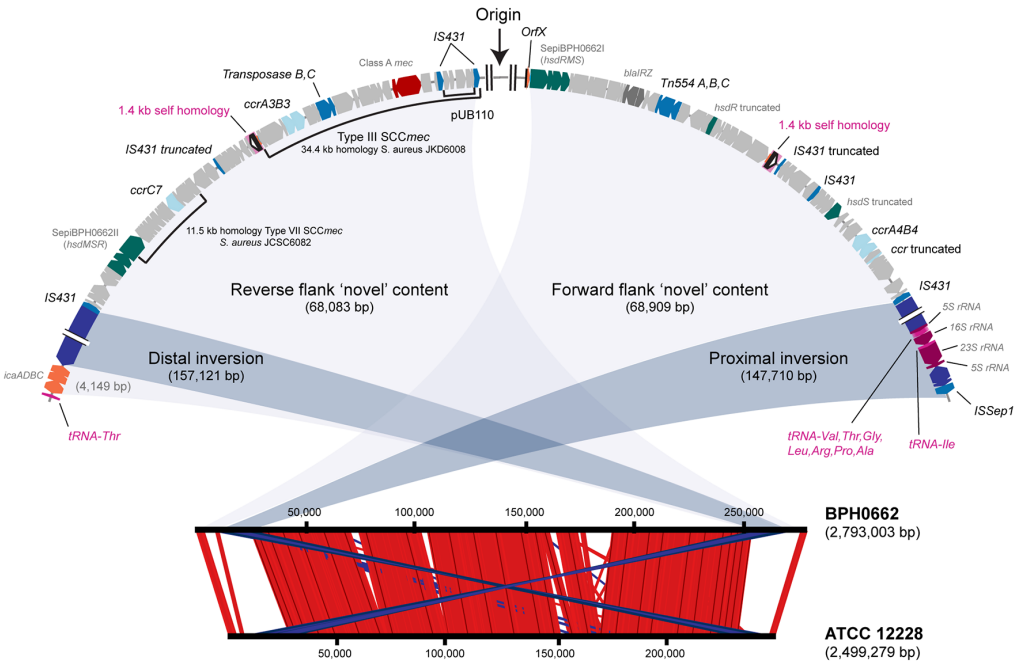
DNA methylation of the target strain enabling transformation and subsequent genetic manipulation [216,217].

Much of the current molecular knowledge about *S. aureus* was determined through the genetic manipulation of laboratory strain RN4220 that was derived from the clinically significant, hospital dominant, CC8 lineage. Its parental *S. aureus* strain NCTC8325 was cured of three prophages then subjected to UV and chemical mutagenesis [218] that rendered the isolate amenable to plasmid transformation through the introduction of a non-sense mutation in *hsdR* [219]. By complementing the *hsdR* mutation back to WT, Waldron and Lindsay demonstrated the type I RM system to be a barrier to horizontal gene transfer and the major mechanism by which *S. aureus* controls the uptake of foreign DNA [220]. However, despite inactivation of *hsdR*, subsequent studies demonstrated that some *S. aureus* isolates were refractory to transformation with plasmid isolated from cloning strains of *E. coli* (such as K-12), suggesting the presence of at least one other barrier [221,222]. In 2010, Corvaglia *et al.*, identified the second barrier as a type IV system responsible for the detection and degradation of cytosine methylated DNA, which is present on *E. coli* K-12 DNA [222]. There are currently no known *S. epidermidis* isolates representing the dominant hospital lineage (ST2) that are amenable to manipulation. Identification of such an isolate, or a development of a method for the specific manipulation of *S. epidermidis* would be critical in advancing the study of the species.

1.12 Project aims

The following chapters adhere to a narrative arc that was determined by the experimental outcomes. As such, the specific aims for each chapter shall be outlined in its introduction in the context of the work preceding. Using functional genomics, the primary aims of this thesis were to advance scientific and clinical understanding of *S. epidermidis*, with respect to global molecular epidemiology; antibiotic resistance mechanisms; and treatment recommendations specifically pertaining to the commonly used combination of vancomycin and rifampicin. To address these objectives, the genetic manipulation of clinical lineages of *S. epidermidis* that had hitherto proven refractory was required. Therefore, this project also sought to establish an efficient methodology for the genetic manipulation of any *S. epidermidis* isolate of interest, and identification of a candidate laboratory strain that was both clinically relevant, and tractable.

CHAPTER 2. Functional analysis of the first complete genome sequence of a multidrug resistant sequence type 2 *Staphylococcus epidermidis*



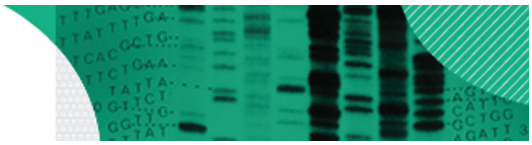
Reproduced from Lee *et al.* [223]

2.1 Introduction

In this chapter, a clinical case of severe *S. epidermidis* CNS infection caused by a hospital-adapted, multidrug-resistant ST2 strain, BPH0662, is presented. Although the isolate tested susceptible to vancomycin by standard diagnostic methods, the patient failed vancomycin therapy. In order to elucidate the mechanisms underlying this treatment failure and better characterise ST2 *S. epidermidis*, this work had the following objectives: 1. Establish the first ST2 *S. epidermidis* reference genome; 2. Demonstrate that BPH0662 is representative of international clinical ST2 *S. epidermidis* isolates; 3. Correlate the BPH0662 *in silico* resistome with phenotype to determine resistance mechanisms; 4. Characterise the ST2 *S. epidermidis* methylome. This work was published in Microbial Genomics. Supplementary materials for the publication are included in 2.3 Supplementary Appendix.

2.2 Published manuscript

MICROBIAL GENOMICS
Bases to Biology



Research Paper

Functional analysis of the first complete genome sequence of a multidrug resistant sequence type 2 *Staphylococcus epidermidis*

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Staphylococcus epidermidis is a significant opportunistic pathogen of humans. The ST2 lineage is frequently multidrug-resistant and accounts for most of the clinical disease worldwide. However, there are no publically available, closed ST2 genomes and pathogenesis studies have not focused on these strains. We report the complete genome and methylome of BPH0662, a multidrug-resistant, hospital-adapted, ST2 *S. epidermidis*, and describe the correlation between resistome and phenotype, as well as demonstrate its relationship to publically available, international ST2 isolates. Furthermore, we delineate the methylome determined by the two type I restriction modification systems present in BPH0662 through heterologous expression in *Escherichia coli*, allowing the assignment of each system to its corresponding target recognition motif. As the first, to our knowledge, complete ST2 *S. epidermidis* genome, BPH0662 provides a valuable reference for future genomic studies of this clinically relevant lineage. Defining the methylome and the construction of these *E. coli* hosts provides the foundation for the development of molecular tools to bypass restriction modification systems in this lineage that has hitherto proven intractable.

Keywords: *Staphylococcus epidermidis*; antibiotic resistance; comparative genomics; methylome.

Abbreviations: BMD, broth microdilution; ccr, cassette chromosome recombinase; CoNS, coagulase negative staphylococci; CSF, cerebrospinal fluid; EVD, external ventricular drain; *hsdMS1*, SepiBPH0662I *hsdMS* genes; *hsdMS2*, SepiBPH0662II *hsdMS* genes; IS, insertion sequence; MET, macromethod Etest; MLST, multilocus sequence type; PAM, plasmid artificial modification; PAP, population analysis profile; RM, restriction modification; SCC, staphylococcal cassette chromosome; SMRT, single molecule real-time; ST, sequence type.

Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files.

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Data Summary

All raw sequence data (reads and assembled genomes) for the *S. epidermidis* genomes analysed in this publication are publically available in the European Nucleotide Archive (ENA) under study accession numbers PRJEB12090 and PRJEB13975.

http://www.ebi.ac.uk/ena/data/view/PRJEB12090

1. *S. epidermidis* BPH0662 Illumina reads (ERS1019848)

http://www.ebi.ac.uk/ena/data/view/ERS1019848

2. *S. epidermidis* BPH0663 Illumina reads (ERS1019849)

http://www.ebi.ac.uk/ena/data/view/ERS1019849

http://www.ebi.ac.uk/ena/data/view/PRJEB13975

3. *S. epidermidis* BPH0662 PacBio reads (ERS1153932)

http://www.ebi.ac.uk/ena/data/view/ERS1153932

4. *E. coli* DC10B-MS1 PacBio reads (ERS1153933)

http://www.ebi.ac.uk/ena/data/view/ERS1153933

5. *E. coli* DC10B-MS2 PacBio reads (ERS1153934)

http://www.ebi.ac.uk/ena/data/view/ERS1153934

6. *S. epidermidis* BPH0662 complete genome assembly (GCA_900086615.1)

http://www.ebi.ac.uk/ena/data/view/GCA_900086615

Impact Statement

Staphylococcus epidermidis is a major nosocomial pathogen in humans, responsible for a significant proportion of implant- and device-associated infections that globally pose a significant burden on health-care systems. One multilocus sequence type, ST2, which is often multidrug-resistant, dominates *S. epidermidis* hospital-associated infections. However, the genetic structure and content of this clone are poorly understood. Additionally, because of limited understanding of the genetic barriers to the transformation of ST2 isolates, no molecular tools are available to manipulate and study this clone. Through in-depth genomic analysis of ST2 *S. epidermidis* we provide important insights into the genome structure, resistance mechanisms and barriers to DNA transformation and their relationship to other *S. epidermidis* clones. The generation of *E. coli* strains expressing ST2 *S. epidermidis* adenine methylation profiles provides the basis for the development of tools to genetically manipulate this globally dominant clone in the laboratory.

Introduction

Staphylococcus epidermidis is a significant nosocomial pathogen, particularly in the setting of high-acuity medicine and prosthetic devices, where the presence of a foreign body provides a platform for bacterial colonisation. Identified by the Centers for Disease Control and Prevention as the leading cause of central-line-associated bloodstream infections, second ranked cause of surgical site infections and third most reported pathogen for hospital-acquired infections (Sievert *et al.*, 2013), *S. epidermidis* and other coagulase-negative staphylococci (CoNS) are estimated to cost $2 billion a year in the USA alone (Otto, 2009). Despite this significant burden imposed on healthcare systems, relatively little is understood about the mechanisms of pathogenesis and optimal treatment of *S. epidermidis*, with many assumptions extrapolated from *Staphylococcus aureus*-based studies.

S. epidermidis is the most genetically diverse species within the genus *Staphylococcus* (Becker *et al.*, 2014), with diversity also reported within the colonising strains of individual human hosts (Conlan *et al.*, 2012). In spite of this, hospital-based investigations utilising pulsed-field gel electrophoresis or multilocus sequence typing (MLST) typically demonstrate the predominance of one or two multidrug-resistant clones of *S. epidermidis* within the institutions studied (Gordon *et al.*, 2012; Krediet *et al.*, 2004; Widerström *et al.*, 2012). A single lineage, clonal complex 2, for which ST2 is the founder, accounts for 74 % of nosocomial isolates internationally (Miragaia *et al.*, 2007). The global dissemination of the ST2 lineage suggests its successful adaptation to the hospital environment in which favourable circumstances enable opportunistic infections. Knowledge of the genomics of ST2 strains has been limited by the lack of a closed ST2 reference genome, and the inability to genetically manipulate this lineage. The dearth of dedicated molecular tools and tractable clinical *S. epidermidis* strains has meant that pathogenesis studies have been performed on isolates that do not represent this clinically relevant clone that dominates worldwide.

The proportion of methicillin resistance in *S. epidermidis* has been reported to be as high as 92 % in some institutions (Krediet *et al.*, 2004), and is frequently associated with co-resistance to other antibiotic classes (Conlan *et al.*, 2012; Mendes *et al.*, 2012). Due to limited treatment options, vancomycin, a glycopeptide antibiotic considered one of the last-line agents for the treatment of staphylococci, is often utilised for serious infections.

The phenomenon of vancomycin intermediate heteroresistance is well described in *S. aureus* and is characterised by the presence of bacterial subpopulations capable of growth within the intermediate range despite testing as vancomycin-susceptible by standard laboratory methods. Such isolates are recognised as precursors to vancomycin intermediate *S. aureus* (VISA) and are associated with treatment failure (Tenover & Moellering, 2007). Unlike *S. aureus*, the definition and clinical implications of heterogenous vancomycin resistance in *S. epidermidis* is poorly understood. A limited number of studies have described the phenomena in *S. epidermidis* specifically (Gazzola *et al.*, 2013; Sieradzki *et al.*, 1999) or CoNS in general (Ma *et al.*,

2011; Nakipoglu *et al.*, 2005; Sieradzki *et al.*, 1998), however the mechanisms behind this resistance are unknown.

Restriction–modification (RM) systems are proposed to have evolved as a form of bacterial immunity that targets and degrades incoming DNA from viruses and other foreign donors (Casadesús & Low, 2006). Type I and type IV RM systems in particular, present strong barriers to the exchange of DNA in both *S. epidermidis* and *S. aureus* (Monk *et al.*, 2012). Type I RM systems are comprised of three host specificity of DNA (*hsd*) genes, that encode a specificity protein (HsdS), a modification protein (HsdM) and a restriction endonuclease (HsdR). The presence of adenine methylation on DNA sequences, determined by HsdS, termed target recognition motifs (TRMs), inhibits the restriction endonuclease complex. Unless methylated at appropriate sites, DNA is recognised as foreign and restricted (Murray, 2000). The type IV RM system of *S. epidermidis* consists of a single restriction endonuclease (formerly *mcrR*, now renamed *SepRPI*) that recognises cytosine-methylated DNA (Monk *et al.*, 2012). Plasmid artificial modification (PAM) is a means to overcome the barrier imposed by these RM systems, whereby vectors are constructed to express *hsdMS* genes that mimic the DNA methylation pattern of the bacterium into which they are transformed. A method of PAM in which *hsdMS* genes from *S. aureus* strains of interest were chromosomally integrated into a DC10B *E. coli* background, deficient of cytosine methylation, was recently described (Monk *et al.*, 2015). Plasmids isolated from the resulting *E. coli* mutants were capable of bypassing both type I and type IV RM systems of *S. aureus* strains from which their *hsdMS* genes were cloned.

In 2012, a case of persistent, post-neurosurgical infection with a strain of *S. epidermidis* exhibiting multidrug resistance, manifesting as a cerebral abscess associated with an external ventricular drain (EVD), occurred at our institution. Other than a single intraoperative dose of prophylactic cephazolin, the patient had no prior history of antibiotic therapy or relevant hospitalisation. Resistant to commonly encountered agents including flucloxacillin, erythromycin, clindamycin and cotrimoxazole; it was of concern that the isolate also demonstrated resistance to rifampicin, fusidic acid, ciprofloxacin and teicoplanin (a glycopeptide). Extended glycopeptide-susceptibility testing performed in the context of failing vancomycin therapy, showed that the isolate was vancomycin heteroresistant, prompting investigations to further characterise this hospital adapted strain.

Here we use Pacific Biosciences single molecule real-time (SMRT) sequencing to describe the first complete ST2 *S. epidermidis* genome sequence and its methylome, and demonstrate its function as an improved reference for the bioinformatic analysis of international strains belonging to this clinically relevant lineage.

Methods

Media and reagents. *S. epidermidis* and *S. aureus* were routinely cultured at 37 °C in brain heart infusion (BHI) broth (Difco). *E. coli* was routinely cultured in L broth (1 % tryptone, 0.5 % yeast extract, 0.5 % NaCl). Broth microdilution (BMD) MICs were performed in cation-adjusted Mueller–Hinton (Difco) medium. For growth on agar, BHI or L broth were solidified with 1.5 % agar, to yield BHIA and L agar, respectively. The following antibiotics were purchased from Sigma Aldrich and used at the specified concentrations: chloramphenicol (Cm) 15 μg ml^{-1} in *E. coli*; ampicillin (Amp) 100 μg ml^{-1} in *E. coli*; kanamycin (Kan) 50 μg ml^{-1} in *E. coli*. The following antibiotics were used at variable concentrations for susceptibility testing: rifampicin (Rif) (Sigma Aldrich); vancomycin (Hospira).

Oligonucleotides were purchased from Integrated DNA Technologies and are listed in Table 1. Genomic DNA for routine use was isolated with the DNeasy Blood and Tissue Kit (Qiagen). To weaken the cell wall of *S. epidermidis* prior to DNA extraction, harvested cells were washed with PBS, lysostaphin (Ambi) was added to the Gram-positive lysis buffer (final concentration 100 μg ml^{-1}) and incubated at 37 °C for 30 min. Genomic DNA for SMRT sequencing was isolated using the Qiagen Genomic-tip 100 G (Qiagen). Plasmids were purified with a QIAprep Spin Miniprep Kit (Qiagen). PCR products and gel extractions were purified using a QIAquick Gel Extraction Kit (Qiagen). Restriction enzymes and Phusion DNA polymerase were purchased from New England Biolabs. Phire Hotstart DNA polymerase was purchased from Thermofisher. Colony PCR was performed as previously described (Monk *et al.*, 2012).

Bacterial isolates. Bacterial strains used in this study are listed in Table 1. Phenotypic comparator strains consisted of three reference strains: *S. epidermidis* ATCC 12228, *S. aureus* ATCC 700698 [Mu3] and ATCC 700699 [Mu50].

Antibiotic susceptibility testing. Vitek 2 (bioMerieux) susceptibilities were tested as per the manufacturer's instructions. BMD MICs for vancomycin and rifampicin were determined as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012). Extended glycopeptide susceptibilities were determined by the macromethod Etest (MET) with vancomycin and teicoplanin Etest strips (bioMerieux) using a 2.0 McFarland inoculum and prolonged incubation time of 48 h; vancomycin population analysis profiles (PAPs) were performed as previously described (Wootton *et al.*, 2001). With the exception of Vitek 2 and Etests, all antibiotic-susceptibility testing of strains was performed in triplicate.

Genome sequencing. Sequencing of BPH0662 and BPH0663 was performed using the Illumina MiSeq platform with Nextera XT libraries constructed as per the manufacturer's instructions. SMRT sequencing on a PacBio *RS* instrument with subsequent *de novo* assembly using HGAP2 algorithm, and base modification and motif detection for methylome analysis using SMRT Analysis v1.3.1 was performed for *S. epidermidis* BPH0662. *E. coli* mutants DC10B-MS1 and DC10B-MS2 were assembled and analysed as previously described (Monk *et al.*, 2015). RP62a was used as

Table 1. Strains, plasmids and oligonucleotides used in this study

Bacterial strain, plasmid or oligonucleotide	Description	Reference or source
E. coli strains		
DC10B	DH10B with Δ*dcm* mutation	Monk *et al.* (2015)
DC10B-MS1	DC10B with SepiBPH0662I *hsdMS* integrated between *ybbD* and *ylbG*	This study
DC10B-MS2	DC10B with SepiBPH0662II *hsdMS* integrated between *essQ* and *cspB*	This study
S. epidermidis strains		
ATCC 12228	Non-clinical reference strain	Zhang *et al.* (2003)
BPH0662	Clinical strain. Index patient, isolated day 12	This study
BPH0663	Clinical strain. Index patient, isolated day 23	This study
S. aureus strains		
Mu3	Heteroresistant vancomycin intermediate *S. aureus* reference strain	Hiramatsu *et al.* (1997a)
Mu50	Vancomycin intermediate *S. aureus* reference strain	Hiramatsu *et al.* (1997b)
Plasmids		
pKD4	Plasmid for amplification of FRT-*kan*-FRT; Amp^R, Kan^R	Datsenko & Wanner (2000)
pKD46	*E. coli* temperature-sensitive plasmid containing λ red recombinase genes under the control of an arabinose-inducible promoter; Amp^R	Datsenko & Wanner (2000)
pCP20	*E. coli* temperature-sensitive plasmid expressing *flp* enzyme for *flp*-catalysed excision of *kan* marker; Amp^R, Cm^R	Cherepanov & Wackernagel (1995)
Cloning *hsdMS* genes		
IM199	CCCAAACTGCACCCAAGAGTCAGAACACAGTTTTTCAAGAGTACAAAGGGGTAAAC TAAAATAAATATTGACACTCTATCATTG	This study
IM200	AAACTAAAATAAATATTGACACTCTATCATTGATAGAGTATAATTAAAATAAGGAG-GAAATTAATGGCAACTATTGGATTTGAAG	This study
IM201	TTAAATTGAAAGTTCATCACTATTCACCTC	This study
IM202	GGTGAATAGTGATGAACTTTCAATTTAAGTGTAGGCTGGAGCTGCTTC	This study
IM203	GCTAACCATTGTGGTGAAGTGCAGGTTTGCTGCATGAATAGTTTTACGGTCCATATGAA TATCCTCCTTAG	This study
IM204	ACTGAGAAAAGACATGTCGGCTATTGTGTAAAGCCATATAGCTCAGACGACATAAAAAA TTTATTTGCTTTCAGG	This study
IM205	CATAAAAAATTTATTTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGA-GAGAGGAGTTATGTCAACGACGGAAAAACAAAGAC	This study
IM206	CTACACAAACATCTTCTGTAAAAAGCC	This study
IM207	GCTTTTTACAGAAGATGTTTGTGTAGGTGTAGGCTGGAGCTGCTTCG	This study
IM208	TTCTATGTAAACTCTCTGACTGTTCATTTTATTTGTTGTTTCAGGGTCGGGGTCCATA TGAATATCCTCCTTAG	This study
Recombineering		
IM177	TCGAAGTCGTCAACTTCGTAGTGAGG	This study
IM178	TGCCGCTGGTTTTCCGCTAATGG	This study
IM179	CGGCCATTTATACAGGAAAAGCCTA	This study
IM180	GTTACCTTCTCTATAGAGAGTGGTG	This study

Amp^R, ampicillin resistant; Kan^R, kanamycin resistant; Cm^R, chloramphenicol resistant

the methylation reference for BPH0662, while DH10B was used as a reference for the *E. coli* strains. Final assembly was validated by reference to a high-resolution *Nco*I chromosome optical map using MapSolver (v3.2.0, OpGen), as previously described (Gao *et al.*, 2015). BPH0662 Illumina reads were used to correct homopolymer errors in the PacBio assembled BPH0662 genome. BPH0662 Illumina reads were assembled using SPAdes v3.7.1 (Bankevich *et al.*, 2012) and the resulting contigs were screened for small plasmids that would be lost during PacBio size selection.

Genome analysis. Variant calling between the closed BPH0662 genome and BPH0663 Illumina reads was confirmed by two methods, using Snippy v3.0 (Seemann, 2016a) and Nesoni (Harrison, 2014). A one-way comparison of gene content between each of the four published,

closed *S. epidermidis* genomes and BPH0662 was performed using Blast Ring Image Generator (BRIG) (Alikhan *et al.*, 2011). Annotated prophages in BPH0662 were identified using PHage Search Tool (PHAST) (Zhou *et al.*, 2011). The presence of CRISPRs was screened for with CRISPRfinder (Grissa *et al.*, 2007). *In silico* MLST was performed on the *de novo* assemblies (Seemann, 2016b).

All publically available, assembled *S. epidermidis* genomes in NCBI GenBank were downloaded and analysed (4th April 2016). Selection criteria for inclusion as a comparator strain were as follows: *in silico* MLST categorisation as ST2. The exclusion criteria were: sequencing performed on a Roche 454 or Ion Torrent platform (per GenBank metadata); Illumina sequencing with depth <25× (per GenBank metadata); assembled genome size >120 % of the BPH0662 genome (surrogate marker for mixed bacterial samples); organism not *S. epidermidis* determined by Kraken v0.10.5beta (Wood & Salzberg, 2014). This resulted in selection of 32 potential comparator genomes from 307 assemblies. For isolates where core-SNP phylogeny of the 32 strains indicated clonal groupings, a single representative isolate from each group was included. The resulting 15 unique comparator genomes with metadata are listed in Table S1 (available in the online Supplementary Material).

Genome annotation was performed with Prokka v1.12 (Seemann, 2014). Maximum-likelihood core-SNP phylogeny of *S. epidermidis* isolates using the newly closed BPH0662 genome as a reference, was determined using Snippy v3.0 (Seemann, 2016a) to generate an alignment of core SNPs, PhyML v3.1 (Guindon *et al.*, 2010) was then used to reconstruct a maximum-likelihood tree. Pairwise SNP analysis was performed using pairwise_snp_differences (Goncalves da Silva, 2015). For pangenome analysis, protein ortholog clustering was performed using Proteinortho v5.11(Lechner *et al.*, 2011), alignment of the resultant CDS orthologs to BPH0662 and visualisation of the pangenome was performed using FriPan (Powell, 2015).

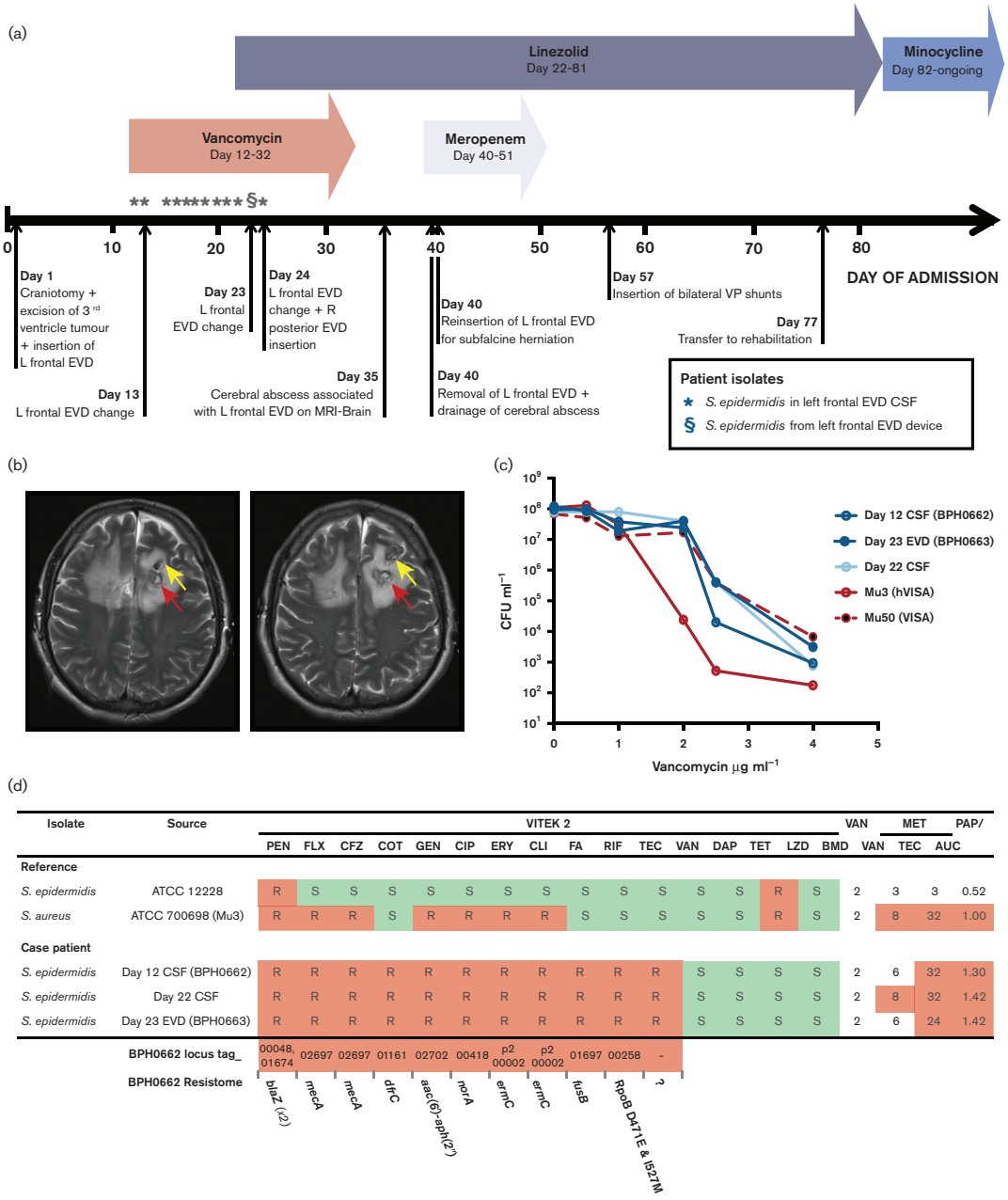
Construction of *E. coli* DC10B-MS1 and DC10B-MS2. *E. coli* mutants expressing the BPH0662 type I RM systems in a DC10B background were created using the method previously described by Monk *et al.* (2015). *E. coli* DC10B-MS1, which expressed SepiBPH0662I *hsdMS (hsdMS1)* was constructed as follows (Fig. S1). The *hsdMS1* system from BPH0662 was amplified with primers IM205 (incorporating PN25 promoter)/IM206. Plasmid pKD4 (Datsenko & Wanner, 2000) was used as a template for the amplification of the Kan resistance marker flanked by flippase recognition target (FRT) sites with primers IM207/IM208 (product pKD4-1). The hsdMS1 and pKD4-1 products were gel-extracted and joined by spliced overlap extension (SOE) PCR using primers IM204/208 (primer tails contained 50 bp homology for integration into the intergenic region between *ybbD* and *ylbG* in the DC10B chromosome). The hsdMS1–pKD4-1 linear amplicon was pellet paint (Novagen) precipitated and electroporated into DC10B containing pKD46 (Datsenko & Wanner, 2000), made competent as previously described (Monk *et al.*, 2012). Transformants were selected for on L agar Cm10. Colony PCR with primers IM177/IM178 was used to screen for recombination at the integration site, positive clones were grown overnight at 43 °C to promote loss of pKD46. To excise the pKD4-1 product, strains were transformed with pCP20 (Cherepanov & Wackernagel, 1995) at 28 °C, single-colony purified at 28 °C, then grown overnight at 43 °C to promote plasmid loss. To confirm excision of the Kan resistance marker and the loss of pCP20, cells were patch plated on L agar Kan and L agar Cm, respectively. The resulting Kan^S, Cm^S strain containing *hsdMS1* (with PN25 promoter) was named DC10B-MS1.

Using the same protocol, the *E. coli* DC10B-MS2 mutant was constructed from *E. coli* DC10B using the following primer sets: IM200 (incorporating Pxyl/tetO promoter)/IM201 for the amplification of SepiBPH0662II *hsdMS (hsdMS2)*; IM202/IM203 for amplification of FRT-*kan*-FRT from pKD4 (product named pKD4-2); IM199/IM203 (containing tails with 50 bp homology for integration into the intergenic region between *essQ* and *cspB* in the DC10B chromosome) for SOE PCR of hsdMS2-pKD4-2; IM179/IM180 to confirm recombination at integration site.

Results & Discussion

Identification of a clinical multidrug-resistant *S. epidermidis* displaying vancomycin heteroresistance

While multidrug resistance in clinical isolates of *S. epidermidis* is increasingly common, standard antimicrobial susceptibility testing demonstrated BPH0662 was more broadly resistant than usually reported (resistant to rifampicin, fusidic acid and teicoplanin). Despite testing as susceptible to vancomycin with a MIC of 2 μg ml^{-1} using the gold standard method of BMD, the patient failed vancomycin therapy (Fig. 1d). Phenotypically identical *S. epidermidis* were repeatedly isolated from both EVD cerebrospinal fluid (CSF) and sterile surgical site samples from the patient, on 12 occasions over 13 days (Fig. 1a, d). To investigate the mechanism of treatment failure, extended glycopeptide susceptibility testing using vancomycin and teicoplanin METs and vancomycin PAPs was performed. Although no diagnostic criteria has been defined for the classification of vancomycin heteroresistance in *S. epidermidis*, if the criteria used for *S. aureus* were applied (>8 μg ml^{-1} for both vancomycin and teicoplanin MET, or >12 μg ml^{-1} for teicoplanin MET regardless of vancomycin MET; PAP:AUC of >0.9 compared with Mu3 standard for vancomycin PAP) then all of the patient's *S. epidermidis* isolates were found to be vancomycin-heteroresistant by both testing methods (Fig. 1c, d). This demonstrated that the true antibiogram of BPH0662 was more complex than initially appreciated, with the isolates approaching pan-drug resistance. Vancomycin is a last-line agent in the treatment of staphylococci, therefore evolution of resistance to this antimicrobial left no conventional therapeutic options. In spite of this, the patient



(d)

Isolate	Source	VITEK 2																VAN	MET		PAP/
		PEN	FLX	CFZ	COT	GEN	CIP	ERY	CLI	FA	RIF	TEC	VAN	DAP	TET	LZD	BMD	VAN	TEC	AUC	
Reference																					
S. epidermidis	ATCC 12228	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		2	3	3	0.52
S. aureus	ATCC 700698 (Mu3)	R	R	R	S	R	R	R	R	S	S	S	S	S	R	S		2	8	32	1.00
Case patient																					
S. epidermidis	Day 12 CSF (BPH0662)	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S		2	6	32	1.30
S. epidermidis	Day 22 CSF	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S		2	8	32	1.42
S. epidermidis	Day 23 EVD (BPH0663)	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S		2	6	24	1.42
	BPH0662 locus tag_	00048, 01674	02697	02697	01161	02702	00418	p2 00002	p2 00002	01697	00258	-									
	BPH0662 Resistome	*blaZ (x2)*	*mecA*	*mecA*	*dfrC*	*aac(6')-aph(2'')*	*norA*	*ermC*	*ermC*	*fusB*	*RpoB D471E & I527M*	*?*									

Fig. 1. BPH0662 - a clinically significant, multidrug-resistant *S. epidermidis*. (a) Summary of case patient's clinical course. (b) Magnetic resonance imaging (MRI) slices of the patient's brain, demonstrating a large cerebral abscess (red arrow) formed around the tip of an external ventricular drain (EVD) device (yellow arrow). (c) Vancomycin population analysis profile of three *S. epidermidis* isolates from the case patient compared with reference strains Mu3 [heterogeneous vancomycin intermediate *S. aureus* (hVISA)] and Mu50 [vancomycin intermediate *S. aureus* (VISA)]. (d) Correlation of phenotypic susceptibility testing for case patient isolates with the BPH0662 resistome. L, left; R,

right; VP, ventriculoperitoneal; CSF, cerebrospinal fluid; PEN, penicillin; FLX, flucloxacillin; COT, cotrimixazole; GEN, gentamicin; CIP, ciprofloxacin; ERY, erythromycin; CLI, clindamycin; FA, fusidic acid; RIF, rifampicin; TEC, tecoplanin; VAN, vancomycin; DAP, daptomycin; TET, tetracycline; LZD, linezolid; BMD, broth microdilution; MET, macromethod Etest; PAP/AUC, population analysis profile/ area under the curve (compared with Mu3 hVISA reference).

was successfully treated with a combination of surgical source control and change in therapy to linezolid, followed by life-long suppressive minocycline therapy.

Establishing a complete ST2 reference genome

To confirm that these isolates represented persistent infection with the same strain and to further investigate the molecular characteristics underlying the near pan-resistant phenotype of these isolates, we initially performed Illumina whole-genome sequencing of BPH0662 (initial CSF isolate, day 12 of admission) and BPH0663 (intraoperative isolate from EVD device, day 23). Analysis indicated that both were ST2 *S. epidermidis*. Despite being the globally dominant hospital lineage, no complete reference sequence of an ST2 *S. epidermidis* strain was available. Therefore, the genome of BPH0662 was analysed using SMRT sequencing (Fig. 2), and validated by comparison to an optical map generated for the same isolate (Fig. 3a). The *S. epidermidis* BPH0662 genome comprises a 2 793 003 bp circular chromosome with 32.0 % DNA G+C content, and three circular plasmids. The largest plasmid (pBPH0662-01) is 45 807 bp with 30.0 % DNA G+C content, the second (pBPH0662-02) only 2366 bp with 31.1 % DNA G+C content, and the third (pBPH0662-03) is 13 569 bp with 28.6 % DNA G+C content. The chromosome was predicted to contain 2653 coding genes, with an additional 40 genes located on pBPH0662-01. Containing only two coding regions, *repL* and *ermC*, pBPH0662-02 shared 99 % DNA sequence similarity with the naturally occurring, 2355 bp plasmid pNE131 (Genbank M12730.1) previously described in clinical *S. epidermidis* strains (Lampson & Parisi, 1986a). Notably, pBPH0662-02 possesses the 107 bp deletion in the 5′ regulatory region of *ermC* to which the constitutive expression of macrolide–lincosamide–streptogramin B resistance in pNE131 has been attributed (Lampson & Parisi, 1986b). Furthermore, plasmids genetically identical (Genbank AB982225.1) to pBPH0662-02 have been described in Russian, ST8, community-acquired methicillin-resistant *S. aureus*

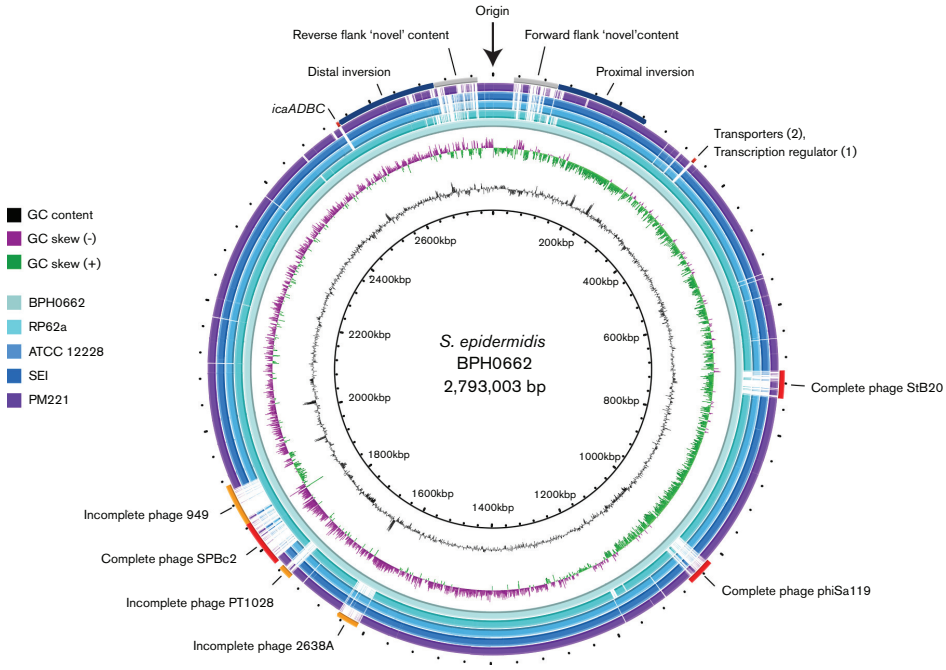


Fig. 2. Hospital-evolved strain BPH0662 possesses novel gene content acquired through horizontal gene transfer. A one-way comparison of gene content between the four published *S. epidermidis* genomes and BPH0662 was generated using BRIG (Alikhan *et al.*, 2011). Prophages are annotated in the outermost ring.

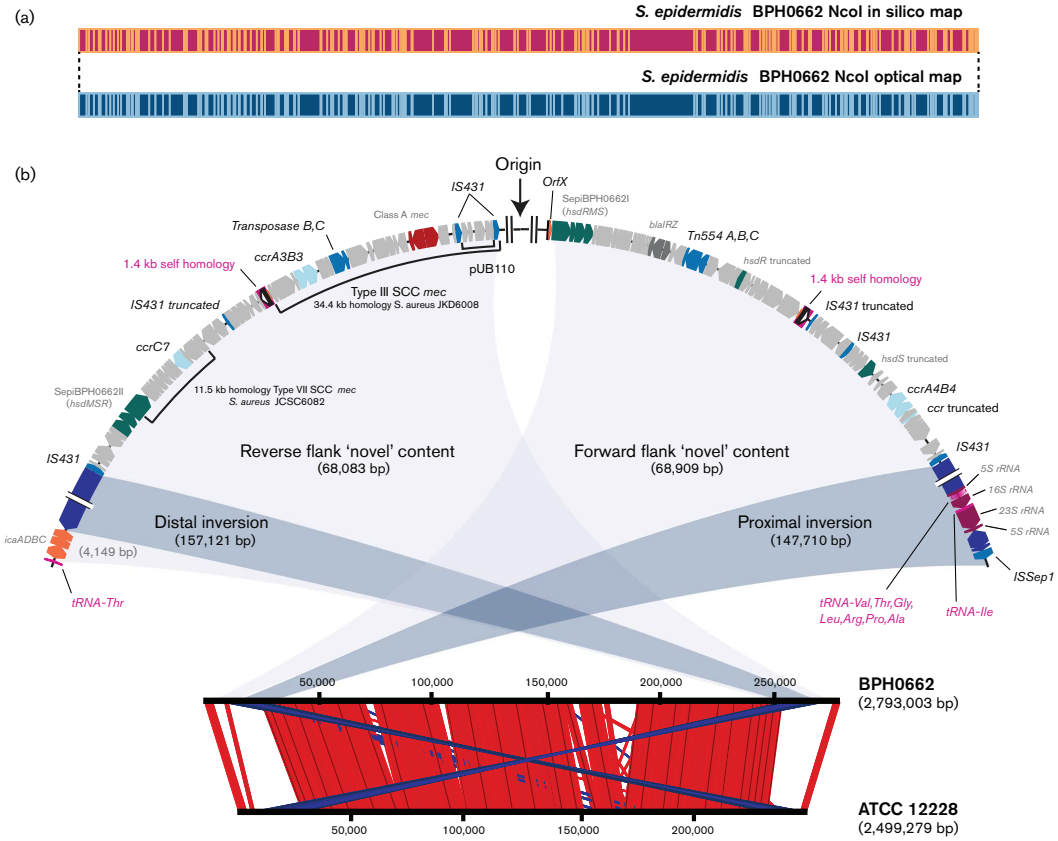


Fig. 3. The novel structure of *S. epidermidis* BPH0662 with unusual location of a chimeric SCC*mec*. (a) *In silico* NcoI map of the BPH0662 genome assembly aligned with NcoI optical map of BPH0662 genome. (b) The closed genome of BPH0662 aligned to the *S. epidermidis* ATCC 12228 reference genome, demonstrating the inversion associated with the unusual location and orientation of the SCC*mec* element in BPH0662.

strains (Khokhlova *et al.*, 2015). A further 14 predicted coding genes were on pBPH0662-03, including the antiseptic resistance gene *qacA* and its regulator. Analysis of the Illumina-sequenced BPH0663 genome compared with the closed BPH0662 genome indicated that the isolates were clonal, with no detectable SNPs between them.

Comparisons between BPH0662 and the four existing, complete, *S. epidermidis* genomes are shown in (Fig. 2) and Table 2. Similar to the biofilm-forming, drug-resistant clinical strain RP62a (Gill *et al.*, 2005), BPH0662 possessed the *icaADBC* operon and *mecA*. Analysis indicated that the majority of novel gene content in BPH0662 was acquired by horizontal gene transfer. Three complete and three partial prophages were detected, accounting for 9.3 % of the genome. Further novel content was concentrated in the regions flanking the origin of replication, which contained multiple resistance determinants, drug transporters and regulatory genes, interspersed between numerous insertion-sequence (IS) elements (particularly IS*431*) and transposases. No clustered regularly interspaced short palindromic repeat (CRISPR) loci were identified in the genome. The phenotypic susceptibility testing of BPH0662 correlated with the resistome is shown in (Fig. 1d). Genetic determinants accounting for the phenotypic resistance observed for BPH0662 were identified for all antimicrobial agents with the exception of teicoplanin and vancomycin, both glycopeptide antibiotics. In the related species *S. aureus*, rifampicin-resistance mutations in *rpoB* have been demonstrated to result in vancomycin heteroresistance (Gao *et al.*, 2013; Matsuo *et al.*, 2011), leading us to speculate that the same phenomenon may also occur in *S. epidermidis*. Dual D471E and I527M mutations were identified in the BPH0662 RpoB, associated with rifampicin resistance (MIC of 64 μg ml^{-1}). The same dual substitutions have previously

Table 2. Comparison of completed *S. epidermidis* genomes

Strain	Chromosome (Mb)	Plasmids	GC %	Genes	Source	*In silico* MLST	Reference
BPH0662	2.79	3	32.0	2709	Clinical	2	This study
RP62a/ ATCC 35984	2.61	1	32.2	2662	Clinical	10	Gill *et al.* (2005)
SEI/ ATCC 49134	2.50	1	32.0	2397	Non-clinical	*-	Zhang *et al.* (2012)
ATCC 12228	2.50	6	32.1	2558	Non-clinical	8	Zhang *et al.* (2003)
PM221	2.49	4	31.9	2461	Bovine mastitis	184	Savijoki *et al.* (2014)

*Published reference strain SEI was not classifiable by existing multi-locus sequence type (MLST) schema.

been described as the RpoB mutations most commonly associated with high-level rifampicin resistance in a *S. epidermidis* prosthetic joint infection study (Hellmark *et al.*, 2009).

BPH0662 contained an unusual variant of staphylococcal cassette chromosome *mec* (SCC*mec*) type III, as it was located in reverse orientation in the region upstream of the origin of replication (Fig. 3b). The element was not associated with the typical integration site *orfX* [corresponding to the last 15 nucleotides of the coding sequence of the rRNA large subunit methytransferase (Boundy *et al.*, 2013)], located approximately 32 kb downstream from the origin of replication in BPH0662. Despite the unexpected location on the chromosome, the BPH0662 SCC*mec* element contained *ccrA3B3* and a prototypical class A *mec* complex, with 34.4 kb of the element sharing 99.8 % nucleotide homology with the previously described, 37 kb type III SCC*mec* of ST239 *S. aureus* strains JKD6008 (GenBank CP0021020) and Sa0059 (GenBank JQ412578), including the integrated plasmid pUB110 (harbouring genes encoding kanamycin and bleomycin resistance). Based on the *orfX* sequence in BPH0662, a perfect inverted repeat, and imperfect direct repeat, were present in the distal (upstream) end of the SCC*mec* element in reverse orientation, in keeping with the reverse orientation of the entire element (Fig. S2), suggesting initial integration of the SCC*mec* element at *orfX* with subsequent chromosomal rearrangement. Two 1.4 kb regions of DNA homology potentially explained this rearrangement. These regions mirrored one another on either side of the origin of replication: one located 59.7 kb downstream; the other 71.8 kb at the reverse flank, which incorporated the upstream direct repeat of the SCC*mec* element (Fig. 3b).

Located 20 kb upstream of SCC*mec* was a *ccrC7* in close proximity to a complete type I RM, flanked at both ends by IS*431* [also known as IS*257* (Rouch & Skurray, 1989)] elements. The carriage of *hsdRMS* genes in association with *ccrC* is described in both type V and VII SCC*mec* in *S. aureus* [Berglund *et al.*, 2008; International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009]. Indeed, 11.5 kb of this region in BPH0662, encompassing *ccrC7* extending into *hsdR2*, displayed 98 % homology with joining regions 2 and 3 and the *ccr* gene complex of the prototypical, type VII SCC*mec* described in *S. aureus* strain JCSC6082 (GenBank AB373032). Forward of the origin of replication, another chimeric SCC element was present, containing a *ccrA4B4* complex as well as one complete and a truncated type I RM system, in the absence of a *mec* complex (see Fig. 3b). Remnants of an additional truncated *ccr* gene were in close proximity.

The assortment of *ccr* genes observed in BPH0662 was in keeping with the reported diversity in coagulase-negative staphylococci (Hanssen & Sollid, 2007), suggested to be the donor species in which novel and composite SCC elements arise and are then transferred to the more genetically conserved *S. aureus* (Barbier *et al.*, 2010). The presence of a near complete type III SCC*mec* in BPH0662, identical to that previously described in ST239 *S. aureus* [a globally disseminated, healthcare-associated, clonal lineage of methicillin-resistant *S. aureus* (Harris *et al.*, 2010)] reinforces the probable role of these recombinases in the horizontal transfer of DNA between staphylococcal species.

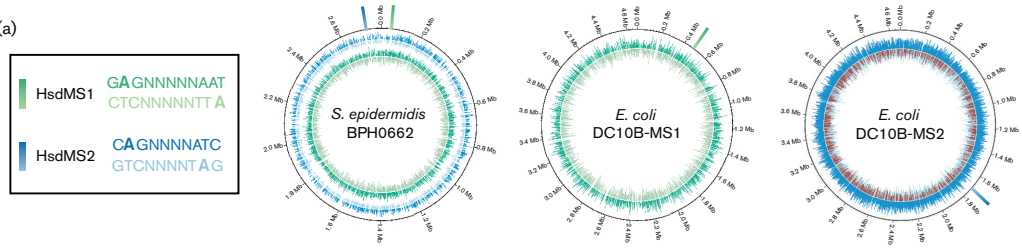
Compared with the closed reference genome of *S. epidermidis* ATCC 12228, the overall structure of BPH0662 was suggestive of chromosomal rearrangement resulting from two inversions around the origin of replication (Fig. 3b). The inversion points closest to the origin, potentially attributable to homologous recombination between the regions of self-homology as described above, and the medial inversions, possibly explained by the nearby presence of tRNA genes, which have been demonstrated to be associated with chromosomal inversions in other bacterial species (DeBoy *et al.*, 2006). Relative to ATCC 12228, RP62a demonstrates a single inversion around the origin of replication (Gill *et al.*, 2005; Lindsay & Holden, 2007), corresponding to the most medial inversions observed in BPH0662 (with a series of tRNA genes and an adjacent ISSep element at one inversion point, and a lone tRNA-Thr adjacent to the *icaADBC* operon at the other). Large-scale chromosomal inversions and deletions near the origin of replication have also been described in other CoNS, associated with homologous recombination of IS elements (Watanabe *et al.*, 2007). While this arrangement around the origin with a double

inversion is unusual, the chromosome assembly of BPH0662 was confirmed to be correct by optical mapping (Fig. 3a). The importance of independent verification of genome assembly for the identification of large chromosomal rearrangements associated with biological consequences has recently been described (Gao *et al.*, 2015). Furthermore, the value of a closely related reference genome for comparative genomics is becoming increasingly evident (Pightling *et al.*, 2014). In view of the global dominance of the ST2 lineage within the hospital environment, as the first, to our knowledge, completed ST2 genome, BPH0662 will be invaluable for future genomic studies of clinically relevant *S. epidermidis* isolates.

The BPH0662 methylome

The pattern of adenine-methylated DNA detected by SMRT sequencing of BPH0662 indicated the presence of two functional type I RM systems, corresponding with two independent TRMs (Fig. 4a), formally named SepiBPH0662I and SepiBPH0662II, as per standard nomenclature (Roberts *et al.*, 2003). An incomplete third system with a predicted non-functional partial *hsdS* was noted in the genome. To determine the assignment of each *hsdMS* system with its associated TRM, two DC10B-derived *E. coli* hosts were constructed, each containing an individual *hsdMS* system from BPH0662 (DC10B-MS1 and DC10B-MS2). Integration of the BPH0662 *hsdMS* systems into DC10B at the expected sites was confirmed by SMRT sequencing of DC10B-MS1 and DC10B-MS2. Functional expression of the systems by the *E. coli* hosts was demonstrated by the acquisition of chromosomal adenine methylation at a high proportion (89.65–99.90 %) of predicted target recognition domains (TRDs) determined by the integrated BPH0662 *hsdS* (Fig. 4b). Notably, the 5′-G**A**TNNNNCTG-3′ TRM of DC10B-MS2 was predicted to overlap with endogenous *dam* (5′-G**A**TC-3′) methylation at

(a)



(b)

S. epidermidis	Motif	% Motifs Detected	# Motifs Detected	# Motifs in Genome	Mean Modification QV‡	Mean Motif Coverage§
BPH0662						
HsdS1	GAGNNNNNAAT	99.87	1582	1584	110.7	75.9
	ATTNNNNNCTC	99.56	1577	1584	105.1	76.1
HsdS2	CAGNNNNATC	100.00	1319	1319	125.4	76.4
	GATNNNNCTG	97.80	1290	1319	104.3	76.2
E. coli						
DC10B-MS1						
	GATC	98.36	37954	38588	59.7	33.7
HsdS1	GAGNNNNNAAT	90.45	1573	1739	55.8	33.2
	ATTNNNNNCTC	89.65	1559	1739	54.6	32.7
DC10B-MS2						
	GATC	99.52	38405	38592	70.4	40.4
HsdS2	CAGNNNNATC	99.41	4185	4210	70.8	39.7
	GATDNNNCTG	98.18	3183	3242	68.4	40.2
	GATCNNNCTG*	99.90	967	968	60.7	39.8

Fig. 4. The type I restriction modification (RM) systems of BPH0662. (a) Two functional type I RM systems, encoding SepiBPH0662I (HsdMS1) and SepiBPH0662II (HsdMS2), were identified in the *S. epidermidis* BPH0662 genome. *E. coli* hosts expressing HsdMS1 (DC10B-MS1) and HsdMS2 (DC10B-MS2) were engineered and SMRT sequencing was used to determine their methylomes, allowing correlation of the BPH0662 target recognition domains (TRDs) with their corresponding HsdMS systems. The chromosomal position of each HsdMS system and its associated methylated adenine residues (represented by a line whose length corresponds with the interpulse duration of the read) for BPH0662 and the two *E. coli* host strains were plotted using Circos (Krzywinski *et al.*, 2009). (b) Using the SMRT Suite Pipeline v2.2.0/Motif Finder v1.3.1 to analyse reads, the conserved adenine-methylated residues (in bold type) and TRMs associated with the HsdS alleles of *S. epidermidis* BPH0662 and the *E. coli* host strains DC10B-MS1 and DC10B-MS2 were identified. ‡Mean modification QV is defined as the quality value of the base calls within the motif; §Mean motif coverage is defined as the average depth of read coverage within a motif; *The HsdMS2 TRM (GATCNNNCTG) overlapped with endogenous *dam* (GATC) methylation in the *E. coli* host.

968 sites in the *E. coli* genome, SMRT sequencing indicated that 967 of these sites were appropriately methylated. Construction of these two *E. coli* hosts sets the groundwork for future experiments where a second integration event (either SepiBPH0662II *hsdMS* into DC10B-MS1 or SepiBPH0662I *hsdMS* into DC10B-MS2), resulting in a single *E. coli* host expressing both BPH0662 type I RM systems, should enable utilisation of plasmid artificial modification to overcome the strong restriction barrier present in *S. epidermidis* isolates from the ST2 lineage, facilitating the genetic manipulation of these clinically relevant clones that have hitherto proven refractory.

BPH0662 in relation to international ST2 *S. epidermidis*

At the time of analysis, 307 partially assembled *S. epidermidis* genomes were publically available from NCBI Genbank. *In silico* MLST identified 87 of these as ST2 isolates. Due to the large number of genomes that were sequenced as part of microbiome projects, a large proportion had poor sequencing coverage and/or contained reads mixed with other species, both of which resulted in questionable assemblies. Furthermore, some genomes represented other bacterial species incorrectly classified as *S. epidermidis*. Using the outlined selection/exclusion criteria, a curated list of 15 genomes from three different projects [two published (Roach *et al.*, 2015; Walsh *et al.*, 2015), one unpublished PRJNA246628] was obtained (Table S1). To determine how the newly closed BPH0662 isolate related to international ST2 strains, analysis of these 15 representative, publically available, draft ST2 genomes together with the four existing complete *S. epidermidis* genomes was performed using BPH0662 as a reference (Fig. 5a). A maximum-likelihood core-SNP-based phylogeny for these 20 strains was performed, aligned with their respective complete coding sequence content (pangenome analysis) (Fig. 5b). Phylogenetically, the BPH0662 reference sits within

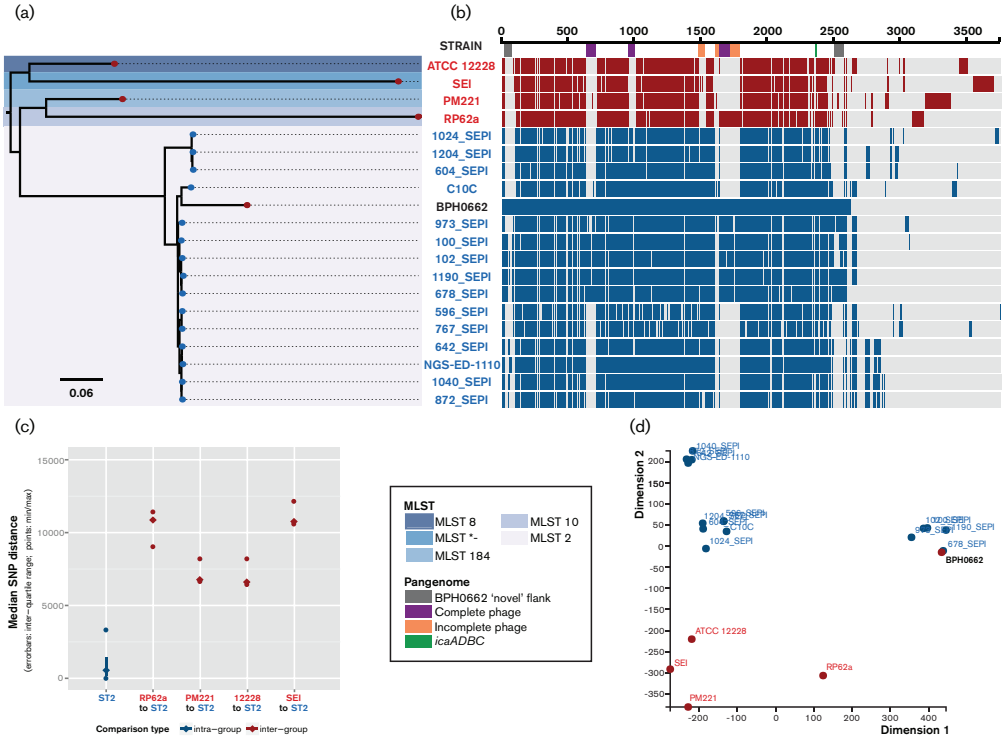


Fig. 5. Comparative genomics of BPH0662 in relation to existing sequenced genomes and published international ST2 *S. epidermidis* isolates. (a) Maximum-likelihood, core-SNP-based phylogeny of 15 representative international ST2 isolates, the four existing sequenced strains and the new BPH0662 ST2 reference strain (scale bar indicates nucleotide substitutions per site); aligned with (b) Pangenome. (c) Intra-group comparison of pairwise core-SNPs for BPH0662 together with the international ST2 strains; and inter-group comparison of pairwise core-SNPs for the existing references, compared with the ST2 strains. (d) Multidimensional scaling of pangenome coding sequence orthologs. *Published reference strain SEI was not classifiable by existing multi-locus sequence type (MLST) schema.

the characterised, international ST2 *S. epidermidis* clade, with an observed 559 median SNPs difference within the ST2 intragroup (Fig. 5c). The *icaADBC* operon was observed to be present in all ST2 strains and RP62a, but none of the non-human-host reference strains (Fig. 5b). Methicillin-resistance determinant *mecA*, was present in 14 of the 15 ST2 clinical comparators, with the exception of 642_SEPI.

Interestingly, five of the 15 international ST2 isolates grouped closely to BPH0662 when coding sequence ortholog presence/absence was analysed by multidimensional scaling (Fig. 5d). While the exact structures of these isolates could not be determined from their draft assemblies, detailed analyses of contigs of interest revealed that all five possessed over 27.5 kb of the BPH0662 type III SCC*mec* core region, spanning the class A *mec*, J2 region and *ccrA3B3* (Fig. S2c). Due to contig breaks corresponding with an IS*431* insertion sequence in the BPH0662 SCC*mec*, it is not known whether these five isolates also possessed the same J3 region, with an integrated pUB110. However, all but one (102_SEPI) contained pUB110 as a single contig. Intriguingly, the J1 region of three of the isolates (1190_SEPI, 678_SEPI and 102_SEPI) spanned 15 kb containing the full SepiBPH0662I type I RM system with identical downstream genes to BPH0662 (Fig. S2a, c). Furthermore, two of these isolates (1190_SEPI and 678_SEPI) also possessed a 26 kb contig composed of the SepiBPH0662II system associated with *ccrC7*, with identical intervening gene arrangement as BPH0662 (Fig. S2c). A single isolate, 100_SEPI, possessed only the SepiBPH0662I system, in the same position as BPH0662, adjacent to *orfX*. While 973_SEPI contained only SepiBPH0662II (Fig. S2c). Despite similar total content, the composite structure of these ST2 comparators highlights the role of mobile elements in the translocation and horizontal transfer of novel gene clusters.

Like BPH0662, these five ST2 isolates were all collected from critically ill patients in intensive care (Roach *et al.*, 2015). In view of the gene content common to these strains, including resistance determinants such *mecA*, *aadD* and *aac(6′)-aph(2″)* together with the same type I RM systems, and the circumstances in which they were collected, it could be proposed that BPH0662 and the closely clustered isolates may represent a successful hospital-adapted sublineage of ST2. This reinforces the relevance of BPH0662 as a representative ST2 strain and the potential applicability of the constructed *E. coli* hosts for manipulation of international ST2 *S. epidermidis* isolates.

Notably, RP62a, a ST10 strain frequently utilised as a reference isolate in *S. epidermidis* studies was observed to be particularly divergent from ST2 isolates based on median pairwise SNP difference, with a predicted 10 8612 SNPs between the groups (Fig. 5c). Furthermore, shared ortholog clustering indicated that RP62a and the other three existing reference genomes are divergent from ST2 isolates (Fig. 5d), overall indicating that RP62a may not be the most appropriate reference strain for studies of clinically relevant phenotypes.

Conclusions

Evolving within the hospital environment, analysis of the BPH0662 genome indicates that this isolate has undergone chromosomal rearrangements and multiple horizontal gene transfer events resulting in the accumulation of a broad range of resistance determinants enabling its establishment as a successful multidrug-resistant hospital clone. Furthermore, the likely role of *S. epidermidis* as a potential donor species for the generation and transfer of resistance elements to other staphylococci is highlighted by the structure of the BPH0662 genome, which contains multiple examples of chimeric mobile genetic elements. As the first complete genome of an ST2 *S. epidermidis* strain, BPH0662 provides an ideal reference genome for future bioinformatic analysis of clinical *S. epidermidis* isolates, which worldwide are dominated by the ST2 lineage. Characterisation of the BPH0662 methylome, which has been demonstrated to be representative of the ST2 lineage internationally, and construction of *E. coli* hosts that express the type I RM systems of BPH0662 will assist the development of molecular methods to bypass the restriction barrier in clinical ST2 *S. epidermidis* isolates.

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2.3 Supplementary Appendix

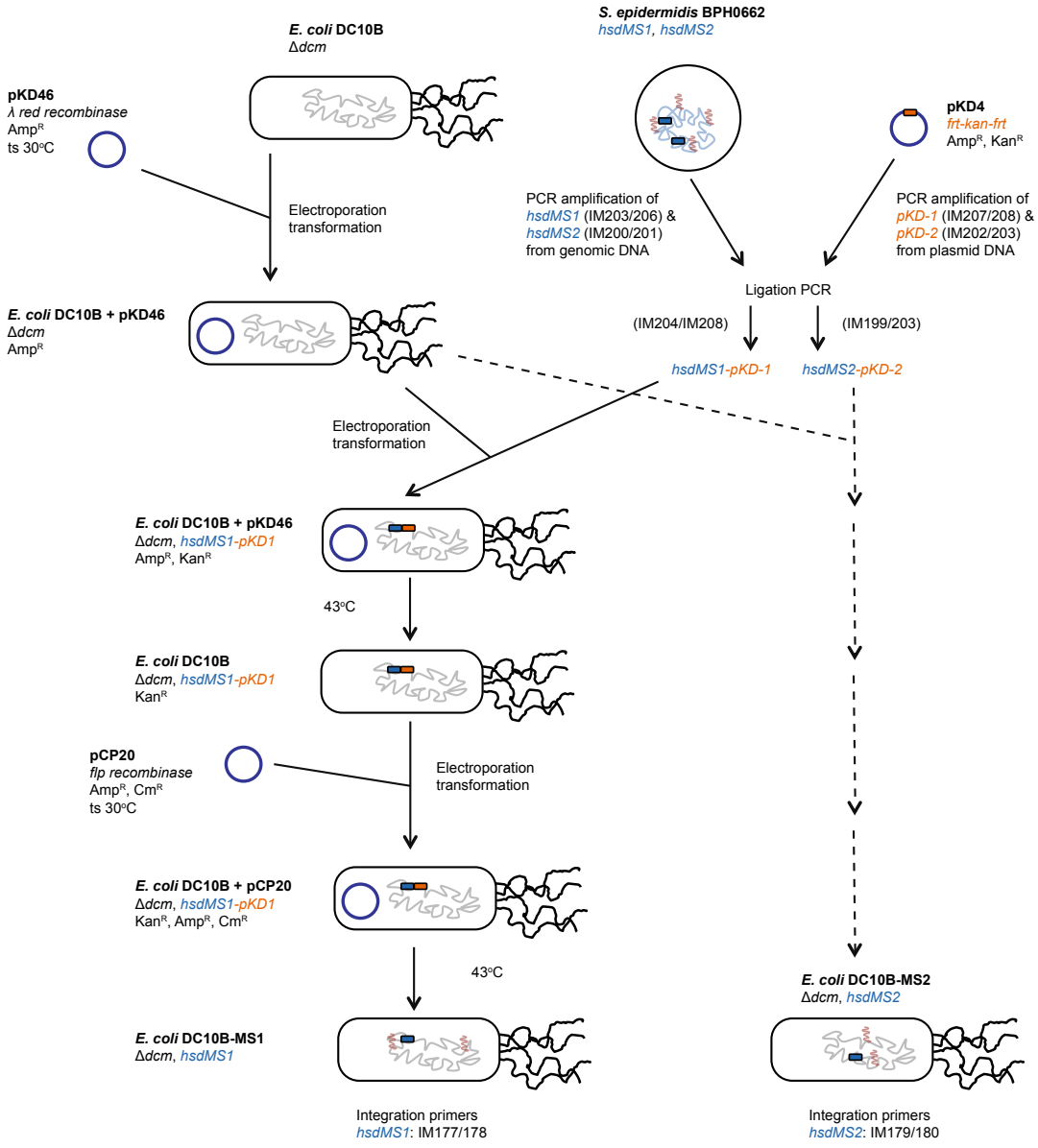


Figure S1. Methodology for construction of *E. coli* hosts expressing the type I restriction modification systems of BPH0662. A schematic representation of the methodology for the construction of *E. coli* host strains DC10B-MS1 (which expressed SepiBPH0662I *hsdMS* (*hsdMS1*) from *S. epidermidis* BPH0662) and DC10B-MS2 (which expressed SepiBPH0662II *hsdMS* (*hsdMS2*) from *S. epidermidis* BPH0662). Amp^R = ampicillin resistant; ts = temperature sensitive; Kan^R = kanamycin resistant.

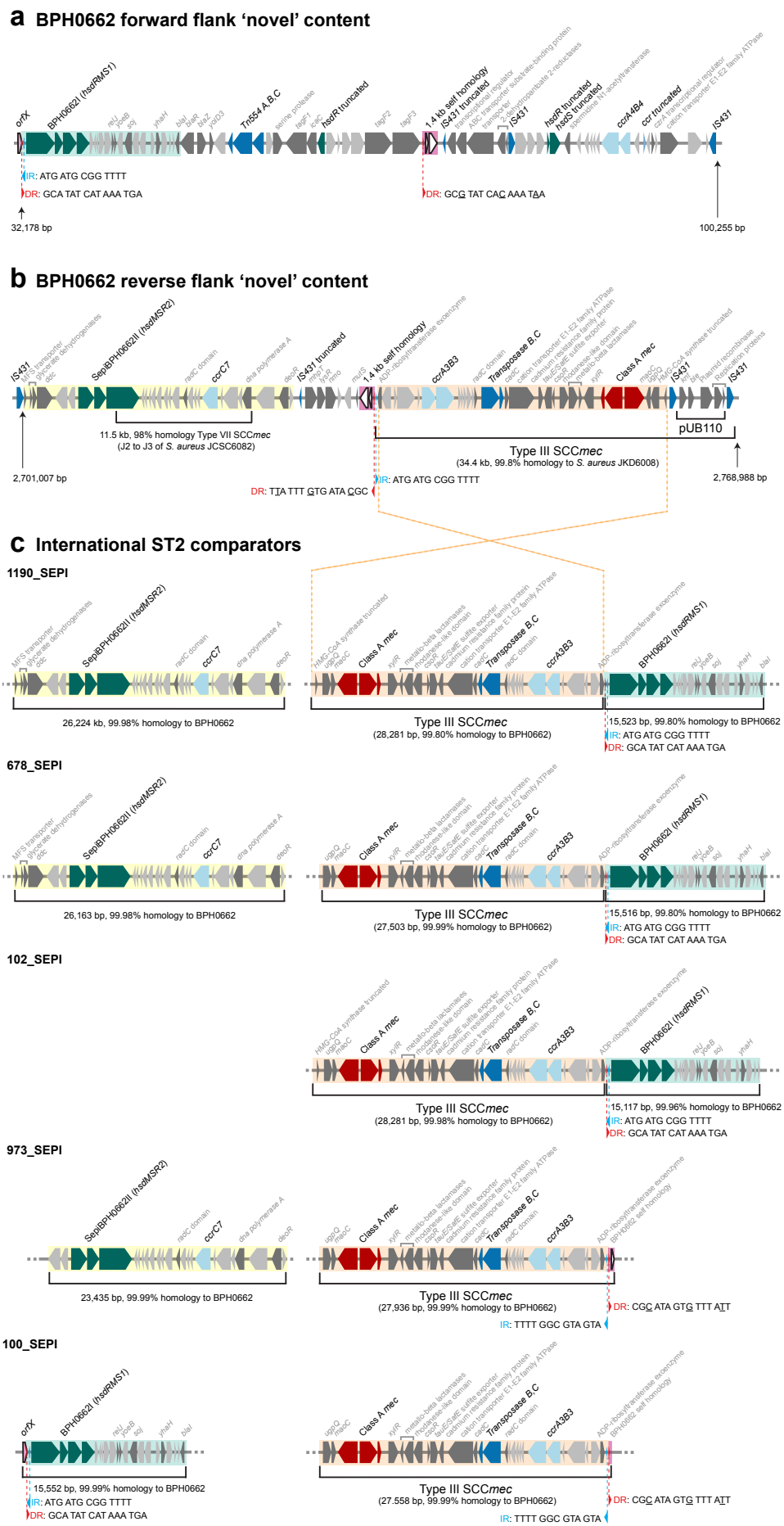
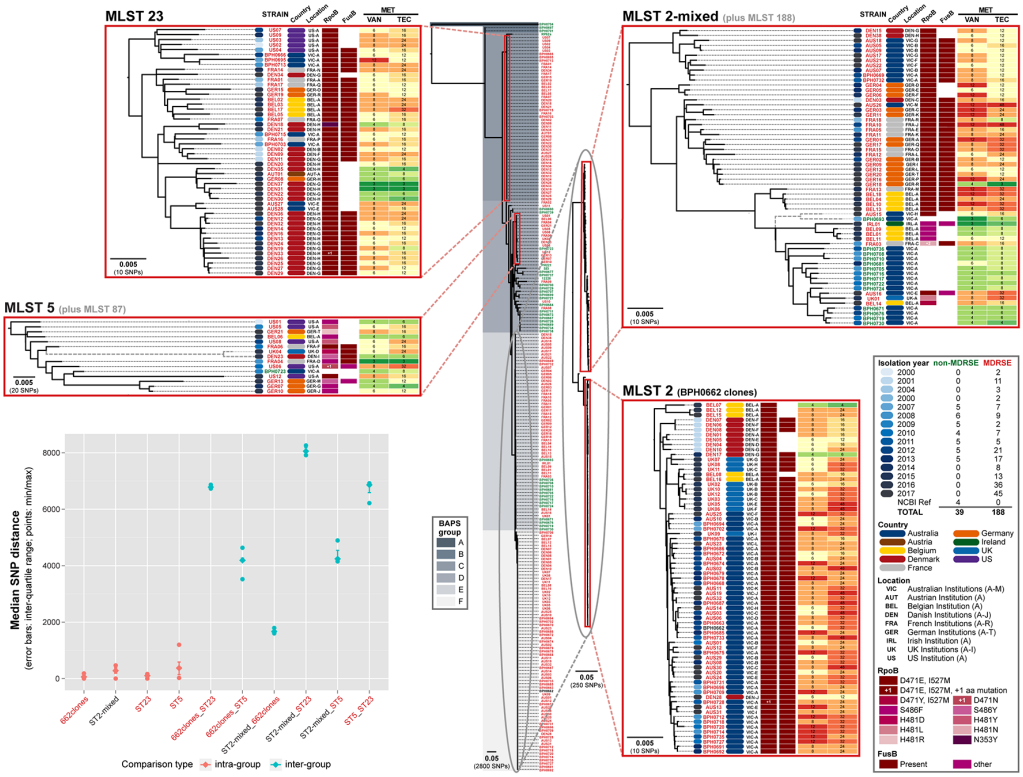

a BPH0662 forward flank 'novel' content
orfX
BPH0662I (hsdRMS1)
Tn554 A,B,C
hsdR truncated
1.4 kb self homology
IS431 truncated
IS431
hsdR truncated
hsdS truncated
ccr4484
ccr truncated
IS431
IR: ATG ATG CGG TTTT
DR: GCA TAT CAT AAA TGA
DR: GCG TAT CAC AAA TAA
32,178 bp
100,255 bp
b BPH0662 reverse flank 'novel' content
IS431
SepiBPH0662II (hsdMSR2)
ccrC7
IS431 truncated
1.4 kb self homology
ccrA3B3
Transposase B,C
Class A mec
IS431
IS431
pUB110
11.5 kb, 98% homology Type VII SCCmec
(J2 to J3 of S. aureus JCSC6082)
Type III SCCmec
(34.4 kb, 99.8% homology to S. aureus JKD6008)
2,701,007 bp
2,768,988 bp
IR: ATG ATG CGG TTTT
DR: TTA TTT GTG ATA CGC
c International ST2 comparators
1190_SEPI
SepiBPH0662II (hsdMSR2)
ccrC7
Class A mec
ccrA3B3
Transposase B,C
BPH0662I (hsdRMS1)
26,224 kb, 99.98% homology to BPH0662
Type III SCCmec
(28,281 bp, 99.80% homology to BPH0662)
15,523 bp, 99.80% homology to BPH0662
IR: ATG ATG CGG TTTT
DR: GCA TAT CAT AAA TGA
678_SEPI
SepiBPH0662II (hsdMSR2)
ccrC7
Class A mec
ccrA3B3
Transposase B,C
BPH0662I (hsdRMS1)
26,163 bp, 99.98% homology to BPH0662
Type III SCCmec
(27,503 bp, 99.99% homology to BPH0662)
15,516 bp, 99.80% homology to BPH0662
IR: ATG ATG CGG TTTT
DR: GCA TAT CAT AAA TGA
102_SEPI
Class A mec
ccrA3B3
Transposase B,C
BPH0662I (hsdRMS1)
Type III SCCmec
(28,281 bp, 99.98% homology to BPH0662)
15,117 bp, 99.96% homology to BPH0662
IR: ATG ATG CGG TTTT
DR: GCA TAT CAT AAA TGA
973_SEPI
SepiBPH0662II (hsdMSR2)
ccrC7
Class A mec
ccrA3B3
Transposase B,C
23,435 bp, 99.99% homology to BPH0662
Type III SCCmec
(27,936 bp, 99.99% homology to BPH0662)
DR: CGC ATA GTG TTT ATT
IR: TTTT GGC GTA GTA
100_SEPI
orfX
BPH0662I (hsdRMS1)
15,552 bp, 99.99% homology to BPH0662
IR: ATG ATG CGG TTTT
DR: GCA TAT CAT AAA TGA
Class A mec
ccrA3B3
Transposase B,C
Type III SCCmec
(27.558 bp, 99.99% homology to BPH0662)
DR: CGC ATA GTG TTT ATT
IR: TTTT GGC GTA GTA

Figure S2. Detailed gene content of the 'novel' chromosomal regions flanking the origin of replication in BPH0662. (a) Forward flank. **(b)** Reverse flank. **(c)** International ST2 *S. epidermidis* comparator isolates that share the 'novel' content regions of BPH0662. DR = direct repeat, IR = inverted repeat.

Table S1. International *S. epidermidis* ST2 comparator isolates

Isolate	Sequencer	Depth	Assembler	Contigs	Genome (bp)	ok	Ns	Gaps	Min Contig Length	Avg Contig Length	Max Contig Length	N50	GC%	MLST	BioProject	BioSample	GenBank	Reference
100_SEPI	Illumina HiSeq	25x	ABYSS v. 1.3.5	122	2702163	2702163	0	0	200	22148	196587	64548	31.80	2	PRJNA267549	SAMN03196961	JWGV00000000.1	Roach et al., 2015
102_SEPI	Illumina HiSeq	30x	ABYSS v. 1.3.5	98	2677737	2677637	100	0	202	27323	190265	67289	31.80	2	PRJNA267549	SAMN03196982	JWGA00000000.1	Roach et al., 2015
1024_SEPI	Illumina HiSeq	37x	ABYSS v. 1.3.5	140	2512765	2512765	0	0	214	17948	156482	50251	31.80	2	PRJNA267549	SAMN03196988	JWFU00000000.1	Roach et al., 2015
1040_SEPI	Illumina HiSeq	52x	ABYSS v. 1.3.5	118	2603795	2603695	100	0	202	22066	196593	62363	31.90	2	PRJNA267549	SAMN03197006	JWFC00000000.1	Roach et al., 2015
1190_SEPI	Illumina HiSeq	35x	ABYSS v. 1.3.5	102	2737632	2737523	109	0	200	26839	191328	70302	31.80	2	PRJNA267549	SAMN03197150	JVZO00000000.1	Roach et al., 2015
1204_SEPI	Illumina HiSeq	31x	ABYSS v. 1.3.5	145	2491124	2491124	0	0	206	17180	159304	48890	32.00	2	PRJNA267549	SAMN03197166	JVYY00000000.1	Roach et al., 2015
596_SEPI	Illumina HiSeq	29x	ABYSS v. 1.3.5	180	2535005	2534477	528	0	218	14083	96274	31445	31.80	2	PRJNA267549	SAMN03197795	JVAT00000000.1	Roach et al., 2015
604_SEPI	Illumina HiSeq	25x	ABYSS v. 1.3.5	73	2527247	2526847	400	0	228	34619	208195	69295	32.10	2	PRJNA267549	SAMN03197804	JVAK00000000.1	Roach et al., 2015
642_SEPI	Illumina HiSeq	30x	ABYSS v. 1.3.5	85	2561559	2560757	802	0	204	30135	150058	67270	31.90	2	PRJNA267549	SAMN03197843	JUYX00000000.1	Roach et al., 2015
678_SEPI	Illumina HiSeq	109x	ABYSS v. 1.3.5	135	2682365	2682356	9	0	204	19869	189766	53486	31.90	2	PRJNA267549	SAMN03197880	JUXM00000000.1	Roach et al., 2015
767_SEPI	Illumina HiSeq	75x	ABYSS v. 1.3.5	196	2556195	2554792	1403	0	204	13041	82486	26206	31.80	2	PRJNA267549	SAMN03197976	JUTU00000000.1	Roach et al., 2015
872_SEPI	Illumina HiSeq	104x	ABYSS v. 1.3.5	149	2585892	2585892	0	0	204	17354	177645	53543	31.90	2	PRJNA267549	SAMN03198079	JUPV00000000.1	Roach et al., 2015
973_SEPI	Illumina HiSeq	41x	ABYSS v. 1.3.5	123	2707637	2707537	100	0	200	22013	177663	56080	31.80	2	PRJNA267549	SAMN03198193	JULL00000000.1	Roach et al., 2015
C10C	Illumina MiSeq	32x	CLC Genomic Workbench v. 2014	63	2540116	2540116	0	0	550	40319	283658	76639	31.80	2	PRJNA246628	SAMN02996614	JQHC00000000.1	Chong et al., 2014. *S. epidermidis* C10C genome sequence. Unpublished.
NGS-ED-1110	Illumina MiSeq	200x	SPAdes v. FEB-2015	45	2664995	2664995	0	0	1116	59222	207733	96796	31.90	2	PRJNA255947	SAMN03396286	JZUM00000000.1	Walsh et al., 2015

CHAPTER 3. Global spread of three multidrug resistant lineages of *Staphylococcus epidermidis*



Reproduced from Lee *et al.* [224].

3.1 Introduction

The work in this chapter follows what began as a local outbreak investigation of into the increasing prevalence of clinical cases of serious MDRSE infections that failed vancomycin therapy, for which the BPH0662 isolate characterised in Chapter 2 was the index case. The initial aim of the project was to characterise the local epidemiology of *S. epidermidis* within Austin Health, a 800-bed tertiary centre in Melbourne, Australia, and determine if an outbreak of ST2 MDRSE had occurred. However, the unexpected finding of three endemic MDRSE lineages (two ST2 and one ST23) that shared a common D471E and I527M RpoB dual substitution that was associated with high level rifampicin resistance and a vancomycin heteroresistant phenotype, led to a much larger international project with the following aims: 1. Characterise the global molecular epidemiology of MDRSE with a focus of rifampicin resistant isolates and determine the extent of dissemination of each of the three MDRSE lineages; 2. Characterise the diversity of rifampicin resistance mutations in *S. epidermidis*; 3. Determine if the dual D471E and I527M RpoB substitution is the most common cause of clinical rifampicin resistance; 4. Develop a method to enable allelic exchange in ST2 *S. epidermidis* that had hitherto been refractory to genetic manipulation; in order to, 5. Demonstrate that the dual D471E and I527M RpoB substitution in *S. epidermidis* causes both high-level rifampicin resistance and confers cross-resistant to vancomycin. The resulting work was published in Nature Microbiology.

Supplementary materials for the publication are included in 3.3 Supplementary Appendix I. Supplementary tables that consisted of large metadata spreadsheets in Excel format have been printed and are located in Appendix A. An updated version of Figure 1.4 from Chapter 1 of this thesis has been amended to include the mutations identified in Chapter 3 and can be found in 3.4 Supplementary Appendix II.

3.2 Published manuscript

nature microbiology

ARTICLES

https://doi.org/10.1038/s41564-018-0230-7

Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*

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***Staphylococcus epidermidis* is a conspicuous member of the human microbiome, widely present on healthy skin. Here we show that *S. epidermidis* has also evolved to become a formidable nosocomial pathogen. Using genomics, we reveal that three multidrug-resistant, hospital-adapted lineages of *S. epidermidis* (two ST2 and one ST23) have emerged in recent decades and spread globally. These lineages are resistant to rifampicin through acquisition of specific *rpoB* mutations that have become fixed in the populations. Analysis of isolates from 96 institutions in 24 countries identified dual D471E and I527M RpoB substitutions to be the most common cause of rifampicin resistance in *S. epidermidis*, accounting for 86.6% of mutations. Furthermore, we reveal that the D471E and I527M combination occurs almost exclusively in isolates from the ST2 and ST23 lineages. By breaching lineage-specific DNA methylation restriction modification barriers and then performing site-specific mutagenesis, we show that these *rpoB* mutations not only confer rifampicin resistance, but also reduce susceptibility to the last-line glycopeptide antibiotics, vancomycin and teicoplanin. Our study has uncovered the previously unrecognized international spread of a near pan-drug-resistant opportunistic pathogen, identifiable by a rifampicin-resistant phenotype. It is possible that hospital practices, such as antibiotic monotherapy utilizing rifampicin-impregnated medical devices, have driven the evolution of this organism, once trivialized as a contaminant, towards potentially incurable infections.**

Staphylococcus epidermidis is universally present on human skin[1]. The shift in medicine towards invasive procedures has favoured its emergence as a significant nosocomial pathogen, particularly in the setting of prosthetic devices[2]. The ability of the bacterium to form biofilms over foreign bodies, in which bacteria are protected from antibiotics and the immune system, is key in the evolution of disease[3]. *S. epidermidis* and other coagulase-negative staphylococci (CoNS) are leading causes of surgical site and central-line-associated bloodstream infections[4], with major economic implications[5]. In spite of this impact, relatively little is understood about the mechanisms of pathogenesis and optimal treatment of *S. epidermidis*. Many of the clinical decisions made when treating this species are based on assumptions from studies in *Staphylococcus aureus*.

A single *S. epidermidis* lineage, multilocus sequence type (MLST) 2, dominates in the hospital environment. MLST eBURST analysis of a global strain collection found 74% of nosocomial isolates belong to clonal complex 2, for which ST2 is the founder[6]. The *S. epidermidis* population structure, as assessed by MLST, comprises six genetic clusters (GCs)[7,8]. Isolates from GC5 (encompassing ST2 and ST23) are almost exclusively nosocomial[8] and significantly enriched for antibiotic resistance and biofilm production, suggesting hospital adaptation[7]. Methicillin resistance in *S. epidermidis* has been reported to be as high as 70–92% in some institutions[9], and is frequently associated with co-resistance to other antibiotic classes[10,11].

We recently reported the first complete genome and methylome of BPH0662, an ST2 *S. epidermidis* exhibiting near pan-drug resistance, including intermediate heteroresistance to vancomycin, responsible for a serious post-neurosurgical infection[12]. Vancomycin intermediate heteroresistance is characterized by the presence of bacterial subpopulations capable of growth within the intermediate range, despite testing as vancomycin-susceptible by standard laboratory methods. Few studies have described the phenomenon in *S. epidermidis*[13,14] or CoNS collectively[15–17]. The diagnostic definitions and clinical implications of *S. epidermidis*

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vancomycin heteroresistance are poorly understood, and resistance mechanisms remain unknown.

Ongoing cases of similarly pan-resistant *S. epidermidis* infections in unrelated patients within our hospital suggested dissemination of a multidrug-resistant lineage. We began investigations using comparative and functional genomics to characterize the molecular epidemiology and resistance mechanisms of *S. epidermidis* linked to clinically significant infections, initially within our institution and then globally.

Results

Increasing prevalence of multidrug-resistant *S. epidermidis*. To address the hypothesis that a multidrug-resistant strain was spreading at Austin Health (an 800-bed tertiary hospital in Melbourne, Australia), the antibiotic susceptibilities of clinical *S. epidermidis* isolates from 2007 to 2013 were explored (see Methods for clinical definition). We observed an increase in teicoplanin resistance from 3.6% in 2007 to 35.3% in 2013 (Fig. 1a). The proportion of isolates that exhibited dual resistance to rifampicin and fusidic acid also progressively rose from 10.9% in 2007 to 31.4% in 2013 (Fig. 1b). Rifampicin resistance correlated with teicoplanin resistance. Like our previously described *S. epidermidis* BPH0662 index case isolate[12], these isolates were also resistant to β-lactams, macrolides, quinolones, aminoglycosides and sulfonamides, indicating the emergence of multidrug-resistant *S. epidermidis* (MDRSE) within our institution. Using rifampicin and fusidic acid resistance as markers for MDRSE, 52 clinically significant MDRSE isolates were identified, of which 33 were available for further testing. Thirty-five clinically significant non-MDRSE comparator strains, two non-MDRSE reference strains (ATCC 12228[18] and RP62a[19]), BPH0662 and BPH0663 (subsequent isolate from the index case) were also included in analyses (full antibiograms are shown in Supplementary Fig. 1). In *S. aureus*, specific rifampicin resistance mutations in *rpoB* result in vancomycin heteroresistance[20,21]. We postulated that MDRSE vancomycin heteroresistance might also be explained by rifampicin resistance mutations. Macromethod Etests (METs) are a sensitive method for the detection of reduced glycopeptide susceptibility in staphylococci[22]. METs performed on these 72 isolates revealed a significant association with heteroresistance to vancomycin (Fig. 1c) and teicoplanin (Fig. 1d) for MDRSE compared to non-MDRSE ($P<0.0001$ for both).

International clonal expansion of endemic, multidrug-resistant, ST2 and ST23 *S. epidermidis* lineages resulting in clinical disease. To investigate the relationship between clinically significant isolates of MDRSE and non-MDRSE at our institution, whole-genome comparisons were made of the 72 isolates plus two additional published, finished *S. epidermidis* genomes. A maximum-likelihood phylogeny was inferred from an alignment of 54,493 core genome single nucleotide polymorphisms (SNPs) (Fig. 2). Bayesian analysis of population structure (BAPS), derived from core-SNP analysis, categorized the 74 genomes into five groups (named A–E). The largest (group E) represented ST2, accounting for 59% of isolates and consisted of two dominant sublineages, one MDRSE ($n=27$), to which the BPH0662 reference belonged, the other predominantly non-MDRSE ($n=16$), of which two were MDRSE (henceforth referred to as the ST2-mixed lineage). A third cluster of five MDRSE isolates belonging to the ST23 lineage (BAPS group C) was also identified (Fig. 2). The presence of isolates from three distinct lineages across at least six years indicated that each was endemic within our institution, resulting in repeated, clinically significant, nosocomial infections.

We next sought to determine the broader distribution of these three MDRSE lineages across Australia and globally. A further 32 rifampicin-resistant clinical *S. epidermidis* isolates from 13 institutions in Australia, and 121 global isolates from Austria ($n=1$), Belgium ($n=18$), Denmark ($n=38$), France ($n=18$), Germany ($n=21$), Republic of Ireland ($n=1$), United Kingdom ($n=12$) and the United States ($n=12$) originating from 61 institutions were collected, totalling 227 isolates (Supplementary Table 1a).

A maximum-likelihood phylogeny for the 227 isolates was inferred from an alignment of 56,756 core genome SNPs and revealed the same basic population structure as before, with the majority of isolates clustered into three MDRSE lineages (Fig. 3 and Supplementary Fig. 2a). Overall, core-SNP-based comparisons suggested high genomic identity within the BPH0662 lineage, with a median pairwise SNP difference of 62 SNPs (interquartile range (IQR) 41–82). In contrast, the median difference within the ST2-mixed clade was 281.5 SNPs (IQR 190.25–378), indicating more genetic diversity among ST2-mixed isolates. Compared to one another, the two ST2 lineages were distinct with a median of 1,669 SNPs (IQR 1,649–1,703) between them (Fig. 3b,c). The ST23 MDRSE also demonstrated high intra-lineage identity with a median difference of 103 SNPs (IQR 88–116), while the ST5 lineage was composed of heterogeneous isolates with a median intra-group difference of 374 SNPs (IQR 141–587) (Fig. 3c).

The BPH0662 ST2 MDRSE lineage, dominant in Australia, was also identified from 25 institutions in four countries (Australia, Belgium, Denmark and the United Kingdom) and persisted within two Australian sites for at least 8 years, while the ST2-mixed lineage was identified in 33 institutions within seven countries (Australia, Belgium, Denmark, France, Germany, Ireland and the United Kingdom). The ST23 lineage was present in 17 institutions across seven countries (Australia, Austria, Belgium, Denmark, France, Germany and the United States); this lineage persisted up to 17 years in Denmark and 15 years at a single site in the United States. Co-circulation of all three clones was observed in two of the European sites (BEL-A and DEN-G). While isolates from the same country tended to cluster within each of the three MDRSE lineages, genotype intermingling was also observed across countries, consistent with the international dissemination of three individual successful clones (Fig. 3 and Supplementary Fig. 2).

Pan-genome analysis identified 5,692 orthologue gene clusters. Minimal variability in gene content was observed within the BPH0662 ST2 clade (Supplementary Fig. 2c). The accessory gene content specific to this lineage was the same as previously characterized for BPH0662, and clustering based on genome content aligned strongly with core genome phylogeny (Supplementary Fig. 2d), the MDRSE ST2-mixed lineage contained similar accessory gene content (Supplementary Fig. 2c). All ST2 and subclustered ST188 ($n=133$) and ST23 ($n=50$) isolates possessed the *icaRADBC* operon, which was not present in any of the ST5 or subclustered ST87 ($n=15$) isolates. The *ica* operon was highly conserved among 193 isolates (Supplementary Fig. 2c). Orthologue clustering clearly demonstrated that RP62a (ST10), a reference isolate frequently used in laboratory experiments, is an outlier compared to other clinical isolates (Supplementary Fig. 2d).

Evolutionary phylogeny suggests independent coevolution of two ST2 and one ST23 multidrug-resistant *S. epidermidis* lineages. To model the evolutionary trajectory of *S. epidermidis* and date the emergence of the drug-resistant ST23 and ST2 lineages, we used core genome SNP diversity and year of isolation to infer a time-scaled phylogeny in a Bayesian framework (Supplementary Fig. 3). Overall, this analysis suggested that each of the three drug-resistant lineages emerged independently in the 1980s (BPH0662 ST2 clade: 1982 (95% highest posterior density interval (HPDI) 1972–1991); the major rifampicin-resistant subclade of ST2-mixed: 1987 (95% HPDI 1978–1998); ST23 clade: 1984 (95% HPDI 1974–1992)). This analysis also suggested the first substantial diversification within the ST2 lineage around 1958 (95% HDPI 1940–1974).

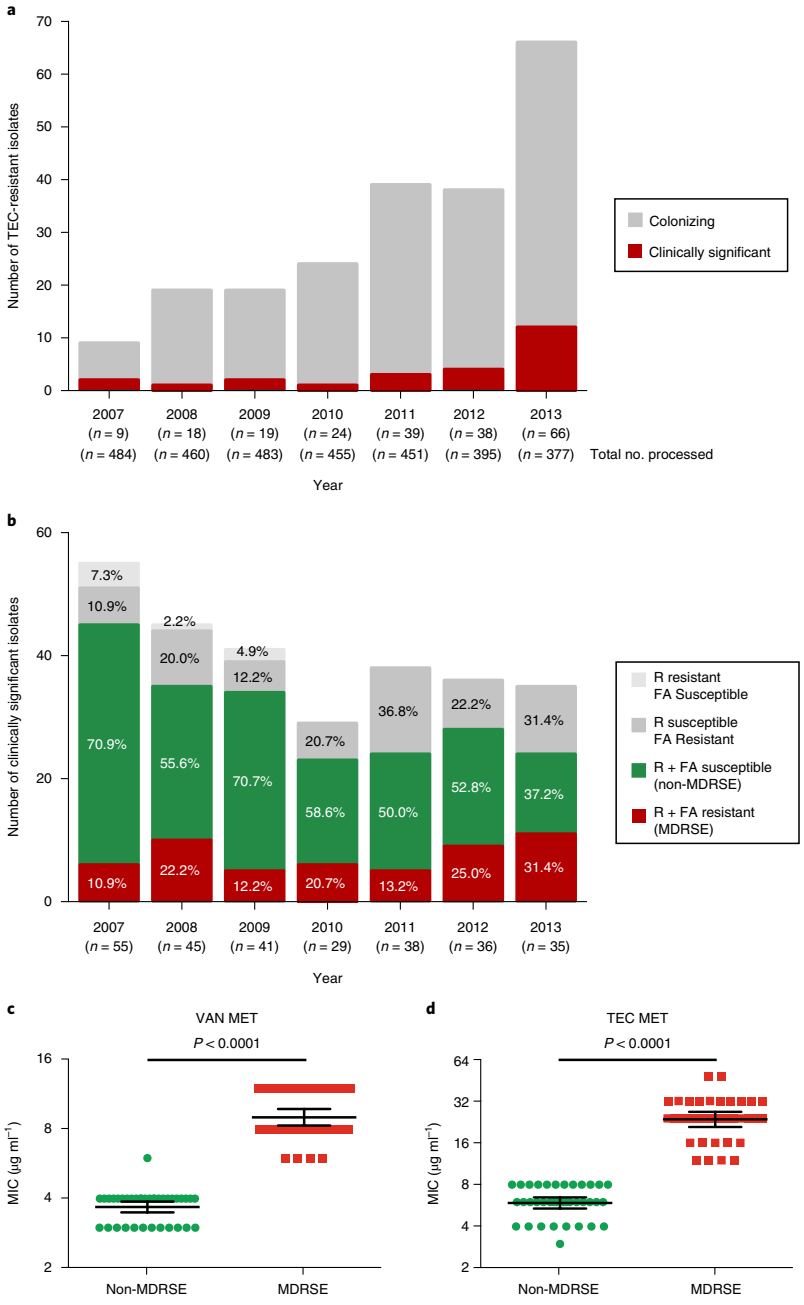

Fig. 1 | Increasing prevalence of multidrug-resistant *S. epidermidis* (MDRSE). The prevalence of antibiotic-resistant *S. epidermidis* isolates at a single Australian institution over a seven-year period is shown. Susceptibilities were performed on a Vitek 2 (bioMérieux) platform and interpreted using CLSI criteria. **a**, Prevalence of teicoplanin (TEC)-resistant isolates. **b**, Prevalence of clinically significant isolates exhibiting rifampicin (R) and fusidic acid (FA) resistance. **c**,**d**, Vancomycin (VAN) (**c**) and teicoplanin (TEC) (**d**) METs were performed on 70 clinically significant *S. epidermidis* strains (35 rifampicin- and fusidic acid-susceptible (non-MDRSE); 35 rifampicin- plus fusidic acid-resistant (MDRSE)) plus two non-MDRSE reference isolates (RP62a and ATCC 12228); $n = 72$. The y axis is plotted on a $\log_2$ scale. Error bars represent geometric mean + 95% confidence interval (CI). Null hypothesis (no difference between means) was rejected for $P < 0.05$ (two-tailed Mann–Whitney U test of $\log_2$ transformed MICs).

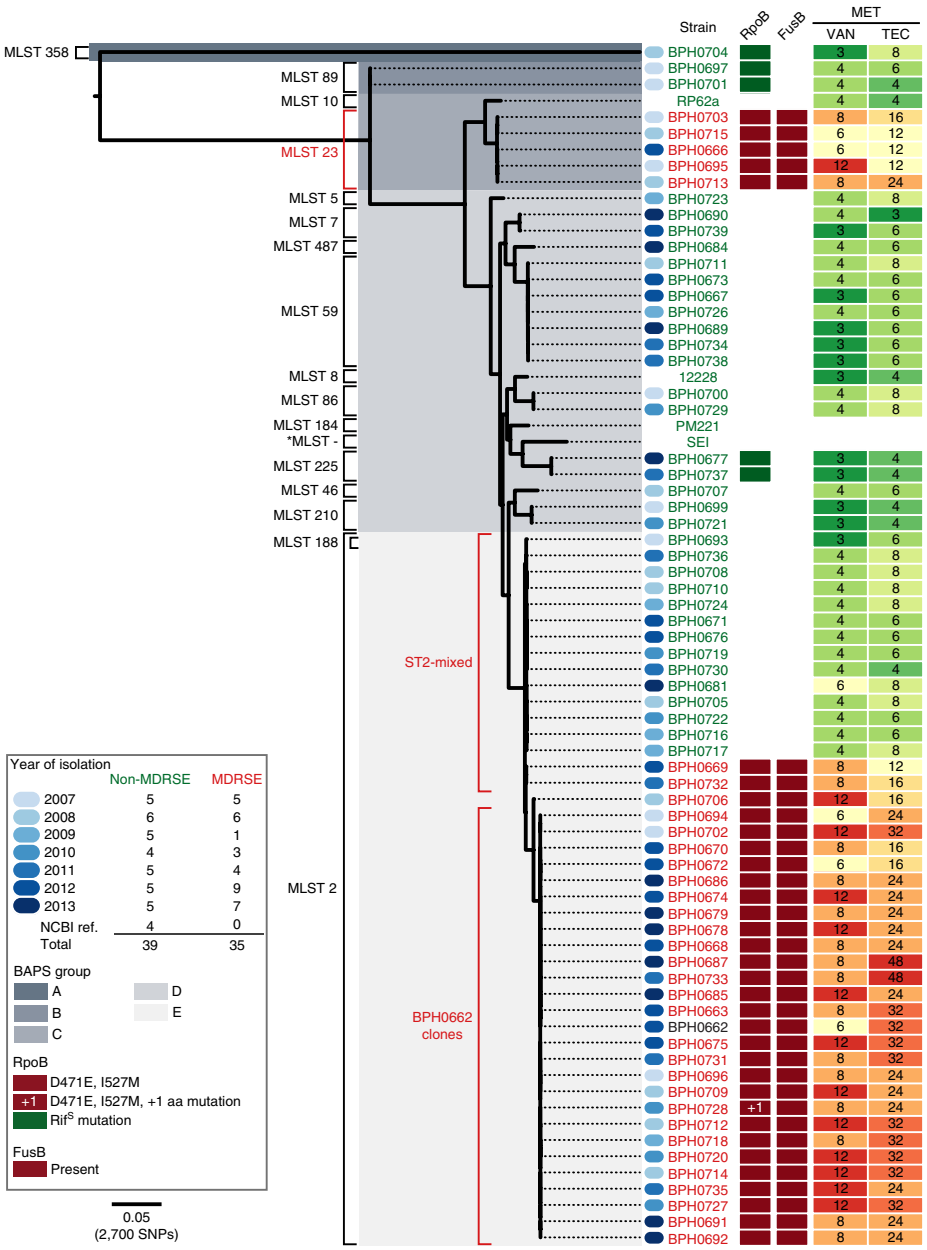

Fig. 2 | Clonal expansion of endemic, multidrug-resistant ST2 and ST23 *S. epidermidis* lineages resulting in clinical disease within a single institution. Maximum-likelihood core-SNP-based phylogeny for 69 clinically significant strains collected from 2007 to 2013 (34 multidrug *S. epidermidis* (MDRSE); 35 non-MDRSE); four closed, published *S. epidermidis* genomes; and the closed BPH0662 genome from the MDRSE index case (used as the reference strain); $n=74$. Overlaid are the results of in silico MLST, BAPS, the determinants of rifampicin (RpoB mutations) and fusidic acid (*fusB*) resistance, and heatmap of vancomycin heteroresistant phenotype testing, as determined by VAN and TEC METs. The scale bar indicates number of nucleotide substitutions per site (bold), with an approximation of SNP rate (in parentheses). *Published reference strain SEI was not classifiable by the existing MLST scheme.

Genetic determinants of antibiotic resistance. Ninety-nine percent of the 133 ST2 isolates possessed *mecA* (Supplementary Table 1d), which correlated with the antibiogram data (Supplementary Fig. 1). Among these, 106 possessed the full *mecA*, *mecR1* and *mecI* complex as a typical class A *mec*. The *fusB* gene was present in 139 of the 143 fusidic-acid-resistant isolates, in keeping with previous reports[23,24],

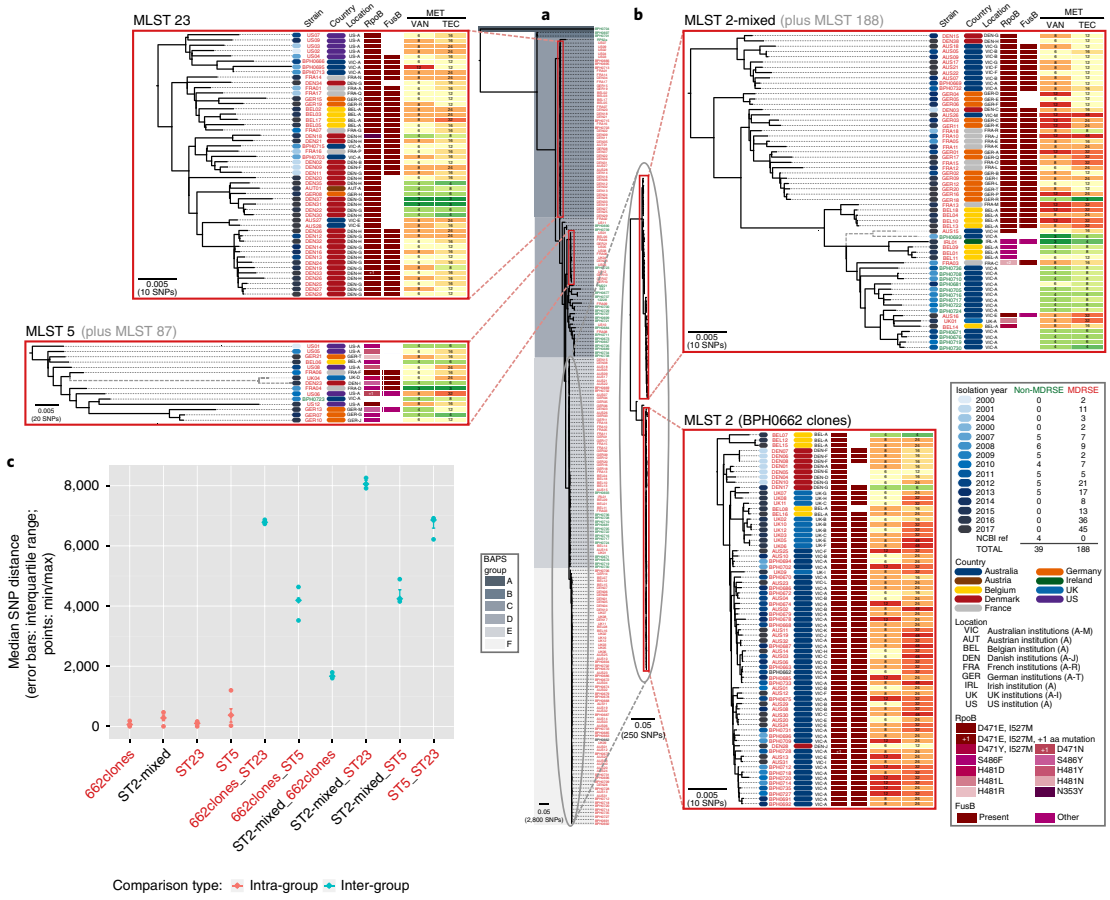

Fig. 3 | International clonal expansion of endemic, multidrug-resistant ST2 and ST23 *S. epidermidis* lineages resulting in clinical disease. a, Maximum-likelihood, core-SNP-based phylogeny of 227 clinical isolates originating from 77 institutions in 10 countries, using BPH0662 as the reference genome, with BAPS groups overlaid. **b**, Subset analyses of the 133 ST2 isolates (plus ST188, a single locus variant of ST2). Insets show subset analyses for each of the four main MDRSE lineages: ST2-mixed ($n=60$); ST2 BPH0662 clones ($n=71$); ST23 ($n=50$); ST5 ($n=15$). Overlaid are the results of the determinants of rifampicin (RpoB mutations) and fusidic acid (*fusB*) resistance, and heatmap of vancomycin heteroresistant phenotype testing, as determined by VAN and TEC METs. BPH0662 was used as reference for all core-SNP subset analyses. Scale bars indicate number of nucleotide substitutions per site (bold), with an approximation of SNP rate (in parentheses). **c**, Pairwise-SNP analyses of the four main MDRSE lineages (sample sizes as in **b**).

the majority in chromosomal phage resistance islands: SeRI$_{fusB\text{-}704}$ (accession no. JF808725) or SeRI$_{fusB\text{-}5907}$ (accession no. JF777506) both attached downstream of *groEL* ($n=121$); or SeRI$_{fusB\text{-}7778}$ (JF808726), attached downstream of *rpsR* ($n=13$). Eighteen linezolid-resistant isolates were identified, all European (Germany $n=14$, France $n=4$, Ireland $n=1$). At least one of three recognized genetic determinants of linezolid resistance were identified for each isolate, including acquisition of *cfr*; the G2576T substitution in domain V of 23S rRNA (equivalent to G2602T in *S. epidermidis*)[25,26], or insertion of a glycine residue ($_{71}$GR$_{72}$ to $_{71}$GGR$_{72}$) in ribosomal protein L4[26] (Supplementary Table 2). All five of the *cfr*-positive isolates were from Germany, three of which had acquired *S. epidermidis* plasmid p12-02300 (accession no. KM521837) previously associated with an outbreak of linezolid resistance in German hospitals[25], one had a previously undescribed plasmid. Twelve isolates were resistant to daptomycin, two of which (AUS14 and FRA09) had an MprF S295L substitution, known to cause daptomycin resistance[27]. Due to the pleiotropic nature of daptomycin resistance, other potential causative mutations were not identified.

Two co-occurring mutations in RpoB, D471E and I527M, explained rifampicin resistance for 163 of the 187 isolates in this study. Two isolates had alternative D471 substitutions. The remaining had at least one RpoB substitution occurring at site H481 ($n=7$), S486 ($n=13$), V135 ($n=2$) and N353 ($n=1$) (Supplementary Figs. 2 and 4). Based on alignment with the known sequence defined for other bacterial species, the rifampicin resistance determining region (RRDR) in *S. epidermidis* spanned amino-acid positions 462 to 488 (inclusive). Three critical positions known to form covalent bonds with rifampicin in other species (at which most rifampicin-resistant mutations occur)[28,29] were determined to correspond to D471, H481 and S486. Although outside the RRDR, sites V135 and I527 aligned with positions also known to be associated with rifampicin resistance in other bacterial species[28,29]. Glycopeptide susceptibility testing found the majority of isolates with the dual RpoB

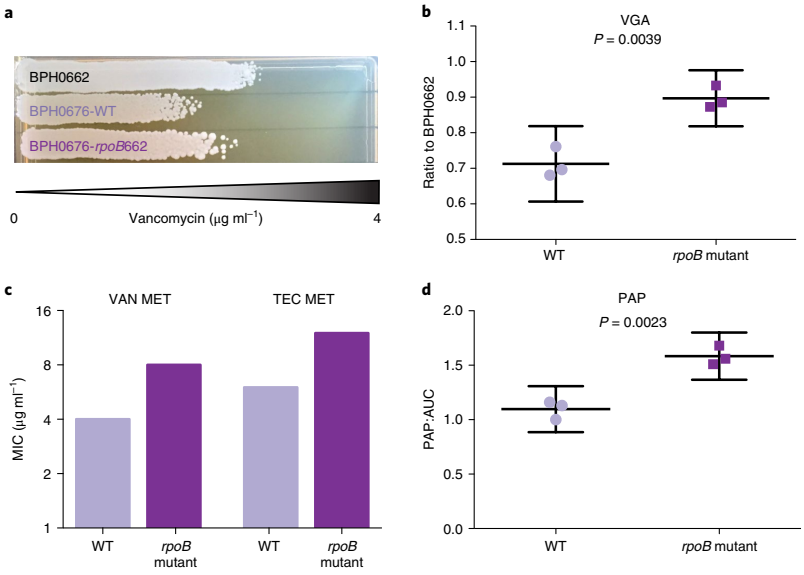

Fig. 4 | RpoB mutations confer vancomycin heteroresistance in *S. epidermidis*. **a**, An example of a VGA for BPH0676-WT, BPH0676-*rpoB*662 and the BPH0662 control strain. **b**, Biological triplicate data for the BPH0676-WT and BPH0676-*rpoB*662 mutant pair compared by VGA. Error bars represent mean ± 95% CI of three independent experiments. Null hypothesis (no difference between means) was rejected for $P < 0.05$ (two-tailed, unpaired Student's *t*-test). **c**, Data for a single set of VAN and TEC METs for BPH0676-WT and the BPH0676-*rpoB*662 mutant pair. The *y* axis for METs is plotted on a $\log_2$ scale. **d**, Biological triplicate data for the BPH0676-WT and BPH0676-*rpoB*662 mutant pair compared by vancomycin PAP. Error bars represent mean ± 95% CI of three independent experiments. Null hypothesis (no difference between means) was rejected for $P < 0.05$ (two-tailed, unpaired Student's *t*-test).

D471E and I527M mutations (96.9%) or single RpoB H481D/L/R/Y substitutions (100%) were vancomycin heteroresistant (Fig. 3 and Supplementary Fig. 2).

Dual D471E and I527M substitutions are the most common RpoB mutations in *S. epidermidis* worldwide. To confirm that the dominant RpoB mutations observed in our study were representative of other *S. epidermidis* globally, we performed an analysis of all publicly available *S. epidermidis* sequence data. Using the selection/exclusion criteria outlined in the Methods, a curated list of 160 clinical isolates (see Supplementary Table 1 for full metadata) downloaded from NCBI Sequence Read Archive (SRA) were assembled, and the *rpoB* genes analysed in conjunction with the four NCBI reference strains and the 222 isolates from this study (BPH0663 was excluded as an in vivo serial isolate of BPH0662); data from a Swedish study by Hellmark et al.[30] in which the *rpoB* gene of 33 *S. epidermidis* strains isolated from prosthetic joint infections were sequenced and correlated with phenotype were also included. Of the 419 isolates analysed, 251 were identified as having at least one of 40 putative amino-acid substitutions in RpoB, occurring across 32 sites. The dual D471E and I527M substitutions were the most common, present in 187 of the isolates, accounting for 73.9% of the observed mutations, and 86.6% of the mutations occurring within the RRDR of RpoB (Supplementary Fig. 4). Interestingly, neither D471E nor I527M were observed as solitary substitutions. Analysis of metadata indicated that isolates possessing the dual mutations originated from 68 different institutions in 11 countries, and in silico MLST demonstrated ST2 (62.9%) and ST23 (33.1%), accounted for 96.0% of isolates with the dual D471E and I527M substitutions.

Dual D471E and I527M mutations in RpoB confer co-resistance to rifampicin and vancomycin in *S. epidermidis*. To confirm a causal link between these mutations and vancomycin heteroresistance we swapped the D471E and I527M *rpoB* allele from BPH0662 into four rifampicin-susceptible *S. epidermidis* backgrounds—two clinical, non-MDRSE ST2 strains, BPH0676 and BPH0736; and reference strains RP62a (ST10) and ATCC 12228 (ST8)—using plasmid artificial modification and allelic exchange. Acquisition of rifampicin resistance enabled positive screening of mutants. Whole-genome sequencing confirmed the presence of only the intended two substitutions in *rpoB*, with the exception of 12228-*rpoB*662, which acquired an additional spontaneous SNP leading to a M305L substitution in a hypothetical regulatory protein (locus_tag SE2129). To the best of our knowledge, this is the first successful report of allelic exchange in ST2 *S. epidermidis*.

Compared to the four wild-type (WT) parental strains—BPH0676, BPH0736, RP62a and ATCC 12228—all *rpoB*662 mutants acquired high-level rifampicin resistance equivalent to that of BPH0662 (64 μg ml^{-1}). The mutants were not identified as glycopeptide resistant using standard testing with vancomycin broth microdilution (BMD) or Vitek 2. To determine the role of these mutations in the evolution of glycopeptide heteroresistance, extended glycopeptide testing by vancomycin gradient assay (VGA), vancomycin and teicoplanin METs and a vancomycin population analysis profile to area under the curve ratio (PAP:AUC) was performed for each of the WT and *rpoB*662 mutant pairs. Experiments for the BPH0676-WT and BPH0676-*rpoB*662 pair are shown in Fig. 4. Compared to its parental strain, the BPH0676-*rpoB*662 mutant had a statistically significant increase in mean ratio to BPH0662 for VGA ($P = 0.0039$) and PAP:AUC ($P = 0.0023$). Furthermore, the gains observed in both vancomycin and teicoplanin MET MICs for the BPH0676-*rpoB*662 mutant achieved the criteria for vancomycin heteroresistance. Statistically significant increases in glycopeptide resistance were also observed for the other *rpoB*662 mutants

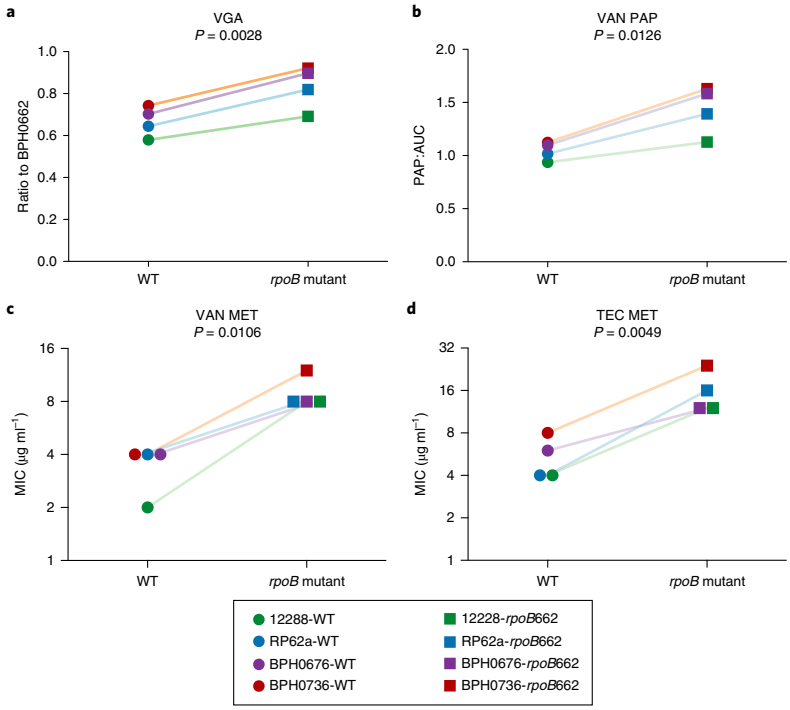

Fig. 5 | RpoB D471E and I527M mutations cause vancomycin heteroresistance in four different *S. epidermidis* backgrounds. a–d, RpoB D471E and I527M dual mutations were introduced into four different, rifampicin-susceptible *S. epidermidis* strains: two clinically significant ST2 strains, BPH0676 and BPH0736, and reference strains RP62a (ST10) and ATCC 12228 (ST8). The gains in vancomycin tolerance of these *rpoB*662 mutants compared to their respective WT parental strains were individually validated (Supplementary Fig. 4), then summary data analysed. Three different phenotypic methods for the detection of vancomycin heteroresistance were used: VGA (**a**), VAN PAP (**b**), and VAN (**c**) and TEC (**d**) METs. The *y* axis for METs is plotted on a $\log_2$ scale. Data points for VGA and VAN PAP represent the mean of three independent experiments for each strain. Null hypothesis (no difference between means) was rejected for $P < 0.05$ (two-tailed, paired Student's *t*-test).

compared with their respective parental strains (Supplementary Fig. 5). Having independently validated each mutant compared to their parental strain, the pairs were then analysed collectively. For each phenotypic testing method, a statistically significant increase in mean glycopeptide resistance was observed for the *rpoB*662 mutant group compared to their paired parental strains (Fig. 5). These experiments show that D471E and I527M RpoB substitutions cause the vancomycin-heteroresistant phenotype.

Mutants containing dual D471E and I527M RpoB substitutions outcompete WT *S. epidermidis* in the presence of vancomycin. To assess the advantage conferred by the dual D471E/I527M RpoB substitutions in the presence of vancomycin, 1:1 competition assays for BPH0736-WT and its *rpoB*662 mutant were conducted. In the absence of vancomycin, the D471E/I527M RpoB allele posed a substantial fitness cost, with a significant shift to the WT population at 48 h (Fig. 6). However, with the introduction of vancomycin the population dynamics reversed and at 48 h with 4 μg ml^{-1} vancomycin, the population was 100% *rpoB*662 mutant (Fig. 6). Individual growth curves for each of the WT and *rpoB*662 mutant pairs were not significantly different in the absence of antibiotics (Supplementary Fig. 6). These experiments confirm that the D471E and I527M RpoB substitutions in *S. epidermidis* were capable of generating both high-level rifampicin resistance as well as vancomycin heteroresistance in *S. epidermidis* strains from divergent backgrounds.

Discussion

We have uncovered here the emergence of three genetically distinct MDRSE lineages, with common dual D471E and I527M RpoB substitutions, that have evolved within the hospital environment to exhibit near pan-drug resistance. The presence of the same mutations in multiple phylogenetically distant lineages is consistent with independent emergence of these mutations with subsequent fixation, presumably due to their favourable antibiotic-resistant phenotype. The international prevalence of these dual mutations, as determined in this study, suggests that a high proportion of rifampicin-resistant clinical *S. epidermidis* isolates possess mutations that also confer glycopeptide heteroresistance, not detectable by standard diagnostic methods. Although dominance may vary by region, the same three lineages, with low intra-group genomic diversity, were demonstrated to be endemic and co-circulate within multiple institutions worldwide, suggesting their unrecognized presence in other establishments. The diminishing pool of antibiotics available for the treatment of infections due to such isolates, and the increasing catalogue of complex genetic interactions that contribute to antimicrobial resistance between unrelated drug classes, reinforces the need for the judicious utilization of remaining therapeutic options.

In particular, this research has implications for how rifampicin is utilized in clinical practice. Device impregnation with rifampicin is of particular concern, as low-level rifampicin monotherapy released in this situation could predispose colonizing

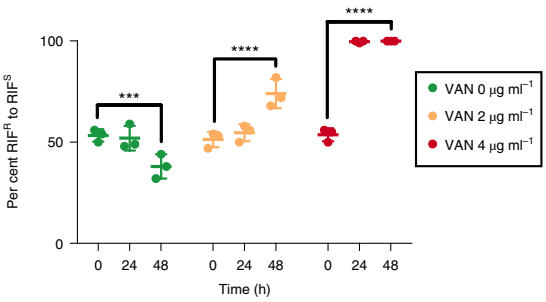

Fig. 6 | Mutants containing dual D471E and I527M RpoB substitutions outcompete WT *S. epidermidis* in the presence of vancomycin. Competition assays for the BPH0736-WT and *rpoB*662 mutant pair performed in the presence of VAN 0, 2 and 4 μg ml^{-1}, with the percentage of rifampicin resistant (RIF^R) to rifampicin susceptible (RIF^S) isolates determined by plate count. All data points for biological triplicate experiments are displayed. Horizontal lines depict mean and error bars show standard deviation. Null hypothesis (no difference between means) was rejected for $P < 0.05$. Differences assessed using two-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons. ***$P < 0.001$, ****$P < 0.0001$.

S. epidermidis to develop rifampicin resistance and concomitant vancomycin heteroresistance. To avoid generating resistance with monotherapy, combination therapy is recommended when using rifampicin for the treatment of staphylococci[31,32]. Historical studies describing dual therapy with vancomycin and rifampicin for *S. epidermidis* prosthetic valve endocarditis[33] have informed current staphylococcal clinical guidelines[31]. However, the emergence of rifampicin resistance when using this treatment combination for serious infections has been reported for both *S. epidermidis*[34] and *S. aureus*[35–37]. Our results suggest that co-prescription of vancomycin and rifampicin will promote the generation of resistance to both agents, although additional experiments are required to test this hypothesis. Widespread loss of vancomycin as a first-line treatment option against *S. epidermidis* would have significant clinical impact, especially in view of the limited available substitutes. While alternative agents such as daptomycin, ceftaroline, lipoglycopeptides and linezolid exist, staphylococcal resistance to all has been described[36]. Considered in this context, our confirmation of a causal association between rifampicin resistance mutations and vancomycin heteroresistance in *S. epidermidis*, together with clinical reports demonstrating lack of cross-protection, suggest that current clinical recommendations warrant review. Alarmingly, our study has identified that near untreatable isolates already exist, with two German vancomycin heteroresistant isolates, one ST2-mixed (GER16) the other an ST23 clone (GER15), also testing as resistant to both daptomycin and linezolid, with tetracycline the only antibiotic option remaining (Supplementary Fig. 1).

Surveillance is not routinely performed for *S. epidermidis*, and consequently there are no robust data on the prevalence of rifampicin resistance in this species. Reference laboratories may be sent MDRSE isolates of clinical concern, but no denominator data exist to determine the background number of susceptible isolates. The data from individual centres contributing to this study suggested local variation in the annual prevalence of *S. epidermidis* rifampicin resistance at each institution between 2012 and 2017: 10% increasing to 20% for Erasme Hospital, Belgium; 10% for Monash Health, Australia; 6% for Hvidovre Hospital, Denmark; and 3% for Mayo Clinic, Rochester, MN, USA. These rates included all *S. epidermidis* isolated from clinical samples for each institution, and almost certainly underestimated the prevalence in clinically significant isolates. This was highlighted by our Australian data, which demonstrated that despite only 10.8% of all *S. epidermidis* isolated from blood cultures in the state of Victoria over the month of March 2017 testing rifampicin-resistant, 30.0% of the clinically significant isolates were rifampicin-resistant (all of which were vancomycin-heteroresistant). In view of the ubiquitous nature of *S. epidermidis* and difficulties distinguishing colonizing from clinically significant isolates, determining the true prevalence of these MDRSE lineages is likely to remain a challenge. Therefore, rifampicin resistance is a potentially useful marker for identifying these clinical *S. epidermidis* clones.

The retrospective nature of the isolate collection we used, which was biased towards the acquisition of multidrug-resistant *S. epidermidis*, is a limitation of this study, and sampling may have missed some less prevalent genotypes. Furthermore, we did not have access to data regarding the treatment received or clinical outcomes of the patients from whom isolates were obtained. Notwithstanding these limitations, we obtained a good representation of the international diversity of rifampicin resistance in clinical *S. epidermidis*, which was the main focus of this study. Having identified the existence of three MDRSE *S. epidermidis* lineages with international presence, future research could include more geographically diverse, prospective sampling of isolates with associated clinical data to include treatment and outcomes, which could better delineate the global extent of these clones and offer insight into the clinical impact of these lineages. Our findings indicate that similar glycopeptide resistant isolates are likely to be present within other institutions but unrecognized by standard phenotypic testing methods, highlighting a need for clinical vigilance. These data challenge current assumptions that cross-protection is afforded by the co-prescription of rifampicin and vancomycin for the treatment of staphylococci, indicating that a review of clinical guidelines is warranted to avoid the further selection of near untreatable clones.

Methods

Media and reagents. *S. epidermidis* and *S. aureus* were routinely cultured at 37 °C in BBL brain heart infusion (BHI) broth (Becton Dickson). For electroporation, *S. epidermidis* was cultured in B medium (1% peptone, 0.5% yeast extract, 0.5% NaCl, 0.1% K_2HPO_4, 0.1% glucose). *Escherichia coli* was routinely cultured in L broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl). BMD minimum inhibitory concentrations (MICs) were performed in cation-adjusted Mueller Hinton (MH) broth (Difco). For growth on agar, BHI or L broth were solidified with 1.5% agar, to yield BHIA and LBA, respectively. The following antibiotics were purchased from Sigma Aldrich and used at the specified concentrations: chloramphenicol (Cm) 10 μg ml^{-1} for *S. epidermidis*, 15 μg ml^{-1} for *E. coli*; ampicillin (Amp) 100 μg ml^{-1} for *E. coli*; kanamycin (Kan) 50 μg ml^{-1} for *E. coli*. The following antibiotics were used at variable concentrations for susceptibility testing: rifampicin (Sigma Aldrich) and vancomycin (Hospira).

Oligonucleotides were purchased from Integrated DNA Technologies and are listed in Supplementary Table 3. Genomic DNA was isolated with DNeasy Blood and Tissue Kit (Qiagen). To weaken the cell wall of *S. epidermidis* before DNA extraction, harvested cells were washed with PBS. Lysostaphin (Ambi) was added to the Gram-positive lysis buffer (final concentration 100 μg ml^{-1}) and

incubated at 37 °C for 30 min. Plasmids were purified with QIAprep Spin Miniprep Kit (Qiagen). PCR products and gel extractions were purified using QIAquick Gel Extraction Kit (Qiagen). Restriction enzymes and Phusion DNA polymerase were purchased from New England Biolabs. Phire Hotstart DNA polymerase was purchased from Thermofisher. Colony PCR was performed as previously described[38].

Bacterial isolates. Bacterial strains used in this study are listed in Supplementary Table 3. Comparator strains consisted of four reference strains (*S. epidermidis* ATCC 12228, ATCC 35984 [RP62a]; *S. aureus* ATCC 700698 [Mu3], ATCC 700699 [Mu50]); 69 clinically significant *S. epidermidis* isolates (34 MDRSE; 35 non-MDRSE) collected at Austin Health, Victoria, Australia, from 1 January 2007 to 31 December 2013 (all available MDRSE were included as comparators; for non-MDRSE comparators, five strains were randomly selected for each year, with the exception of 2010, for which only four clinically significant non-MDRSE were available); 10 clinically significant MDRSE collected from 1 January 2010 to 31 December 2013 originating from three hospitals within the Monash Health network in Victoria, Australia; 22 rifampicin-resistant blood culture isolates originating from 12 different hospitals submitted to the Microbiological Diagnostic Unit Public Health Laboratory, Victoria, Australia, collected during the month of March 2017 as part of a state-wide *S. epidermidis* survey. For Australian isolates, clinical significance was defined as repeated culture (≥2 occasions) of isolates with identical antibiograms as determined by Vitek 2 (bioMérieux), from the same patient, from bloodstream or sterile site samples. International comparator strains consisted of 18 rifampicin-resistant blood culture isolates collected between 2015 and 2017 at Erasme Hospital, Brussels, Belgium; 27 rifampicin-resistant blood culture isolates collected between 2012 and 2017 submitted to the Department of Clinical Microbiology, Hvidovre University Hospital, Copenhagen, Denmark, originating from four institutions; 11 rifampicin-resistant clinical isolates submitted to Statens Serum Institut, Copenhagen, Denmark in 2001, originating from six institutions (including Hvidovre Hospital); 18 rifampicin-resistant clinical isolates submitted to the Institute for Infectious Agents Department of Bacteriology French National Reference Centre for Staphylococci, Lyon, France, between 2004 and 2016 originating from 18 different hospitals; 22 clinically significant rifampicin-resistant isolates submitted to the National Reference Centre for Staphylococci and Enterococci, Robert Koch Institute, Wernigerode, Germany from 2012 to 2017, originating from 20 different institutions in Germany and one from Austria; 13 rifampicin-resistant clinically significant isolates submitted to the Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, National Infection Service, Public Health England, between November 2015 and November 2016, originating from 10 different hospitals in the United Kingdom and the Republic of Ireland; 12 rifampicin-resistant clinically significant isolates collected between 2000 and 2016 at the Mayo Clinic, Rochester, MN, United States. To maximize recruitment, rifampicin resistance alone was used as a marker for MDRSE since prescription of fusidic acid is subject to regional practice and use in the United States is still undergoing trials, seeking Food and Drug Administration approval.

Antibiotic susceptibility testing. The background antibiogram of *S. epidermidis* for Austin Health from 2007 to 2013 was determined by collating all routine isolates that had susceptibility testing performed (interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria), excluding duplicate strains obtained from the same patient. Comparator isolates underwent susceptibility testing with a Vitek 2 Gram Positive ID card (AST-P612; bioMérieux) on a Vitek 2 (bioMérieux) according to the manufacturer's instructions. BMD MICs for vancomycin and rifampicin were performed as recommended by CLSI[39]. Rifampicin Etests (bioMérieux) were performed according to the manufacturer's instructions. Extended glycopeptide susceptibilities were determined by MET with vancomycin and teicoplanin Etest strips (bioMerieux) using a 2.0 McFarland inoculum and prolonged incubation time of 48 h. *S. aureus* criteria for vancomycin heteroresistance were used (vancomycin and teicoplanin MET of >8; teicoplanin MET alone of >12), as no criteria have been defined for *S. epidermidis*. Vancomycin PAPs were performed as previously described[40], but overnight *S. epidermidis* cultures were adjusted to an optical density at 600 nm (OD_{600}) of 0.4. For VGAs, 50 ml of BHIA supplemented with vancomycin 4 μg ml^{-1} was set in a 10 cm square Petri dish with one side elevated at a 5° angle (to form a two-dimensional gradient); the Petri dish was then placed level and a second 50 ml BHIA layer was set over the vancomycin layer. Plates were used within 48 h. Overnight cultures of tested strains were standardized to an OD_{600} of 0.08 in BHI and a 10 μl loop was used to streak the strain across the plate from vancomycin 0 to 4 μg ml^{-1}. Distance of growth was measured in mm after 48 h incubation at 37 °C, and expressed as a ratio to growth of control strain, BPH0662. With the exception of Vitek 2 and Etests, all antibiotic susceptibility testing of strains was performed in biological triplicate. Interpretation of all phenotypic testing was blinded, with the exception of VGAs due to the pairing of mutant and parental isolates on the same gradient plate to ensure consistent experimental conditions.

Genome sequencing. Draft genomes of the 222 clinical comparator strains, the isolates of RP62a and ATCC 12228 used to create mutant strains, four *rpoB* mutant strains and Ec_Se662I-II *E. coli* were sequenced on either an Illumina MiSeq or NextSeq platform, as indicated in Supplementary Table 1c, with Nextera XT libraries, according to the manufacturer's instructions.

Genome analysis. Illumina paired-end reads were trimmed using Trimmomatic v0.36[41], and overlapping pairs merged with PEAR v0.9.10[42]. Draft genomes were de novo assembled with SPAdes v3.7.1[43] using the unassembled R1 and R2, and merged read pairs as input. Genome annotation was performed with Prokka v1.12[44]. An alignment of core genome SNPs across the *S. epidermidis* isolates was determined using Snippy v3.2[45] using the closed BPH0662 genome as a reference. iqtree v1.5.3[46] using model finder[47] and ultrafast bootstrap[48] was then used to produce a maximum-likelihood phylogenetic tree. Pairwise SNP analysis was performed using pairwise_snp_differences[49]. For pan-genome analysis, protein orthologue clustering was performed using Proteinortho v5.11[50], alignment of the resultant CDS orthologues to BPH0662 and visualization of the pan-genome were performed using FriPan[51]. De novo assemblies were genotyped using in silico MLST[52], and the resistome and virulome were characterized with abricate[53] screened against the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (NCBI BARRGD, PRJNA313047) and Virulence Factors of Pathogenic Bacteria Database (VFDB)[54], respectively. The tree was clustered using a hierarchical Bayesian Analysis of Population Structure (hierBAPS) model[55]. To infer the evolutionary phylogeny of ST2 *S. epidermidis*, regions of phage insertion and recombination were masked to ensure modelling was only informed by spontaneous SNPs within the core genome. Five (BPH0704, BPH0697, BPH0677, BPH0737 and SEI) of the 227 isolates were omitted from final analyses based on their extreme divergence, which suggested either excessive natural selection, thereby violating the assumption of evolutionary neutrality used in the model, or separation of these lineages so remotely in the past as to be beyond the abilities of this method to analyse correctly. Two historical, non-MDRSE Australian clinical isolates (BPH0823 isolated in 1989 and BPH0825 isolated 1990) were included to improve temporal sampling depth. Snippy v3.2[45] was used to generate an alignment of the 224 relevant isolates to the full BPH0662 chromosome. Phage elements in the BPH0662 reference were identified using PHASTER[56] and masked from the alignment with BEDtools[57]. Gubbins v2.2.0[58] was used to filter recombination and identify core-SNPs from filtered alignment, with RAxML v8.2.10[59] for tree building. A final maximum-likelihood tree was produced with iqtree v1.5.3 using model finder and ultrafast bootstrap. BEAST v2.4.3[60] was used to generate a timed phylogeny (chronogram) assuming a relaxed clock and using coalescent tree prior. The assumption of a molecular clock was tested using root-to-tip distance in R v3.4[61] using the Analysis of Phylogenetics and Evolution (APE) package v4.1[62].

All publicly available *S. epidermidis* genome raw reads in NCBI SRA were downloaded (4 November 2016) and assembled as described above. Exclusion criteria were as follows: sequencing performed on a Roche 454 or Ion Torrent platform (per Genbank metadata); single end reads; sequencing depth of <30×; isolates sequenced as part of this study; mutagenesis isolate pairs; organism not *S. epidermidis* or sequencing of mixed bacterial species as determined by Kraken v0.10.15beta[63]; >300 assembled contigs; raw reads not associated with publications and lacking metadata. When isolates were identified as exact genetic replicates, a single representative was used in analysis. To determine the diversity of mutations in RpoB, the RpoB translation for ATCC 12228 (accession no. NP_763861) was used as reference for a tblastn query on the combined NCBI SRA and study isolate assemblies using BLAST+ v2.6.0[64], the output of which was aligned with MUSCLE v3.8.1551[65]; this was then manually collated with the RpoB mutations reported in ref. [30]. To determine the RRDR of RpoB for *S. epidermidis* the RpoB translations for ATCC 12228 and BPH0662 were aligned with the published 27-amino-acid RRDR defined for *E. coli* and *M. tuberculosis*[28,29] and *S. aureus*[29]. Using the above method, the following reference nucleic acid/gene translations from ATCC 12228 were used to screen for the presence of mutations conferring linezolid resistance: 23S rRNA (GeneID 3190203), L3 (NP_765379), L4 (NP_765378) and L22 (NP_765374). For Mprf, the gene translation from RP62a was used as the reference (WP_002456532).

Bacterial transformation. Overnight cultures of *S. epidermidis* grown in 10 ml of B medium were diluted to an OD_{600} of 0.5 in 100 ml of fresh, prewarmed B medium. Cultures were then reincubated for 40 min and chilled in an ice slurry for 10 min. All subsequent steps were performed as previously described[38], with the exception of post-electroporation recovery in which cells were incubated in 1 ml of BHI supplemented with 500 mM sucrose (filter sterilized) at 28 °C for 1 h before plating on BHIA Cm10.

Construction of *E. coli* Ec_Se662I-II for transformation of ST2 *S. epidermidis*. The *E. coli* Ec_Se662I-II mutant expressing both BPH0662 type I RM systems in a DC10B background was constructed using a previously described protocol and primers[12]. The DC10B-MS1 mutant with *hsdMS1* integrated between *essQ* and *cspB* was subjected to a second recombination event to integrate *hsdMS2* between *ybbD* and *ylbG*.

Allelic exchange of *rpoB* using pIMAY. To construct pIMAY(*rpoB*662), pIMAY[38] was linearized with KpnI and gel extracted; the vector backbone was then amplified from within the multiple cloning site (MCS) with primers IM1/IM2. The entire

rpoB gene of rifampicin-resistant, reference strain BPH0662 was amplified using primers rpoB3/rpoB4 (incorporating flanking KpnI sites), and the product gel extracted. Seamless ligation cloning extract (SLiCE) was used to insert the *rpoB* construct into the pIMAY vector backbone, as previously described[66,67]. After transformation into *E. coli* Ec_Se662I-II, the *rpoB* insert was verified by Sanger sequencing and the vector pIMAY(*rpoB*662) subsequently electroporated into four rifampicin-susceptible *S. epidermidis* strains: BPH0676, BPH0736, RP62a and ATCC 12228. Transformants were selected for on BHIA plus Cm after 2 days at 28 °C. Integration and excision of pIMAY(*rpoB*662) was performed as previously described[38], with the exception of concurrent (rather than sequential) colony PCRs, for loss of replicating plasmid (with MCS primers IM3/IM4), and chromosomal integration of the plasmid upstream (*rpoB* out forward/IM3) and downstream (IM4/rpoB out reverse). Plasmid excision in favour of mutant creation was selected for on BHIA plus ATc1 (Sigma) with rifampicin at 4, 8, 16 or 32 μg ml^{-1}, large colonies were patch-plated on BHIA plus Cm10 and BHIA plus rifampicin at 4, 8, 16 or 32 μg ml^{-1} and grown at 37 °C overnight, with successful mutants bearing a Cm^{S}, Rif^{R} phenotype. Potential mutants were selected from the plate containing the lowest rifampicin concentration. Stability of Rif^{R} mutants was confirmed by overnight growth on BHIA without antibiotics, with subsequent restreaking on BHIA plus rifampicin 32. Draft genomes of successfully created mutant strains (BPH676-*rpoB*662, BPH0736-*rpoB*662, RP62a-*rpoB*662 and 12228-*rpoB*662,) and the parental strains from which they were derived (BPH0676, BPH0736, RP62a and ATCC 12228, respectively) were sequenced to determine if any unintended mutations were present. Variant calling between the *rpoB*662 mutants to their respective WT parental strains was cross-checked by two methods, using Snippy[45] and Nesoni[68]. Mutants and their corresponding parental strains were subjected to phenotypic testing to determine the role of rifampicin resistance mutations (D471E and I527M) in RpoB on the evolution of glycopeptide heteroresistance in *S. epidermidis*.

Growth curves and vancomycin competition assay. For growth curves, overnight cultures were diluted to an OD_{600} of 0.05 in 50 ml of prewarmed BHI and grown at 37 °C with aeration at 200 r.p.m. The OD_{600} values were measured hourly for 12 h. Growth curves were performed in biological triplicate. The doubling time for each strain was calculated as previously described[69], using the 2 and 5 h exponential phase time points. For vancomycin co-culture assays, overnight cultures of WT and the corresponding *rpoB*662 mutant strain were diluted to an OD_{600} of 0.5 in BHI, 0.5 ml of each culture was added to 49 ml of BHI containing vancomycin at 0, 2 or 4 μg ml^{-1} (starting OD_{600} of co-cultures = 0.01). Cultures were incubated at 37 °C with aeration at 200 r.p.m. For vancomycin 0 and 2 μg ml^{-1}, co-cultures reached saturation by 24 h and the medium was refreshed with a 1/1,000 dilution of the 24 h co-culture in 50 ml of BHI containing vancomycin at 0 and 2 μg ml^{-1}, respectively. Cultures were incubated at 37 °C with aeration at 200 r.p.m, for a further 24 h (48 h total). The vancomycin 4 μg ml^{-1} co-culture did not reach saturation until 48 h, so the medium was not refreshed at 24 h. Serial dilutions of each co-culture were performed at times 0, 24 and 48 h, plated on BHIA and incubated at 37 °C overnight; colonies were then patched onto BHIA plus rifampicin 32 μg ml^{-1} to determine the proportion of WT to *rpoB*662 mutant.

Statistical analysis. Etest MICs were $\log_2$-transformed to normalize the distribution before statistical analysis. All statistical analyses (Mann–Whitney *U* test, unpaired or paired Student's *t*-test, two-way ANOVA with Tukey's test) were performed with GraphPad Prism 7 for Mac OS X (GraphPad Software), with the exception of the pairwise SNP analysis which was performed using pairwise_snp_differences[69] in R[81].

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The data sets supporting the results of this Article are available from the European Nucleotide Archive under Bioproject no. PRJEB12090 (Austin Hospital isolates and *rpoB*662 mutant sequencing) and from NCBI under BioProject nos. PRJNA470534 (Victorian *S. epidermidis* survey isolates) and PRJNA470752 (all other isolates).

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Author contributions

B.P.H. and T.P.S. conceived the project, which was supervised by B.P.H., T.P.S. and I.R.M. J.Y.H.L. performed all experimental work, with assistance from I.R.M. J.Y.H.L., A.G.d.S., T.S., T.P.S. and B.P.H. analysed data, including analysis of genome sequence data. K.Y.L.C., A.K., R.H., N.W., M.D.B., B.S., F.L., M.D., A.D., R.P., A.R.L. and T.M.K. established and analysed clinical and reference isolate data sets and performed susceptibility testing. J.Y.H.L., I.R.M., B.P.H. and T.P.S. drafted the manuscript. All authors reviewed and contributed to the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
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☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
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☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	Trimmomatic v0.36 (Reference 46) PEAR v0.9.10 (Reference 47) SPAdes v3.7.1 (Reference 48) Prokka v1.12 (Reference 49) Snippy v3.2 (Reference 50) iqtree v1.5.3 (Reference 51) model finder (Reference 52) ultrafast bootstrap (Reference 53) pairwise_snp_differences (Reference 54) Proteinortho v5.11 (Reference 55) FriPan (Reference 56) in silico MLST (Reference 57) hierarchical Bayesian Analysis of Population Structure (hierBAPS) (Reference 58) PHASTER (Reference 59) BEDtools (Reference 60) Gubbins v2.2.0 (Reference 61) RAxML v8.2.10 (Reference 62)

BEAST v2.4.3 (Reference 63)
R v3.4 (Reference 64)
Analysis of 739 Phylogenetics and Evolution (APE) package v4.1 (Reference 65)
Kraken v0.10.15beta (Reference 66)
BLAST+ v2.6.0 (Reference 67)
MUSCLE v3.8.1551 (Reference 68)
Nesoni (Reference 71)
GraphPad Prism 7 for Mac OS X (GraphPad Software, La Jolla California USA)

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data sets supporting the results of this article are available in the European Nucleotide Archive, under Bioproject number PRJEB12090 (Austin Health isolates and rpoB662 mutant sequencing), and NCBI under BioProject IDs PRJNA470534 (Victorian S. epidermidis survey isolates) and PRJNA470752 (all other isolates).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. This research was based on genomic interpretation of a retrospective survey of a global collection of bacteria that were samples of convenience. We did not establish this study to test the effect of an experimental intervention or specific hypothesis. Thus, there is no null hypothesis to reject and no sample size calculation was performed. To present an accurate global description of the isolates responsible for clinical S. epidermidis infection, broad sampling was performed to maximise both temporal and geographical representation. The 419 isolates analysed were sampled from 96 institutions in 24 countries, isolated over a 62-year period spanning 1955 to 2017. Furthermore, all but two of the reference isolates were of clinical origin to ensure the relevance of results to clinical practice.
Data exclusions	S. epidermidis RpoB mutation frequency analysis: 324 of 484 S. epidermidis raw reads available on NCBI were excluded as per the criteria listed in the methods section of the manuscript. These isolates were sequenced as part of microbiome projects, with resultant poor sequencing coverage and/or contaminated reads mixed with other species; or sequenced using older chemistry known to be error prone. The low quality assemblies produced from these raw reads would have confounded analyses with artificial mutations, therefore these data were not included.
Replication	To demonstrate a causal relationship between the dual D471E and I527M RpoB substitutions and a vancomycin heteroresistant phenotype in S. epidermidis, mutants possessing these substitutions were made in four different backgrounds, from 3 different lineages (ST2, ST8 and ST10). Extended glycopeptide susceptibility testing for the four mutant and wild-type pairs was performed using three different methods (vancomycin population analysis profile, vancomycin plus teicoplanin macromethod Etests and vancomycin gradient assay); testing was performed in biological triplicate. A statistically significant increase in glycopeptide tolerance was demonstrated for each mutant compared to its respective parental isolate by each of the three phenotypic methods, thereby proving the hypothesis that the dual D471E and I527M substitution in RpoB confers both rifampicin resistance and glycopeptide heteroresistance in S. epidermidis.
Randomization	Randomisation was not relevant to this study as all isolates were subjected to the same phenotypic and genomic analyses in order to fully characterise the dataset.
Blinding	Interpretation of all phenotypic testing was blinded with the exception of vancomycin gradient assays due to the pairing of mutant and parental isolates on the same plate (as shown in Figure 4 and Supplementary Figure 5) to ensure consistent experimental conditions. All four mutant and wild-type pairs were subject to a two additional phenotypic methods to characterise glycopeptide heteroresistance (vancomycin population analysis profile and vancomycin plus teicoplanin macromethod Etests) the interpretation of which were blinded to ensure no bias.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

3.3 Supplementary Appendix I

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SUPPLEMENTARY INFORMATION

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In the format provided by the authors and unedited.

Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*

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Supplementary information for:

Title: Global spread of three-multidrug resistant lineages of *Staphylococcus epidermidis*

Running Title: Genomics of drug-resistant *S. epidermidis*

Authors: Jean YH Lee[1], Ian R Monk[1], Anders Gonçalves da Silva[2,3], Torsten Seemann[3,4], Kyra YL Chua[5], Angela Kearns[6], Robert Hill[6], Neil Woodford[6], Mette D Bartels[7], Birgit Strommenger[8], Frederic Laurent[9], Magali Dodémont[10], Ariane Deplano[10], Robin Patel[11], Anders R Larsen[12], Tony M Korman[13], Timothy P Stinear[#1,3], Benjamin P Howden*[#2,3,14]

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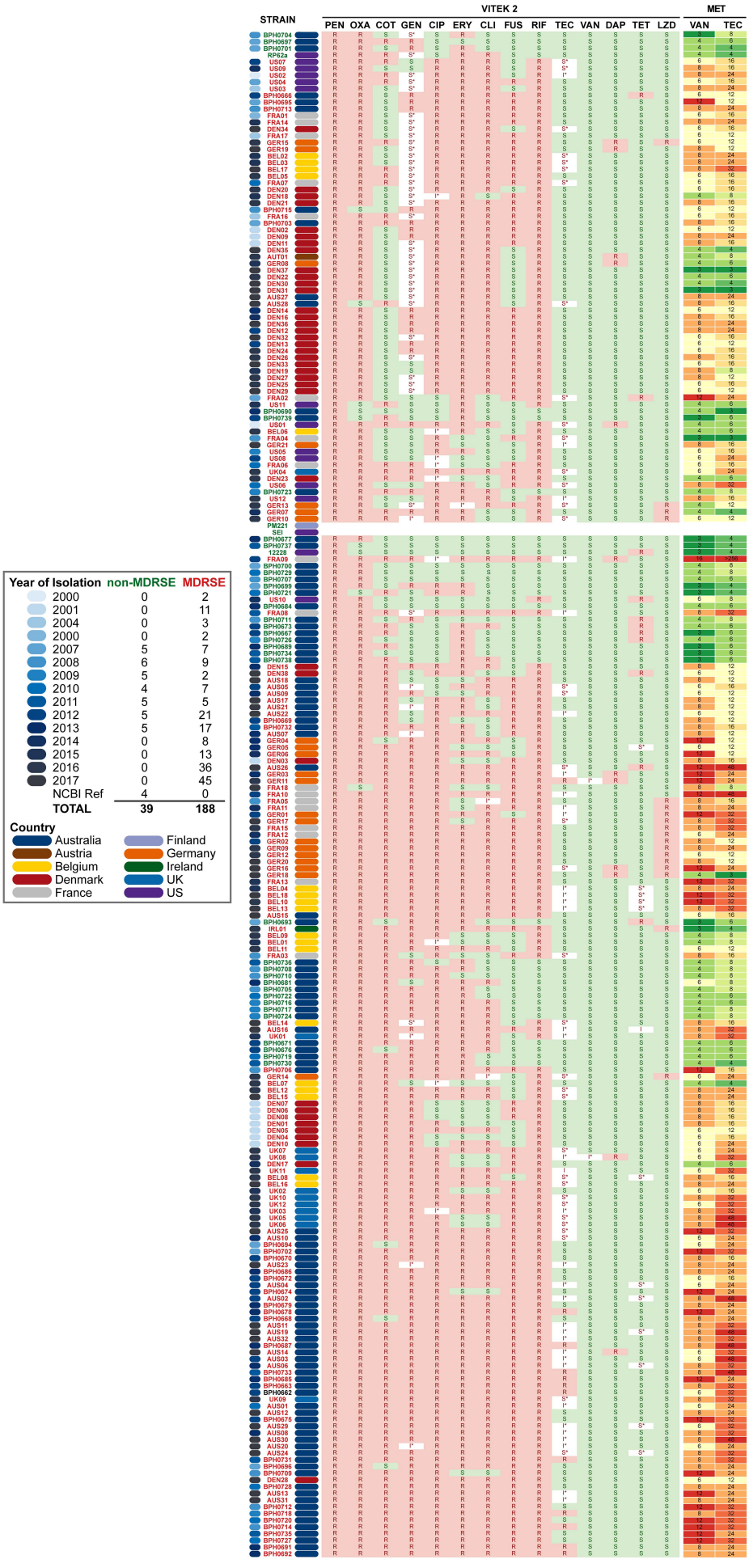
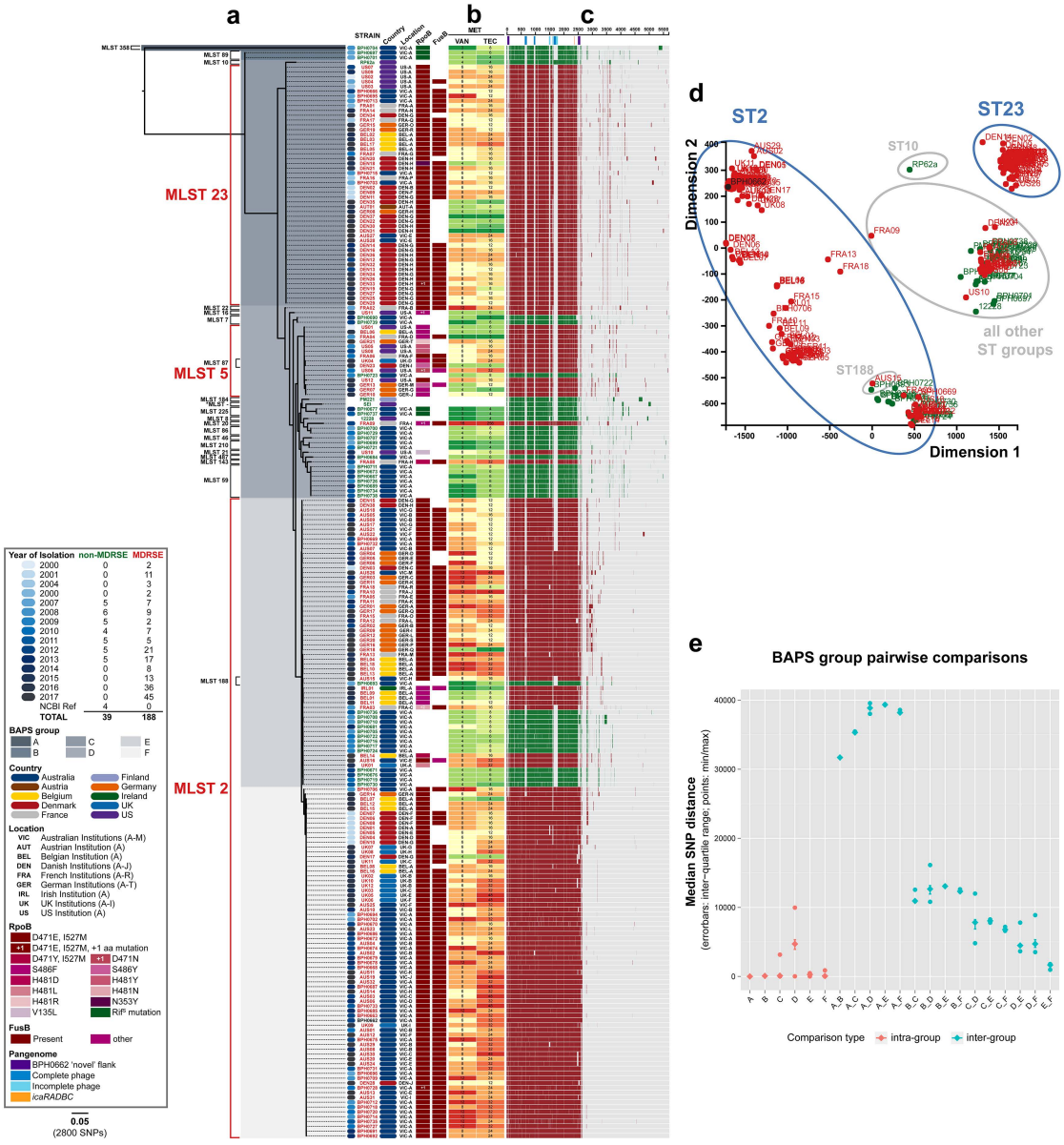
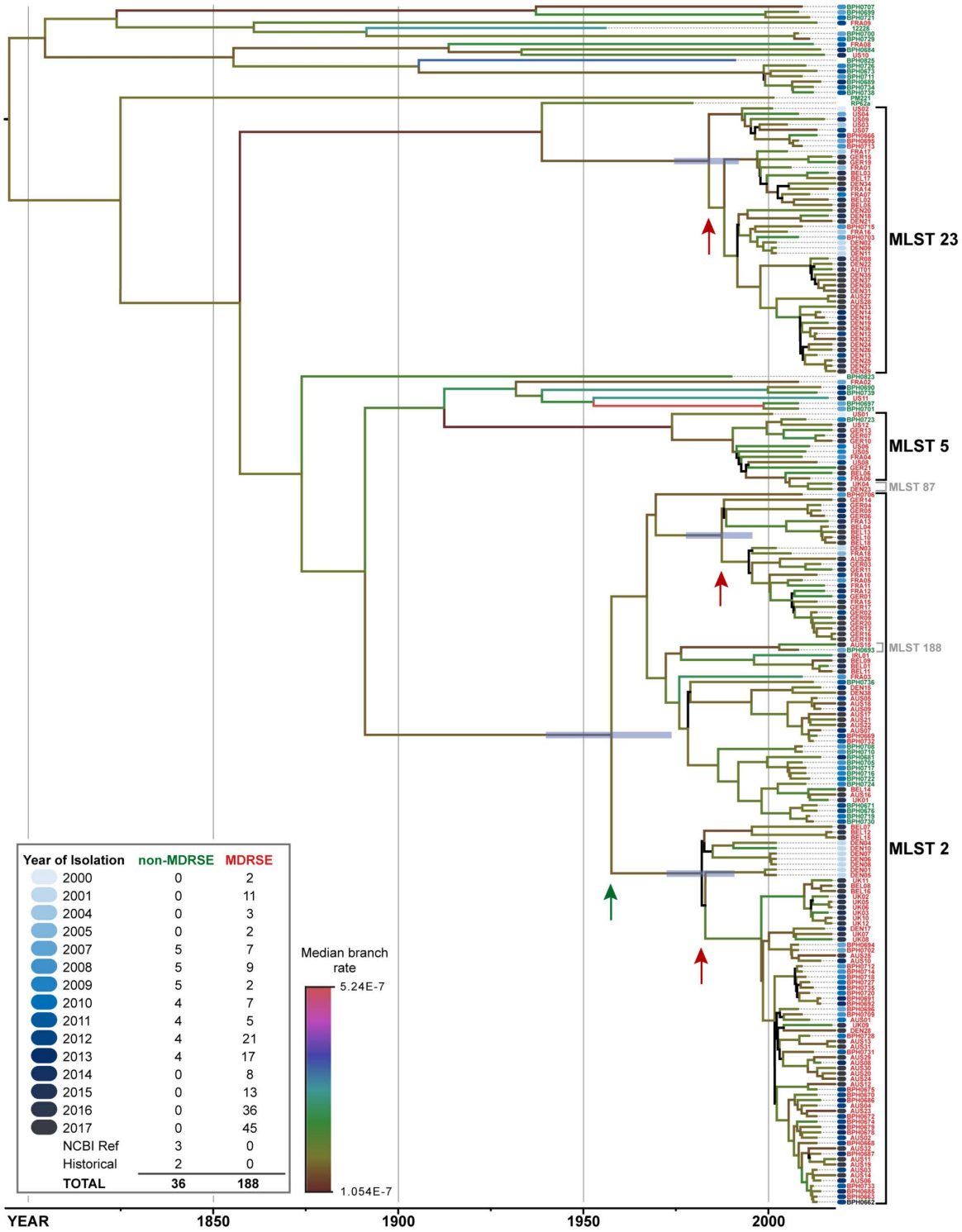

VITEK 2
MET
STRAIN
PEN OXA COT GEN CIP ERY CLI FUS RIF TEC VAN DAP TET LZD
VAN TEC
Year of Isolation non-MDRSE MDRSE
2000 0 2
2001 0 11
2004 0 3
2000 0 2
2007 5 7
2008 6 9
2009 5 2
2010 4 7
2011 5 5
2012 5 21
2013 5 17
2014 0 8
2015 0 13
2016 0 36
2017 0 45
NCBI Ref 4 0
TOTAL 39 188
Country
Australia
Austria
Belgium
Denmark
France
Finland
Germany
Ireland
UK
US

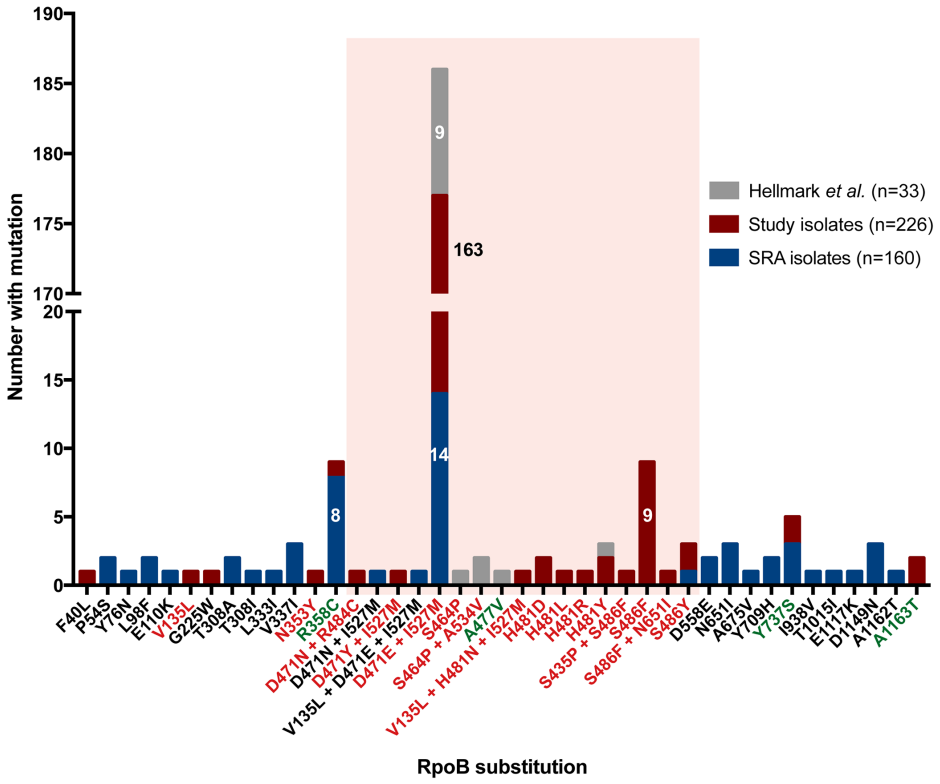
Supplementary Figure 1. Antibiogram of 227 *S. epidermidis* study isolates. Vitek 2 susceptibilities; and heatmap of vancomycin heteroresistant phenotype testing, as determined by vancomycin (VAN) and teicoplanin (TEC) macromethod Etests (MET). Isolates are ordered according to the phylogeny presented in Supplementary Figure 2. For Vitek 2 results: S = susceptible by both CLSI and EUCAST 2017 breakpoints; R = resistant by both CLSI and EUCAST 2017 breakpoints; S* = susceptible by CLSI but resistant by EUCAST; I* = intermediate by CLSI but resistant by EUCAST.


a
b
c
STRAIN
Country
Location
RpoB
FusB
MET
VAN
TEC
MLST 358
MLST 89
MLST 10
MLST 23
MLST 22
MLST 16
MLST 7
MLST 87
MLST 5
MLST 394
MLST 225
MLST 8
MLST 90
MLST 46
MLST 210
MLST 45
MLST 143
MLST 99
MLST 188
MLST 2
Year of Isolation non-MDRSE MDRSE
2000 0 2
2001 0 11
2004 0 3
2000 0 2
2007 5 7
2008 6 9
2009 5 2
2010 4 7
2011 5 5
2012 5 21
2013 5 17
2014 0 8
2015 0 13
2016 0 36
2017 0 45
NCBI Ref 4 0
TOTAL 39 188
BAPS group
A
B
C
D
E
F
Country
Australia
Austria
Belgium
Denmark
France
Finland
Germany
Ireland
UK
US
Location
VIC Australian Institutions (A-M)
AUT Austrian Institution (A)
BEL Belgian Institution (A)
DEN Danish Institutions (A-J)
FRA French Institutions (A-R)
GER German Institutions (A-T)
IRL Irish Institution (A)
UK UK Institutions (A-I)
US US Institution (A)
RpoB
D471E, I527M
+1 D471E, I527M, +1 aa mutation
D471Y, I527M
+1 D471N
S486F
S486Y
H481D
H481Y
H481L
H481N
H481R
N353Y
V135L
RifR mutation
FusB
Present
other
Pangenome
BPH0662 'novel' flank
Complete phage
Incomplete phage
icaRADBC
0.05
(2800 SNPs)
d
ST2
ST23
ST10
ST188
RP62a
FRA09
FRA13
FRA18
FRA15
BEL16
all other
ST groups
Dimension 1
Dimension 2
e
BAPS group pairwise comparisons
Median SNP distance
(errorbars: inter−quartile range; points: min/max)
Comparison type
intra-group
inter-group

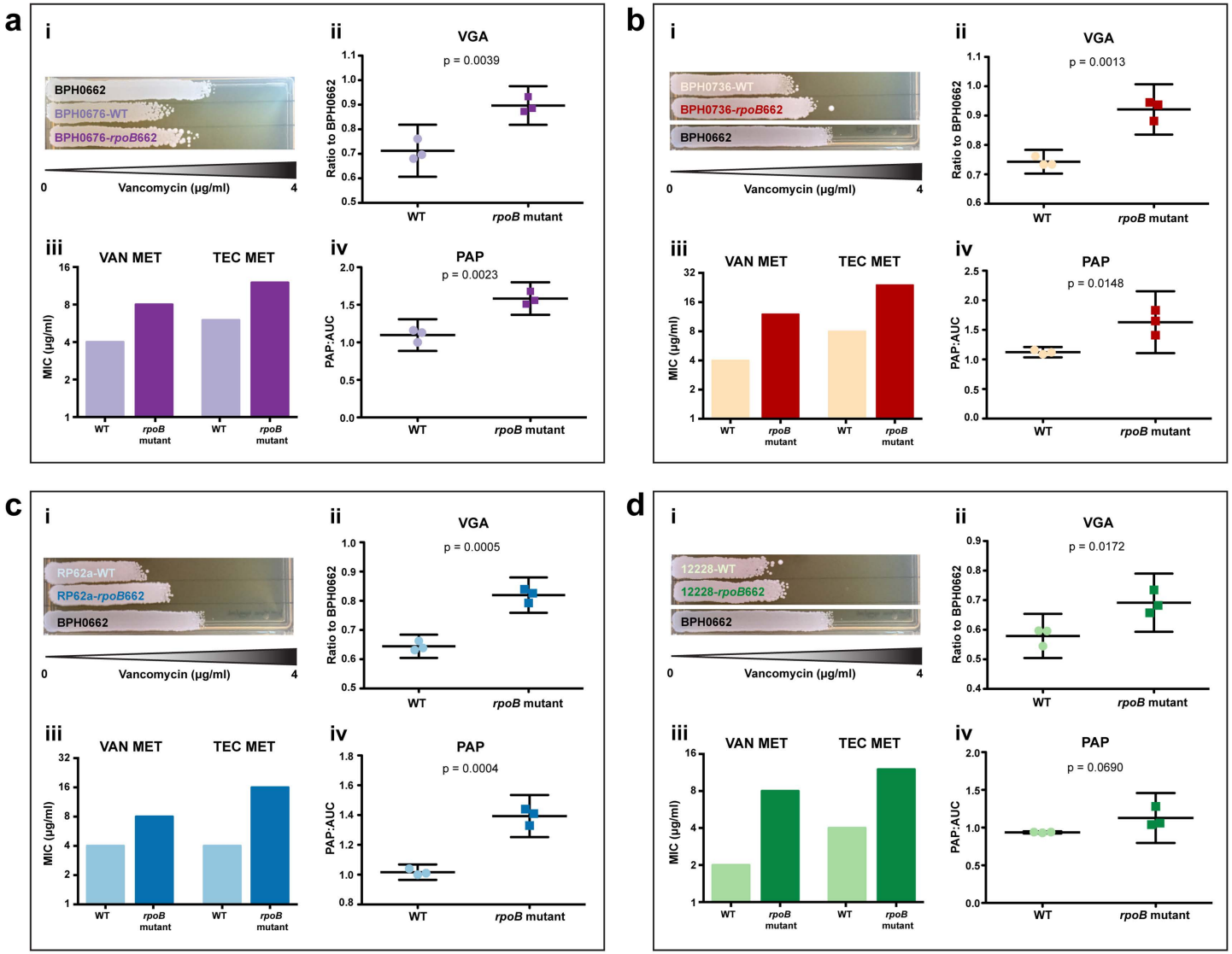
Supplementary Figure 2. Comparative genomics of international *S. epidermidis* isolates. a. Maximum-likelihood, core-SNP-based phylogeny of 227 clinical isolates originating from 77 institutions in ten countries using BPH0662 as the reference genome, overlaid with *in silico* multi-locus sequence typing (MLST) and Bayesian analysis of population structure (BAPS) groups (scale bar indicates number of nucleotide substitutions per site (bold), with an approximation of SNP rate [in brackets]). Aligned with: determinants of rifampicin (RpoB mutations) and fusidic acid (*fusB*) resistance; **b.** Heatmap of vancomycin heteroresistant phenotype testing, as determined by vancomycin (VAN) and teicoplanin (TEC) macromethod Etests (MET); **c.** Pangenome ordered according to the BPH0662 chromosome concatenated with its three plasmids (pBPH0662-01, pBPH0662-02 and pBPH0662-03 in sequential order); **d.** Multidimensional scaling of pangenome coding sequence orthologs; **e.** Intra-group and inter-group comparison of pairwise core-SNPs for BAPS groups: A (n = 1), B (n = 2), C (n = 51), D (n = 40), E (n = 60), F (n = 73). *Published reference strain SEI was not classifiable by the existing MLST scheme.


MLST 23
MLST 5
MLST 87
MLST 188
MLST 2
Year of Isolation non-MDRSE MDRSE
2000 0 2
2001 0 11
2004 0 3
2005 0 2
2007 5 7
2008 5 9
2009 5 2
2010 4 7
2011 4 5
2012 4 21
2013 4 17
2014 0 8
2015 0 13
2016 0 36
2017 0 45
NCBI Ref 3 0
Historical 2 0
TOTAL 36 188
Median branch rate
5.24E-7
1.054E-7
YEAR
1850
1900
1950
2000

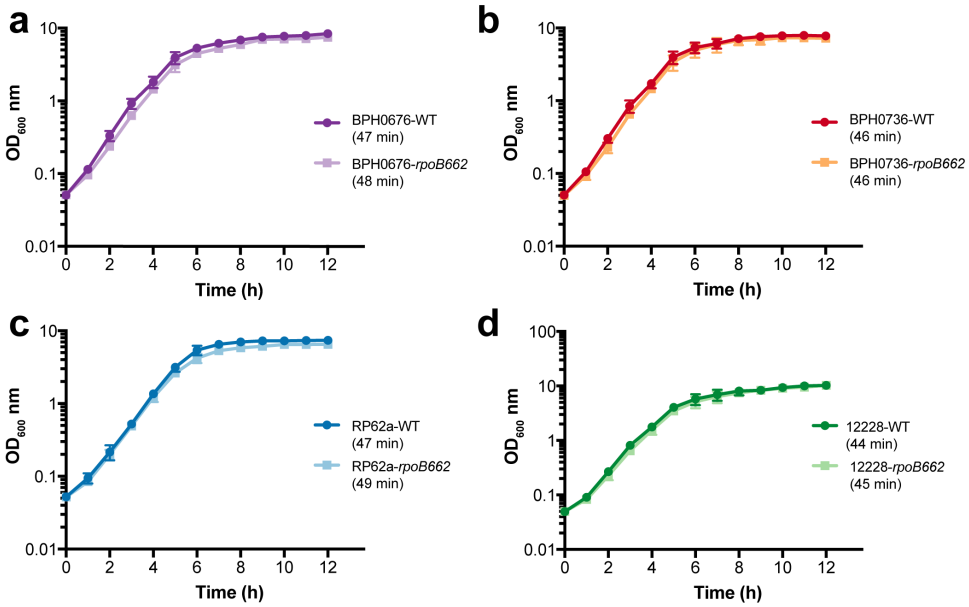
Supplementary Figure 3. Evolutionary phylogeny predicts independent coevolution of two ST2 multidrug resistant *S. epidermidis* clades. Phage element and recombination filtered core-SNP evolutionary phylogeny incorporating 224 clinical isolates originating from 77 institutions in ten countries, using BPH0662 as the reference genome. Branches are coloured according to median substitution rate. The predicted date of the most recent common ancestor for the BPH0662 and ST2-mixed clades (green); and emergence of the ST23 and BPH0662 clades, and ST2-mixed subclade, possessing the RpoB D471E plus I527M dual substitutions (red) are indicated with arrows. Blue bars indicate 95% highest posterior density interval for predicted node date.



Supplementary Figure 4. Dual D471E and I527M substitutions are the most common RpoB mutations in *S. epidermidis* worldwide. The frequency of various RpoB mutations in 419 isolates from 96 institutions in 24 countries, sampled from at least 77 sequence types (STs). Isolates originated from: Australia (n = 101), Austria (n = 1), Belgium (n = 18), Denmark (n = 38), France (n = 18), Finland (n = 1), Germany (n = 22), Republic of Ireland (n = 1); UK (n = 12) and US (n = 14); clinical *S. epidermidis* genomes with DNA reads in the NCBI sequence read archive (SRA); a study correlating *rpoB* gene mutations and phenotype in prosthetic joint infections (Hellmark *et al.*)[1]. Total number of isolates from each source is in brackets. No ST data was available for Hellmark isolates. When known, the phenotype associated is indicated in red font for resistant mutations, and green font for substitutions that do not confer a rifampicin resistant phenotype.


a
i
BPH0662
BPH0676-WT
BPH0676-rpoB662
0
Vancomycin (µg/ml)
4
ii
VGA
p = 0.0039
Ratio to BPH0662
WT
rpoB mutant
iii
VAN MET
TEC MET
MIC (µg/ml)
WT
rpoB mutant
iv
PAP
p = 0.0023
PAP:AUC
b
i
BPH0736-WT
BPH0736-rpoB662
BPH0662
ii
VGA
p = 0.0013
iii
iv
PAP
p = 0.0148
c
i
RP62a-WT
RP62a-rpoB662
BPH0662
ii
VGA
p = 0.0005
iii
iv
PAP
p = 0.0004
d
i
12228-WT
12228-rpoB662
BPH0662
ii
VGA
p = 0.0172
iii
iv
PAP
p = 0.0690

Supplementary Figure 5. RpoB D471E and I527M mutations confer reduced glycopeptide susceptibility in four different *S. epidermidis* backgrounds. Glycopeptide susceptibility testing for each of the rifampicin susceptible wildtype (WT) and rifampicin resistant (*rpoB*662) mutant pairs derived from: **a.** BPH0676 (ST2); **b.** BPH0736 (ST2); **c.** RP62a (ST10); and **d.** ATCC 12228 (ST8). Inserts for each pair show: **i.** An example vancomycin gradient assay (VGA) comparing the WT and *rpoB*662 mutant pair with the BPH0662 control strain; **ii.** Biological triplicate data for the WT and *rpoB*662 mutant pair compared by VGA. Error bars represent mean +/- 95% confidence interval of three independent experiments; Null hypothesis (no difference between means) was rejected for $p < 0.05$ (two-tailed, unpaired Student's t-test); **iii.** Data for a single set of vancomycin (VAN) and teicoplanin (TEC) macromethod Etests (METs) for the WT and *rpoB*662 mutant pair. Y-axis for METs plotted on $\log_2$ scale; **iv.** Biological triplicate data for the WT and *rpoB*662 mutant pair compared by vancomycin population analysis profile (PAP). Error bars represent mean +/- 95% confidence interval of three independent experiments; Null hypothesis (no difference between means) was rejected for $p < 0.05$ (two-tailed, unpaired Student's t-test).



Supplementary Figure 6. Mutants containing dual D471E and I527M RpoB substitutions do not demonstrate a fitness cost in enriched growth conditions. Growth curves for each wild-type (WT) and rifampicin resistant (*rpoB*662) mutant pair, performed in BHI for: **a.** BPH0676 (ST2), **b.** BPH0736 (ST2), **c.** RP62a (ST10) and **d.** ATCC 12228 (ST8). Data shown represents three independent experiments for each WT and mutant pair. Error bars indicate mean ± 95% confidence interval at each time point derived from three independent experiments. Doubling time shown below each strain was calculated at t1 = 2 h and t2 = 5 h.

Supplementary Table 1. Isolate metadata. sheet a. Clinical metadata; **sheet b.** Accession information; **sheet c.** Sequencing and assembly statistics; **sheet d.** Resistome data; **sheet e.** SRA strain metadata.

Provided in a separate excel spreadsheet.

Supplementary Table 2. Genetic determinants of linezolid resistance.

Isolate	Year	MLST	*cfr*	23S rRNA	L3	L4	L22
FRA05	2008	2	.	G2602T*	.	.	.
FRA11	2014	2	.	C165T, G2602T*	M156T	.	.
FRA12	2014	2	.	C165T, G2602T*	G137V, M156T	$_{71}$GGR$_{72}$	.
FRA15	2016	2	.	G267T, G2602T*	Q136L, M156T	$_{71}$GGR$_{72}$	.
GER01	2012	2	Y	G267T, G2602T*	.	.	.
GER02	2012	2	Y	G267T, G2602T*	.	$_{71}$GGR$_{72}$	.
GER07	2014	5	.	.	H146Q, V154L, A157R	$_{71}$GGR$_{72}$, N158S	.
GER09	2016	2	.	G267T, G2602T*	.	$_{71}$GGR$_{72}$	.
GER10	2016	5	.	C165T	H146Q, V154L, A157R	$_{71}$GGR$_{72}$, N158S	.
GER12	2016	2	.	G267T, G2602T*	.	$_{71}$GGR$_{72}$	.
GER13	2016	5	Y	.	H146Q, V154L, A157R	$_{71}$GGR$_{72}$, N158S	.
GER14	2016	2	.	G131A, G267T, G2602T*	.	.	.
GER15	2016	23	Y	G1554A	.	N158S	.
GER16	2017	2	.	G267T, G2602T*	.	$_{71}$GGR$_{72}$	.
GER17	2017	2	Y	G267T, G2602T*	.	.	.
GER18	2017	2	.	G267T, G2602T*	.	$_{71}$GGR$_{72}$	.
GER20	2017	2	.	G267T, G2602T*	.	$_{71}$GGR$_{72}$	.
IRL01	2016	2	.	G2602T*	.	$_{71}$GGR$_{72}$	.

MLST = multi-locus sequence typing.
*Corresponds to G2576T in *E. coli*

Supplementary Table 3. Genetically modified strains, plasmids and oligonucleotides used in this study.

Bacterial strain, plasmid or oligonucleotide	Description	Reference or Source
***E. coli* strains**		
DC10B	DH10B with Δ*dcm* mutation	2
DC10B-MS1	DC10B with BPH0662 *hsd*MS-1 integrated between *ybbD* and *ylbG*	3
DC10B-MS1-MS2	DC10B with BPH0662 *hsd*MS-1 integrated between *ybbD* and *ylbG*, BPH0662 *hsd*MS-2 integrated between *essQ* and *cspB*	This study
***S. epidermidis* strains**		
ATCC 12228	Non-clinical reference strain; Rif^S	4
12228-*rpoB*662	Rif^R mutant of ATCC 12228 (RpoB D471E and I527M)	This study
BPH0662	Clinical strain. Case patient, isolated from CSF. Rif^R (RpoB D471E and I527M)	3
BPH0676	Clinical strain, isolated from blood culture; Rif^S	This study
BPH0676-*rpoB*662	Rif^R mutant of BPH0676 (RpoB D471E and I527M)	This study
BPH0736	Clinical strain, isolated from blood culture; Rif^S	This study
BPH0736-*rpoB*662	Rif^R mutant of BPH0736 (D471E and I527M)	This study
RP62a	Clinical reference strain, isolated from blood culture; Rif^S	5
RP62a-*rpoB*662	Rif^R mutant of RP62a (RpoB D471E and I527M)	This study
Plasmids		
pIMAY	Temperature sensitive allelic exchange plasmid for staphylococci; Cm^R	6
pIMAY(*rpoB*662)	pIMAY with Rif^R *rpoB* amplified from *S. epidermidis* BPH0662 (RpoB D471E and I527M) in MCS	This study
pIMAY(*rpoB*12228)	pIMAY with Rif^S *rpoB* amplified from *S. epidermidis* ATCC 12228 in MCS	This study
***S. epidermidis* mutants**		
IM3	AATACCTGTGACGGAAGATCACTTCG	This study
IM4	TACATGTCAAGAATAAACTGCCAAAGC	This study
rpoB3	CCTCACTAAAGGGAACAAAAGCTGGGTACCTTGGCA GGTCAAGTTGTCCAATATGG	This study
rpoB4	CGACTCACTATAGGGCGAATTGGAGCTCTTAATCAG TAGTTTCTTTTTGTGATTCTG	This study
rpoB out F	CAATTTTTGAGGGGTGAATCTG	This study
rpoB out R	TAGCATTCCATACCTCTAAGGTTTCAG	This study

Rif^S = rifampicin susceptible; Rif^R = rifampicin resistant; CSF = cerebrospinal fluid; Cm^R = chloramphenicol resistant; MCS = multiple cloning site.

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3.4 Supplementary Appendix II

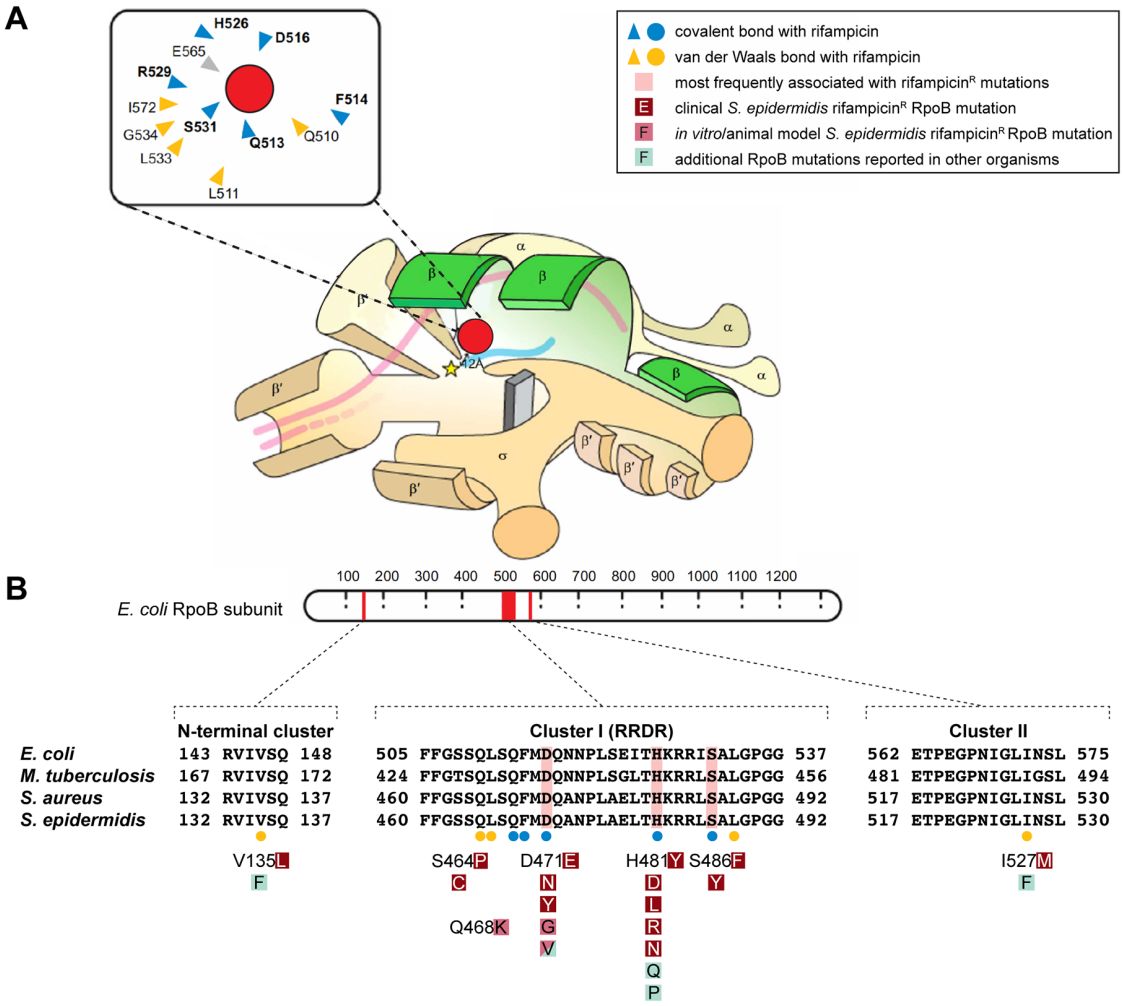
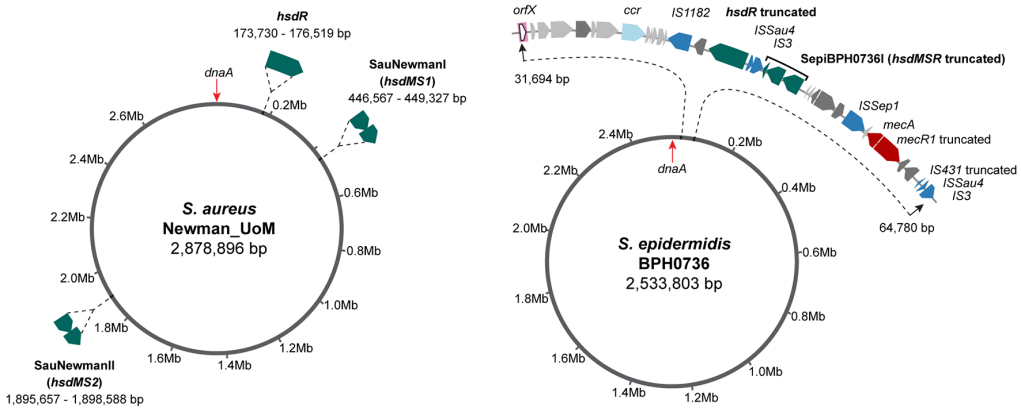


Figure 3.1 Schematic representation of the rifampicin resistance determining region (RRDR) of RpoB updated with mutations identified in Chapter 3. **A.** Cartoon of rifampicin interacting with the structural elements of bacterial RNA polymerase adapted from Koch *et al.* [161]. A rifampicin molecule (red circle) is bound in the active site (yellow star) of the RpoB subunit (green) to inhibit transcription. DNA (pink lines) is shown as double-stranded entering the active site, then a single strand of template DNA once unwound. The growing RNA chain is represented by the blue line. Inset shows the rifampicin binding pocket of RpoB, highlighting the residues that interact with rifampicin, colour coded by the nature of the chemical bond (blue and yellow triangles). The grey triangle (*E. coli* RpoB E565) is the single interacting residue not known to be associated with a rifampicin resistance mutation. **B.** Alignment of the rifampicin resistance mutation hotspots in RpoB for various bacterial species, with reported mutations [159,161,168,169] and those newly identified in Chapter 3 overlaid.

CHAPTER 4. Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification



Reproduced from Lee *et al.* [225].

4.1 Introduction

To address the Chapter 3 hypothesis that the dual D471E and I527M RpoB substitutions found to dominate in *S. epidermidis* were the cause of the vancomycin heteroresistant phenotype observed in the three MDRSE lineages, a method for the genetic manipulation of ST2 and other lineages of *S. epidermidis* needed to be established. While developing this methodology, it was observed that electroporation experiments did not yield the expected results if the conventional assumption that *S. epidermidis* type I RM systems followed lineage specificity [226], as demonstrated in *S. aureus* [220], was true. These observations led to the hypothesis that *S. epidermidis* type I RM systems are not strictly conserved within lineages. To address this, PacBio RSII sequencing and methylome characterisation of seven clinically significant *S. epidermidis* genomes was performed, followed by the first systematic genomic analyses of the type I RM systems in *S. aureus* and *S. epidermidis*. This work aimed to: 1. Confirm that *S. aureus* type I RM systems are lineage specific; 2. Demonstrate that *S. epidermidis* type I RM systems do not adhere to lineage specificity; 3. Identify the underlying differences in the type I RM systems between the two species that explain why they behave differently. The results of these analyses were used to demonstrate how genomics can assist in predicting the functionality of type I RM systems and competence of strains. An approach for the efficient manipulation of any given *S. epidermidis* strain of interest, including those from backgrounds that have hitherto been intractable was then demonstrated. The resulting work was published in the journal mBio.

Supplementary materials for the publication are included in 4.3 Supplementary Appendix. Supplementary tables that consisted of large metadata spreadsheets in Excel format have been printed and are located at the end of the thesis in Appendix B.

4.2 Published manuscript

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RESEARCH ARTICLE
Molecular Biology and Physiology

Mining the Methylome Reveals Extensive Diversity in *Staphylococcus epidermidis* Restriction Modification

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ABSTRACT *Staphylococcus epidermidis* is a significant opportunistic pathogen of humans. Molecular studies in this species have been hampered by the presence of restriction-modification (RM) systems that limit introduction of foreign DNA. Here, we establish the complete genomes and methylomes for seven clinically significant, genetically diverse *S. epidermidis* isolates and perform the first systematic genomic analyses of the type I RM systems within both *S. epidermidis* and *Staphylococcus aureus*. Our analyses revealed marked differences in the gene arrangement, chromosomal location, and movement of type I RM systems between the two species. Unlike *S. aureus*, *S. epidermidis* type I RM systems demonstrate extensive diversity even within a single genetic lineage. This is contrary to current assumptions and has important implications for approaching the genetic manipulation of *S. epidermidis*. Using *Escherichia coli* plasmid artificial modification (PAM) to express *S. epidermidis hsdMS*, we readily overcame restriction barriers in *S. epidermidis* and achieved electroporation efficiencies equivalent to those of modification-deficient mutants. With these functional experiments, we demonstrated how genomic data can be used to predict both the functionality of type I RM systems and the potential for a strain to be electroporation proficient. We outline an efficient approach for the genetic manipulation of *S. epidermidis* strains from diverse genetic backgrounds, including those that have hitherto been intractable. Additionally, we identified *S. epidermidis* BPH0736, a naturally restriction-defective, clinically significant, multidrug-resistant ST2 isolate, as an ideal candidate for molecular studies.

IMPORTANCE *Staphylococcus epidermidis* is a major cause of hospital-acquired infections, especially those related to implanted medical devices. Understanding how *S. epidermidis* causes disease and devising ways to combat these infections have been hindered by an inability to genetically manipulate clinically significant hospital-adapted strains. Here, we provide the first comprehensive analyses of the barriers to the uptake of foreign DNA in *S. epidermidis* and demonstrate that these are distinct from those described for *S. aureus*. Using these insights, we demonstrate an efficient approach for the genetic manipulation of *S. epidermidis* to enable the study of clinical isolates for the first time.

KEYWORDS DNA methylation, *Staphylococcus aureus*, coagulase-negative staphylococci, generalized transduction, genetic manipulation, genome analysis, type I restriction modification

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Staphylococcus epidermidis is a ubiquitous colonizer of human skin (1). Invasive medical procedures, specifically, insertion of prosthetic devices on which the bacteria can form a biofilm, enable evasion of both antibiotics and the host immune system, which has contributed to its increasing importance as a significant nosocomial pathogen. A leading cause of surgical-site- and central-line-associated bloodstream infections (2), *S. epidermidis* poses a major economic burden (3). In the hospital environment, two multilocus sequence types (MLSTs), ST2 and ST23, account for most clinical disease (4, 5). Three hospital-adapted clones (two ST2 and one ST23) were recently demonstrated to be globally disseminated and to have evolved to become untreatable with first-line agents though the acquisition of multiple antibiotic resistance determinants and resistance-conferring mutations (5). Efforts to increase knowledge concerning the molecular genetics, pathogenesis, and treatment of *S. epidermidis* have been limited by barriers preventing the genetic manipulation of clinically relevant isolates and the assumption that the characteristics of *S. epidermidis* are similar to those of *S. aureus*.

Restriction-modification (RM) systems have evolved as a form of bacterial immunity that degrades incoming DNA from foreign donors such as bacteriophage (6). Type I and IV RM systems represent a significant barrier to genetic manipulation of staphylococci. Type I RM systems are comprised of three host specificity for DNA (*hsd*) genes that encode (i) a specificity protein (HsdS), (ii) a modification protein (HsdM), and (iii) a restriction endonuclease (HsdR). Together, these function as a single protein complex in which HsdS determines the DNA target recognition motif (TRM) in which adenine residues are methylated by HsdM, while HsdR cleaves unmodified and non-self-modified DNA (7, 8). Type IV RM systems consist of a single restriction endonuclease that cleaves DNA with inappropriate modification (8).

Plasmid artificial modification (PAM) is a method to overcome the barrier imposed by RM systems where plasmid DNA is passaged through a cytosine methylation-deficient *Escherichia coli* host (DC10B) that has been engineered to heterologously express the *hsdMS* system of the staphylococcal strain to be transformed. Plasmid DNA extracted from this *E. coli* host mimics the DNA methylation profile of the target strain, thus enabling introduction of plasmid DNA and subsequent genetic manipulation (9).

Type I RM systems of staphylococci are best understood in *S. aureus*. The distribution of *hsdS* alleles corresponds to clonal complex (CC) for the 10 dominant *S. aureus* lineages (10). Far less is known about the type I RM systems in *S. epidermidis*. A recent study suggested that *S. epidermidis* type I RM systems adhered to lineage-specific groupings like *S. aureus*. However, this inference was based on analysis of only four new *S. epidermidis* methylomes (11) plus the one methylome that had already been characterized; namely, the ST2 reference genome of strain BPH0662 (12).

Here, we present the first systematic genomic analyses of the type I RM systems in *S. aureus* and *S. epidermidis* and demonstrate how these data can be used to predict functionality of type I RM systems and associated competence of strains. We show that PAM is a highly efficient method to enable genetic manipulation of *S. epidermidis*, particularly hospital-adapted isolates that possess multiple functional type I RM systems.

RESULTS AND DISCUSSION

***S. aureus* type I RM systems are lineage specific.** We began this study by testing the notion that *S. aureus* type I RM systems are lineage specific. We compared 128 publicly available finished *S. aureus* genome sequences (see Table S1A in the supplemental material) and confirmed that the chromosomal location and structure of type I RM systems in *S. aureus* are highly conserved. A total of 110 genomes had a single *hsdR* gene and two copies of *hsdMS* with the first in forward orientation located in the alpha pathogenicity island and the second in the opposite orientation and located within the beta pathogenicity island (10, 13) (Fig. 1A). The remaining 18 strains possessed *hsdR* and a single copy of forward oriented *hsdMS* in the alpha pathogenicity island. Five of the 128 strains possessed a third type I RM system at a nonmobile chromosomal

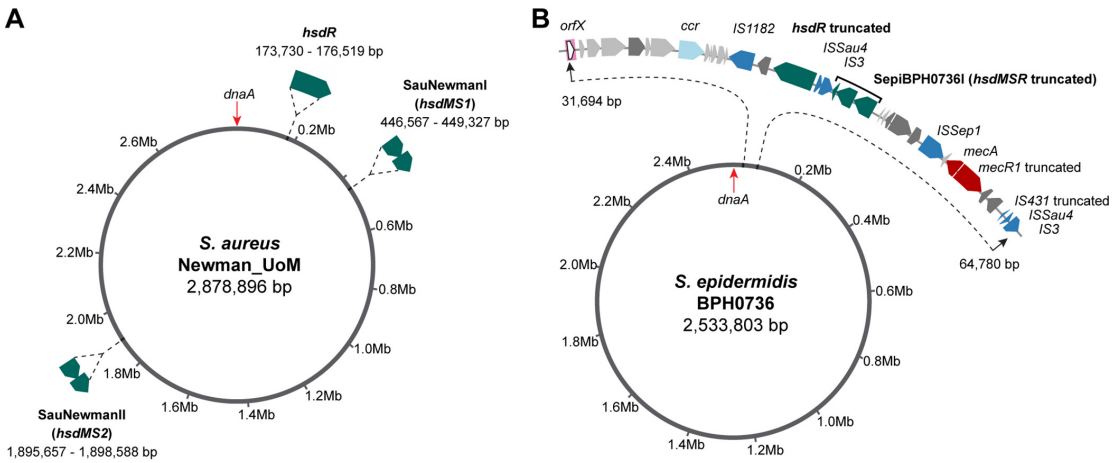

FIG 1 Comparison of the structures and chromosomal locations of *S. aureus* and *S. epidermidis* type I restriction-modification systems. (A) *S. aureus* Newman_UoM (29, 32). (B) *S. epidermidis* BPH0736. For consistency, the chromosome is orientated forwards starting at the start codon of *dnaA*, and native type I RM systems are sequentially numbered.

location downstream of *lacA*. Twenty-three strains carried an additional type I RM system on a mobile genetic element, 22 were on staphylococcal cassette chromosome (SCC) elements (Fig. 2; see also Fig. S1 in the supplemental material), and 1 carried a type I RM system on a plasmid (strain HUV05). Single variants of both HsdR (NCBI protein accession no. WP_000331347.1; $n = 127$) and HsdM (WP_000028628.1; $n = 222$) were demonstrated for the type I RM systems situated in nonmobile chromosomal locations, indicating stable vertical inheritance. Interruptions in *hsdR* and *hsdMS* due to horizontal gene transfer were rarely seen in *S. aureus*. A solitary example of *hsdS* truncation due to insertion of a bacteriophage was noted in strain Sa17_S6. Most changes were due to single nucleotide polymorphisms (SNPs) leading to amino acid substitutions ($n = 35$) or nonsense mutations ($n = 31$) in *hsdS* (Fig. 2). See Table 1 for a comparison of *S. aureus* and *S. epidermidis* type I RM systems.

We next established a high-resolution phylogeny using 144,727 core genome SNPs for the 128 *S. aureus* genomes covering 40 STs (Fig. 2). Occurrences of *hsdMS* genes were mapped across the phylogeny for each genome. A total of 48 HsdS subunits were identified with associated TRMs (Table 2; see also Table S2A [https://melbourne.figshare.com/articles/Sa_HsdS_48_fasta/7986956]). Although the same *hsdMS* genes were present in genetically distinct lineages, the combinations of *hsdMS* genes were conserved within each lineage (Fig. 2). For example, the same two HsdMS products were present in ST250 and ST254, which are single-locus variants of ST8. A notable exception to the lineage specificity was represented by the type I RM systems carried on SCC elements (Fig. S1B), which may have been acquired from coagulase-negative staphylococci (CoNS). Complete *S. epidermidis* BPH0736 *hsdMSR* genes (nondisrupted, identical sequences) were observed in four *S. aureus* strains from three different STs (ST5, ST59, and ST338), suggesting gene transfer between the species (Fig. S1).

***S. epidermidis* type I RM systems are carried on mobile genetic elements.** Seven complete *S. epidermidis* reference genomes were publicly available at the beginning of this study (Table S1). Of these, only BPH0662 (12) and RP62a had characterized type I RM system motifs. However, the RP62a methylome was determined independently (11) of the finished genome (14). The methylomes of *S. epidermidis* isolates 1457 (15) and 14.1.R1 (16) confirmed that, consistent with the absence of *hsdM* genes, neither possessed a functional type I RM system. To improve understanding of the type I RM systems in *S. epidermidis*, we conducted PacBio SMRT sequencing and established complete genomes and adenine methylomes for six additional *S. epidermidis* strains

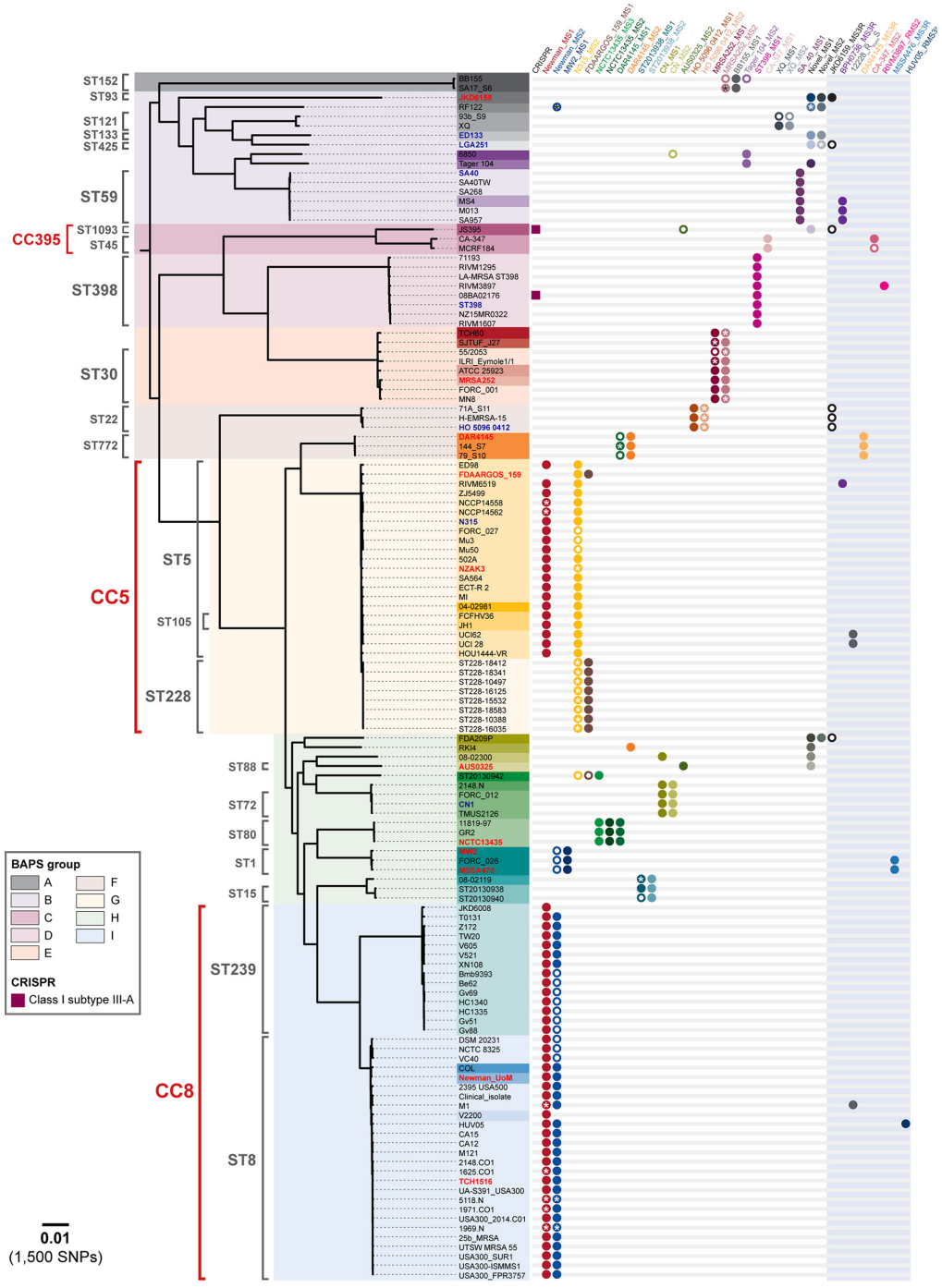

FIG 2 *S. aureus* native type I restriction-modification systems are lineage specific. The figure shows a maximum likelihood core SNP-based phylogeny of 128 closed *S. aureus* genomes originating from 40 STs, using Newman_UoM as the reference genome. Overlaid are the results

(Continued on next page)

TABLE 1 Comparison of *S. aureus* and *S. epidermidis* type I restriction-modification systems

***Staphylococcus aureus* type I RM system characteristics**	***Staphylococcus epidermidis* type I RM system characteristics**
RM system organized as a single *hsdR* gene separated from one, two, or three distant *hsdMS* gene pairs	RM system organized as complete three-gene operon (*hsdRMS* or *hsdMSR*)
Conserved, stable chromosomal location for each gene	Close proximity to *ccr* genes integrated at *orfX*, located in a highly plastic region of the genome
Most strains have two type I RM systems	Most strains have a single type I RM system
All strains have at least one type I RM system	Many (38.1%) strains have no type I RM system
Up to three functional type I RM systems per isolate	Up to three functional type I RM systems per isolate
99.7% amino acid pairwise identity for all native HsdRs	At least five identified variants of HsdR
99.3% amino acid pairwise identity for all native HsdMs	At least six identified variants of HsdM
At least 48 different variants of HsdS (8 likely imported from coagulase-negative staphylococci)	At least 31 different variants of HsdS
Relative conservation of HsdS present within ST groups	No clear conservation of HsdS according to ST group
Conservation of HsdM provides redundancy, enabling interaction with multiple different HsdS	Each HsdS is capable of interacting only with the corresponding paired HsdM; therefore, not all orphan *hsdS* genes are functional
Complete three-gene *hsdRMS*/*MSR* type I systems carried on SCC elements do not adhere to lineage specificity and are likely imported from coagulase-negative staphylococci	

from ST2, ST5, ST59, and ST358 and we resequenced RP62a (ST10) (See Table S3 for metadata).

The typical chromosomal arrangement of the type I RM system in *S. epidermidis* is shown for BPH0736 (ST2) (Fig. 1B). Unlike *S. aureus* (Fig. 1A), type I RM systems in *S. epidermidis* are arranged as a complete three-gene operon in either an *hsdRMS* or *hsdMSR* organization, unless interrupted (Fig. S1). Analyses of the 11 closed *S. epidermidis* genomes containing type I RM systems demonstrated their co-occurrence with cassette chromosome recombinase (*ccr*) genes (with or without the presence of *mecA*). The 16 type I RM systems present within these 11 genomes were located a mean distance of 11.5 kb (minimum, 2.3 kb; maximum, 51.0 kb) from the nearest *ccr* (Fig. S1A). Similarly, in the 22 *S. aureus* genomes with a SCC-associated type I RM system, the mean distance between *hsdRMS*/and *hsdMSR* and the nearest *ccr* was 6.9 kb (minimum, 1.6 kb; maximum, 20.9 kb) (Fig. S1B).

Cassette chromosome recombinases typically integrate at *orfX* (corresponding to the last 15 nucleotides of the rRNA large subunit methyltransferase [17]), which is located at 31.6 kb in the *S. epidermidis* chromosome (33.3 kb in *S. aureus*). This represents the start of a highly plastic region of the chromosome, in which multiple antibiotic resistance genes and genes corresponding to drug transporters and insertion sequence (IS) elements have accumulated (12) (Fig. S1). All 11 *S. epidermidis* and 22 *S. aureus* genomes with *ccr*-associated type I RM systems were integrated at *orfX* (Fig. S1). For type I RM system variants present in multiple isolates, conservation of genes surrounding the system and *ccr* was observed, consistent with the mobilization of an entire element (Fig. S1). Preserved cassette structure between isolates and in both species (Fig. S1B) led us to hypothesize that the movement of type I RM systems in *S. epidermidis* is mediated by *ccr*, enabling mobilization on SCC elements between strains and to other staphylococcal species. Localization in this region of the genome also predisposes *S. epidermidis* type I RM systems to disruption, potentially rendering variants restriction deficient. This was seen with interruption of *hsdR* by IS elements in BPH0736 (Fig. 1B).

***S. epidermidis* type I RM systems are not strictly conserved within lineages.** To expand the *S. epidermidis* data set, we added short-read data from 234 publicly

FIG 2 Legend (Continued)
of *in silico* multilocus sequence type (MLST), clonal cluster (CC), Bayesian analysis of population structure (BAPS), presence of CRISPR-Cas systems, and type I restriction-modification system HsdS variants. Bold red font indicates isolates with PacBio-characterized methylomes. Bold blue font indicates isolates with methylomes determined by DNA cleavage with purified enzyme. Boxes around strain names are colored according ST type. Open circles represent amino acid substitutions present in HsdS. An asterisk (*) indicates a truncated HsdS subunit. The scale bar indicates the number of nucleotide substitutions per site (bold) with an approximation of SNP rate (in parentheses).

TABLE 2 Diversity of *S. aureus* type I restriction-modification system methylation profiles[d]

	Representative HsdS	Motif	N° isolates	MLST	HsdS NCBI Protein Accession
1.	**Newman_UoM_MS1**	AGG(N)$_5$GAT	60	CC5 [5 (225, 105)], CC8 [239, 8 (250, 254, 923)]	WP_000072584.1
2.	**Newman_UoM_MS2**	CCAY(N)$_6$TGT	42	151, 1, CC8 [8 (250, 254, 923)]	WP_000072576.1
3.	**MW2_MS1**	CCAY(N)$_5$TTAA	3	1	WP_000072566.1
4.	**N315_MS2**	CCAY(N)$_6$GTA	30	CC5 [5 (225, 105), 228]	WP_000072627.1
5.	**FDAARGOS_159_MS1**	CCAY(N)$_5$GAT	10	CC5 [5, 228], 25	WP_000072629.1
6.	**NCTC13435_MS3**	TCTA(N)$_6$RTTC	4	25, 72	WP_000072557.1
7.	**NCTC13435_MS2**	GAC(N)$_8$TTYG	3	72	WP_000072638.1
8.	**DAR4145_MS1**	CTA(N)$_7$TAG	6	772, 72	WP_000072592.1
9.	**DAR4145_MS2**	GAAG(N)$_6$TTRG	4	772, 27	WP_000072555.1
10.	ST20130938_MS1	predict ?-RTGA	3	582, 15	WP_000066763.1
11.	ST20130938_MS2	predict GGHA-?-TTYG	3	582, 15	WP_000072556.1
12.	**CN1_MS1**	GARA(N)$_6$RTGT	5	7, unclassified, 72	WP_000072573.1
13.	**CN1_MS2**	GGA(N)$_7$TGC	5	50, unclassified, 72	WP_000072558.1
14.	**AUS0325_MS2**	GAG(N)$_6$TCG	2	1093, 88	WP_069992008.1
15.	**HO 5096 0412_MS1**	AGG(N)$_6$TGAR	3	22	WP_000072565.1
16.	HO 5096 0412_MS2$_{trunc}$	not functional	3	22	WP_000323907.1
17.	**MRSA252_MS1**	GWAG(N)$_5$GAT	8	unclassified, 30 (36, 243), 433	WP_000072632.1
18.	**MRSA252_MS2**	GGA(N)$_7$TCG	10	unclassified, 30 (36, 243), 433	WP_000072622.1
19.	BB155_MS1	predict AGG-?	2	152	WP_054190421.1
20.	Tager 104_MS2	predict ?-RTTC	3	152, 50, 49	WP_000072571.1
21.	**S0385(ST398_MS1)**	ACC(N)$_5$RTGA	8	398	WP_000072568.1
22.	**CA-347_MS1**	GWAG(N)$_8$TAAA	2	45	WP_000072579.1
23.	XQ_MS1	GGA(N)$_6$CCT	2	121	WP_058008355.1
24.	XQ_MS2	GAC(N)$_6$TAYG	2	121	WP_058008121.1
25.	**SA40_S**	GGA(N)$_6$RTGT	6	59 (338)	WP_000072559.1
26.	**JKD6159_MS1**	CAG(N)$_6$TTC	1	93	WP_000072616.1
27.	**JKD6159_MS2**	GGHA(N)$_7$TCG	1	93	WP_000072554.1
28.	RF122_MS1	unknown	1	151	WP_000072567.1
29.	RF122_MS2$_{trunc}$	not functional	1	151	WP_070007671.1[a]
30.	**ED133_MS1**	CAG(N)$_5$RTGA	1	133	WP_000072617.1
31.	**ED133_MS2**	GGA(N)$_5$TTRG	1	133	WP_000072562.1
32.	**LGA251_MS1**	GWAG(N)$_5$RTGA	1	425	WP_000072635.1
33.	**LGA251_MS2$_{trunc}$**	not functional	1	425	WP_044122248.1[b]
34.	**Tager 104_S1**	predict GGA-?-RTGA	1	49	WP_000072560.1
35.	**JS395_MS1**	predict CAG-?	1	1093	WP_000072352.1[c]
36.	FDA209P_MS1	CCAY(N)$_6$RTC	1	464	WP_000072586.1
37.	FDA209P_MS2	predict CCAY-?-TTYG	1	464	WP_047210362.1
38.	RKI4_MS1	TCTA(N)$_6$TTAA	1	27	WP_000072580.1
39.	08-02300_MS2	predict ?-RTTC	1	7	WP_000072588.1
40.	**AUS0325_MS1**	ACC(N)$_5$RTGT	1	88	WP_000072613.1
Imported systems					
41.	**JKD6159_MS3R**	GAAG(N)$_5$TAC	7	93, 425, 1093, 22, 464	WP_000394004.1
42.	**BPH0736_MS3R**	GAT(N)$_4$CTTA	4	59 (338), 5	WP_000456234.1
43.	**12228_R$_{trunc}$S3**	GAA(N)$_8$CTTA	3	5, 8	WP_001631029.1
44.	**DAR4145_MS3R**	TTAC(N)$_5$TAC	3	772	WP_000394606.1
45.	CA-347_MS2	unknown	2	45	WP_000809140.1
46.	RIVM3897_MS2R	unknown	1	398	WP_060585130.1
47.	MSSA476_MS3R	unknown	2	1	WP_000085808.1
48.	HUV05_RMS3*	unknown	1	8	WP_048520805.1

[a]Truncation at amino acid 203.
[b]Truncation at amino acid 249.
[c]Truncation at amino acid 8. Full amino acid translations of all 48 HsdS variants are accessible at Figshare (https://melbourne.figshare.com/articles/Sa_HsdS_48_fasta/7986956).
[d]Isolate HsdS motifs were collated from publications by Monk et al. (9) and Cooper et al. (18) and from the REBASE database (33). HsdS names in bold black font have motifs determined by PacBio sequencing of the isolate after which the representative HsdS was named. HsdS names in bold blue font have motifs determined by DNA cleavage with purified restriction enzyme. The multilocus sequence types (MLSTs) in which each HsdS was found are listed according to the order in which they appear in the Fig. 2 phylogeny (top to bottom). STs within the same clonal complex (CC) are listed within square brackets; STs within parentheses represent single-locus variants of the ST group they are listed after. trunc, truncated; A (red), methylated adenine residue; T, complementary partner to methylated adenine residue. *, HUV05_RMS3 is carried on a plasmid, not integrated in the chromosome.

available *S. epidermidis* genomes to the 13 finished genomes (Table S1). In contrast to the *S. aureus* data, variability was noted in both the HsdR and HsdM subunits for *S. epidermidis*. Across the 247 genomes, 183 intact HsdR genes were identified, including five major HsdR variants (<90% amino acid pairwise identity threshold) (Table S2 [https://melbourne.figshare.com/articles/Se_HsdR_5_fasta/7986893]). The two variants of HsdR in strain BPH0662 shared only 22% amino acid identity. Similarly, 178 complete HsdM genes were identified with six major variants (Table 3; see also Table S2)

TABLE 3 Diversity of *S. epidermidis* type I restriction modification methylation profiles[g]

	Representative HsdS	Motif	N° isolates	MLST	HsdS NCBI Protein Accession	Interacting HsdM
1.	12228-S[a]	GAA(N)$_6$CTTA	64	22, 5, 6, 8, 59, 22, 2 (185)	WP_001631029.1	BPH0662-M1
2.	**BPH0662-S2**	CAG(N)$_4$ATC	52	23, 35, 2	WP_002504701.1	BPH0662-M2
3.	**BPH0662-S1**	ATT(N)$_5$CTC	43	2	WP_002504637.1	BPH0662-M1
4.	**BPH0736-S**	TAAG(N)$_4$ATC	18	210, 16, 2	WP_000456234.1	BPH0662-M2
5.	**BPH0723-S**	GAAY(N)$_5$TGC	13	32, 46, 83, 5, 210, 21	WP_002469391.1	BPH0662-M2
6.	**RP62a-S**	GAG(N)$_7$TAC	9	230, 71, 23, 10, 40	WP_002489618.1	BPH0662-M1
7.	**BPH0697-S**	ACA(N)$_5$GTG	4	89, 4, 22	WP_002505893.1	BPH0697-M1
8.	**BPH0711-S**	GGA(N)$_6$TAG	2	59	WP_002437522.1	BPH0662-M2
9.	**BPH0704-S**	CYYA(N)$_6$CGT	1	358	WP_061544233.1	BPH0662-M1
10.	**BPH0747-S**	CNAC(N)$_4$RTTA	1	2	WP_100481761.1	BPH0662-M2
11.	UCLA-14-S	unknown	7	186, 2	WP_002504281.1	BPH0662-M2
12.	**1457-S$_{trunc}$**	non-functional	4	86	WP_079118799.1[c]	unknown
13.	972_SEPI-S	unknown	4	5, 6, 20	WP_049387567.1	BPH0662-M2
14.	288_GRE41-S	unknown	3	83	WP_049366366.1	BPH0662-M2
15.	300_ITL34-S	unknown	3	66, 57	WP_021298801.1[d]	BPH0662-M1
16.	308_MEX35-S$_{trunc}$	non-functional	2	328, 559	WP_080035763.1	unknown
17.	332_1340N-S1$_{trunc}$	non-functional	2	402	novel	BPH0662-M2
18.	332_1340N-S2	unknown	2	402	novel	332_1340N-M
19.	BPH0710-S$_{trunc}$	non-functional	2	2	WP_064584491.1[e]	BPH0710-M
20.	**14.1.R1-S**[b]	unknown	1	unclassified	WP_102841642.1	unknown
21.	248_DEN19-S	unknown	1	1	novel	BPH0662-M1
22.	271_ICE87-S	unknown	1	40	novel	BPH0662-M1
23.	290_TAW113-S	unknown	1	85	novel	BPH0662-M1
24.	298_JAP60-S1	unknown	1	33	novel	unknown
25.	298_JAP60-S2	unknown	1	33	novel	298_JAP60-M
26.	307_HFA173B-S	unknown	1	37	novel	BPH0662-M2
27.	313_93N-S1	unknown	1	329	WP_104992793.1	BPH0662-M1
28.	313_93N-S2$_{trunc}$	non-functional	1	329	novel	unknown
29.	315_148 N-S	unknown	1	390	WP_107639500.1[f]	BPH0662-M2
30.	327_790N-S	predict ?-ATC	1	406	WP_080352270.1	BPH0662-M2
31.	328_803NLR2-S	unknown	1	595	WP_002490392.1	BPH0662-M1

[a]The ATCC 12228 type I RM system is nonfunctional, with a truncated *hsdR* gene, a complete *hsdS* gene, and no *hsdM* gene. All 64 isolates possessed the same incomplete type I RM system. The motif was identified based on the methylome determined for NIH4008 due to the presence of an HsdM protein capable of interacting with 12228 HsdS.

[b]14.1.R1 type I RM system is nonfunctional, with truncated *hsdR*, complete *hsdS*, and no *hsdM*.

[c]L1M substitution.

[d]First 81 amino acids truncated.

[e]S295P substitution.

[f]Eleven amino acid substitutions (K26E, I56V, E59K, E171K, K174R, K175T, E178A, I193V, D201N, Y386F, and V434I). Amino acid translations of all 31 HsdS variants (https://melbourne.figshare.com/articles/Se_HsdS_31_fasta/7986911) and their interacting HsdMs (https://melbourne.figshare.com/articles/Se_HsdM_6_fasta/7986827) are accessible through Figshare.

[g]Isolate HsdS motifs were collated from methylomes newly characterized in this study and from publications by Lee et al. (12) and Costa et al. (11). HsdS names in bold black font have motifs determined by PacBio sequencing of the isolate after which the representative HsdS was named. The multilocus sequence types (MLSTs) in which each HsdS was found are listed according to the order in which they appear in the Fig. 3 phylogeny (clockwise). ST185 is a single-locus variant of ST2. trunc, truncated; A (red), methylated adenine residue; T, complementary partner to methylated adenine residue.

(https://melbourne.figshare.com/articles/Se_HsdM_6_fasta/7986827). The amino acid sequences of these six variants were markedly divergent. The two variants of HsdM present in BPH0662 shared only 31% amino acid identity.

A maximum likelihood phylogeny for the 247 *S. epidermidis* genomes, derived from 83,210 core SNPs and sampled from 72 STs, was established, and the 31 different *S. epidermidis* HsdS subunits identified were overlaid (Fig. 3). Where known, their associated TRMs are shown in Table 3 with NCBI protein accession numbers (see also Table S2). Amino acid sequences of all 31 HsdS are available from Figshare (https://melbourne.figshare.com/articles/Se_HsdS_31_fasta/7986911). The distribution of *S. epidermidis* HsdS proteins within the population differed markedly from that observed within *S. aureus*, with no strict concordance to lineage specificity. For example, HsdS from BPH0723 (BPH0723-S; Table 3) was present in 13 isolates from five STs (ST5, ST21, ST46, ST210, and one unclassified ST), while BPH0662-S2 was identified in 52 isolates from three STs (ST2, ST23, and ST35). Although a high proportion of ST2 isolates shared the same predicted methylome (Fig. 3), the majority of these were known to be clones of internationally disseminated, multidrug-resistant strain BPH0662 (5). However, even within this highly clonal group (n = 36), some predicted

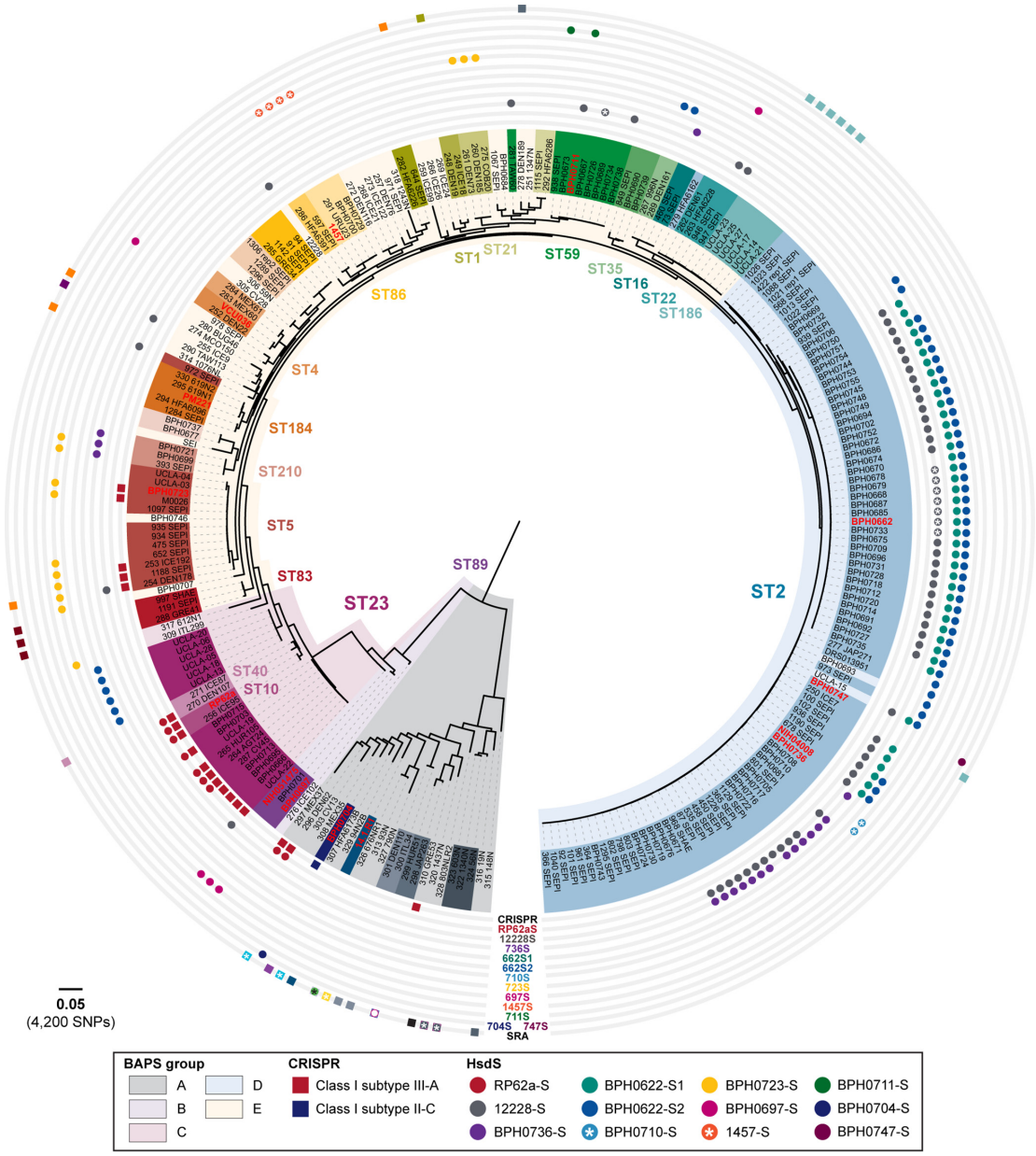

FIG 3 *S. epidermidis* type I restriction-modification systems are not conserved within lineages. The figure shows a maximum likelihood, core SNP-based phylogeny for 247 *S. epidermidis* genomes, including 7 newly closed reference genomes, 6 existing reference genomes, 156 genomes curated from the NCBI sequence read archive (SRA), 75 isolates from a study by Lee et al. (5); and the 3 draft genomes with methylation data (11). BPH0736 was used as the reference genome for analyses. Overlaid are the results of *in silico* multilocus sequence type (MLST), Bayesian analysis of population structure (BAPS), presence of CRISPR-Cas systems, and type I restriction-modification system HsdS variants. Bold red font indicates isolates with characterized methylomes. Isolates were from 70 recognized and two unclassified MLST groups. Boxes around strain names are colored according ST type; where background color is same as that of the BAPS group, the result indicates an ST represented by a single isolate. An asterisk (*) represents a truncated HsdS subunit. The scale bar indicates the number of nucleotide substitutions per site (bold) with an approximation of SNP rate (in parentheses).

methylation variation existed. For example, BPH0662-S2 was absent from two isolates, six isolates (including BPH0662) had a truncation in the 12228-S orphan system, and two isolates were missing the 12228-S orphan system completely. Furthermore, within ST2, seven different variants of HsdS were identified in 11 arrangements, including the absence of any type I RM system (Fig. 3). Of the 247 *S. epidermidis* genomes analyzed, 38% did not contain any *hsdS* alleles and were predicted to be restriction deficient.

Methylation analysis performed using our Pacbio-sequenced, Illumina read-corrected RP62a_UoM genome as a reference indicated the presence of a single type I RM system with a G**A**GN$_7$TAC TRM (Table 3). Although this motif was consistent with that previously reported by Costa et al. (11), the three additional motifs previously described (lacking apparent associated genes) were not detected by our methylome analysis; the low complexity of the motifs (e.g., GGBNNH) and the low frequency of detected methylation (12% to 29%) (11) suggest that these might have represented artifacts rather than true motifs. Similarly, the three additional low-complexity and low-frequency motifs reported for VCU036 (11) probably represented artifacts. Although the ST type was not specified, VCU036, which shared the same methylome with ST89 isolate NIH051475, was reported as CC89 by Costa et al., leading to the conclusion that *S. epidermidis* type I RM systems follow *S. aureus*-like lineage specificity (11). We performed *in silico* MLST by two independent methods and determined that VCU036 belongs to ST4. Furthermore, our analysis of the 247 *S. epidermidis* genomes demonstrated VCU036 to be phylogenetically distinct from ST89 (Fig. 3).

Overall, our analyses demonstrated that, in contrast to current assumptions (11), the type I RM systems of *S. epidermidis* do not adhere to the lineage-specific distribution observed in *S. aureus*. These differences are attributable to the arrangement of *S. epidermidis* type I RM systems as complete three-gene operons that reside within a highly mobile region of the chromosome, the movement of which we hypothesize to be mediated by *ccr*.

Recombinant target recognition domains generate HsdS variants with low conservation of amino acid identity. The structure of a typical type I RM system HsdS allele is shown in Fig. S2A and is composed of two highly variable target recognition domains (TRDs) flanked and separated by conserved regions (CRs) that collectively determine the methylation of the TRM by HsdM. Recombinant pairings of TRDs result in different variants of HsdS (13, 18). Alignments of the range of *S. aureus* and *S. epidermidis* HsdS proteins identified in this study are shown in Fig. S2B and S3, respectively. Within our *S. aureus* and *S. epidermidis* collections, 77 variants of HsdS that shared only 24% pairwise identity were identified. This low level of conservation poses a potential challenge to the high-throughput bioinformatic screening for HsdS variants within genomic data sets. However, using HsdS from ATCC 12228 as the reference translation with our described method, we were able to detect the partial if not complete presence of all HsdS variants in both species. Of note, 12228-S was the only HsdS variant found within both species that clustered with the majority of *S. aureus* variants. In comparison, RP62a-S captured only 18 of the 31 *S. epidermidis* HsdS variants and fragments of fewer than half of the *S. aureus* HsdS variants.

***S. epidermidis* HsdS variants interact only as part of a specific complex.** The arrangement of some *S. epidermidis* type I RM systems, with the presence of a truncated *hsdS* gene between complete *hsdR* and *hsdMS* genes, suggested the occurrence of recombination of component genes (e.g., *S. epidermidis* BPH0662I [SepiBPH0662I], SepiRP62aI, and SepiBPH0704I; Fig. S1). Analyses of the 247 genomes indicated that each variant of *hsdS* in *S. epidermidis* was always associated with a specific *hsdR* gene and *hsdM* gene, with a gene arrangement that was conserved (unless interrupted), frequently with the same surrounding genes found in association with *ccr* (Fig. S1A). These observations support our hypothesis of a role for SCC elements in the mobilization of *S. epidermidis* type I RM systems.

The presence of an orphan *hsdS* gene without a partner *hsdM* gene in *S. epidermidis* introduces additional complexity to the prediction of type I RM system functionality. This was demonstrated by the presence of 12228-S, the most prevalent HsdS within the data set, in 64 *S. epidermidis* isolates (Table 3) (Fig. 3) and three *S. aureus* isolates (Table 2 and Fig. 2). All examples of this *hsdS* variant followed a truncated *hsdR* gene, without an *hsdM* gene. We determined that 12228-S was expressed only when the corresponding specific interacting variant of *hsdM* (BPH0662-M1; WP_002504638.1) was also present (see Table S4 for full explanation). In contrast, conservation of a single variant of *hsdM* present twice within the same *S. aureus* genome provides redundancy for the expression of type I RM methylation. This is consistent with previous findings where the product of a single copy of the conserved *S. aureus hsdM* allele could functionally interact with both CC8 HsdS products when heterologously expressed in *E. coli* (9).

Plasmid artificial modification to overcome the type I RM systems in *S. epidermidis* provides electroporation efficiency equivalent to deletion of functional type I systems. To determine the restriction barrier posed by type I RM systems in *S. epidermidis* and assess the efficiency of PAM as a means of bypassing restriction barriers (Fig. 4), Δ*hsdS* mutants and *E. coli* hosts for PAM were constructed for *S. epidermidis* isolates BPH0662, RP62a, and BPH0736. Two different plasmids (pRAB11 [19] and pIMAY [8]) were used in electroporation experiments, as each carried a different number of TRMs recognized by the type I RM systems present in each isolate (Fig. 4D). A clinical ST2 isolate, BPH0662-WT (BPH0662 wild type), was found to have an intractable restriction barrier unless both functional type I RM systems were overcome by complete bypass with PAM in an *E. coli* host (Ec_Se662I-II) or by deletion of both complete *hsdS* genes (BPH0662 Δ*hsdSI* Δ*hsdSII*) or by a combination of the two approaches (plasmid from Ec_Se662I transferred into BPH0662 Δ*hsdSII* or plasmid from Ec_Se662II transferred into BPH0662 Δ*hsdSI*) (Fig. 4A).

Using our protocol, the type I restriction barrier in RP62a-WT was found to be incomplete. Low numbers of transformants (10^1 CFU/ml) were obtained with plasmid DNA isolated from DC10B, indicating that bypassing the type IV restriction barrier alone was sufficient to allow genetic manipulation of this strain (Fig. 4B) as previously demonstrated (8). Complete bypass of the single type I RM system in this isolate with *E. coli* host Ec_SeRP62aI significantly improved electroporation efficiency to 10^4 CFU/ml, which was equivalent to the complete absence of a functional type I RM system as determined with the RP62a Δ*hsdS* mutant (Fig. 4B). In contrast, when expressing the RP62a *hsdMS* genes from a plasmid in DC10B, Costa et al. were unable to completely bypass the type I RM barrier. This discrepancy was attributed to the presence of additional RM systems with low-frequency methylation (11). However, our results showed that only one type I RM system is present in RP62a, suggesting that the heterologous expression of type I RM systems on a plasmid in DC10B rather than from a single copy of the genes integrated into the chromosome may be suboptimal. Previously, we found that plasmid-based expression of *hsdMS* was unstable and that cells were unable to tolerate the high level of expression required for complete methylation of the target DNA (9).

Clustered regularly interspaced short palindromic repeat (CRISPR) loci confer sequence-directed immunity against phages and other foreign DNA and represent another recognized barrier to horizontal gene transfer in *S. epidermidis* (20). Our analysis of the CRISPR spacers for RP62a (Table S3D) did not demonstrate the presence of any targets on pSK236 (5.6 kb) as used by Costa et al. or on pRAB11 (6.4 kb) or on pIMAY (5.7 kb) as used in this study that would account for the fact that their electroporation efficiency (10^2 CFU/ml per 5 μg plasmid DNA) (11) was lower than that determined by our protocol (10^4 CFU/ml per 5 μg plasmid DNA for both pRAB11 and pIMAY).

Isolate BPH0736-WT was predicted to be naturally restriction deficient due to the interruption of *hsdR* by IS elements (Fig. 1B), but PacBio sequencing demonstrated that it retained functional methylation conferred by an intact *hsdMS* system. Due to

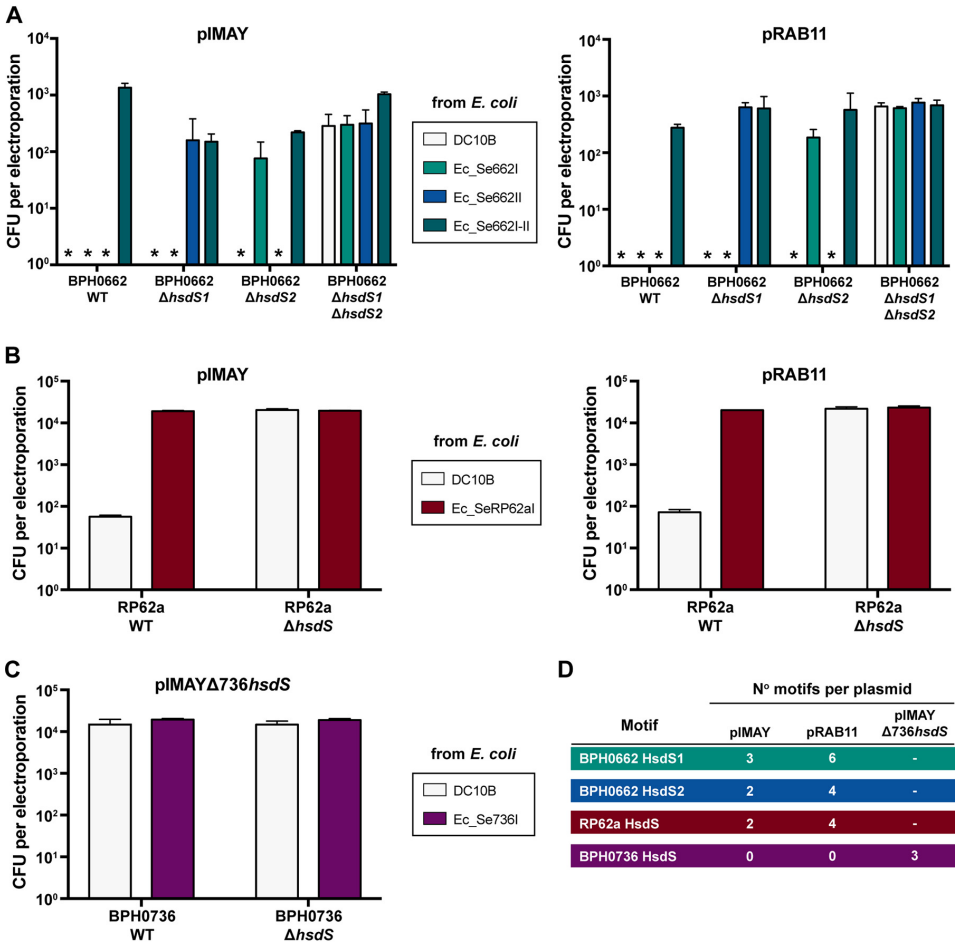

FIG 4 Plasmid artificial modification to overcome the type I RM systems in *S. epidermidis*. Biological triplicate data were determined for 5 μg of plasmid passaged through DC10B *E. coli* compared to the relevant *E. coli* PAM construct and transformed into *S. epidermidis* wild-type (WT) and Δ*hsdS* mutant strains. Error bars represent means ± standard deviations of results from three independent experiments. *, no transformants. (A) Electroporation of BPH0622-WT, BPH0662 Δ*hsdS1*, BPH0662 Δ*hsdS2*, and BPH0662 Δ*hsdS1* Δ*hsdS2* with plasmid pIMAY (left) or pRAB11 (right) isolated from DC10B and strain-specific *E. coli* Ec_Se662I (expressing BPH0662*hsdMS1*), Ec_Se662II (expressing BPH0662*hsdMS2*), and Ec_Se662I-II (expressing both BPH0662*hsdMS1* and BPH0662*hsdMS2*). (B) Electroporation of RP62a-WT and RP62a Δ*hsdS* with plasmid pIMAY (left) or pRAB11 (right) isolated from DC10B and strain-specific *E. coli* Ec_SeRP62aI (expressing RP62a*hsdMS*). (C) Electroporation of BPH0736-WT and BPH0736 Δ*hsdS* with plasmid pIMAY Δ736*hsdS* isolated from DC10B and strain-specific *E. coli* Ec_Se736I (expressing BPH0736*hsdMS*). Note that pIMAY Δ736*hsdS* was used because neither pIMAY nor pRAB11 possessed any TRMs. (D) Number of *S. epidermidis* strain-specific HsdS TRMs present on each plasmid.

the complex and infrequently occurring TRM dictated by the single *hsdS* (Table 3), neither pIMAY nor pRAB11 had any BPH0736-S TRMs present. Therefore, pIMAY bearing the Δ736*hsdS* insertion (pIMAY Δ736*hsdS*) was used as this contained three TRMs (Fig. 4D). BPH0736-WT was functionally confirmed to be restriction deficient, with the same electroporation efficiency (10^4 CFU/ml) demonstrated for both BPH0736-WT and BPH0736 Δ*hsdS* using plasmid isolated from nonspecific *E. coli* host DC10B and PAM-tailored mutant Ec_Se736I (Fig. 4C). Further supporting our bioinformatic predictions, like BPH0736, ATCC 12228 (truncated *hsdR* and no *hsdM*; ST8), 1457 (truncated HsdS containing only one TRD, a truncated *hsdR*, and no *hsdM*; ST86), and BPH0710 (truncation at amino acid 81 of HsdS; ST2) were all

predicted to have no functional restriction barrier. Similarly to BPH0736, these three strains were transformable at levels on the order of 10^4 CFU/ml with plasmid isolated from DC10B, suggesting that this was the maximum electroporation efficiency expected for our protocol. A clinical ST2 strain, BPH0676, was also predicted to have no restriction barrier and the complete absence of a type I RM system; however, similarly to BPH0662, the maximum electroporation efficiency achieved was only 10^3 CFU/ml, suggesting that inherent strain-dependent factors other than type I RM systems, e.g., cell wall thickness (21), impacted the electroporation of these isolates.

Although the data presented above demonstrate that PAM is an efficient method to overcome the type I restriction barrier of *S. epidermidis*, we observed potential instability with the integration of multiple *S. epidermidis hsdMS* genes of particular TRMs in a DC10B *E. coli* background. With serial passage of Ec_Se662I-II, the electroporation efficiency of plasmid isolated from this *E. coli* host into BPH0662 declined from 10^3 to 10^1 CFU/ml despite all other experimental parameters remaining the same. This was not observed for any of the *E. coli* PAM mutants expressing a single *hsdMS* gene, including Ec_Se662I and Ec_Se662II, which maintained high-level methylation (89.65% to 99.90%) of motifs within the genome (12) (Table S3). Illumina sequencing of the Ec_Se662I-II genome confirmed integration of both *hsdMS* genes at the expected chromosomal sites but loss of approximately half of the coding sequence of both *hsdS* genes for the majority of the population sequenced. This instability was hypothesized to be due to the burden of excessive DNA methylation (10,930 sites of heterologous expression of two BPH0662 *S. epidermidis* type I RM systems in addition to 38,592 sites of endogenous *E. coli dam* methylation) that may interfere with normal cellular function, rendering expression toxic in *E. coli*. The same likely accounts for the poor electroporation efficiency seen in experiments using PAM for NIH4008 (100-fold lower than that observed for isolates with only a single type I RM system) as reported previously by Costa et al. (11). NIH4008 possesses the same type I RM systems as BPH0662, without the truncation of the orphan *hsdS* gene (Fig. 3). Furthermore, although stable chromosomal integration of three *S. aureus hsdMS* systems in *E. coli* DC10B (IM93B) was described previously by Monk et al., decreased efficiency of methylation was observed, with only 10,135 of a total of 14,602 TRM sites demonstrating detectable methylation (9).

Collectively, our current and previous (9, 12) data suggest that DC10B *E. coli* is unlikely to consistently maintain heterologous expression of staphylococcal type I RM systems in the setting of high-frequency methylation (>10,000 sites). This limitation should not impact plasmid electroporation for mutant creation by allelic exchange, which theoretically requires only a single transformant. However, should high-efficiency electroporation be sought (e.g., for direct transposon mutant library selection), then suitable strains can be predicted using genomic data to identify restriction-deficient isolates, such as our newly described reference isolate BPH0736, representing a clinically significant ST2 isolate. Clinical metadata, genome characteristics, CRISPR spacers (when present), *in silico* resistome, an Vitek 2 antibiogram representing clinically relevant antibiotics, and common plasmid selection markers for the seven new reference isolates and BPH0662 are shown in Table S3. Metadata and sequencing accession numbers for mutant isolates are listed in Table S5.

Phage transduction of plasmid is subject to type I restriction. Phage transduction is an alternative method for the genetic manipulation of *S. epidermidis*. In particular, *S. aureus* ST395 lineage-specific Φ187 shares wall teichoic acid (WTA) receptors with *S. epidermidis* (22, 23). Depending on the incidental packaging of plasmid introduced into a restriction-deficient intermediary host, *S. aureus* PS187 Δ*hsdR* Δ*sauPSI*, with Φ187 phage machinery (24), the method can be used to transduce a number of CoNS strains but is not universally applicable to all *S. epidermidis* isolates (23). The observed ability of ST395 *S. aureus* to exchange DNA with some CoNS strains led

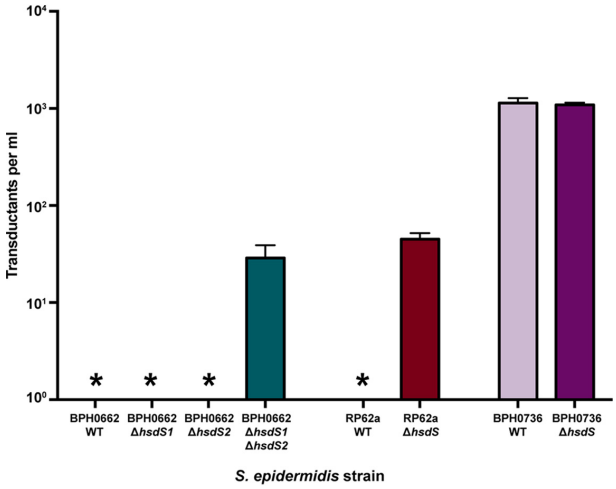

FIG 5 *S. epidermidis* phage transduction is subject to type I restriction. Biological triplicate data were determined for phage transduction of Φ187-pRAB11 lysate transduced into *S. epidermidis* wild-type (WT) and Δ*hsdS* mutant strains. Error bars represent means ± standard deviations of results from three independent experiments. *, no transductants.

Winstel et al. to conclude that overlap of the DNA methylation of ST395 *S. aureus* and that of CoNS strains that share the same WTA receptors may exist (22, 23). Results of phage Φ187 transduction experiments performed using our WT isolates and Δ*hsdS* mutants for BPH0662, RP62a, and BPH0736 representing the transfer of pRAB11 are shown in Fig. 5. These experiments demonstrated that even if successfully transduced into a *S. epidermidis* isolate, plasmids are still subject to degradation by type I RM systems if they bear a recognized TRM. However, in BPH0736 (absent type I restriction) or in mutant strains in which systems have been rendered inactive, transduced plasmid remains viable. The methylome for ST395 *S. aureus* has not been characterized; however, the draft genome sequence for PS187 (GCA_000452885.1) indicates that the two type I RM systems in this isolate are identical to those in *S. aureus* isolate JS395 (ST1093, belonging to CC395 [25]). We predicted the methylome of the isolate to include G**A**GN$_6$TCG (same as AUS0325-MS2) and another unknown TRM (Fig. 2) (Table 2). The results of our experiments and analyses of the diversity of *S. epidermidis* type I RM systems suggest that successful phage transduction of some *S. epidermidis* isolates with Φ187 is more likely related to the absence of a functional system than to the presence of a methylome shared with ST395 *S. aureus*. This is further supported by data from experiments performed by Winstel et al. (23) in which Φ187 was found to be able to transduce pTX15 (26) only into RP62a and not into pKOR1 (27). On the basis of our characterized RP62a TRM, we determined that pTX15 possesses no RP62a motifs whereas pRAB11 and pKOR1 each bear four motifs, explaining why neither plasmid is transducible into RP62a-WT.

A temperature-sensitive plasmid, pIMAY, is frequently used for allelic exchange in staphylococci due to the presence of inducible *secY* antisense counterselection and the lower likelihood of unintended mutations (that occurs with pKOR1) as integrants are selected at 37°C instead of 43°C (28). However, we found that Φ187 was not capable of transducing pIMAY into any of the tested strains, including the Δ*hsdS* mutants and naturally restriction-deficient BPH0736. We hypothesized this was due to the low copy number of pIMAY in staphylococci, resulting in low levels of incidental packaging of the plasmid within Φ187, compared to high-copy-number plasmid pRAB11. Other limitations of Φ187 transduction include a recommendation to use plasmids of <10 kb (24); however, that should not have impacted pIMAY (5.7 kb), which is smaller than pRAB11

(6.4 kb). Although a simplified harvesting and infection protocol was used compared to that described by Winstel et al. (24), we achieved an efficiency of 10^4 transductants per ml with pRAB11, equivalent to their anticipated results of 10^1 to 10^4 (24), in restriction-deficient *S. epidermidis* strain BPH0736. Of note, the levels of efficiency of both Δ*hsdS* mutants, BPH0662 Δ*hsdSI* Δ*hsdSII* and RP62a Δ*hsdS*, were 2 logs lower than those measured for BPH0736 (Fig. 5), further supporting the theory that strain-dependent factors beyond the barriers posed by type I RM systems and WTA are present in these backgrounds.

Conclusions. Our results demonstrate marked differences between the type I RM systems in *S. aureus* and *S. epidermidis*, which had hitherto been assumed to share the same characteristics (11). These differences are predominantly attributable to the arrangement and genome location of the *S. epidermidis* type I system as a complete three-gene operon, which we hypothesize to be mobilized by *ccr*. Localization of the operon in a highly plastic region of the chromosome increases the likelihood of horizontal transfer of these complete systems between *S. epidermidis* strains as well as to other staphylococci. This results in a lack of lineage specificity and a higher probability of spontaneous interruption of component genes. This is in contrast to *S. aureus*, where the type I systems are typically arranged as one *hsdR* and two *hsdMS* genes located apart from one another in stable regions of the chromosome. The evolutionary impact of these differences in the type I RM systems of *S. epidermidis* and *S. aureus* are unknown and warrant future research. The diversity of *S. epidermidis* type I RM systems that do not strictly adhere to ST/CC groupings indicates that genetic manipulation of *S. epidermidis* requires tailoring for each isolate of interest. Attempting electroporation without genomic analysis of the methylome could be successful, as our analyses found that 38% of *S. epidermidis* strains did not possess a type I RM system, and not all systems pose an intractable barrier (e.g., RP62a). However, some isolates such as internationally disseminated, almost pan-drug-resistant clone BPH0662 have complex and absolute type I restriction barriers.

We have demonstrated that PAM using a DC10B *E. coli* host is a simple and effective means to bypass the type I RM barrier in *S. epidermidis*, with plasmid transfer efficiency equivalent to that seen in the complete absence of type I RM systems. The decreasing cost and ready availability of whole-genome sequencing has made the sequencing of isolates planned for mutagenesis and their mutant derivatives commonplace and a practice that is recommended to ensure the absence of acquired secondary mutations (29). If the genome sequence of an isolate is known, then its methylome and ability to be transformed can be predicted as follows. (i) Does the isolate possess an intact type I RM system? If not, type I methylation would not be expressed and the isolate should be inherently transformable. (ii) Each complete type I RM system within a genome should be functional. For an HsdS protein with known TRMs, the presence of the TRMs on a vector would likely prevent electroporation. (iii) Orphaned, complete *hsdS* genes may be expressed in the absence of an adjacent *hsdM* if the associated *hsdM* allele is present elsewhere in the genome. In view of the guidelines presented above, when designing an *E. coli* PAM host, to ensure complete recapitulation of the endogenous type I methylome, we recommend including all complete *hsdMS* genes and any complete orphan *hsdS* genes from the *S. epidermidis* strain to be manipulated.

The 247 genomes that we analyzed are by no means an exhaustive representation of all *S. epidermidis*, and additional examples of type I RM systems will undoubtedly be catalogued as further sequencing of this organism is performed. However, this genomic sampling and our functional data were sufficient to draw the conclusions presented above. In view of the identified complexities associated with the genetic manipulation of *S. epidermidis*, the BPH0736 reference isolate should prove particularly useful. A clinical ST2 isolate that is representative of international circulating clones (5), BPH0736 is naturally type I restriction deficient due to the spontaneous interruption of *hsdR*, rendering it highly amenable to both electroporation and phage transduction and making it an ideal strain for future molecular studies.

MATERIALS AND METHODS

Media and reagents. Bacterial strains, plasmids, and oligonucleotides used in this study are listed in Table S6 in the supplemental material. *S. epidermidis* were routinely cultured at 37°C in brain heart infusion (BHI) broth (Difco). See Text S1 in the supplemental material for detailed descriptions of culture media, antibiotics, and enzymes.

Genome sequencing and analysis. The genome sequencing and analysis procedures are described in Text S1.

Electroporation. Early (8-h)-stationary-phase cultures of *S. epidermidis* grown in 10 ml of B media (BM) were added to 90 ml of fresh, prewarmed BM. Cultures were reincubated to an optical density at 600 nm (OD_{600}) of between 0.8 and 0.9 and chilled in an ice slurry for 10 min. Cells were harvested at 3,900 × *g* for 5 min at 4°C in a swinging bucket rotor, and the cell pellet was resuspended in 100 ml of autoclaved, ice-cold water. Centrifugation was repeated, and the pellet was resuspended in 50 ml of autoclaved ice-cold water. Cells were centrifuged and successively resuspended in 20 ml, 10 ml, and 250 μl of autoclaved ice-cold 10% (wt/vol) glycerol. Equal aliquots (50 μl) were frozen at −80°C. Prior to electroporation, cells were thawed on ice for 5 min and then at room temperature for 5 min. Following centrifugation at 5,000 × *g* for 1 min, cells were resuspended in 50 μl of 10% glycerol–500 mM sucrose (filter sterilized). Pellet paint (Novagen) precipitated plasmid DNA was added to the cells, and then the cells were transferred into a 1-mm-path length electroporation cuvette (Bio-Rad) and pulsed at 21 kV/cm, 100 Ω, and 25 μF at room temperature. Routinely, 5 μg of plasmid DNA was used, with concentrations determined by fluorometric assay (Qubit 2.0; Life Technologies). Cells were incubated in 1 ml of BHI broth supplemented with 500 mM sucrose (filter sterilized) at 28°C for 2 h prior to plating on BHI agar (BHIA) containing chloramphenicol (10 μg/ml).

Construction of Ec_Se736I and Ec_SeRP62aI *E. coli* hosts. *E. coli* mutants expressing the relevant *S. epidermidis* type I RM systems in a DC10B background were created as previously described (8, 9, 12) using the primers listed in Table S6. Details of the methodology are provided in Text S1.

Construction of *S. epidermidis* Δ*hsdS* mutants. The pIMAY(Δ*hsdS*) vectors were constructed using amplification by overlap extension PCR (30) with the A/B/C/D primer sets specified for each strain in Table S6, cloning into the pIMAY vector backbone, and subsequent cloning of the insertion into the vector. Mutant selection and screening were conducted as previously described (5). Details of the methodology are provided in Text S1.

Harvesting Φ187 plus pRAB11/pIMAY lysate from *S. aureus* PS187 Δ*hsdR* Δ*sauPSI*. Φ187 containing pRAB11/pIMAY was harvested from *S. aureus* PS187 Δ*hsdR* Δ*sauPSI* using a protocol adapted from Winstel (24). See Text S1 for detailed methodology.

Φ187 plus pRAB11/pIMAY transduction of *S. epidermidis*. A phage transduction protocol was adapted a method described previously by Foster et al. (31). Details of the methodology are provided in Text S1.

Data accessibility. Isolate BPH0662 has been deposited with the NCTC (NCTC accession no. 14219). The data sets supporting the results of this article are available from NCBI (BioProject PRJNA532483) and ENA (BioProject PRJEB35032) (sequencing and closed genome assemblies) and Figshare (https://melbourne.figshare.com/articles/Sa_HsdS_48_fasta/7986956 [*S. aureus* HsdS]; https://melbourne.figshare.com/articles/Se_HsdS_31_fasta/7986911 [*S. epidermidis* HsdS]; https://melbourne.figshare.com/articles/Se_HsdM_6_fasta/7986827 [*S. epidermidis* HsdM]; https://melbourne.figshare.com/articles/Se_HsdR_5_fasta/7986893 [*S. epidermidis* HsdR]).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.02451-19.

TEXT S1, DOCX file, 0.04 MB.
FIG S1, PDF file, 1.6 MB.
FIG S2, PDF file, 0.9 MB.
FIG S3, PDF file, 0.6 MB.
TABLE S1, XLSX file, 0.1 MB.
TABLE S2, XLSX file, 0.04 MB.
TABLE S3, XLSX file, 0.03 MB.
TABLE S4, DOCX file, 0.02 MB.
TABLE S5, XLSX file, 0.02 MB.
TABLE S6, DOCX file, 0.04 MB.

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The project was conceived by J.Y.H.L., T.P.S., B.P.H., and I.R.M. The experimental work was performed by J.Y.H.L. and I.R.M., with PacBio sequencing performed by G.P.C. and

S.J.P. Bioinformatic analysis was performed by J.Y.H.L. with assistance from T.S., R.G., and A.G.D.S. J.Y.H.L., T.J.F., B.P.H., T.P.S., and I.R.M. drafted the manuscript; all of us reviewed and contributed to the final manuscript.

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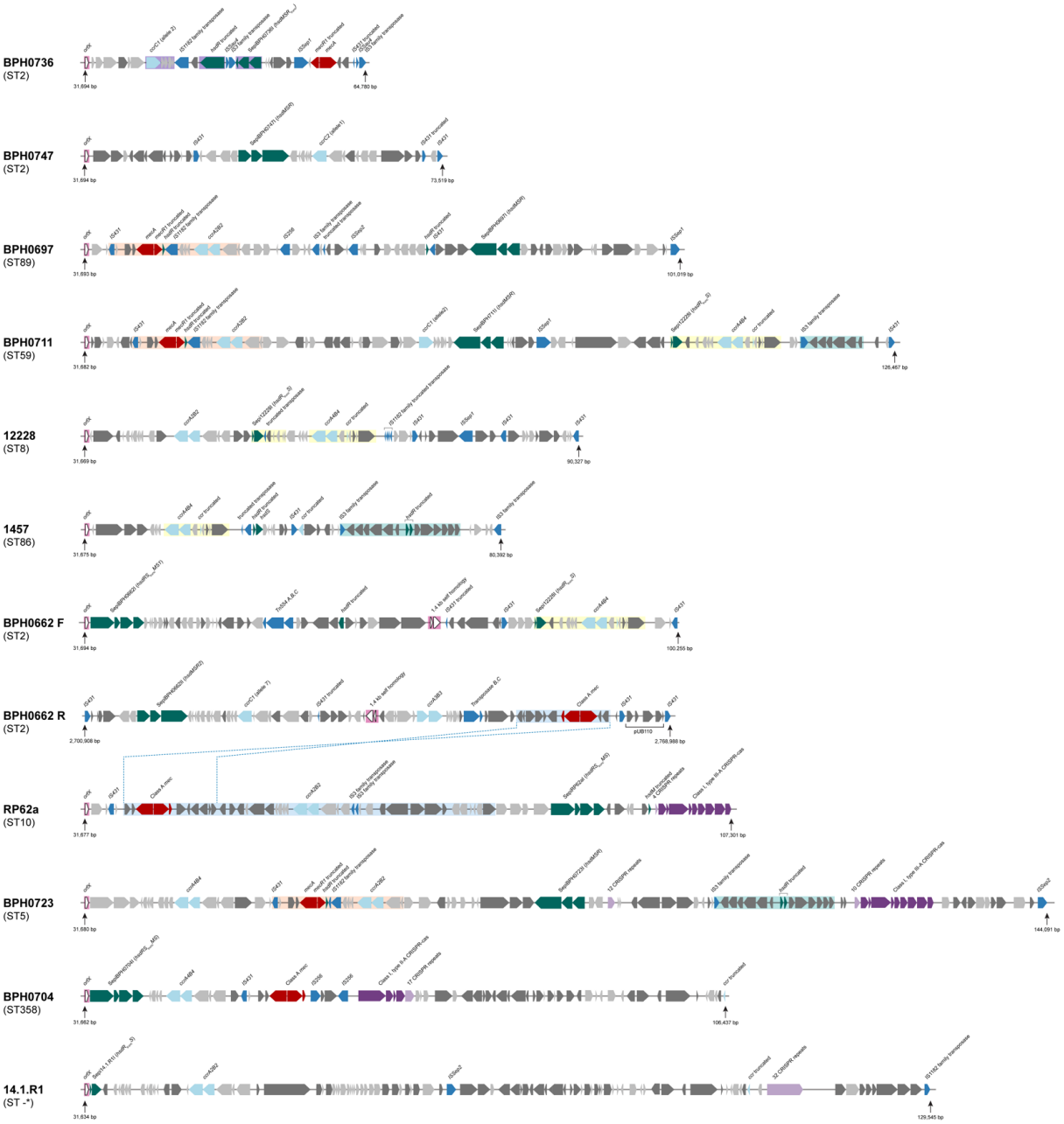
A. *Staphylococcus epidermidis* complete genomes type I RM systems



B. *Staphylococcus aureus* imported type I RM systems

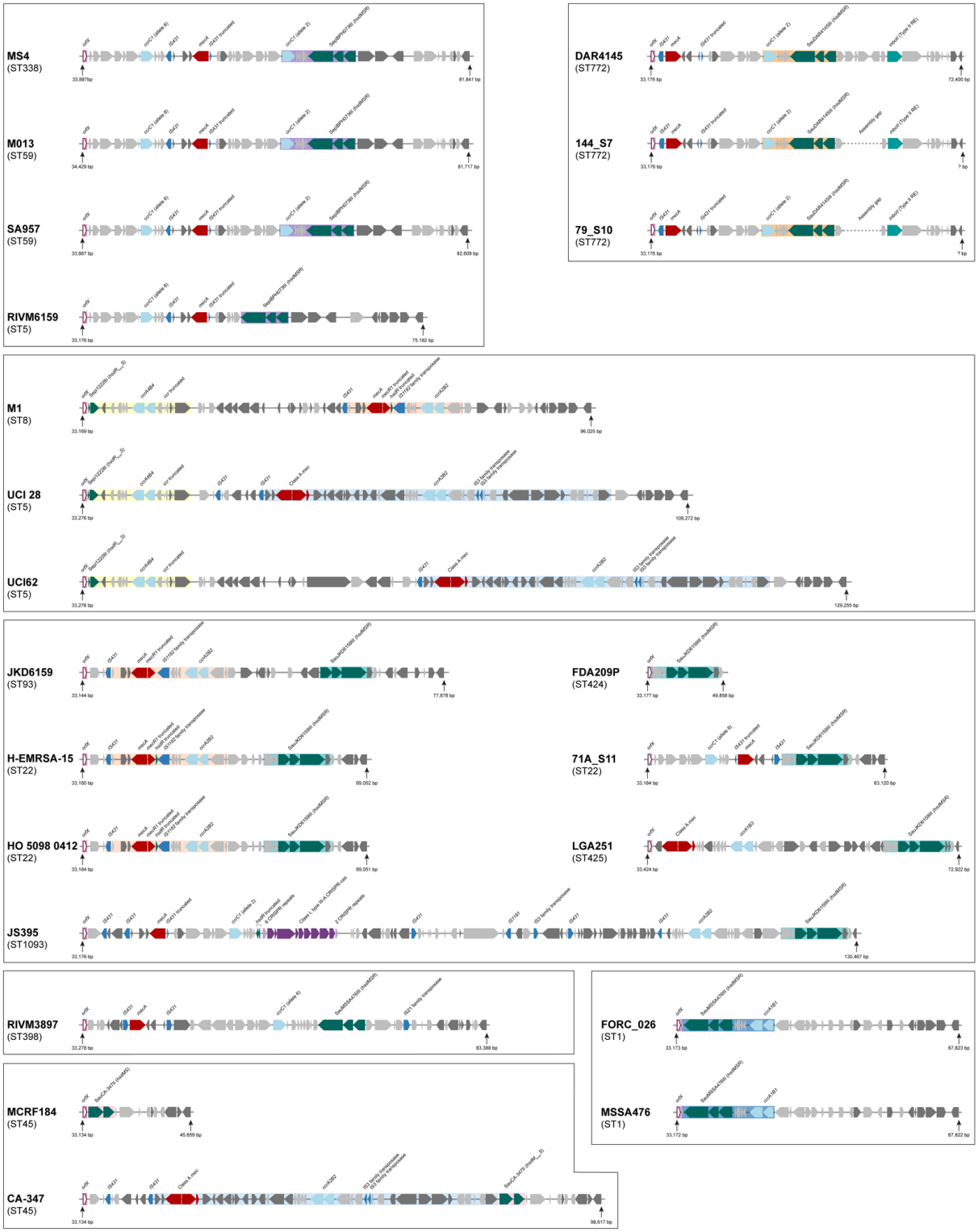


Figure S1. A hypothesised role for cassette chromosome recombinase (*ccr*) in the mobilisation of *S. epidermidis* and *S. aureus* type I restriction modification systems. A. Complete *S. epidermidis* genomes with type I RM systems. **B.** *S. aureus* genomes with imported type I RM systems. Genomes are orientated forwards starting at *dnaA*. #NCBI uploaded genome does not start at *dnaA*. *SEI not classifiable by existing MLST scheme.

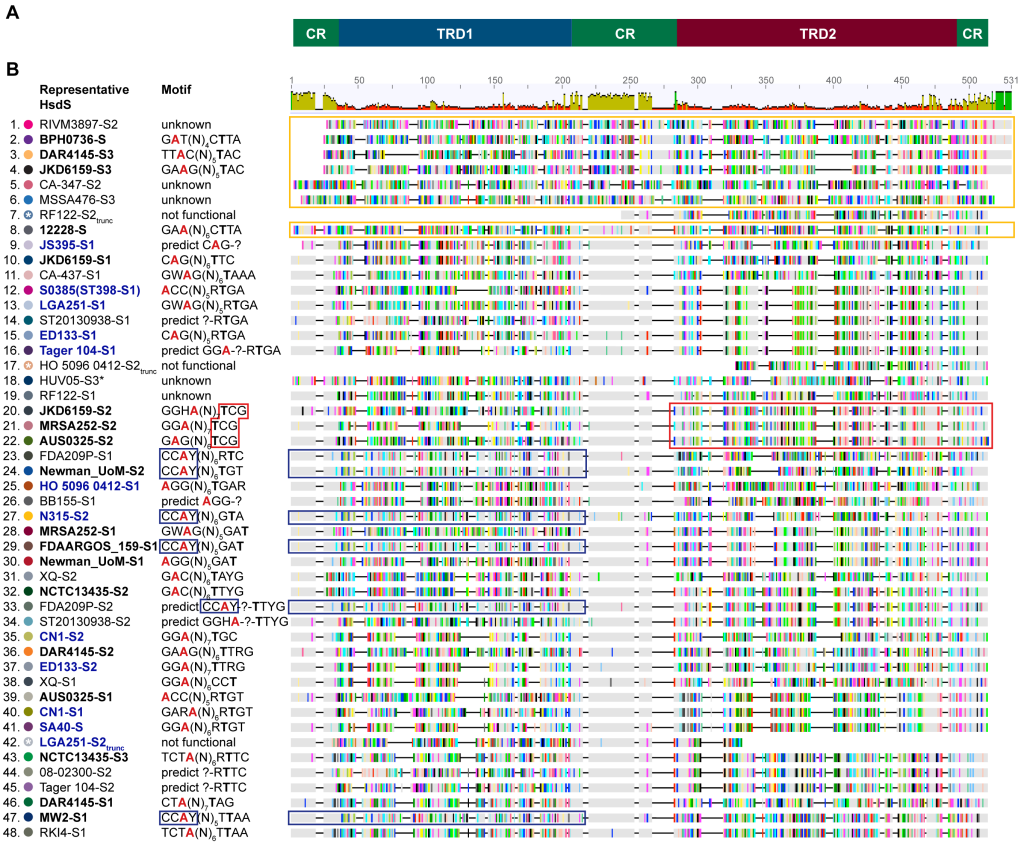

A
CR
TRD1
CR
TRD2
CR
B
Representative HsdS
Motif
1 50 100 150 200 250 300 350 400 450 500 531
1. RIVM3897-S2 unknown
2. BPH0736-S GAT(N)5CTTA
3. DAR4145-S3 TTAC(N)6TAC
4. JKD6159-S3 GAAG(N)5TAC
5. CA-347-S2 unknown
6. MSSA476-S3 unknown
7. RF122-S2trunc not functional
8. 12228-S GAA(N)5CTTA
9. JS395-S1 predict CAG-?
10. JKD6159-S1 CAG(N)5TTC
11. CA-437-S1 GWAG(N)5TAAA
12. S0385(ST398-S1) ACC(N)5RTGA
13. LGA251-S1 GWAG(N)5RTGA
14. ST20130938-S1 predict ?-RTGA
15. ED133-S1 CAG(N)5RTGA
16. Tager 104-S1 predict GGA-?-RTGA
17. HO 5096 0412-S2trunc not functional
18. HUV05-S3* unknown
19. RF122-S1 unknown
20. JKD6159-S2 GGHA(N)7TCG
21. MRSA252-S2 GGA(N)7TCG
22. AUS0325-S2 GAG(N)7TCG
23. FDA209P-S1 CCAY(N)5RTC
24. Newman_UoM-S2 CCAY(N)5TGT
25. HO 5096 0412-S1 AGG(N)5TGAR
26. BB155-S1 predict AGG-?
27. N315-S2 CCAY(N)5GTA
28. MRSA252-S1 GWAG(N)5GAT
29. FDAARGOS_159-S1 CCAY(N)5GAT
30. Newman_UoM-S1 AGG(N)5GAT
31. XQ-S2 GAC(N)5TAYG
32. NCTC13435-S2 GAC(N)5TTYG
33. FDA209P-S2 predict CCAY-?-TTYG
34. ST20130938-S2 predict GGHA-?-TTYG
35. CN1-S2 GGA(N)7TGC
36. DAR4145-S2 GAAG(N)5TTRG
37. ED133-S2 GGA(N)7TTRG
38. XQ-S1 GGA(N)5CCT
39. AUS0325-S1 ACC(N)5RTGT
40. CN1-S1 GARA(N)5RTGT
41. SA40-S GGA(N)5RTGT
42. LGA251-S2trunc not functional
43. NCTC13435-S3 TCTA(N)5RTTC
44. 08-02300-S2 predict ?-RTTC
45. Tager 104-S2 predict ?-RTTC
46. DAR4145-S1 CTA(N)7TAG
47. MW2-S1 CCAY(N)5TTAA
48. RKI4-S1 TCTA(N)5TTAA

Figure S2. Alignment of *S. aureus* HsdS variants. A. Structure of an HsdS allele with conserved regions (CRs) flanking two variable regions known as target recognition domains (TRD1 & TRD2). **B.** Each TRD typically specifies three to four defined base pairs including a methylated adenine residue (red **A**; **T** = complementary partner to methylated adenine residue); with a four to seven base pair non-specific spacer (N) between the two defined halves, collectively these TRDs determine the full target recognition motif (TRM) specified by an HsdS variant. HsdS names in bold black font have motifs determined by PacBio sequencing of the isolate after which the representative HsdS was named. HsdS names in bold blue font have motifs determined by DNA cleavage with purified restriction enzyme. Alignments of the identified variants of *S. aureus* HsdS are shown adjacent to their TRMs, each formed by a different TRD pairing. Scale above alignments indicates the position in the consensus alignment with mean pairwise identity at each site graphed (green = 100% identity; khaki = 30-100%; red <30%). Blue (TRD1) and red (TRD2) outlines highlight examples of TRDs that recur within the alignments and the TRM base pairs they define. Yellow boxes highlight alignments of HsdS imported into *S. aureus* on Staphylococcal cassette chromosome elements.

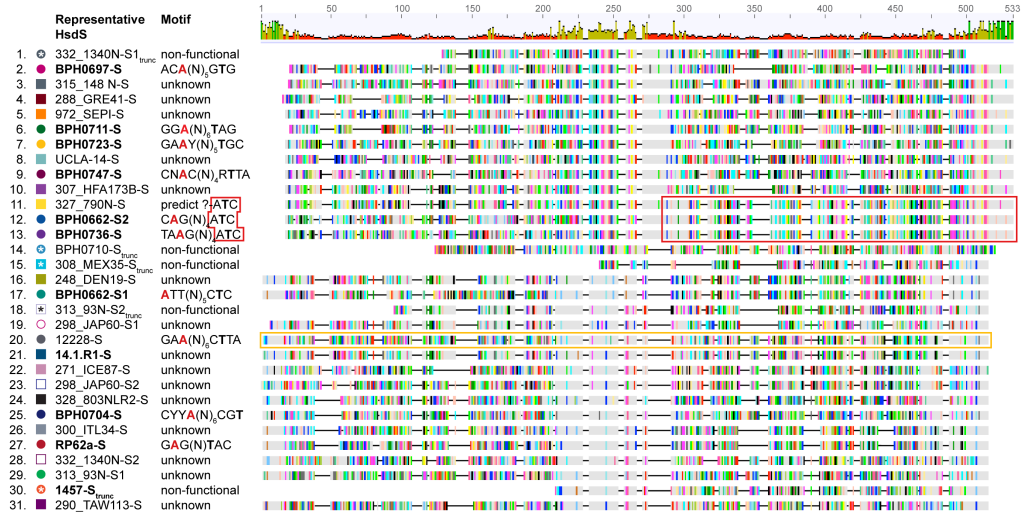


Figure S3. Alignment of *S. epidermidis* HsdS variants. HsdS names in bold black font have motifs determined by PacBio sequencing of the isolate after which the representative HsdS was named. Target recognition motifs (TRMs) (when known) and amino acid alignments are shown adjacent. Scale above alignments indicates the position in the consensus alignment with mean pairwise identity at each site graphed (green = 100% identity; khaki = 30-100%; red <30%). Red outline highlights an example of a target recognition domains that recurs within the alignments and the TRM base pairs they define. Yellow box highlights the 12228-S alignment, this HsdS variant was found to be present in both *S. aureus* and *S. epidermidis* and shared conserved regions with the *S. aureus* HsdS variants located within stable chromosomal islands (Figure S2).

Table S1. Metadata associated with pre-existing *S. aureus* and *S. epidermidis* sequencing. **A.** *S. aureus* reference genomes; **B.** *S. epidermidis* reference genomes; **C.** *S. epidermidis* SRA isolate metadata; **D.** Lee *et al*., 2018 isolate metadata; **E.** Costa *et al*., 2017 isolate metadata.

Provided in a separate excel spreadsheet.

Table S2. *S. aureus* and *S. epidermidis* type I restriction modification systems subunit protein accession numbers and references. A. *S. aureus* HsdS; **B.** *S. aureus* HsdR and HsdM; **C.** *S. epidermidis* HsdS; **D.** *S. epidermidis* HsdR and HsdM.

Provided in a separate excel spreadsheet.

Table S3. *S. epidermidis* reference isolates metadata. A. Clinical metadata; **B.** Closed genome statistics; **C.** PacBio methylation statistics; **D.** CRISPR; **E.** Vitek 2 susceptibilities; **F.** Resistome; **G.** Susceptibilities to commonly used plasmid selection markers.

Provided in a separate excel spreadsheet.

Table S4. Determining the HsdM variant that interacts with 12228 HsdS.

1. Three type I systems were identified in NIH4008 (1) without assignment of the TRM to their associated *hsdS*, for which only draft genome sequencing was available (2).
2. Expression of 12228-S by this isolate indicated that at least one of the two HsdM present were capable of interacting with 12228-S.
3. The same three type I RM systems are present in closed reference genome BPH0662 (3), for which the TRM of the two complete type I RM systems were assigned through heterologous expression in an *E. coli* host (3).
4. The 12228-S is non-functional in BPH0662 due to a SNP (64ΔA) resulting in truncation after the first 21 amino acids.
5. Our analysis of the BPH0711 methylome indicated expression of only one TRM despite 12228-S also being present, since BPH0711-M has 98.0% pairwise identity with BPH0622-M2, and was not able to interact with 12228-S, BPH0662-M1 was determined to interact with 12228-S.

Table S5. *S. epidermidis* Δ*hsdS* and *E. coli* plasmid artificial modification mutant sequencing accession data. A. PacBio sequencing; **B.** PacBio methylation; **C.** Illumina sequencing.

Provided in a separate excel spreadsheet.

Table S6. Strains, plasmids and oligonucleotides used in this study.

Bacterial strain, plasmid or oligonucleotide	Description	Reference or Source
***E. coli* strains**		
DC10B	DH10B with Δ*dcm* mutation	(4)
Ec_Se662I (previously DC10B-MS1)	DC10B with BPH0662 *hsdMS1* integrated between *ybbD* and *ylbG*. PB	(3)
Ec_Se662II (previously DC10B-MS2)	DC10B with BPH0662 *hsdMS2* integrated between *essQ* and *cspB.* PB	(3)
Ec_Se662I-II	DC10B with BPH0662 *hsdMS1* integrated between *ybbD* and *ylbG*, BPH0662 *hsdMS2* integrated between *essQ* and *cspB*	(5)
Ec_Se736I	DC10B with BPH0736 *hsdMS* integrated between *essQ* and *cspB.* PB	This study
Ec_SeRP62aI	DC10B with RP62a *hsdMS* integrated between *gidB* and *atpI*	This study
S. aureus strains		
PS187Δ*hsdR*Δ*sauPSI*	ST395 *S. aureus* deficient in type I and IV RM systems	(6)
***S. epidermidis* strains**		
ATCC 12228	Non-clinical reference strain; non-functional type I RM system; ST8	(7)
BPH0662	Clinical reference strain, isolated from CSF; two functional type I RM systems ST2; PB $Cm10^S$, $Tet5^S$, $Ery30^R$, $Kan50^R$	(3)
BPH0662Δ*hsdSI*	Mutant of BPH0662 with *hsdS1*deletion	This study
BPH0662Δ*hsdSII*	Mutant of BPH0662 with *hsdS2* deletion	This study
BPH0662Δ*hsdSI*Δ*hsdSII*	Mutant of BPH0662 with both *hsdS1* and *hsdS2* deleted	This study
BPH0676	Clinical strain, isolated from blood; no type I RM systems; ST2	(5)
BPH0697	Clinical strain, isolated from blood; one functional type I RM system; ST89; PB $Cm10^R$, $Tet5^S$, $Ery30^S$, $Kan50^R$	This study
BPH0704	Clinical strain, isolated from blood; one functional type I RM system; ST358; PB $Cm10^S$, $Tet5^S$, $Ery30^R$, $Kan50^R$	This study
BPH0710	Clinical strain, isolated from blood; non-functional type I RM system; ST2	This study
BPH0711	Clinical strain, isolated from blood; one functional type I RM system; ST59. PB $Cm10^S$, $Tet5^R$, $Ery30^S$, $Kan50^R$	This study
BPH0723	Clinical strain, isolated from blood; one functional type I RM system; ST5. PB $Cm10^S$, $Tet5^S$, $Ery30^R$, $Kan50^R$	This study
BPH0736	Clinical strain, isolated from blood; non-functional type I RM system; ST2. PB $Cm10^S$, $Tet5^S$, $Ery30^R$, $Kan50^S$	This study
BPH0736Δ*hsdS*	Mutant of BPH0736 with *hsdS* deletion	This study
BPH0747	Clinical strain, isolated from blood; one functional type I RM system; ST2. PB $Cm10^R$, $Tet5^S$, $Ery30^S$, $Kan50^R$	This study
RP62a_UoM	Clinical reference strain, isolated from blood; one functional type I RM system; ST10. PB $Cm10^S$, $Tet5^S$, $Ery30^R$, $Kan50^R$	This study
RP62aΔ*hsdS*	Mutant of RP62a with *hsdS* deletion	This study

Plasmids		
pKD4	Plasmid for amplification of FRT-*kan*-FRT; Amp^R, Kan^R	(8)
pKD46	*E. coli* temperature sensitive plasmid containing λ red recombinase genes under the control of an arabinose-inducible promoter; Amp^R	(8)
pCP20	*E. coli* temperature sensitive plasmid expressing *flp* enzyme for *flp*-catalyzed excision of *kan* marker; Amp^R, Cm^R	(9)
pIMAY	Temperature sensitive, low copy number allelic exchange plasmid for staphylococci; Cm^R	(4)
pIMAY(Δ662*hsdSI*)	pIMAY with BPH0662Δ*hsdSI* construct in MCS	This study
pIMAY(Δ662*hsdSII*)	pIMAY with BPH0662Δ*hsdSII* construct in MCS	This study
pIMAY(Δ736*hsdS*)	pIMAY with BPH0736Δ*hsdS* construct in MCS	This study
pIMAY(ΔRP62a*hsdS*)	pIMAY with RP62aΔ*hsdS* construct in MCS	This study
pRAB11	Non-temperature sensitive, high copy number staphylococcal vector; Cm^R	(10)
Phage		
Φ187	*S. aureus* ST395 lineage specific phage	(11)
Cloning *hsdMS* genes		
Ec_Se736I		
IM199 (*essQ* tail + Pxyl/tetO promoter-736MS F)	CCCAAACTGCACCCAAGAGTCAGAACACAGTTTTTCAAGA GTACAAAGGGGTAAACTAAAATAAATATTGACACTCTATC ATTG	(3)
736MS-F (Pxyl/tetO promoter- 736MS F)	AAACTAAAATAAATATTGACACTCTATCATTGATAGAGTA TAATTAAAATAAGGAGGAAATTAATGGCCACTATAGGTTT TGAAGAA	This study
IM201 (736MS R)	TTAAATTGAAAGTTCATCACTATTCACCTC	(3)
IM202 (pKD4 F)	GGTGAATAGTGATGAACTTTCAATTTAAGTGTAGGCTGGA GCTGCTTC	(3)
IM203 (pKD4 R + *cspB* tail)	GCTAACCATTGTGGTGAAGTGCAGGTTTGCTGCATGAATA GTTTTACGGTCCATATGAATATCCTCCTTAG	(3)
Ec_SeRP62aI		
IM365 (RP62aMS F; NcoI/PstI site)	ATATCCATGGCAACGACGGAAAAACAAAG	This study
IM266 (RP62aMS R; NcoI/PstI site)	ATATCTGCAGTTACACAAACATTTTCTGTAGTAATCC	This study
IM367 (*aptI* tail + Phelp promoter-RP62aMS F)	CAAAAAGCGGTCAAATTATACGGTGCGCCCCCGTGATTTC AAACAATAAGTACGGAGCTCCATTATGCTTTGGCAG	This study
IM360 (*SOE to RP62aMS* + pKD4 F)	*CTACAGAAAATGTTTGTGTAACTGCAG*ATATGTGTAGGCT GGAGCTGCTTC	This study
IM366 (pKD4 R + *gidB* tail)	ATAACGTGGCTTTTTTTGGTAAGCAGAAAATAAGTCATTA GTGAAAATATGTCCATATGAATATCCTCCTTAG	This study
Δ*hsdS*		
BPH0662-A1 (pIMAY MCS/KpnI site)	CCTCACTAAAGGGAACAAAAGCTGGGTACCAGTGATATTG AAATTGATATGTTAGGG	This study
BPH0662-B1	AAATCTTAATTCTGGAGTGTTTGTGTG	This study
BPH0662-C1 (*SOE to B*)	*CACACAAACACTCCAGAATTAAGATTT*TAGTTCTGATAAC AGCCTATTATGTC	This study
BPH0662-D1 (pIMAY MCS)	CGACTCACTATAGGGCGAATTGGAGCTCGTGATTCTAGCT TATGGTGCTCAT	This study
BPH0662-A2 (pIMAY MCS/KpnI site)	CCTCACTAAAGGGAACAAAAGCTGGGTACCGGAGAATTCT ATACGCCATCATC	This study
BPH0662-B2	TCCATTAAACTCCATAACCTAGTCC	This study

BPH0662-C2 (*SOE to B*)	*GGACTAGGTTATGGAGTTTAATGGA*AATAGTGATGAACTTTCAATTTAATGAAG	This study
BPH0662-D2 (pIMAY MCS)	CGACTCACTATAGGGCGAATTGGAGCTCGACAACTAAGGTAGGATTATTAAGCATTTG	This study
BPH0736-A (pIMAY MCS/KpnI site)	CCTCACTAAAGGGAACAAAAGCTGGGTACCACAGCAGGATTCGTATTAGCC	This study
BPH0736-B	AGCTACATCACCGAAAAAAATTGT	This study
BPH0736-C (*SOE to B*)	*ACAATTTTTTTCGGTGATGTAGCT*ACAAATAGTAGACAAAGAGTAAAACCAACA	This study
BPH0736-D (pIMAY MCS)	CGACTCACTATAGGGCGAATTGGAGCTCCGATTGTCCTTCATCATCATAGTC	This study
RP62a-A (pIMAY MCS/KpnI site)	CCTCACTAAAGGGAACAAAAGCTGGGTACCAGGAGAATTCTATACACCACAACAG	This study
RP62a-B	AAATCTTAATTCTGGAGTATTTGTGTG	This study
RP62a-C (*SOE to B*)	*CACACAAATACTCCAGAATTAAGATTT*TAATTCTGATAAAGCCCTATTATGTTAATTG	This study
RP62a-D (pIMAY MCS)	CGACTCACTATAGGGCGAATTGGAGCTCCGACGCTATCACAACCTTACTTAAC	This study
Recombineering		
IM179 (*essQ* out F)	CGGCCATTTATACAGGAAAAGCCTA	(3)
IM180 (*cspB* out R)	GTTACCTTCTCTATAGAGAGTGGTG	(3)
IM434 (*atpI* out F)	ACTTTCTTTAAGGCTTAGAGTCAAGC	This study
IM435 (*gidB* out R)	TTTAACGCCACGTTCACTCTTTTGC	This study
IM1 (pIMAY backbone F)	GGTACCCAGCTTTTGTTCCCTTTAGTGAGG	(5)
IM2 (pIMAY backbone R)	GAGCTCCAATTCGCCCTATAGTGAGTCG	(5)
IM3 (pIMAY MCS R)	AATACCTGTGACGGAAGATCACTTCG	(5)
IM4 (pIMAY MCS F)	TACATGTCAAGAATAAACTGCCAAAGC	(5)
BPH0662Δ*hsdSI* out F	CGAAACTTATTTCGAAGGTCAT	This study
BPH0662Δ*hsdSI* out R	GATAAACACACAGACATCCACTTG	This study
BPH0662Δ*hsdSII* out F	AGATGTATTAGGCCGAGTGTATGAG	This study
BPH0662Δ*hsdSII* out R	CCGTCCTTTCGATTTAACAAAC	This study
BPH0736Δ*hsdSI* out F	ATGGCCACTATAGGTTTTGAAG	This study
BPH0736Δ*hsdSI* out R	CACTACGATAGGCAAGCCATT	This study
RP62aΔ*hsdSI* out F	AATTGATATGTTAGGGGATGCTTATG	This study
RP62aΔ*hsdSI* out R	GGTTTTCAAGTGAATTTAAGGTGTG	This study

PB = PacBio sequenced; RM = restriction modification; ST = sequenced type; CSF = cerebrospinal fluid; $Cm10^R$ = chloramphenicol 10 µg/ml resistant; $Cm10^S$ = chloramphenicol 10 µg/ml susceptible; Tet5 = tetracycline 5 µg/ml; Ery30 = erythromycin µg/ml; Kan50 = kanamycin 50 µg/ml; Amp = ampicillin; MCS = multiple cloning site; F = forward; R = reverse; SOE = spliced overlap extension.

Supplementary Methods.

Media and reagents. Bacterial strains, plasmids and oligonucleotides used in this study are listed in Table S6. *S. epidermidis* were routinely cultured at 37°C in brain heart infusion broth (BHI)(Difco). For electroporation, *S. epidermidis* were grown in B media (BM) (1% Bacto™ Proteose Peptone No.3, 0.5% yeast extract, 0.5% NaCl, 0.1% K_2HPO_4, 0.1% glucose). *E. coli* was routinely cultured in L broth (LB) (1% tryptone, 0.5% yeast extract, 0.5% NaCl). Staphylococci infected with Φ187 were routinely cultured in LB. For growth on agar, BHI or LB were solidified with 1.5% agar, to yield BHIA and LBA, respectively. The following antibiotics were purchased from Sigma Aldrich and used at the specified concentrations: chloramphenicol (Cm) 10 μg/ml in *S. epidermidis*, 15 μg/ml in *E. coli*; ampicillin (Amp) 100 μg/ml in *E. coli*; kanamycin (Kan) 50 μg/ml in *E. coli.* Oligonucleotides were purchased from Integrated DNA Technologies. Isolation of genomic DNA, plasmid purification, gel extraction and colony PCR were performed as previously described (5). Restriction enzymes and Phusion DNA polymerase were purchased from New England Biolabs. Phire Hotstart DNA polymerase was purchased from Thermofisher.

Genome sequencing. Single molecule real-time (SMRT) sequencing was performed on a PacBio RS II instrument with subsequent analysis performed using SMRT Analysis v2.3.0.140936. The RS_HGAP_Assembly.3 algorithm was used for *de novo* assembly with trimmed Illumina reads used to correct homopolymer errors using Snippy v3.2 (12). Illumina reads were assembled using SPAdes v3.7.1 (13) and the resulting contigs were screened for small plasmids that would be lost during PacBio size selection. Final assemblies for each new reference genome were independently confirmed using Unicycler v0.3.0 (14). For methylome analysis, each newly closed genome was used as its own reference with the

RS_Modification_and Motif_Analysis.1 algorithm. Draft genomes were sequenced using on an Illumina Nextseq platform with Nextera XT libraries, as per manufacturer's instructions.

Genome analysis. All publicly available, *S. epidermidis* genome raw reads in NCBI SRA were downloaded (4th November 2016). Exclusion criteria were as follows: sequencing performed on a Roche 454 or Ion Torrent platform (per Genbank metadata); single end reads; sequencing depth <30x; mutagenesis isolate pairs; organism not *S. epidermidis* or sequencing of mixed bacterial species as determined by Kraken v0.10.15beta (15); >300 assembled contigs; raw reads not associated with publications and lacking metadata. When isolates were identified as exact genetic replicates, a single representative was used in analyses. Trimming, assembly, genome annotation and *in silico* MLST determination was performed as previously described (5). Isolate VCU036 with a previously unspecified ST (1), was independently reanalysed using a second method, MLST v1.8 (16). CRISPR type and direct repeat and spacer sequences were determined using CRISPRCasFinder (17). Maximum-likelihood core-SNP phylogeny of *S. epidermidis* isolates and hierarchical Bayesian Analysis of Population Structure cluster modelling of the resultant tree was determined as previously described (5), using the closed BPH0736 genome as a reference. All publicly available, closed *S. aureus* genomes available were downloaded (2nd February 2017). Mutant derivatives and repeat sequencing of serial isolates over the course of an infection were excluded from analyses. Sa_Newman_UoM (GCA_900092595.1) (18) was used as the reference genome to generate a maximum likelihood core-SNP phylogeny as described (5).

To determine the diversity of HsdS in *S. epidermidis* and *S. aureus*, each of the ten *S. epidermidis* (Table 3) and 32 *S. aureus* (Table S2) HsdS translations with characterised TRMs were used as a reference for individual tblastn queries against the collected assemblies using

BLAST v2.6.0 (19) with an e-value cutoff of $1e^{-6}$, and low complexity filtering disabled (-F F); the output of which was aligned with Clustal Omega v1.2.4 (20). To validate this approach, all *S. epidermidis* and *S. aureus* genomes were manually screened for the presence of type I RM systems or component genes using Geneious v7.1.8 (21) and type I systems were manually extracted as contiguous operons and full individual genes. The complete HsdS translations from all isolates were aligned with Clustal Omega, a shared amino acid identity of <90% was used to classify separate variants of HsdS. The diversity of HsdR and HsdM in *S. aureus* (Table S2B) and *S. epidermidis* (Table S2D) were determined using this same method. In the case of closed genomes, systems were numbered relative to location after *dnaA*.

Electroporation. Early stationary phase cultures (8 h) of *S. epidermidis* grown in 10 ml of BM were added to 90 ml of fresh, prewarmed BM. Cultures were reincubated to an OD_{600} between 0.8 - 0.9 and chilled in an ice slurry for 10 min. Cells were harvested at 3,900*xg* for 5 min at 4ºC in a swinging bucket rotor and the cell pellet resuspended in 100 ml of autoclaved, ice-cold water. Centrifugation was repeated, and the pellet resuspended in 50 ml of autoclaved ice-cold water. Cells were centrifuged and successively resuspended in 20 ml, 10 ml then 250 µl of autoclaved ice-cold 10% (weight/volume) glycerol. Equal aliquots (50 µl) were frozen at -80ºC. Prior to electroporation, cells were thawed on ice for 5 min, then at room temperature for 5 min. Following centrifugation at 5,000 *xg* for 1 min, cells were resuspended in 50 µl of 10% glycerol with 500 mM sucrose (filter sterilised). Pellet paint (Novagen) precipitated plasmid DNA was added to the cells, and transferred into a 1 mm electroporation cuvette (Bio-Rad) and pulsed at 21 kV/cm, 100 Ω, 25 µF at room temperature. Routinely, 5 µg of plasmid DNA was used, with concentration determined by fluorometric assay (Qubit 2.0; Life Technologies). Cells were incubated in 1 ml of BHI supplemented with 500 mM sucrose (filter sterilised) at 28ºC for 2 h prior to plating on BHIA containing Cm 10 µg/ml.

Construction of Ec_Se736I and Ec_SeRP62aI *E. coli* hosts. *E. coli* mutants expressing the relevant *S. epidermidis* type I RM systems in a DC10B background were created using methods previously described (3, 22). The *hsdMS* system from BPH0736 was amplified with primers 736MS-F (incorporating Pxyl/tetO promoter)/IM201. Plasmid pKD4 (8) was used as a template for the amplification of the Kan resistance marker flanked by flippase recognition target (FRT) sites with primers IM202/IM203 (product pKD4). The *hsdMS* and pKD4 products were gel extracted and joined by spliced overlap extension (SOE) PCR using primers IM199/203 (primer tails contained 50 bp homology for integration into the intergenic region between *essQ* and *cspB* in the DC10B chromosome). The *hsdMS*-pKD4 amplicon was pellet paint (Novagen) precipitated and electroporated into DC10B containing pKD46 (8), made competent as previously described (4). Transformants were selected for on L agar Cm10. Colony PCR with primers IM179/IM180 was used to screen for recombination at the integration site, positive clones were grown overnight at 43ºC to promote loss of pKD46. To excise the pKD4 product, strains were transformed with pCP20 (9) at 28ºC, single colony purified at 28ºC, then grown overnight at 43ºC to promote plasmid loss. To confirm excision of the Kan resistance marker and the loss of pCP20, cells were patch plated on L agar Kan and L agar Cm, respectively. The resulting Kan^S, Cm^S strain containing BPH0736 *hsdMS* (with Pxyl/tetO promoter promoter) was named Ec_Se736I.

The *E. coli* Ec_SeRP62aI mutant was constructed from *E. coli* DC10B using the following primer sets: IM365/266 for the amplification of RP62a *hsdMS* from *S. epidermidis* RP62a, which were then ligated into complementary digested (NcoI/PstI) plasmid pIMK2 (23); Primers IM367/IM266 were used to amplify *hsdMS* with Phelp promoter from pIMK2(*hsdMS*) with subsequent steps following the same protocol described above. Primers IM360/IM366 were used to for amplify the FRT-*kan*-FRT from pKD4 (product pKD4); IM366/IM367

(containing tails with 50 bp homology for integration into the intergenic region between *gidB* and *atpI* in the DC10B chromosome) for SOE PCR of *hsdMS*-pKD4. Primers IM434/IM435 were used to confirm recombination at integration site.

Construction of *S. epidermidis* Δ*hsdS* mutants. The pIMAY(Δ*hsdS*) vectors were constructed as follows: Δ*hsdS* inserts (designed to delete the complete *hsdS* gene) were amplified by overlap extension PCR (24) with the A/B/C/D primer sets specified for each strain in Table S6; cloning into the pIMAY vector backbone and subsequent cloning of the insert into the vector was conducted as previously described (5). Transformation of pIMAY(Δ*hsdS*) into the relevant *E. coli* host for PAM and the subsequent selection of transformants was as previously described (5). Integration and excision of pIMAY(Δ*hsdS*) was performed as previously described (4), with the exception of concurrent (rather than sequential) colony PCRs: for loss of replicating plasmid (with MCS primers IM3/IM4); and chromosomal integration of the plasmid upstream (relevant Δ*hsd* out forward/IM3) and downstream (IM4/relevant Δ*hsd* out reverse). Post 1 μg/ml anhydrotetracycline (ATc1, Sigma) counter selection, large colonies were patch-plated on BHIA plus ATc1, and BHIA containing Cm10, with successful mutants bearing a Cm^S phenotype and loss of the type I restriction barrier. Draft genomes of successfully created mutant strains in *E. coli/S. epidermidis* were sequenced and assembled as above to confirm the insertion/deletion of the *hsdMS/hsdS* at the expected site and determine if any unintended mutations were present. Variant calling between the mutants to their respective parental strains was performed using Snippy v3.2 (12). Mutants and their corresponding parental strains were subject to phenotypic testing to determine the barrier to genetic manipulation presented by each individual type I RM system.

Harvesting Φ187 + pRAB11/pIMAY lysate from *S. aureus* PS187Δ*hsdR*Δ*sauPSI*

To harvest Φ187 containing pRAB11/pIMAY, an overnight culture of *S. aureus* PS187Δ*hsdR*Δ*sauPSI* (6) containing pRAB11 was diluted to an OD_{600} 1:100 in 50 ml of prewarmed LB plus 10 mM $CaCl_2$/Cm10 and grown at 37°C for pRAB11 (28°C for pIMAY) with aeration at 200 rpm for 4 h (final OD_{600} 2.5). In 15 ml tubes, 300 µl aliquots of the 4 h *S. aureus* PS187Δ*hsdR*Δ*sauPSI* containing pRAB11/pIMAY culture were infected with 200 µl of 10^{-1} to 10^{-6} serial dilutions of Φ187 lysate. Tubes were incubated for 45 min at room temperature, then 10 ml of molten top agar LBA plus 10 mM $CaCl_2$/Cm10 (0.35% agar) was added to the infection reaction and the mixture poured over LBA plus 10 mM $CaCl_2$/Cm10 base agar (1.5% agar). Plates were incubated at 37°C overnight without inversion. The top agar layer of plates with cotton wool appearance (10^{-4} to 10^{-5} dilution) were transferred into 15 ml tubes with a sterile spreader and centrifuged at 15,000 *xg* for 10 min at 4°C. Supernatant was filter sterilised through a 0.22 µM filter and stored at 4°C. Phage titer was determined by plaque assay as previously described(6), using LBA rather than BM agar.

Φ187 + pRAB11/pIMAY transduction of *S. epidermidis.* A phage transduction protocol was adapted from Foster (25). A 25 ml overnight culture of the target *S. epidermidis* strain was harvested at 7,000 *xg* for 10 min, then resuspended in 1 ml L broth supplemented with 10 mM $CaCl_2$ (LBC). In 15 ml tubes, 500 µl of suspension was added to: 1.0 ml of LBC and 500 µl Φ187-plasmid lysate (10^{11} plaque forming units); and control of 500 µl of suspension plus 1.5 ml of LBC. Tubes were incubated in a 37°C water bath for 25 min, then at 37°C a further 15 min with aeration at 200 rpm. To each tube, 1 ml of ice cold 0.02 M sodium citrate was added, and the cells were harvested at 7,000 *xg* for 10 min at 4°C. The cell pellet was resuspended in 1 ml of ice cold 0.02 M sodium citrate and incubated on ice for 2 h. Aliquots (100 µl) were

spread plated on BHIA Cm10 containing 0.05% sodium citrate. Plates were incubated 37°C for 24h (pRAB11) or 28°C for 48h (pIMAY) and colony counts performed.

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CHAPTER 5. Discussion

5.1 Introduction

At the commencement of this work, the global dominance of ST2 *S. epidermidis* in the clinical environment had been established for close to a decade [46]. However, the existing MLST scheme lacked the sensitivity to offer any insights into epidemiology beyond this, while the absence of dedicated molecular tools coupled with the genetically intractable nature of *S. epidermidis*, hindered the study of molecular genetics, pathogenesis and treatment. Utilising new sequencing technologies with a functional genomics approach, the works presented here sought to provide new insights into the molecular epidemiology, resistance mechanisms, recommended treatment, and genetic manipulation of *S. epidermidis*, with a focus on clinical ST2 isolates.

5.2 Key findings

In Chapter 2, BPH0662 was introduced as the first complete ST2 reference genome. The largest *S. epidermidis* genome described to date, BPH0662 was demonstrated to have undergone chromosomal rearrangements and multiple horizontal gene transfer events resulting in the accumulation of a broad range of resistance determinants, conferring a phenotype resistant to all standard therapeutic options. Demonstrated to be representative of international ST2 isolates, the BPH0662 genome provides an ideal reference for future bioinformatic analysis of clinical isolates.

The international clonal expansion of three genetically distinct lineages of MDRSE (two ST2, one ST23) with a common dual D471E and I527M RpoB substitution, was presented in Chapter 3. Found to account for 86.6% of mutations occurring within the RRDR of RpoB, this dual D471E and I527M mutation was determined to be the most common cause of rifampicin resistance in *S. epidermidis* and demonstrated to occur almost exclusively (96.0%) within in the ST2 (63.9%) or ST23 (33.1%) lineages. Thereby,

identifying rifampicin resistance as a marker for these clones. By breaching lineage-specific DNA methylation RM barriers and performing site specific mutagenesis, the D471E plus I527M RpoB substitutions were shown to confer both rifampicin resistance and reduced susceptibility to vancomycin in isolates from divergent genetic backgrounds. This was the first successful report of allelic exchange in a ST2 *S. epidermidis* background.

Co-resistance to vancomycin potentially explains the fixation of the D471E and I527M RpoB substitutions in the three lineages and their success within the hospital environment. Once these mutations were present within the lineages, even if the selective pressure of rifampicin exposure were removed, the high prevalence of vancomycin use in clinical practice would ensure preservation of the mutations within the population, and subsequent clonal expansion of strains possessing these mutations. A theory supported by the competition assays performed in Chapter 3, in which the presence of as little as 2 μg/ml of vancomycin was demonstrated to shift the population away from WT in favour of the *rpoB*662 mutant. *S. epidermidis* RpoB sites D471 and I527 correspond with RpoB positions D516 and I572 in *E. coli* (Figures 1.4 and 3.1), which have been demonstrated to have a high frequency of positive epistasis, segregate in natural populations and are associated with compensatory mutations in unrelated alleles that confer resistance to other antibiotics [209]. These mutations are also hypothesised to favour the accumulation of additional mutations that abrogate the cost of a multidrug resistant phenotype ensuring their preservation even in the absence of antibiotic selective pressure [209]. The presence of the same RpoB mutations in not only different *S. epidermidis* genetic backgrounds but also at the equivalent RpoB sites in other species, suggests convergent evolution of a favourable phenotype with effects potentially extending beyond just antibiotic resistance.

The Australian data suggested that local factors within an institution were critical to the expansion of these MDRSE clones and their observed prevalence. This was highlighted by the markedly different prevalence in rifampicin resistance observed between Austin Health and Monash Health. Both institutions are tertiary centres located in Melbourne, Victoria, Australia. All three lineages were demonstrated to be endemic at both sites, and in the Australian isolates sampled, rifampicin resistance was exclusively conferred by the dual D471E and I527M mutations, 100% of which displayed a vancomycin heteroresistant phenotype. Despite being located only 24 km apart, the prevalence of rifampicin resistance increased from 10.9% to 31.4% over seven years at Austin Health, but remained unchanged at 10% at Monash Health. The site-specific factors contributing to local prevalence were not addressed by this study and warrant further investigation that may also offer insight into the mode of transmission.

Austin Health is the only Victorian centre that provides a liver transplant service, it is tempting to hypothesise that increased use of rifaximin (for treatment of hepatic encephalopathy associated with chronic liver disease [227]) may be contributory. A recent study demonstrated that the transient use of rifaximin (600 mg orally preoperatively) for surgical prophylaxis in elective colorectal surgery was associated with the emergence of rifampicin resistant CoNS in 43.2% (32/74) of patients two weeks post treatment, with reversion of resistance after three months [228]. The closely related chemical structures of rifampicin and rifaximin [160] is thought to induce cross resistance between agents [228].

The endemic nature of the three MDRSE lineages within hospitals internationally, suggests that studies attempting to distinguish clinically significant from contaminating *S. epidermidis* strains based on the presence of multiple genetic markers are unlikely to be successful. The pathogenesis of infection is not as simple as some lineages always

colonise, while other always cause infection. Instead, studies suggest that patients become colonised with hospital adapted lineages over the course of their admission [5,41,53], in addition to or replacing their existing isolates, and under favourable circumstances clinically significant disease can occur. Although hospital adapted clones are better equipped and more likely to cause disease, isolates from backgrounds other than ST2 or ST23 can also cause nosocomial infections as evidenced by the data in Chapter 3. While the detection of hospital strains may assist clinical decision making, the reality is that the molecular methods proposed to screen *S. epidermidis* isolates for the presence of invasive genetic markers [74,75,80] are costly and not readily available in a diagnostic setting. Conveniently, this project has identified rifampicin resistance alone as a reliable marker of the three MRSE lineages. Furthermore, the presence of rifampicin resistance in *S. epidermidis* flags probable glycopeptide heteroresistance, and therefore the likely need for intervention in the patient from whom the strain was isolated, such as device removal to achieve source control and/or a change in therapy from first-line agent vancomycin.

The work presented in Chapter 4 dispels the assumption that what is known about *S. aureus* can extrapolated and generally applied to *S. epidermidis*. Using PacBio sequencing, the complete genomes and methylomes of seven clinical *S. epidermidis* isolates were characterised to double the number of complete *S. epidermidis* genomes and expand upon the five isolates for which methylation data was available. This first systematic genomic analysis of the type I RM systems in *S. aureus* and *S. epidermidis* revealed marked differences in gene arrangement, chromosomal location and movement of the systems between the two species. These structural differences accounted for the lineage specificity seen in *S. aureus* type I RM systems and the previously unrecognised, extensive diversity of *S. epidermidis* type I RM systems, that contrary to current

assumptions, do not follow lineage specificity. These results indicated that genetic manipulation of *S. epidermidis* requires tailoring to the isolate of interest, potentially explaining the difficulty with attempts to transform the species thus far. In conjunction with genomic data, PAM was demonstrated as an effective approach to manipulate any given *S. epidermidis* isolate, able to overcome restriction barriers and achieve electroporation efficiencies equivalent to modification-deficient mutants. Additionally, BPH0736, a naturally restriction defective, clinically significant, multidrug-resistant ST2 isolate was identified as an ideal candidate for molecular studies. The work presented in Chapter 4 should prove invaluable in advancing molecular studies in *S. epidermidis*.

5.3 Limitations

Determining the true prevalence of rifampicin resistance in *S. epidermidis* is a challenge due to the lack of denominator data regarding the background number of susceptible isolates. While some sites maintain local data, there are no dedicated surveillance studies. The frequency with which *S. epidermidis* is encountered in diagnostic specimens and challenges associated with distinguishing clinically significant isolates from contaminants hinders the collection of robust data. The ubiquitous nature of the species and clinical perception that *S. epidermidis* is a dismissible contaminant, undoubtedly contributed to the pervasive yet unrecognised spread of the three MDRSE lineages.

The sampling of isolates in Chapter 3 was skewed towards Australia and Europe. It is possible that the dominance of the three MDRSE lineages may not be as pronounced in other geographic regions. Unlike most other sites, no ST2 isolates were sampled from the Mayo Clinic in Minnesota, USA. Furthermore, it was found to have the lowest prevalence of rifampicin resistant clinical *S. epidermidis*, only 3%, unchanging from 2012 to 2017. Despite this, ST23 isolates bearing the dual D471E and I527M mutations were found to persist at the site for 17 years. How representative this data is of other institutions

in the USA and beyond is uncertain. Limited representation of less prevalent genotypes was common to the studies in both Chapter 3 and 4. It is debatable whether wider sampling to include them would have altered outcomes as they are less likely to be of clinical relevance in terms of molecular epidemiology in Chapter 3, while the sampling in Chapter 4 was sufficient to demonstrate the diversity of *S. epidermidis* type I RM systems.

5.4 Future Directions

While the majority of the *S. epidermidis* isolates sampled in Chapter 3 were known to cause serious disease manifestations, the retrospective nature of the data collection meant the full clinical impact of these rifampicin resistant clones was uncertain, as was their hospital reservoir and mode of transmission. Prospective studies that include more geographically diverse isolate sampling from both clinical and environmental sources (including healthcare workers) with correlated treatment and outcome data would better delineate the global extent of these clones, their true clinical impact, and offer insights into transmission.

How these rifampicin resistant clones prevailed when implementation of rigorous hand-hygiene has seemingly resulted in the decline of in-hospital MRSA transmission [229,230], also bears investigation. The high level of biocide resistance observed in these clones [231,232] was possibly contributory and consistent with reports of increasing chlorhexidine resistance [233,234] and alcohol tolerance [235] in hospital adapted bacteria, that has been attributed to hand-hygiene products, and prompted questions about the need for biocide stewardship.

In the setting of misinformed clinical practice and liberal antibiotic use, it would seem that these clones have surpassed being merely hospital-adapted to become hospital-thriving. Current guidelines that recommend adjunctive rifampicin and vancomycin for the treatment of serious methicillin resistant staphylococcal prosthetic infections have almost certainly resulted in the generation of resistance and contributed to the prevalence of the three MDRSE lineages. There is an apparent lack of knowledge regarding optimal antimicrobial combinations for *S. epidermidis*, and a need for dedicated research.

Furthermore, the work in this thesis emphasises that the evolution of antibiotic resistance is more complex than just breakthrough resistance while on treatment. Unintentional collateral exposure of colonising *S. epidermidis* when treating other organisms would also have contributed to the expansion of the three MDRSE lineages and their accumulation of multiple resistance determinants (e.g. vancomycin prescribed for *S. aureus* and *Enterococcus*; rifampicin for tuberculosis and *S. aureus*; rifaximin for small bowel overgrowth, *C. difficile* and hepatic encephalopathy). Alarmingly, Chapter 3 identified two near untreatable MDRSE German clones, both rifampicin and vancomycin heteroresistant with acquired resistance to daptomycin and linezolid, tetracycline their only remaining treatment option. Whether other colonising bacterial species have evolved similarly unrecognised multidrug-resistant phenotypes, remains to be seen.

Beyond its role in bacterial immunity, type I RM systems have been demonstrated to influence gene expression in some species, with downstream effects on virulence [236-238]. In *Streptococcus pyogenes*, a mutant strain expressing no adenosine methylation due to deletion of its type I RM system, was demonstrated to have significant down regulation of genes affecting virulence, surface adhesins, and immune evasion factors [236]. Compared to WT, the mutant strain had reduced survival in human neutrophils,

diminished adherence to human epithelial cells and was unable to supress the host immune response in a murine subcutaneous infection model [236]. Whereas in *Streptococcus pneumoniae*, that possesses three copies of *hsdS*, site-specific recombination occurring between the *hsdS* genes has been demonstrated to generate changes in methylome, gene transcription and virulence [237,238]. Reversible inversions among the three *hsdS* served as a switch between two distinct and well recognised colony morphologies (phase variants). The first, an opaque phenotype that contains more polysaccharide capsule, demonstrates better evasion of opsonophagocytic killing and causes more virulent systemic infection in animal models [237]. The second, a transparent colony with higher cell wall teichoic acid content and faster autolysis, that demonstrates higher levels of nasopharyngeal colonisation and lower animal model virulence [237]. Paired with their respective parental strains, the five methylation-deficient Δ*hsdS* mutants introduced in Chapter 4 would be ideal candidates to determine if similar epigenetic regulation occurs in *S. epidermidis* and any effects of methylation on virulence in this species.

Utilising the methodology outlined in Chapter 4, the genetic manipulation of any *S. epidermidis* strain of interest is now possible, and should advance scientific understanding of antibiotic resistance mechanisms, pathogenesis and virulence in the species. It is likely that future research shall uncover new examples of complex bacterial resistance mechanisms and other drugs with previously unrecognised cross resistance. In the interim, clinical vigilance and good antimicrobial stewardship with judicious prescribing of all agents, not just those saved for salvage therapy, is key to limiting the spread of resistance and preserving our remaining antibiotic options.

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APPENDIX A.

Chapter 3. Global spread of three multidrug resistant lineages of *Staphylococcus epidermidis* – Supplementary Tables

STRAIN	*in silico* MLST	Year	Country	Institution location	LOCATION	Host	Clinical Presentation	Sample	Reference	DOI
12228	8	1955	United States	-	US	Non-clinical	Not applicable	Not applicable	Zhang et al., 2003	10.1046/j.1365-2958.2003.03671.x
12228-rpoB662	8	2014	Australia	Melbourne, Victoria	-	Not applicable	Not applicable	Laboratory derived mutant	This study	
AUS01	2	2010	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS02	2	2012	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS03	2	2012	Australia	Melbourne, Victoria	VIC-C	Human	Bacteraemia	Blood culture	This study	
AUS04	2	2012	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS05	2	2012	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS06	2	2013	Australia	Melbourne, Victoria	VIC-D	Human	Bacteraemia	Blood culture	This study	
AUS07	2	2013	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS08	2	2013	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS09	2	2013	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS10	2	2013	Australia	Melbourne, Victoria	VIC-B	Human	Bacteraemia	Blood culture	This study	
AUS11	2	2017	Australia	Melbourne, Victoria	VIC-K	Human	Bacteraemia	Blood culture	This study	
AUS12	2	2017	Australia	Melbourne, Victoria	VIC-F	Human	Colonising isolate	Blood culture	This study	
AUS13	2	2017	Australia	Melbourne, Victoria	VIC-E	Human	Colonising isolate	Blood culture	This study	
AUS14	2	2017	Australia	Melbourne, Victoria	VIC-H	Human	Bacteraemia	Blood culture	This study	
AUS15	188	2017	Australia	Melbourne, Victoria	VIC-H	Human	Colonising isolate	Blood culture	This study	
AUS16	2	2017	Australia	Melbourne, Victoria	VIC-E	Human	Colonising isolate	Blood culture	This study	
AUS17	2	2017	Australia	Melbourne, Victoria	VIC-G	Human	Colonising isolate	Blood culture	This study	
AUS18	2	2017	Australia	Melbourne, Victoria	VIC-G	Human	Colonising isolate	Blood culture	This study	
AUS19	2	2017	Australia	Melbourne, Victoria	VIC-J	Human	Colonising isolate	Blood culture	This study	
AUS20	2	2017	Australia	Melbourne, Victoria	VIC-E	Human	Bacteraemia	Blood culture	This study	
AUS21	2	2017	Australia	Melbourne, Victoria	VIC-F	Human	Colonising isolate	Blood culture	This study	
AUS22	2	2017	Australia	Melbourne, Victoria	VIC-F	Human	Colonising isolate	Blood culture	This study	
AUS23	2	2017	Australia	Melbourne, Victoria	VIC-L	Human	Colonising isolate	Blood culture	This study	
AUS24	2	2017	Australia	Melbourne, Victoria	VIC-E	Human	Colonising isolate	Blood culture	This study	
AUS25	2	2017	Australia	Melbourne, Victoria	VIC-F	Human	Colonising isolate	Blood culture	This study	
AUS26	2	2017	Australia	Melbourne, Victoria	VIC-M	Human	Colonising isolate	Blood culture	This study	
AUS27	23	2017	Australia	Melbourne, Victoria	VIC-E	Human	Colonising isolate	Blood culture	This study	
AUS28	23	2017	Australia	Melbourne, Victoria	VIC-E	Human	Colonising isolate	Blood culture	This study	
AUS29	2	2017	Australia	Melbourne, Victoria	VIC-B	Human	Colonising isolate	Blood culture	This study	
AUS30	2	2017	Australia	Melbourne, Victoria	VIC-C	Human	Colonising isolate	Blood culture	This study	
AUS31	2	2017	Australia	Melbourne, Victoria	VIC-I	Human	Colonising isolate	Blood culture	This study	
AUS32	2	2017	Australia	Melbourne, Victoria	VIC-A	Human	Colonising isolate	Blood culture	This study	
AUT01	23	2016	Austria	Austria	AUT-A	Human	Unknown	Liquor aspirate	This study	
BEL01	2	2015	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL02	23	2015	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL03	23	2015	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL04	2	2015	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL05	23	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL06	5	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL07	2	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL08	2	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL09	2	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL10	2	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL11	2	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL12	2	2016	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL13	2	2017	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL14	2	2017	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL15	2	2017	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL16	2	2017	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL17	23	2017	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BEL18	2	2017	Belgium	Brussels	BEL-A	Human	Bacteraemia	Blood culture	This study	
BPH0662	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	CNS infection	CSF	Lee et al., 2016	10.1099/mgen.0.000077
BPH0663	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	CNS infection	CSF	This study	
BPH0666	23	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0667	59	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0668	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0669	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0670	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0671	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0672	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0673	59	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0674	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0675	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0676	2	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0676-rpoB662	2	2014	Australia	Melbourne, Victoria	-	Not applicable	Not applicable	Laboratory derived mutant	This study	
BPH0677	225	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0678	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	
BPH0679	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study	

BPH0681	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0684	487	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0685	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0686	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0687	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0689	59	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0690	7	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0691	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0692	2	2013	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0693	188	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0694	2	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0695	23	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0696	2	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0697	89	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0699	210	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0700	86	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0701	89	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0702	2	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0703	23	2007	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0704	368	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0705	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0706	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0707	46	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0708	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0709	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0710	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0711	59	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0712	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0713	23	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0714	2	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0715	23	2008	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0716	2	2009	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0717	2	2009	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0718	2	2009	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0719	2	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0720	2	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0721	210	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0722	2	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0723	5	2009	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0724	2	2009	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0726	59	2009	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0727	2	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0728	2	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0729	86	2010	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0730	2	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0731	2	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0732	2	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0733	2	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0734	59	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0735	2	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0736	2	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0736-rpoB662	2	2014	Australia	Melbourne, Victoria	-	Not applicable	Not applicable	Laboratory derived mutant	This study
BPH0737	225	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0738	59	2011	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
BPH0739	7	2012	Australia	Melbourne, Victoria	VIC-A	Human	Bacteraemia	Blood culture	This study
DEN01	2	2001	Denmark	Jutland	DEN-A	Human	Unknown	Unknown	This study
DEN02	23	2001	Denmark	Copenhagen	DEN-B	Human	Unknown	Unknown	This study
DEN03	2	2001	Denmark	Copenhagen	DEN-C	Human	Unknown	Unknown	This study
DEN04	2	2001	Denmark	Copenhagen	DEN-D	Human	Unknown	Unknown	This study
DEN05	2	2001	Denmark	Jutland	DEN-E	Human	Unknown	Unknown	This study
DEN06	2	2001	Denmark	Copenhagen	DEN-F	Human	Unknown	Unknown	This study
DEN07	2	2001	Denmark	Copenhagen	DEN-F	Human	Unknown	Unknown	This study
DEN08	2	2001	Denmark	Copenhagen	DEN-F	Human	Unknown	Unknown	This study
DEN09	23	2001	Denmark	Copenhagen	DEN-F	Human	Unknown	Unknown	This study
DEN10	2	2001	Denmark	Copenhagen	DEN-G	Human	Unknown	Unknown	This study
DEN11	23	2001	Denmark	Copenhagen	DEN-G	Human	Unknown	Unknown	This study
DEN12	23	2012	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study
DEN13	23	2012	Denmark	Copenhagen	DEN-H	Human	Colonising isolate	Blood culture	This study
DEN14	23	2013	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study
DEN15	2	2013	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study
DEN16	23	2014	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study

DEN17	2	2014	Denmark	Copenhagen	DEN-G	Human	CVC Infection	Blood culture	This study	
DEN18	23	2015	Denmark	Copenhagen	DEN-H	Human	Colonising isolate	Blood culture	This study	
DEN19	23	2015	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study	
DEN20	23	2016	Denmark	Copenhagen	DEN-H	Human	CVC Infection	Blood culture	This study	
DEN21	23	2016	Denmark	Copenhagen	DEN-H	Human	CVC Infection	Blood culture	This study	
DEN22	23	2016	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study	
DEN23	87	2016	Denmark	Copenhagen	DEN-I	Human	Colonising isolate	Blood culture	This study	
DEN24	23	2016	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study	
DEN25	23	2016	Denmark	Copenhagen	DEN-G	Human	CVC Infection	Blood culture	This study	
DEN26	23	2016	Denmark	Copenhagen	DEN-H	Human	CVC Infection	Blood culture	This study	
DEN27	23	2016	Denmark	Copenhagen	DEN-G	Human	CVC Infection	Blood culture	This study	
DEN28	2	2017	Denmark	Copenhagen	DEN-J	Human	Colonising isolate	Blood culture	This study	
DEN29	23	2017	Denmark	Copenhagen	DEN-G	Human	CVC Infection	Blood culture	This study	
DEN30	23	2017	Denmark	Copenhagen	DEN-H	Human	CVC Infection	Blood culture	This study	
DEN31	23	2017	Denmark	Copenhagen	DEN-H	Human	Colonising isolate	Blood culture	This study	
DEN32	23	2017	Denmark	Copenhagen	DEN-H	Human	Colonising isolate	Blood culture	This study	
DEN33	23	2017	Denmark	Copenhagen	DEN-H	Human	Colonising isolate	Blood culture	This study	
DEN34	23	2017	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study	
DEN35	23	2017	Denmark	Copenhagen	DEN-H	Human	Colonising isolate	Blood culture	This study	
DEN36	23	2017	Denmark	Copenhagen	DEN-H	Human	Skin and Soft Tissue Infection	Blood culture	This study	
DEN37	23	2017	Denmark	Copenhagen	DEN-G	Human	Colonising isolate	Blood culture	This study	
DEN38	2	2017	Denmark	Copenhagen	DEN-H	Human	CVC Infection	Blood culture	This study	
FRA01	23	2005	France	Saint Quentin	FRA-A	Human	Bacteraemia	Blood culture	This study	
FRA02	22	2007	France	Lyon	FRA-B	Human	Bacteraemia	Blood culture	This study	
FRA03	2	2008	France	Dole	FRA-C	Human	Septic arthiritis	Synovial fluid	This study	
FRA04	5	2008	France	Cahors	FRA-D	Human	Unknown	Eye	This study	
FRA05	2	2008	France	Nancy	FRA-E	Human	Bacteraemia	Blood culture	This study	
FRA06	5	2010	France	Metz	FRA-F	Human	Bacteraemia	Catheter	This study	
FRA07	23	2010	France	Giens	FRA-G	Human	Septic arthiritis	Synovial fluid	This study	
FRA08	143	2011	France	Angouleme	FRA-H	Human	Skin and Soft Tissue Infection	Abcess	This study	
FRA09	20	2012	France	Fort de France	FRA-I	Human	Bacteraemia	Blood culture	This study	
FRA10	2	2012	France	Romans	FRA-J	Human	Bacteraemia	Catheter	This study	
FRA11	2	2014	France	Lille	FRA-K	Human	Bacteraemia	Catheter	This study	
FRA12	2	2014	France	Clermont Ferrand	FRA-L	Human	Bacteraemia	Blood culture	This study	
FRA13	2	2015	France	Amiens	FRA-M	Human	Bacteraemia	Blood culture	This study	
FRA14	23	2015	France	Vienne	FRA-N	Human	Bone and joint infection	Bone	This study	
FRA15	2	2016	France	Bayonne	FRA-O	Human	Bacteraemia	Catheter	This study	
FRA16	23	2004	France	Neuilly	FRA-P	Human	Bacteraemia	Blood culture	This study	
FRA17	23	2004	France	Chartes	FRA-Q	Human	Bacteraemia	Blood culture	This study	
FRA18	2	2005	France	Mantes la Jolie	FRA-R	Human	Bacteraemia	Blood culture	This study	
GER01	2	2012	Germany	Bavaria	GER-A	Human	Bacteraemia	Blood culture	This study	
GER02	2	2012	Germany	Hamburg	GER-B	Human	Bacteraemia	Blood culture	This study	
GER03	2	2013	Germany	Saxony-Anhalt	GER-C	Human	Bacteraemia	Blood culture	This study	
GER04	2	2013	Germany	Lower Saxony	GER-D	Human	Bacteraemia	Blood culture	This study	
GER05	2	2013	Germany	Saxony	GER-E	Human	Wound infection	Biopsy	This study	
GER06	2	2014	Germany	Bavaria	GER-F	Human	Urinary tract infection	Urine	This study	
GER07	5	2014	Germany	North Rhine-Westphalia	GER-G	Human	Bacteraemia	Catherter tip	This study	
GER08	23	2015	Germany	Mecklenburg-West Pomerania	GER-H	Human	Bacteraemia	Blood Culture	This study	
GER09	2	2016	Germany	Mecklenburg-West Pomerania	GER-I	Human	Bacteraemia	Blood Culture	This study	
GER10	5	2016	Germany	North Rhine-Westphalia	GER-J	Human	Bacteraemia	Blood Culture	This study	
GER11	2	2016	Germany	North Rhine-Westphalia	GER-K	Human	Peritonitis	Aspirate	This study	
GER12	2	2016	Germany	North Rhine-Westphalia	GER-L	Human	Skin and Soft Tissue Infection	Swab	This study	
GER13	5	2016	Germany	Lower Saxony	GER-M	Human	Bacteraemia	Blood culture	This study	
GER14	2	2016	Germany	Baden-Württemberg	GER-N	Human	Urinary tract infection	Urine	This study	
GER15	23	2016	Germany	Saxony-Anhalt	GER-O	Human	Bacteraemia	Blood culture	This study	
GER16	2	2017	Germany	Lower Saxony	GER-P	Human	Bacteraemia	Blood culture	This study	
GER17	2	2017	Germany	North Rhine-Westphalia	GER-Q	Human	Wound infection	Swab	This study	
GER18	2	2017	Germany	North Rhine-Westphalia	GER-Q	Human	Bacteraemia	Blood culture	This study	
GER19	23	2017	Germany	Rhineland-Palatinate	GER-R	Human	Osteomyelitis	Osteosynthesis biopsy	This study	
GER20	2	2017	Germany	Lower Saxony	GER-S	Human	Bacteraemia	Blood culture	This study	
GER21	5	2017	Germany	Baden-Württemberg	GER-T	Human	Osteomyelitis	Swab	This study	
IRL01	2	2016	Republic of Ireland	Republic of Ireland	IRL-A	Human	Bacteraemia	Blood culture	This study	
PM221	184	1999-2002	Finland	Unknown	FIN-A	Bovine	Mastitis	Mastitis	Savijoki et al., 2014	10.1021/pr500322d
RP62a	10	1979-1980	United States	Memphis, Tennessee	US-B	Human	Bacteraemia	Blood culture	Gill et al., 2005	10.1128/JB.187.7.2426-2438.2005
RP62a-rpoB662	10	2014	Australia	Melbourne, Victoria	-	Not applicable	Not applicable	Laboratory derived mutant	This study	
SEI	-	Unknown	United States	Unknown	US	Non-clinical	Not applicable	Not applicable	Davenport et al., 2014	10.1128/genomeA.01059-14
UK01	2	2015	United Kingdon	East, England	UK-A	Human	Bacteraemia	Blood culture	This study	
UK02	2	2015	United Kingdon	S East, England	UK-B	Human	CNS infection	CSF	This study	
UK03	2	2015	United Kingdon	E Midlands, England	UK-C	Human	Osteomyelitis	Spinal tissue	This study	
UK04	87	2016	United Kingdon	Yorkshire & Humber England	UK-D	Human	Bacteraemia	Blood culture	This study	
UK05	2	2016	United Kingdon	London, England	UK-E	Human	Bacteraemia	CVP tip	This study	

Isolate	Sample unique name	Uploaded to	Study accession	Sample accession
AUS11	AUSMDU00010940	NCBI SRA	PRJNA470534	SAMN09093616
AUS12	AUSMDU00010947	NCBI SRA	PRJNA470534	SAMN09093617
AUS13	AUSMDU00010951	NCBI SRA	PRJNA470534	SAMN09093618
AUS14	AUSMDU00010956	NCBI SRA	PRJNA470534	SAMN09093619
AUS15	AUSMDU00010984	NCBI SRA	PRJNA470534	SAMN09093620
AUS16	AUSMDU00010995	NCBI SRA	PRJNA470534	SAMN09093621
AUS17	AUSMDU00011002	NCBI SRA	PRJNA470534	SAMN09093622
AUS18	AUSMDU00011006	NCBI SRA	PRJNA470534	SAMN09093623
AUS19	AUSMDU00011014	NCBI SRA	PRJNA470534	SAMN09093624
AUS20	AUSMDU00011024	NCBI SRA	PRJNA470534	SAMN09093625
AUS21	AUSMDU00011032	NCBI SRA	PRJNA470534	SAMN09093626
AUS22	AUSMDU00011033	NCBI SRA	PRJNA470534	SAMN09093627
AUS23	AUSMDU00007545	NCBI SRA	PRJNA470534	SAMN09093628
AUS24	AUSMDU00007547	NCBI SRA	PRJNA470534	SAMN09093629
AUS25	AUSMDU00007550	NCBI SRA	PRJNA470534	SAMN09093630
AUS26	AUSMDU00007553	NCBI SRA	PRJNA470534	SAMN09093631
AUS27	AUSMDU00008046	NCBI SRA	PRJNA470534	SAMN09093632
AUS28	AUSMDU00008047	NCBI SRA	PRJNA470534	SAMN09093633
AUS29	AUSMDU00007904	NCBI SRA	PRJNA470534	SAMN09093634
AUS30	AUSMDU00007905	NCBI SRA	PRJNA470534	SAMN09093635
AUS31	AUSMDU00007924	NCBI SRA	PRJNA470534	SAMN09093636
AUS32	AUSMDU00007933	NCBI SRA	PRJNA470534	SAMN09093637
12228-rpoB662	Se_12228-rpoB662	ENA	PRJEB12090	ERS1019846
12228	Se_12228-WT	ENA	PRJEB12090	ERS1019847
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BPH0677	Se_BPH0677	ENA	PRJEB12090	ERS1019862
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UK02	Se_BPH0744	NCBI SRA	PRJNA470752	SAMN09103954
UK03	Se_BPH0745	NCBI SRA	PRJNA470752	SAMN09103955
UK04	Se_BPH0746	NCBI SRA	PRJNA470752	SAMN09103956
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UK06	Se_BPH0749	NCBI SRA	PRJNA470752	SAMN09103958
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UK10	Se_BPH0753	NCBI SRA	PRJNA470752	SAMN09103962
UK11	Se_BPH0754	NCBI SRA	PRJNA470752	SAMN09103963
UK12	Se_BPH0755	NCBI SRA	PRJNA470752	SAMN09103964
AUS01	Se_BPH0771	NCBI SRA	PRJNA470752	SAMN09103846
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AUS04	Se_BPH0795	NCBI SRA	PRJNA470752	SAMN09103849
AUS05	Se_BPH0801	NCBI SRA	PRJNA470752	SAMN09103850
AUS06	Se_BPH0808	NCBI SRA	PRJNA470752	SAMN09103851

AUS07	Se_BPH0809	NCBI SRA	PRJNA470752	SAMN09103852
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AUS09	Se_BPH0816	NCBI SRA	PRJNA470752	SAMN09103854
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RP62a-rpoB662	Se_RP62a-rpoB662	ENA	PRJEB12090	ERS1019920
RP62a	Se_RP62a-WT	ENA	PRJEB12090	ERS1019921

Isolate	Sample ID	Sequencer	Reads	Yield	GC	MinLen	AvgLen	MaxLen	ModeLen	Phred	AvgQual	Depth	Assembler	No	bp	ok	Ns	Gaps	Min	Avg	Max	N50
AUS11	AUSMDU00010940	Illumina NextSeq	2948496	427878249	34.6	35	145	151	151	33	32.2	150x	SPAdes v 3.7.1	76	2722905	2722905	0	0	504	35827	167410	71333
AUS12	AUSMDU00010947	Illumina NextSeq	2593948	381852962	34.5	35	147	151	151	33	32.1	134x	SPAdes v 3.7.1	69	2732917	2732917	0	0	504	39607	271176	80570
AUS13	AUSMDU00010951	Illumina NextSeq	2691052	397362115	34.7	35	147	151	151	33	32.1	139x	SPAdes v 3.7.1	80	2730646	2730646	0	0	504	34133	185077	71175
AUS14	AUSMDU00010956	Illumina NextSeq	2676688	396157030	34.3	35	148	151	151	33	31.8	139x	SPAdes v 3.7.1	79	2756712	2756712	0	0	504	34895	189912	77684
AUS15	AUSMDU00010984	Illumina NextSeq	2487598	367294531	34.8	35	147	151	151	33	31.9	129x	SPAdes v 3.7.1	69	2590670	2590670	0	0	504	37545	181227	83701
AUS16	AUSMDU00010995	Illumina NextSeq	2180786	324841876	34.4	32	148	151	151	33	31.6	114x	SPAdes v 3.7.1	61	2594709	2594709	0	0	504	42536	239911	103856
AUS17	AUSMDU00011002	Illumina NextSeq	2501908	371405979	34.1	35	148	151	151	33	32.1	130x	SPAdes v 3.7.1	55	2502789	2502789	0	0	590	45505	244548	116627
AUS18	AUSMDU00011006	Illumina NextSeq	2579092	382081652	34.1	33	148	151	151	33	32	134x	SPAdes v 3.7.1	37	2496417	2496417	0	0	504	67470	350332	140663
AUS19	AUSMDU00011014	Illumina NextSeq	2888724	424991745	34.5	31	147	151	151	33	32.1	149x	SPAdes v 3.7.1	74	2786706	2786706	0	0	504	37658	221409	87665
AUS20	AUSMDU00011024	Illumina NextSeq	2928352	429332516	34.3	34	146	151	151	33	32.1	150x	SPAdes v 3.7.1	65	2695681	2695681	0	0	504	41472	271176	87592
AUS21	AUSMDU00011032	Illumina NextSeq	3277692	479384646	34.6	35	146	151	151	33	32	168x	SPAdes v 3.7.1	42	2529630	2529630	0	0	504	60229	380315	120908
AUS22	AUSMDU00011033	Illumina NextSeq	2475760	364698071	34.6	32	147	151	151	33	32.1	128x	SPAdes v 3.7.1	47	2529417	2529417	0	0	504	53817	444438	140660
AUS23	AUSMDU00007545	Illumina NextSeq	2189868	317054551	34.7	30	144	151	151	33	31	111x	SPAdes v 3.7.1	65	2766081	2766081	0	0	504	42555	271176	89281
AUS24	AUSMDU00007547	Illumina NextSeq	2072050	305149599	33.5	32	147	151	151	33	31.2	107x	SPAdes v 3.7.1	67	2693317	2693317	0	0	504	40198	271883	89356
AUS25	AUSMDU00007550	Illumina NextSeq	1430752	209861370	33.9	31	146	151	151	33	30.9	74x	SPAdes v 3.7.1	69	2730534	2730534	0	0	504	39572	257008	82548
AUS26	AUSMDU00007553	Illumina NextSeq	2278504	329369145	34.3	31	144	151	151	33	31.1	115x	SPAdes v 3.7.1	62	2734818	2734818	0	0	504	44109	185028	125738
AUS27	AUSMDU00008046	Illumina NextSeq	2403978	357715751	34.3	35	148	151	151	33	32.3	125x	SPAdes v 3.7.1	62	2483427	2483427	0	0	635	40055	312073	119337
AUS28	AUSMDU00008047	Illumina NextSeq	2408978	358452731	34.5	35	148	151	151	33	32.2	126x	SPAdes v 3.7.1	58	2406216	2406216	0	0	505	41486	312073	123433
AUS29	AUSMDU00007904	Illumina NextSeq	2172226	322343301	34.3	33	148	151	151	33	32.1	113x	SPAdes v 3.7.1	79	2702423	2702423	0	0	504	34207	184441	67537
AUS30	AUSMDU00007905	Illumina NextSeq	1915524	284928797	34.2	35	148	151	151	33	31.9	100x	SPAdes v 3.7.1	81	2689947	2689947	0	0	504	33209	183659	71136
AUS31	AUSMDU00007924	Illumina NextSeq	2053034	304204673	34.3	35	148	151	151	33	32.1	107x	SPAdes v 3.7.1	69	2712565	2712565	0	0	504	39312	189912	80013
AUS32	AUSMDU00007933	Illumina NextSeq	2308826	341782156	34.1	33	148	151	151	33	32.1	120x	SPAdes v 3.7.1	82	2774968	2774968	0	0	504	33841	189912	75810
12228-rpoB662	Se_12228-rpoB662	Illumina NextSeq	1515702	333958994	35.2	35	220	301	301	33	36.7	117x	SPAdes v 3.7.1	116	2538976	2538976	0	0	504	21887	152388	54838
12228	Se_12228-WT	Illumina NextSeq	1289378	290462533	35.5	35	225	301	301	33	36.3	102x	SPAdes v 3.7.1	148	2538595	2538595	0	0	500	17152	113597	39956
BPH0662	Se_BPH0662	Illumina MiSeq	1646975	230230230	33.7	35	139	151	151	33	38.2	81x	SPAdes v 3.7.1	75	2799609	2799609	0	0	504	37328	221409	87844
BPH0663	Se_BPH0663	Illumina MiSeq	4206822	525908666	33.7	35	125	151	151	33	38.2	184x	SPAdes v 3.7.1	84	2804013	2804013	0	0	504	33381	221409	81415
BPH0666	Se_BPH0666	Illumina NextSeq	1938512	287905754	34.8	35	148	151	151	33	32.8	101x	SPAdes v 3.7.1	73	2441668	2441668	0	0	505	33447	312071	76264
BPH0667	Se_BPH0667	Illumina MiSeq	687032	150574538	34.6	35	219	301	214	33	36.0	53x	SPAdes v 3.7.1	74	2460192	2460192	0	0	504	33245	145633	62948
BPH0668	Se_BPH0668	Illumina NextSeq	2259914	335766013	34.3	35	148	151	151	33	32.8	118x	SPAdes v 3.7.1	69	2775172	2775172	0	0	504	40219	201713	86785
BPH0669	Se_BPH0669	Illumina MiSeq	881676	167656908	35.1	35	190	301	214	33	36.2	59x	SPAdes v 3.7.1	104	2471246	2471246	0	0	500	23761	122723	43256
BPH0670	Se_BPH0670	Illumina MiSeq	1360360	230371010	34.8	35	169	301	214	33	36.3	81x	SPAdes v 3.7.1	154	2750775	2750775	0	0	504	17862	118098	36556
BPH0671	Se_BPH0671	Illumina MiSeq	1303138	226314287	34.9	35	173	301	214	33	35.9	79x	SPAdes v 3.7.1	102	2598090	2598090	0	0	500	25471	181009	58113
BPH0672	Se_BPH0672	Illumina MiSeq	1357862	232796526	34.5	35	171	301	215	33	36.1	82x	SPAdes v 3.7.1	76	2738935	2738935	0	0	504	36038	216358	80611
BPH0673	Se_BPH0673	Illumina MiSeq	1831908	312764296	35.1	35	170	301	215	33	36.1	110x	SPAdes v 3.7.1	45	2450384	2450384	0	0	504	54452	267079	125369
BPH0674	Se_BPH0674	Illumina MiSeq	1982404	338574803	34.9	35	170	301	215	33	36.1	119x	SPAdes v 3.7.1	65	2748661	2748661	0	0	504	42287	227261	80331
BPH0675	Se_BPH0675	Illumina MiSeq	1948848	325153308	34.9	35	166	301	215	33	35.8	114x	SPAdes v 3.7.1	77	2740571	2740571	0	0	504	35591	225669	80568
BPH0676	Se_BPH0676	Illumina MiSeq	857140	158310082	34.6	35	184	301	214	33	35.6	55x	SPAdes v 3.7.1	96	2598181	2598181	0	0	504	27064	139697	71191
BPH0676-rpoB662	Se_BPH0676-rpoB662	Illumina NextSeq	1448616	330529534	35.4	35	228	301	301	33	36.8	116x	SPAdes v 3.7.1	104	2588379	2588379	0	0	501	24888	167284	70223
BPH0677	Se_BPH0677	Illumina MiSeq	1348260	250299890	34.5	35	185	301	214	33	36.3	88x	SPAdes v 3.7.1	78	2509146	2509146	0	0	505	32168	234495	59637
BPH0678	Se_BPH0678	Illumina MiSeq	3458424	782725288	33.9	35	226	251	251	33	37.7	274x	SPAdes v 3.7.1	88	2814869	2814869	0	0	504	31987	221409	85738
BPH0679	Se_BPH0679	Illumina MiSeq	1029724	226791381	34.6	35	220	301	214	33	35.7	79x	SPAdes v 3.7.1	78	2729763	2729763	0	0	504	34996	198700	71333
BPH0681	Se_BPH0681	Illumina MiSeq	1894640	345475614	34.5	35	182	301	214	33	36.3	121x	SPAdes v 3.7.1	53	2549438	2549438	0	0	504	48102	204822	130064
BPH0684	Se_BPH0684	Illumina MiSeq	1576776	297376064	34.7	35	188	301	215	33	36.2	104x	SPAdes v 3.7.1	46	2446950	2446950	0	0	504	53194	245809	144699
BPH0685	Se_BPH0685	Illumina MiSeq	1827142	350101274	34.7	35	191	301	215	33	36.2	123x	SPAdes v 3.7.1	79	2786873	2786873	0	0	504	35276	190528	80431
BPH0686	Se_BPH0686	Illumina MiSeq	1875284	327923113	34.8	35	174	301	215	33	36.1	115x	SPAdes v 3.7.1	66	2770154	2770154	0	0	504	41972	216360	88492
BPH0687	Se_BPH0687	Illumina MiSeq	1792360	349923301	34.6	35	195	301	215	33	35.9	123x	SPAdes v 3.7.1	79	2793740	2793740	0	0	504	35363	221409	81415
BPH0689	Se_BPH0689	Illumina MiSeq	1228646	220677703	34.5	35	179	301	214	33	36.1	77x	SPAdes v 3.7.1	48	2443029	2443029	0	0	504	50896	211285	96507
BPH0690	Se_BPH0690	Illumina MiSeq	978916	165994266	34.8	35	169	301	214	33	36.2	58x	SPAdes v 3.7.1	56	2340119	2340119	0	0	505	41787	193052	66652
BPH0691	Se_BPH0691	Illumina MiSeq	4190182	807890652	33.5	35	192	251	251	33	37.9	283x	SPAdes v 3.7.1	62	2740484	2740484	0	0	504	44201	216459	89085
BPH0692	Se_BPH0692	Illumina MiSeq	2062758	489552889	34.5	35	237	301	301	33	35.7	171x	SPAdes v 3.7.1	65	2740301	2740301	0	0	504	42158	207199	87844
BPH0693	Se_BPH0693	Illumina MiSeq	2015628	476501974	34.5	35	236	301	301	33	35.6	167x	SPAdes v 3.7.1	48	2601839	2601839	0	0	504	54204	308939	127464
BPH0694	Se_BPH0694	Illumina NextSeq	2243354	330745904	34.6	35	147	151	151	33	33.1	116x	SPAdes v 3.7.1	86	2743179	2743179	0	0	504	31897	207299	73528
BPH0695	Se_BPH0695	Illumina MiSeq	824284	167801131	35.1	35	203	301	215	33	35.2	59x	SPAdes v 3.7.1	135	2471534	2471534	0	0	505	18307	104169	38063
BPH0696	Se_BPH0696	Illumina MiSeq	3064148	710138518	34.3	35	231	251	251	33	37.7	249x	SPAdes v 3.7.1	63	2739504	2739504	0	0	572	43484	226625	89085
BPH0697	Se_BPH0697	Illumina MiSeq	1641686	291714021	34.7	35	177	301	215	33	35.9	102x	SPAdes v 3.7.1	67	2488865	2488865	0	0	502	37147	241253	83062
BPH0699	Se_BPH0699	Illumina MiSeq	1288656	235013488	34.5	35	182	301	215	33	35.7	82x	SPAdes v 3.7.1	57	2460843	2460843	0	0	505	43172	210466	102523
BPH0700	Se_BPH0700	Illumina MiSeq	1604442	287276663	34.4	35	179	301	215	33	34.7	101x	SPAdes v 3.7.1	64	2465908	2465908	0	0	504	38529	194127	75251
BPH0701	Se_BPH0701	Illumina MiSeq	1021294	177204659	34.4	35	173	301	214	33	36.3	62x	SPAdes v 3.7.1	74	2454303	2454303	0	0	504	33166	244635	57667
BPH0702	Se_BPH0702	Illumina MiSeq	944810	159209892	34.4	35	168	301	214	33	36.0	56x	SPAdes v 3.7.1	122	2733030	2733030	0	0	504	22401	182736	42781
BPH0703	Se_BPH0703	Illumina MiSeq	2704480	638861619	34.5	35	236	251	251	33	37.4	224x	SPAdes v 3.7.1	172	2557035	2557035	0	0	622	14866	311814	132476
BPH0704	Se_BPH0704	Illumina MiSeq	1317556	212050431	34.8	35	160	301	214	33	36.1	74x	SPAdes v 3.7.1	86	2493209	2493209	0	0	501	28990	208652	55448
BPH0705	Se_BPH0705	Illumina MiSeq	1294582	272511125	34.9	35	210	301	215	33	35.7	95x	SPAdes v 3.7.1	62	2593608	2593608	0	0	504	41832	185179	82986
BPH0706	Se_BPH0706	Illumina MiSeq	1519164	282116923	34.8	35	185	301	215	33	36.0	99x	SPAdes v 3.7.1	65	2717009	2717009	0	0	504	41800	220691	94308
BPH0707	Se_BPH0707	Illumina MiSeq	1787056	346131507	34.6	35	193	301	215	33	36.0	121x	SPAdes v 3.7.1	47	2472202	2472202	0	0	501	52600	213503	105951
BPH0708	Se_BPH0708	Illumina MiSeq	1323952	250872727	34.4	35	189	301	215	33	35.9	88x	SPAdes v 3.7.1	49	2662546	2662546	0	0	504	54337	287867	97277
BPH0709	Se_BPH0709	Illumina NextSeq	2137046	319190030	33.7	25	149	151	151	33	32.7	112x	SPAdes v 3.7.1	60	2732976	2732976	0	0	504	45549	271176	87844
BPH0710	Se_BPH0710	Illumina NextSeq	2238186	332079426	34.5	35	148	151	151	33	32.9	116x	SPAdes v 3.7.1	63	2654541	2654541	0	0	504	42135	365465	83092
BPH0711	Se_BPH0711	Illumina MiSeq	1240732	240683540	34.6	35	193	301	214	33	36.2	84x	SPAdes v 3.7.1	58	2516281	2516281	0	0	503	43384	267180	96507
BPH0712	Se_BPH0712	Illumina NextSeq	2120058	314060912	33.9	35	148	151	151	33	33.1	110x	SPAdes v 3.7.1	61	2734147	2734147	0	0	504	44822	226625	87844
BPH0713	Se_BPH0713	Illumina MiSeq	2650490	489056589	35.0	35	184	301	215	33	36.0	171x	SPAdes v 3.7.1	69	2485264	2485264	0	0	505	36018	271087	127939
BPH0714	Se_BPH0714	Illumina MiSeq	1358422	222084982	34.5	35	163	301	215	22	35.9	78x	SPAdes v 3.7.1	84	2740680	2740680	0	0	500	32627	226105	66141
BPH0715	Se_BPH0715	Illumina MiSeq	2305354	403723544	34.7	35	175	301	215	33	36.0	141x	SPAdes v 3.7.1	66	2476034	2476034	0	0	505	37515	186066	93798
BPH0716	Se_BPH0716	Illumina MiSeq	2137038	495111059	34.7	35	231	301	301	33	35.6	173x	SPAdes v 3.7.1	61	2586852	2586852	0	0	504	42407	204822	85009
BPH0717	Se_BPH0717	Illumina MiSeq	848962	196879102	34.5	35	231	301	215	33	34.9	69x	SPAdes v 3.7.1	63	2593819	2593819	0	0	504	41171	198812	83091
BPH0718	Se_BPH0718	Illumina MiSeq	1189744	213814783	34.0	35	179	301	215	33	35.5	75x	SPAdes v 3.7.1	83	2745492	2745492	0	0	500	33078	197645	62203
BPH0719	Se_BPH0719	Illumina NextSeq	1258832	185676332	31.7	35	147	151	151	33	32.6	65x	SPAdes v 3.7.1	49	2574274	2574274	0	0	504	52536	521025	149749
BPH0720	Se_BPH0720	Illumina MiSeq	1387394	266850272	34.5	35	192	301	214	33	36.3	93x	SPAdes v 3.7.1	85	2738524	2738524	0	0	500	32217	183843	80012
BPH0721	Se_BPH0721	Illumina MiSeq	1281838	250504008	34.4	35	195	301	214	33	36.1	88x	SPAdes v 3.7.1	41	2398618	2398618	0	0	505	58502	213976	99445
BPH0722	Se_BPH0722	Illumina MiSeq	929496	179486597	34.6	35	193	301	214	22	35.9	63x	SPAdes v 3.7.1	121	2563756	2563756	0	0	504	21188	95536	43280
BPH0723	Se_BPH0723	Illumina MiSeq	1403638	298738854	34.8	35	212	301	215	33	35.8	105x	SPAdes v 3.7.1	56	2548736	2548736	0	0	505	45513	307924	95395
BPH0724	Se_BPH0724	Illumina MiSeq	1924104	421912231	34.8	35	203	301	215	33	36.1	148x	SPAdes v 3.7.1	56	2558707	2558707	0	0	504	45691	264885	120988
BPH0726	Se_BPH0726	Illumina MiSeq	1243748	245606935	34.7	35	197	301	215	33	35.8	86x	SPAdes v 3.7.1	100	2482849	2482849	0	0	505	24828	327470	102523
BPH0727	Se_BPH0727	Illumina MiSeq	999748	203410081	34.5	35	203	301	214	33	35.5	71x	SPAdes v 3.7.1	98	2739374	2739374	0	0	538	27952	183829	52025
BPH0728	Se_BPH0728	Illumina MiSeq	811344	168381690	34.4	35	207	301	214	33	35.7	59x	SPAdes v 3.7.1	119	2723567	2723567	0	0	504	22887	209918	41360
BPH0729	Se_BPH0729	Illumina MiSeq	781414	156409916	34.9	35	200	301	214	33	36.2	55x	SPAdes v 3.7.1	115	2456937	2456937	0	0	504	21364	79576	39853
BPH0730	Se_BPH0730	Illumina MiSeq	751644	151753781	34.7	35	201	301	214	33	36.1	53x	SPAdes v 3.7.1	159	2542154	2542154	0	0	504	15988	97664	33434
BPH0731	Se_BPH0731	Illumina MiSeq	1406286	281543899	34.3	35	200	301	215	33	36.3	99x	SPAdes v 3.7.1	63	2731907	2731907	0	0	504	43363	226149	87844
BPH0732	Se_BPH0732	Illumina MiSeq	1355242	267030244	34.6	35	197	301	215	33	36.1	94x	SPAdes v 3.7.1	39	2531475	2531475	0	0	504	64909	283819	136301
BPH0733	Se_BPH0733	Illumina MiSeq	1965162	368816353	34.4	35	187	301	215	33	36.2	129x	SPAdes v 3.7.1	76	2787111	2787111	0	0	504	36672	221409	87733
BPH0734	Se_BPH0734	Illumina MiSeq	1471548	301489643	34.8	35	204	301	215	33	36.0	106x	SPAdes v 3.7.1	37	2422377	2422377	0	0	504	65469	292413	157876
BPH0735	Se_BPH0735	Illumina MiSeq	702656	145802756	34.8	35	207	301	214	33	36.0	51x	SPAdes v 3.7.1	161	2701420	2701420	0	0	506	16779	175263	31488
BPH0736	Se_BPH0736	Illumina MiSeq	1121508	226575555	34.8	35	202	301	214	33	36.3	79x	SPAdes v 3.7.1	89	2529190	2529190	0	0	504	28417	149749	50466
BPH0736-rpoB662	Se_BPH0736-rpoB662	Illumina NextSeq	1284014	295332624	35.7	35	230	301	301	33	36.8	103x	SPAdes v 3.7.1	107	2484471	2484471	0	0	504	23219	160501	45051
BPH0737	Se_BPH0737	Illumina NextSeq	1258832	185676332	31.7	35	147	151	151	33	32.6	65x	SPAdes v 3.7.1	74	2511073	2511073	0	0	502	33933	211032	82569
BPH0738	Se_BPH0738	Illumina NextSeq	2300312	339766212	34.1	35	147	151	151	33	33.2	119x	SPAdes v 3.7.1	29	2429410	2429410	0	0	504	83772	487209	211391
BPH0739	Se_BPH0739	Illumina MiSeq	2399586	475805579	34.5	35	198	301	215	33	36.1	167x	SPAdes v 3.7.1	54	2425519	2425519	0	0	505	44917	659150	122119
UK01	Se_BPH0743	Illumina NextSeq	3049548	450748227	33.8	30	147	151	151	33	31.6	158x	SPAdes v 3.7.1	55	2568312	2568312	0	0	504	46696	239817	116445
UK02	Se_BPH0744	Illumina NextSeq	2346856	346171912	33.6	30	147	151	151	33	31.5	121x	SPAdes v 3.7.1	58	2729891	2729891	0	0	504	47067	278416	81312
UK03	Se_BPH0745	Illumina NextSeq	2426780	358990904	33.8	30	147	151	151	33	31.6	126x	SPAdes v 3.7.1	60	2710723	2710723	0	0	504	45178	441996	80013
UK04	Se_BPH0746	Illumina NextSeq	2487722	368090416	33.6	30	147	151	151	33	31.4	129x	SPAdes v 3.7.1	65	2453752	2453752	0	0	505	37750	307416	83082
IRL01	Se_BPH0747	Illumina NextSeq	2271514	336487373	33.5	30	148	151	151	33	31.4	118x	SPAdes v 3.7.1	48	2695604	2695604	0	0	504	56158	713465	122086
UK05	Se_BPH0748	Illumina NextSeq	2493554	371211646	33.8	30	148	151	151	33	31.4	130x	SPAdes v 3.7.1	63	2726974	2726974	0	0	504	43285	271177	78842
UK06	Se_BPH0749	Illumina NextSeq	2490668	364628898	33.9	30	146	151	151	33	31.8	128x	SPAdes v 3.7.1	66	2728502	2728502	0	0	504	41340	239150	80449
UK07	Se_BPH0750	Illumina NextSeq	2290592	339805541	33	30	148	151	151	33	31.5	119x	SPAdes v 3.7.1	68	2695610	2695610	0	0	743	39641	189764	80195
UK08	Se_BPH0751	Illumina NextSeq	2580912	382363574	33.7	30	148	151	151	33	31.6	134x	SPAdes v 3.7.1	60	2669105	2669105	0	0	504	44485	258721	87844
UK09	Se_BPH0752	Illumina NextSeq	2226326	329683200	33.8	30	148	151	151	33	31.3	115x	SPAdes v 3.7.1	69	2720313	2720313	0	0	504	39424	206743	86643
UK10	Se_BPH0753	Illumina NextSeq	2291640	341310543	33.5	30	148	151	151	33	31.4	120x	SPAdes v 3.7.1	57	2681833	2681833	0	0	504	47049	278239	87843
UK11	Se_BPH0754	Illumina NextSeq	2676064	395191323	33.7	30	147	151	151	33	31.7	138x	SPAdes v 3.7.1	60	2692275	2692275	0	0	504	44871	271124	87892
UK12	Se_BPH0755	Illumina NextSeq	2297576	340782858	33.6	30	148	151	151	33	31.7	119x	SPAdes v 3.7.1	54	2682944	2682944	0	0	504	49684	278415	96186
AUS01	Se_BPH0771	Illumina NextSeq	2385280	351548176	34.6	34	147	151	151	33	32.2	123x	SPAdes v 3.7.1	69	2730295	2730295	0	0	504	39569	224889	87706
AUS02	Se_BPH0781	Illumina NextSeq	1519582	224559683	34	30	147	151	151	33	32	79x	SPAdes v 3.7.1	100	2703930	2703930	0	0	504	27039	243523	71289
AUS03	Se_BPH0794	Illumina NextSeq	1822170	268190471	34	35	147	151	151	33	32	94x	SPAdes v 3.7.1	70	2738198	2738198	0	0	504	39117	198667	87844
AUS04	Se_BPH0795	Illumina NextSeq	2109178	312646524	34.2	31	148	151	151	33	32.1	110x	SPAdes v 3.7.1	81	2743682	2743682	0	0	504	33872	189912	81415
AUS05	Se_BPH0801	Illumina NextSeq	2359544	345554685	34.5	31	146	151	151	33	32.2	121x	SPAdes v 3.7.1	48	2536611	2536611	0	0	504	52846	336286	105665
AUS06	Se_BPH0808	Illumina NextSeq	1782990	263482646	34.1	35	147	151	151	33	32.1	92x	SPAdes v 3.7.1	66	2735665	2735665	0	0	504	41449	216484	89124
AUS07	Se_BPH0809	Illumina NextSeq	2659468	396878691	33.5	35	149	151	151	33	32.2	139x	SPAdes v 3.7.1	34	2529432	2529432	0	0	504	74395	400545	149750
AUS08	Se_BPH0813	Illumina NextSeq	2139970	313670128	34	30	146	151	151	33	32	110x	SPAdes v 3.7.1	71	2741885	2741885	0	0	504	38618	271177	81415
AUS09	Se_BPH0816	Illumina NextSeq	2565138	376499431	34	35	146	151	151	33	31.6	132x	SPAdes v 3.7.1	39	2507819	2507819	0	0	504	64303	399536	121068
AUS10	Se_BPH0822	Illumina NextSeq	1836098	271586493	34.1	35	147	151	151	33	32	95x	SPAdes v 3.7.1	72	2705757	2705757	0	0	504	37579	278368	67964
BPH0823	Se_BPH0823	Illumina NextSeq	2154054	320230890	33.8	34	148	151	151	33	32.7	112x	SPAdes v 3.7.1	44	2479106	2479106	0	0	505	56343	517154	103250
BPH0825	Se_BPH0825	Illumina NextSeq	2082128	309848273	33.6	35	148	151	151	33	32.4	109x	SPAdes v 3.7.1	44	2465604	2465604	0	0	505	56036	301356	135443
DEN12	Se_BPH0828	Illumina NextSeq	2837482	419604669	34.4	32	147	151	151	33	32.4	147x	SPAdes v 3.7.1	40	2434407	2434407	0	0	505	60860	312073	135242
DEN13	Se_BPH0829	Illumina NextSeq	2540318	377828997	34.4	35	148	151	151	33	32.2	132x	SPAdes v 3.7.1	36	2427005	2427005	0	0	505	67416	312074	135242
DEN14	Se_BPH0830	Illumina NextSeq	2475628	368476427	34.2	33	148	151	151	33	32.3	129x	SPAdes v 3.7.1	44	2476150	2476150	0	0	505	56276	253853	132293
DEN15	Se_BPH0831	Illumina NextSeq	2473464	368100136	34.4	31	148	151	151	33	32.2	129x	SPAdes v 3.7.1	47	2528153	2528153	0	0	504	53790	204629	119372
DEN16	Se_BPH0832	Illumina NextSeq	2881560	429935592	34.3	31	149	151	151	33	32.2	151x	SPAdes v 3.7.1	40	2432873	2432873	0	0	505	60821	312073	135242
DEN17	Se_BPH0833	Illumina NextSeq	2591360	386161991	34	30	149	151	151	33	32.2	135x	SPAdes v 3.7.1	59	2649968	2649968	0	0	504	44914	271178	89190
DEN18	Se_BPH0834	Illumina NextSeq	2435382	364024969	34.3	31	149	151	151	33	32.1	128x	SPAdes v 3.7.1	48	2446777	2446777	0	0	505	50974	233694	101958
DEN19	Se_BPH0835	Illumina NextSeq	2599396	386932127	34.5	31	148	151	151	33	32.2	136x	SPAdes v 3.7.1	40	2419188	2419188	0	0	505	60479	312074	159828
DEN20	Se_BPH0836	Illumina NextSeq	2748154	409115434	34.5	31	148	151	151	33	32.4	143x	SPAdes v 3.7.1	48	2469327	2469327	0	0	505	51444	304175	167627
DEN21	Se_BPH0837	Illumina NextSeq	2490922	372062764	34.2	31	149	151	151	33	32.1	130x	SPAdes v 3.7.1	56	2482802	2482802	0	0	505	44335	311623	86554
DEN22	Se_BPH0838	Illumina NextSeq	2657968	395074549	34.3	31	148	151	151	33	32.4	138x	SPAdes v 3.7.1	44	2440210	2440210	0	0	505	55459	209165	110289
DEN23	Se_BPH0839	Illumina NextSeq	2570788	382443409	34.3	31	148	151	151	33	32.4	134x	SPAdes v 3.7.1	52	2497395	2497395	0	0	505	48026	326193	96492
DEN24	Se_BPH0840	Illumina NextSeq	2713246	404152518	34	31	148	151	151	33	32.5	142x	SPAdes v 3.7.1	38	2424769	2424769	0	0	690	63809	312074	140550
DEN25	Se_BPH0843	Illumina NextSeq	2448160	364803249	34.4	32	149	151	151	33	32.2	128x	SPAdes v 3.7.1	41	2412549	2412549	0	0	505	58842	312074	135184
DEN26	Se_BPH0844	Illumina NextSeq	2675086	397303005	34.4	31	148	151	151	33	32.5	139x	SPAdes v 3.7.1	43	2382297	2382297	0	0	505	55402	293658	132411
DEN27	Se_BPH0845	Illumina NextSeq	2559386	380142217	34.5	30	148	151	151	33	32.5	133x	SPAdes v 3.7.1	40	2411087	2411087	0	0	505	60277	312074	135184
DEN28	Se_BPH0846	Illumina NextSeq	2842596	423103139	34.2	31	148	151	151	33	32.4	148x	SPAdes v 3.7.1	59	2696787	2696787	0	0	504	45708	271177	89480
DEN29	Se_BPH0847	Illumina NextSeq	3135600	464709080	34.3	32	148	151	151	33	32.5	163x	SPAdes v 3.7.1	37	2396044	2396044	0	0	505	64757	312074	135184
DEN30	Se_BPH0848	Illumina NextSeq	2651260	395673185	34.3	31	149	151	151	33	32.3	139x	SPAdes v 3.7.1	46	2438606	2438606	0	0	505	53013	312073	99936
DEN31	Se_BPH0849	Illumina NextSeq	2617698	390999698	33.7	31	149	151	151	33	32.3	137x	SPAdes v 3.7.1	47	2469009	2469009	0	0	505	52532	312073	110314
DEN32	Se_BPH0850	Illumina NextSeq	2756734	412141320	34.1	31	149	151	151	33	32.4	144x	SPAdes v 3.7.1	34	2396362	2396362	0	0	505	70481	312073	147572
DEN33	Se_BPH0851	Illumina NextSeq	2877072	427219393	34.6	30	148	151	151	33	32.4	150x	SPAdes v 3.7.1	46	2429980	2429980	0	0	505	52825	312073	126616
DEN34	Se_BPH0852	Illumina NextSeq	2788308	415921101	34.3	30	149	151	151	33	32.3	146x	SPAdes v 3.7.1	45	2434874	2434874	0	0	505	54108	311800	121117
DEN35	Se_BPH0853	Illumina NextSeq	2883036	429161169	34.3	31	148	151	151	33	32.4	150x	SPAdes v 3.7.1	48	2468769	2468769	0	0	505	51432	311493	101128
DEN36	Se_BPH0854	Illumina NextSeq	2548596	380360966	34.3	33	149	151	151	33	32.3	133x	SPAdes v 3.7.1	49	2461606	2461606	0	0	505	50236	179854	126495
DEN37	Se_BPH0855	Illumina NextSeq	2651136	394936984	34.3	31	148	151	151	33	32.5	138x	SPAdes v 3.7.1	49	2402986	2402986	0	0	505	49040	284645	125903
DEN38	Se_BPH0856	Illumina NextSeq	2528998	378485583	33.8	31	149	151	151	33	32.2	133x	SPAdes v 3.7.1	41	2523265	2523265	0	0	504	61543	358534	127467
US01	Se_BPH0857	Illumina NextSeq	2777582	413821259	34	35	148	151	151	33	32.2	145x	SPAdes v 3.7.1	48	2580668	2580668	0	0	504	53763	327207	119449
US02	Se_BPH0858	Illumina NextSeq	3227762	477888853	34.5	35	148	151	151	33	32.6	167x	SPAdes v 3.7.1	46	2430688	2430688	0	0	505	52841	312074	135242
US03	Se_BPH0859	Illumina NextSeq	3022146	451038770	34.5	31	149	151	151	33	32.3	158x	SPAdes v 3.7.1	48	2411859	2411859	0	0	505	50247	312073	132411
US04	Se_BPH0860	Illumina NextSeq	3168028	469953795	34.3	31	148	151	151	33	32.4	165x	SPAdes v 3.7.1	50	2447782	2447782	0	0	505	48955	284759	101958
US05	Se_BPH0861	Illumina NextSeq	2886082	430090187	34.6	31	149	151	151	33	32.4	151x	SPAdes v 3.7.1	59	2522919	2522919	0	0	504	42761	295707	94761
US06	Se_BPH0863	Illumina NextSeq	2718122	405381985	34.2	30	149	151	151	33	32.3	142x	SPAdes v 3.7.1	57	2424950	2424950	0	0	501	42542	454415	110262
US07	Se_BPH0865	Illumina NextSeq	3293888	488572172	34.6	31	148	151	151	33	32.6	171x	SPAdes v 3.7.1	77	2487108	2487108	0	0	505	32300	312074	89541
US08	Se_BPH0866	Illumina NextSeq	3072148	456706318	34.5	31	148	151	151	33	32.5	160x	SPAdes v 3.7.1	56	2465768	2465768	0	0	504	44031	518820	89617
US09	Se_BPH0867	Illumina NextSeq	3121222	464058730	34.2	31	148	151	151	33	32.5	163x	SPAdes v 3.7.1	52	2458006	2458006	0	0	505	47269	312074	128279
US10	Se_BPH0868	Illumina NextSeq	2845616	424287006	34.2	31	149	151	151	33	32.3	149x	SPAdes v 3.7.1	35	2493250	2493250	0	0	504	71235	416140	135409
US11	Se_BPH0869	Illumina NextSeq	2971958	443332416	34.6	32	149	151	151	33	32.3	155x	SPAdes v 3.7.1	57	2443265	2443265	0	0	504	42864	317713	119831
US12	Se_BPH0870	Illumina NextSeq	2705922	404535415	34.3	31	149	151	151	33	32.3	142x	SPAdes v 3.7.1	48	2566158	2566158	0	0	504	53461	295685	105919
GER01	Se_BPH0871	Illumina NextSeq	2822118	421088054	34.4	35	149	151	151	33	32.6	148x	SPAdes v 3.7.1	58	2752792	2752792	0	0	504	47461	218102	102449
GER02	Se_BPH0872	Illumina NextSeq	3039274	451591341	34.1	35	148	151	151	33	32.8	158x	SPAdes v 3.7.1	60	2713323	2713323	0	0	504	45222	218102	120911
GER03	Se_BPH0873	Illumina NextSeq	2904094	433073195	34.2	35	149	151	151	33	32.8	152x	SPAdes v 3.7.1	51	2659430	2659430	0	0	504	52145	195017	138720
GER04	Se_BPH0874	Illumina NextSeq	3102848	457230010	34.4	31	147	151	151	33	32.8	160x	SPAdes v 3.7.1	57	2669890	2669890	0	0	504	46840	325760	95622
GER05	Se_BPH0875	Illumina NextSeq	3085880	459272396	34.5	33	148	151	151	33	32.8	161x	SPAdes v 3.7.1	50	2655191	2655191	0	0	504	53103	181059	102727
GER06	Se_BPH0876	Illumina NextSeq	3039314	453543211	34.5	35	149	151	151	33	32.7	159x	SPAdes v 3.7.1	53	2674721	2674721	0	0	504	50466	205706	108549
GER07	Se_BPH0877	Illumina NextSeq	3007078	448164587	34.3	34	149	151	151	33	32.7	157x	SPAdes v 3.7.1	65	2551270	2551270	0	0	504	39250	307830	105994
GER08	Se_BPH0879	Illumina NextSeq	3272570	487991669	34.4	35	149	151	151	33	32.7	171x	SPAdes v 3.7.1	46	2442017	2442017	0	0	505	53087	312037	123457
GER09	Se_BPH0880	Illumina NextSeq	3256654	485711249	34.3	35	149	151	151	33	32.8	170x	SPAdes v 3.7.1	61	2718268	2718268	0	0	505	44561	218102	133629
GER10	Se_BPH0881	Illumina NextSeq	2932278	438349221	34.3	35	149	151	151	33	32.7	154x	SPAdes v 3.7.1	67	2514204	2514204	0	0	504	37525	327001	94312
GER11	Se_BPH0882	Illumina NextSeq	2893624	428609259	34.4	35	148	151	151	33	32.8	150x	SPAdes v 3.7.1	62	2661902	2661902	0	0	504	42933	332745	98849

AUT01	Se_BPH0883	Illumina NextSeq	3342906	499587035	34.3	35	149	151	151	33	32.7	175x	SPAdes v.3.7.1	47	2437347	2437347	0	0	505	51858	256374	124013
GER12	Se_BPH0884	Illumina NextSeq	2976034	442624493	34.2	34	148	151	151	33	32.8	155x	SPAdes v.3.7.1	68	2720897	2720897	0	0	511	40013	197660	135638
GER13	Se_BPH0885	Illumina NextSeq	2837442	421930354	34.2	35	148	151	151	33	32.7	148x	SPAdes v.3.7.1	47	2554407	2554407	0	0	505	54349	284741	110262
GER14	Se_BPH0886	Illumina NextSeq	3009962	447922387	34	30	148	151	151	33	32.8	157x	SPAdes v.3.7.1	58	2713035	2713035	0	0	504	46776	250180	87770
GER15	Se_BPH0887	Illumina NextSeq	2664306	398610363	34.1	35	149	151	151	33	32.5	140x	SPAdes v.3.7.1	51	2522925	2522925	0	0	505	49469	311791	127298
GER16	Se_BPH0888	Illumina NextSeq	3037658	432323569	34.6	31	142	151	151	33	33.6	151x	SPAdes v.3.7.1	63	2720706	2720706	0	0	504	43185	197660	91884
GER17	Se_BPH0889	Illumina NextSeq	3166360	461035723	34.5	32	145	151	151	33	33.5	161x	SPAdes v.3.7.1	78	2735622	2735622	0	0	516	35072	197957	93848
GER18	Se_BPH0890	Illumina NextSeq	3919870	572987000	34.5	32	146	151	151	33	33.5	201x	SPAdes v.3.7.1	64	2720269	2720269	0	0	504	42504	218102	128561
GER19	Se_BPH0891	Illumina NextSeq	3490260	511759547	34.8	30	146	151	151	33	33.4	179x	SPAdes v.3.7.1	45	2378699	2378699	0	0	505	52859	311794	93692
GER20	Se_BPH0892	Illumina NextSeq	3622578	530330590	34.4	32	146	151	151	33	33.4	186x	SPAdes v.3.7.1	59	2675107	2675107	0	0	538	45340	218102	120911
GER21	Se_BPH0893	Illumina NextSeq	3788906	557719288	34.7	30	147	151	151	33	33.4	195x	SPAdes v.3.7.1	49	2456280	2456280	0	0	504	50128	508561	119214
BEL01	Se_BPH0894	Illumina NextSeq	3164730	470098687	34.5	35	148	151	151	33	32.8	165x	SPAdes v.3.7.1	46	2633563	2633563	0	0	504	57251	347918	122230
BEL02	Se_BPH0895	Illumina NextSeq	2963000	442409395	34.5	34	149	151	151	33	32.9	155x	SPAdes v.3.7.1	54	2474149	2474149	0	0	505	45817	187630	83750
BEL03	Se_BPH0896	Illumina NextSeq	2830850	422417520	34.6	35	149	151	151	33	32.9	148x	SPAdes v.3.7.1	52	2481971	2481971	0	0	505	47730	311800	101894
BEL04	Se_BPH0897	Illumina NextSeq	2794172	416850347	34.4	32	149	151	151	33	32.8	146x	SPAdes v.3.7.1	58	2692004	2692004	0	0	504	46413	432938	95232
BEL05	Se_BPH0898	Illumina NextSeq	2958794	440697033	34.7	34	148	151	151	33	32.8	154x	SPAdes v.3.7.1	65	2530912	2530912	0	0	505	38937	292606	93906
BEL06	Se_BPH0899	Illumina NextSeq	3184588	468770446	34.9	35	147	151	151	33	32.9	164x	SPAdes v.3.7.1	66	2503702	2503702	0	0	505	37934	518793	77250
BEL07	Se_BPH0900	Illumina NextSeq	4426896	656271122	34.5	33	148	151	151	33	32.8	230x	SPAdes v.3.7.1	54	2731325	2731325	0	0	504	50580	279164	93691
BEL08	Se_BPH0901	Illumina NextSeq	3063972	455640553	34.5	35	148	151	151	33	32.8	160x	SPAdes v.3.7.1	62	2711691	2711691	0	0	504	43736	278401	87844
BEL09	Se_BPH0902	Illumina NextSeq	2924560	435328257	34.3	35	148	151	151	33	32.7	152x	SPAdes v.3.7.1	44	2657707	2657707	0	0	505	60402	428152	138894
BEL10	Se_BPH0903	Illumina NextSeq	3418134	509328174	34.4	35	149	151	151	33	32.8	178x	SPAdes v.3.7.1	60	2690938	2690938	0	0	504	44848	275428	111758
BEL11	Se_BPH0904	Illumina NextSeq	2922906	435836889	34.5	35	149	151	151	33	32.7	153x	SPAdes v.3.7.1	49	2683002	2683002	0	0	530	54755	428062	130745
BEL12	Se_BPH0905	Illumina NextSeq	3091096	460044912	34.4	31	148	151	151	33	32.7	161x	SPAdes v.3.7.1	66	2763845	2763845	0	0	504	41876	258720	87652
BEL13	Se_BPH0906	Illumina NextSeq	3066090	457459402	34.4	34	149	151	151	33	32.6	160x	SPAdes v.3.7.1	58	2691661	2691661	0	0	504	46407	347540	95232
BEL14	Se_BPH0907	Illumina NextSeq	3234392	477595304	34.6	34	147	151	151	33	32.9	167x	SPAdes v.3.7.1	55	2579273	2579273	0	0	504	46895	258810	93692
BEL15	Se_BPH0908	Illumina NextSeq	2943244	437334462	34.4	30	148	151	151	33	32.8	153x	SPAdes v.3.7.1	64	2766283	2766283	0	0	504	43223	442493	86931
BEL16	Se_BPH0909	Illumina NextSeq	2895484	431132104	34.4	33	148	151	151	33	32.7	151x	SPAdes v.3.7.1	62	2732594	2732594	0	0	504	44074	380990	84314
BEL17	Se_BPH0910	Illumina NextSeq	2814478	418733161	34.4	35	148	151	151	33	32.7	147x	SPAdes v.3.7.1	52	2465778	2465778	0	0	505	47418	311824	119454
BEL18	Se_BPH0911	Illumina NextSeq	3120474	465614020	34.5	31	149	151	151	33	32.8	163x	SPAdes v.3.7.1	60	2690970	2690970	0	0	504	44849	292612	95232
DEN01	Se_BPH0912	Illumina NextSeq	3919816	574062164	34.7	31	146	151	151	33	33.4	201x	SPAdes v.3.7.1	51	2671450	2671450	0	0	504	52381	271972	89615
DEN02	Se_BPH0913	Illumina NextSeq	3323870	486283752	34.7	31	146	151	151	33	33.5	170x	SPAdes v.3.7.1	52	2462921	2462921	0	0	505	47363	292515	110226
DEN03	Se_BPH0914	Illumina NextSeq	3523448	511200830	34.6	32	145	151	151	33	33.4	179x	SPAdes v.3.7.1	59	2708030	2708030	0	0	504	45898	325826	120915
DEN04	Se_BPH0916	Illumina NextSeq	3675098	542249433	34.6	30	147	151	151	33	33.2	190x	SPAdes v.3.7.1	60	2737015	2737015	0	0	504	45616	279162	86071
DEN05	Se_BPH0917	Illumina NextSeq	2855514	420801529	34.5	33	147	151	151	33	33.3	147x	SPAdes v.3.7.1	60	2673413	2673413	0	0	504	44556	271176	95344
DEN06	Se_BPH0918	Illumina NextSeq	3249028	479325679	34.6	30	147	151	151	33	33.3	168x	SPAdes v.3.7.1	63	2770264	2770264	0	0	538	43972	279162	87607
DEN07	Se_BPH0919	Illumina NextSeq	3546600	522250739	34.5	30	147	151	151	33	33.3	183x	SPAdes v.3.7.1	59	2784081	2784081	0	0	504	47187	442492	95707
DEN08	Se_BPH0920	Illumina NextSeq	3228028	475514815	34.5	30	147	151	151	33	33.3	167x	SPAdes v.3.7.1	64	2784498	2784498	0	0	504	43507	380693	93692
DEN09	Se_BPH0921	Illumina NextSeq	3329742	489409154	34.6	30	146	151	151	33	33.4	171x	SPAdes v.3.7.1	43	2447042	2447042	0	0	505	56907	311799	132116
DEN10	Se_BPH0922	Illumina NextSeq	3500388	511908359	34.9	32	146	151	151	33	33.4	179x	SPAdes v.3.7.1	54	2721496	2721496	0	0	504	50398	277548	84301
DEN11	Se_BPH0923	Illumina NextSeq	4550982	671355582	34.7	30	147	151	151	33	33.2	235x	SPAdes v.3.7.1	45	2435777	2435777	0	0	505	54128	311791	132411
FRA01	Se_BPH0924	Illumina NextSeq	3591008	530256538	34.7	33	147	151	151	33	33.4	186x	SPAdes v.3.7.1	51	2447873	2447873	0	0	505	47997	226722	102451
FRA02	Se_BPH0925	Illumina NextSeq	2567030	379032894	34.6	31	147	151	151	33	33.2	133x	SPAdes v.3.7.1	73	2526560	2526560	0	0	505	34610	222983	93796
FRA03	Se_BPH0926	Illumina NextSeq	3131680	463900439	34.8	30	148	151	151	33	33.2	163x	SPAdes v.3.7.1	41	2540685	2540685	0	0	504	61967	180843	136045
FRA04	Se_BPH0927	Illumina NextSeq	3247290	479725350	34.8	31	147	151	151	33	33.3	168x	SPAdes v.3.7.1	45	2390449	2390449	0	0	504	53121	518824	119185
FRA05	Se_BPH0928	Illumina NextSeq	3297766	485292636	34.7	32	147	151	151	33	33.3	170x	SPAdes v.3.7.1	55	2685276	2685276	0	0	504	48823	457057	108643
FRA06	Se_BPH0929	Illumina NextSeq	3598432	528881688	34.7	32	146	151	151	33	33.3	185x	SPAdes v.3.7.1	51	2464346	2464346	0	0	505	48320	309204	118563
FRA07	Se_BPH0930	Illumina NextSeq	3512916	510011869	34.8	31	145	151	151	33	33.5	179x	SPAdes v.3.7.1	49	2448829	2448829	0	0	659	49976	200560	125688
FRA08	Se_BPH0931	Illumina NextSeq	3850302	568935314	34.6	30	147	151	151	33	33.2	199x	SPAdes v.3.7.1	50	2501798	2501798	0	0	504	50035	384502	115737
FRA09	Se_BPH0933	Illumina NextSeq	3210312	475421596	34.5	30	148	151	151	33	33	167x	SPAdes v.3.7.1	63	2624184	2624184	0	0	504	41653	291396	113717
FRA10	Se_BPH0934	Illumina NextSeq	3373502	499054546	34.6	31	147	151	151	33	33.3	175x	SPAdes v.3.7.1	53	2680405	2680405	0	0	504	50573	316507	131743
FRA11	Se_BPH0935	Illumina NextSeq	3521188	522354614	33.9	33	148	151	151	33	33.1	183x	SPAdes v.3.7.1	59	2677827	2677827	0	0	504	45386	456779	93692
FRA12	Se_BPH0936	Illumina NextSeq	3520988	518547250	34.4	35	147	151	151	33	33.3	182x	SPAdes v.3.7.1	57	2645177	2645177	0	0	538	46406	197581	118997
FRA13	Se_BPH0937	Illumina NextSeq	3533890	519708750	34.7	30	147	151	151	33	33.3	182x	SPAdes v.3.7.1	54	2591569	2591569	0	0	504	47992	292432	143104
FRA14	Se_BPH0938	Illumina NextSeq	3590122	524360963	34.7	30	146	151	151	33	33.4	184x	SPAdes v.3.7.1	43	2438291	2438291	0	0	505	56704	311801	135241
FRA15	Se_BPH0940	Illumina NextSeq	3689046	543346670	34.6	30	147	151	151	33	33.2	190x	SPAdes v.3.7.1	55	2658571	2658571	0	0	504	48337	216817	120910
FRA16	Se_BPH0941	Illumina NextSeq	3133532	466112577	33.9	30	148	151	151	33	33.1	163x	SPAdes v.3.7.1	47	2477554	2477554	0	0	505	52713	262043	136833
FRA17	Se_BPH0942	Illumina NextSeq	3180380	473145496	34.6	30	148	151	151	33	33.1	166x	SPAdes v.3.7.1	41	2451511	2451511	0	0	505	59792	311799	135144
FRA18	Se_BPH0943	Illumina NextSeq	3144828	466578969	34.6	30	148	151	151	33	33	163x	SPAdes v.3.7.1	47	2552577	2552577	0	0	504	54310	412604	158381
RP62a-rpoB662	Se_RP62a-rpoB662	Illumina NextSeq	1429684	316249401	35.2	35	221	301	301	33	36.8	111x	SPAdes v.3.7.1	107	2584349	2584349	0	0	504	24152	186870	52593
RP62a	Se_RP62a-WT	Illumina NextSeq	2020500	466405092	35.5	35	230	301	301	33	36.4	163x	SPAdes v.3.7.1	91	2576729	2576729	0	0	501	28315	186870	76214

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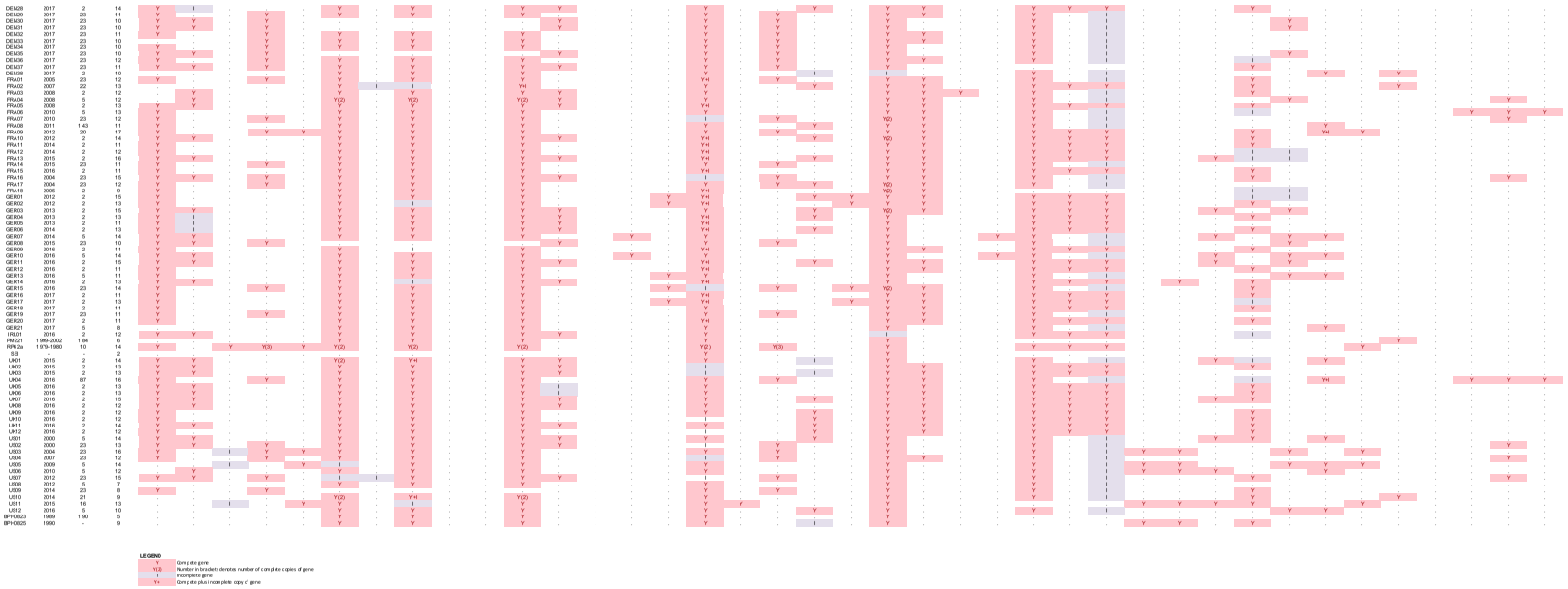

LEGEND

Y	Complete gene
Y(2)	Number in brackets denotes number of complete copies of gene
I	Incomplete gene
Y+I	Complete plus incomplete copy of gene

ISOLATE	BIOSAMPLE	SAMPLE NAME	SRA	ORGANISM	STRAIN	COLLECTED BY	COLLECTION DATE	GEOGRAPHIC LOCATION	ISOLATION SOURCE	LATITUDE & LONGITUDE	HOST	HOST DISEASE	ACCESSION	ID	SEQUENCING METHOD	READS	YIELD	GC	MINLEN	AVGLEN	MODELEN	MAXLEN	AVGQUAL	DEPTH	ASSEMBLER	CONTIGS	BP	OK	Ns	GAPS	MIN	AVG	MAX	N50	Reference	DOI
100_SEPI	SAMN03196961	100_SEPI	SRS746168	Staphylococcus epidermidis	100_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196961	3196961	Illumina Hiseq	1470304	137671202	31.8	19	93	101	101	36	48	SPAdes v3.7.1	78	2712016	2712016	0	0	201	34769	191611	73522	Roach et al., 2015	10.1371/journal.pgen.1005413
101_SEPI	SAMN03196971	101_SEPI	SRS746179	Staphylococcus epidermidis	101_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196971	3196971	Illumina Hiseq	1415016	134256554	31.9	19	94	101	101	35.3	47	SPAdes v3.7.1	100	2627628	2627628	0	0	324	26276	177746	64643	Roach et al., 2015	10.1371/journal.pgen.1005413
1013_SEPI	SAMN03196975	1013_SEPI	SRS746184	Staphylococcus epidermidis	1013_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196975	3196975	Illumina Hiseq	1457560	127076805	33.1	8	87	101	101	33.9	45	SPAdes v3.7.1	101	2606122	2606122	0	0	319	25803	206454	59071	Roach et al., 2015	10.1371/journal.pgen.1005413
102_SEPI	SAMN03196982	102_SEPI	SRS746192	Staphylococcus epidermidis	102_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196982	3196982	Illumina Hiseq	1523558	142081238	31.8	19	93	101	101	35.9	50	SPAdes v3.7.1	72	2704030	2704030	0	0	203	37555	208811	81893	Roach et al., 2015	10.1371/journal.pgen.1005413
1021.rep1_SEPI	SAMN03196984	1021.rep1_SEPI	SRS746191	Staphylococcus epidermidis	1021.rep1_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196984	3196984	Illumina Hiseq	1614092	137918480	33.1	8	85	101	101	34	49	SPAdes v3.7.1	105	2606186	2606186	0	0	335	24820	227697	59082	Roach et al., 2015	10.1371/journal.pgen.1005413
1022_SEPI	SAMN03196986	1022_SEPI	SRS746194	Staphylococcus epidermidis	1022_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196986	3196986	Illumina Hiseq	2256332	192910230	33	8	85	101	101	34.5	68	SPAdes v3.7.1	126	2611572	2611572	0	0	329	20726	206538	57923	Roach et al., 2015	10.1371/journal.pgen.1005413
1023_SEPI	SAMN03196987	1023_SEPI	SRS746195	Staphylococcus epidermidis	1023_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196987	3196987	Illumina Hiseq	1881180	162535076	32.9	8	86	101	101	34.4	57	SPAdes v3.7.1	100	2542191	2542191	0	0	320	25421	196625	71805	Roach et al., 2015	10.1371/journal.pgen.1005413
1026_SEPI	SAMN03196990	1026_SEPI	SRS746198	Staphylococcus epidermidis	1026_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03196990	3196990	Illumina Hiseq	1180544	103384001	32.9	8	87	101	101	34.6	36	SPAdes v3.7.1	77	2555301	2555301	0	0	316	33185	227103	71686	Roach et al., 2015	10.1371/journal.pgen.1005413
1040_SEPI	SAMN03197006	1040_SEPI	SRS746213	Staphylococcus epidermidis	1040_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197006	3197006	Illumina Hiseq	1896172	164176780	33	8	86	101	101	34.8	58	SPAdes v3.7.1	113	2611226	2611226	0	0	316	23108	177766	64674	Roach et al., 2015	10.1371/journal.pgen.1005413
1067_SEPI	SAMN03197029	1067_SEPI	SRS746237	Staphylococcus epidermidis	1067_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197029	3197029	Illumina Hiseq	1854226	159906388	33	8	86	101	101	34.1	56	SPAdes v3.7.1	97	2459827	2459827	0	0	314	25359	198471	81463	Roach et al., 2015	10.1371/journal.pgen.1005413
1088_SEPI	SAMN03197050	1088_SEPI	SRS746258	Staphylococcus epidermidis	1088_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197050	3197050	Illumina Hiseq	1645192	142966913	33	8	86	101	101	34.4	50	SPAdes v3.7.1	123	2566325	2566325	0	0	321	20864	208269	58577	Roach et al., 2015	10.1371/journal.pgen.1005413
1097_SEPI	SAMN03197059	1097_SEPI	SRS746267	Staphylococcus epidermidis	1097_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197059	3197059	Illumina Hiseq	1480548	129039475	33.1	8	87	101	101	34.1	45	SPAdes v3.7.1	87	2449985	2449985	0	0	338	28160	189678	76015	Roach et al., 2015	10.1371/journal.pgen.1005413
1115_SEPI	SAMN03197069	1115_SEPI	SRS746277	Staphylococcus epidermidis	1115_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197069	3197069	Illumina Hiseq	1794454	154746779	33.1	8	86	101	101	34.6	54	SPAdes v3.7.1	92	2442175	2442175	0	0	323	26545	157038	63100	Roach et al., 2015	10.1371/journal.pgen.1005413
1129_SEPI	SAMN03197083	1129_SEPI	SRS746291	Staphylococcus epidermidis	1129_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197083	3197083	Illumina Hiseq	979878	93457829	32.3	19	95	101	101	36.3	33	SPAdes v3.7.1	60	2511785	2511785	0	0	321	41863	196263	82543	Roach et al., 2015	10.1371/journal.pgen.1005413
1142_SEPI	SAMN03197098	1142_SEPI	SRS746305	Staphylococcus epidermidis	1142_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197098	3197098	Illumina Hiseq	899214	85910372	32.2	19	95	101	101	36.3	30	SPAdes v3.7.1	89	2489839	2489839	0	0	317	27975	233894	76908	Roach et al., 2015	10.1371/journal.pgen.1005413
1188_SEPI	SAMN03197147	1188_SEPI	SRS746355	Staphylococcus epidermidis	1188_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197147	3197147	Illumina Hiseq	1259430	119219979	32.2	19	94	101	101	36.2	42	SPAdes v3.7.1	70	2476525	2476525	0	0	328	35378	253806	82440	Roach et al., 2015	10.1371/journal.pgen.1005413
1190_SEPI	SAMN03197150	1190_SEPI	SRS746357	Staphylococcus epidermidis	1190_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197150	3197150	Illumina Hiseq	1149722	109541947	32	19	95	101	101	36.5	39	SPAdes v3.7.1	83	2747960	2747960	0	0	317	33107	189811	75077	Roach et al., 2015	10.1371/journal.pgen.1005413
1191_SEPI	SAMN03197151	1191_SEPI	SRS746359	Staphylococcus epidermidis	1191_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197151	3197151	Illumina Hiseq	1183788	112463290	32	19	95	101	101	36.3	40	SPAdes v3.7.1	78	2542915	2542915	0	0	335	32601	196685	75070	Roach et al., 2015	10.1371/journal.pgen.1005413
1226_SEPI	SAMN03197190	1226_SEPI	SRS746397	Staphylococcus epidermidis	1226_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197190	3197190	Illumina Hiseq	1111406	106006779	32.2	19	95	101	101	36.5	37	SPAdes v3.7.1	86	2523111	2523111	0	0	320	29338	177532	78593	Roach et al., 2015	10.1371/journal.pgen.1005413
1284_SEPI	SAMN03197255	1284_SEPI	SRS746463	Staphylococcus epidermidis	1284_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197255	3197255	Illumina Hiseq	979684	93539793	32.1	19	95	101	101	36.3	33	SPAdes v3.7.1	65	2466656	2466656	0	0	332	37948	268773	79380	Roach et al., 2015	10.1371/journal.pgen.1005413
1289_SEPI	SAMN03197260	1289_SEPI	SRS746467	Staphylococcus epidermidis	1289_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197260	3197260	Illumina Hiseq	1015442	96813478	32.2	19	95	101	101	36.5	34	SPAdes v3.7.1	67	2422096	2422096	0	0	208	36150	233757	75570	Roach et al., 2015	10.1371/journal.pgen.1005413
1295_SEPI	SAMN03197267	1295_SEPI	SRS746475	Staphylococcus epidermidis	1295_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197267	3197267	Illumina Hiseq	968766	92724819	32.1	19	95	101	101	36.5	33	SPAdes v3.7.1	80	2603330	2603330	0	0	318	32541	196342	73147	Roach et al., 2015	10.1371/journal.pgen.1005413
1296_SEPI	SAMN03197268	1296_SEPI	SRS746476	Staphylococcus epidermidis	1296_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197268	3197268	Illumina Hiseq	1017942	96839330	33.8	19	95	101	101	36.3	34	SPAdes v3.7.1	235	2495643	2495643	0	0	314	10619	233872	67393	Roach et al., 2015	10.1371/journal.pgen.1005413
1306.rep2_SEPI	SAMN03197281	1306.rep2_SEPI	SRS746488	Staphylococcus epidermidis	1306.rep2_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197281	3197281	Illumina Hiseq	999056	95076568	32.2	19	95	101	101	36.4	33	SPAdes v3.7.1	72	2425396	2425396	0	0	350	33686	252948	70327	Roach et al., 2015	10.1371/journal.pgen.1005413
248_DEN19	SAMN05207190	248	SRS1525994	Staphylococcus epidermidis	DEN19	ITQB	1997	Denmark	unknown	55.67 N 12.57 E	Homo sapiens	Infection	SAMN05207190	5207190	Illumina Hiseq	3276956	327695600	31.3	100	100	100	100	34.9	115	SPAdes v3.7.1	92	2482742	2482742	0	0	224	26986	184839	70571	Meric et al., 2015	10.1093/gbe/evv066
249_ICE18	SAMN05207191	249	SRS1525993	Staphylococcus epidermidis	ICE18	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207191	5207191	Illumina Hiseq	4319056	431905600	32	100	100	100	100	35.8	152	SPAdes v3.7.1	185	2527316	2527316	0	0	201	13661	198502	57394	Meric et al., 2015	10.1093/gbe/evv066
250_ICE7	SAMN05207192	250	SRS1526005	Staphylococcus epidermidis	ICE7	ITQB	1997	Iceland	Blood	64.13 N 21.82 W	Homo sapiens	Bacteraemia	SAMN05207192	5207192	Illumina Hiseq	3172982	317298200	32.5	100	100	100	100	35.2	112	SPAdes v3.7.1	125	2703929	2703929	0	0	206	21631	177404	65973	Meric et al., 2015	10.1093/gbe/evv066
251_1347N	SAMN05207193	251	SRS1526017	Staphylococcus epidermidis	1347N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207193	5207193	Illumina Hiseq	2211012	221101200	32.3	100	100	100	100	33.8	78	SPAdes v3.7.1	99	2424659	2424659	0	0	313	24491	198492	81635	Meric et al., 2015	10.1093/gbe/evv066
252_DEN22	SAMN05207194	252	SRS1526027	Staphylococcus epidermidis	DEN22	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207194	5207194	Illumina Hiseq	3409886	340988600	32.3	100	100	100	100	35.1	120	SPAdes v3.7.1	116	2490903	2490903	0	0	233	21473	207847	58821	Meric et al., 2015	10.1093/gbe/evv066
253_ICE192	SAMN05207195	253	SRS1526037	Staphylococcus epidermidis	ICE192	ITQB	1998	Iceland	Blood	64.13 N 21.82 W	Homo sapiens	Bacteraemia	SAMN05207195	5207195	Illumina Hiseq	3569974	356997400	32.6	100	100	100	100	35.1	126	SPAdes v3.7.1	120	2456915	2456915	0	0	201	20474	263670	82257	Meric et al., 2015	10.1093/gbe/evv066
254_DEN178	SAMN05207196	254	SRS1526049	Staphylococcus epidermidis	DEN178	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207196	5207196	Illumina Hiseq	3671280	367128000	32.3	100	100	100	100	34.9	129	SPAdes v3.7.1	145	2637079	2637079	0	0	204	18186	263578	82907	Meric et al., 2015	10.1093/gbe/evv066
255_ICE9	SAMN05207197	255	SRS1526060	Staphylococcus epidermidis	ICE9	ITQB	1997	Iceland	Urine	64.13 N 21.82 W	Homo sapiens	UTI	SAMN05207197	5207197	Illumina Hiseq	5217288	521728800	32.9	100	100	100	100	35.7	184	SPAdes v3.7.1	98	2481963	2481963	0	0	218	25326	252334	78522	Meric et al., 2015	10.1093/gbe/evv066
256_ICE95	SAMN05207198	256	SRS1526071	Staphylococcus epidermidis	ICE95	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207198	5207198	Illumina Hiseq	4642176	464217600	32.8	100	100	100	100	35.6	163	SPAdes v3.7.1	100	2435923	2435923	0	0	204	24359	233824	82969	Meric et al., 2015	10.1093/gbe/evv066
257_DEN76	SAMN05207199	257	SRS1526075	Staphylococcus epidermidis	DEN76	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207199	5207199	Illumina Hiseq	4035442	403544200	32.1	100	100	100	100	35.1	142	SPAdes v3.7.1	106	2415933	2415933	0	0	316	22791	185124	78266	Meric et al., 2015	10.1093/gbe/evv066
259_ICE99	SAMN05207201	259	SRS1525996	Staphylococcus epidermidis	ICE99	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207201	5207201	Illumina Hiseq	4956620	495662000	32.6	100	100	100	100	35.3	175	SPAdes v3.7.1	96	2431352	2431352	0	0	202	25326	198589	72163	Meric et al., 2015	10.1093/gbe/evv066
260_DEN185	SAMN05207202	260	SRS1525997	Staphylococcus epidermidis	DEN185	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207202	5207202	Illumina Hiseq	5396584	539658400	32.7	100	100	100	100	35.9	190	SPAdes v3.7.1	141	2558445	2558445	0	0	201	18145	198505	70556	Meric et al., 2015	10.1093/gbe/evv066
261_DEN73	SAMN05207203	261	SRS1525998	Staphylococcus epidermidis	DEN73	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207203	5207203	Illumina Hiseq	4702852	470285200	32.8	100	100	100	100	35.5	166	SPAdes v3.7.1	265	2667187	2667187	0	0	200	10064	215774	67260	Meric et al., 2015	10.1093/gbe/evv066
262_DEN61	SAMN05207204	262	SRS1525999	Staphylococcus epidermidis	DEN61	ITQB	1997	Denmark	Wound	55.67 N 12.57 E	Homo sapiens	Commensal	SAMN05207204	5207204	Illumina Hiseq	2872812	287281200	32	100	100	100	100	34.8	101	SPAdes v3.7.1	91	2429777	2429777	0	0	314	26700	263666	81903	Meric et al., 2015	10.1093/gbe/evv066
263_HFA6228	SAMN05207205	263	SRS1526000	Staphylococcus epidermidis	HFA6228	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207205	5207205	Illumina Hiseq	3651130	365113000	32.8	100	100	100	100	35.1	129	SPAdes v3.7.1	114	2466138	2466138	0	0	202	21632	263664	82908	Meric et al., 2015	10.1093/gbe/evv066
264_AGT24	SAMN05207206	264	SRS1526001	Staphylococcus epidermidis	AGT24	ITQB	1997	Argentina	Blood	34.60 S 58.38 W	Homo sapiens	Bacteraemia	SAMN05207206	5207206	Illumina Hiseq	6120474	612047400	32.7	100	100	100	100	35.7	216	SPAdes v3.7.1	103	2441041	2441041	0	0	203	23699	234095	81914	Meric et al., 2015	10.1093/gbe/evv066
265_HUR105	SAMN05207207	265	SRS1526002	Staphylococcus epidermidis	HUR105	ITQB	1998	Hungary	Sputum	47.50 N 19.04 E	Homo sapiens	Pneumonia	SAMN05207207	5207207	Illumina Hiseq	3883156	388315600	32.5	100	100	100	100	35.1	137	SPAdes v3.7.1	126	2491932	2491932	0	0	201	19777	234400	75404	Meric et al., 2015	10.1093/gbe/evv066
266_ICE26	SAMN05207208	266	SRS1526003	Staphylococcus epidermidis	ICE26	ITQB	1997	Iceland	Blood	64.13 N 21.82 W	Homo sapiens	Bacteraemia	SAMN05207208	5207208	Illumina Hiseq	4093300	409330000	31.8	100	100	100	100	35.2	144	SPAdes v3.7.1	176	2722358	2722358	0	0	205	15467	195608	76698	Meric et al., 2015	10.1093/gbe/evv066
267_966N	SAMN05207209	267	SRS1526004	Staphylococcus epidermidis	966N	ITQB	1998	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207209	5207209	Illumina Hiseq	3148714	314871400	32.4	100	100	100	100	34.4	111	SPAdes v3.7.1	131	2503277	2503277	0	0	215	19108	263583	82913	Meric et al., 2015	10.1093/gbe/evv066
268_ICE21	SAMN05207210	268	SRS1526006	Staphylococcus epidermidis	ICE21	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207210	5207210	Illumina Hiseq	4008034	400803400	32.5	100	100	100	100	35.3	141	SPAdes v3.7.1	99	2597625	2597625	0	0	319	26238	196856	70540	Meric et al., 2015	10.1093/gbe/evv066
269_ICE24	SAMN05207211	269	SRS1526007	Staphylococcus epidermidis	ICE24	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207211	5207211	Illumina Hiseq	5002040	500204000	32.5	100	100	100	100	35.2	176	SPAdes v3.7.1	118	2477811	2477811	0	0	201	20998	207184	82854	Meric et al., 2015	10.1093/gbe/evv066
270_DEN107	SAMN05207212	270	SRS1526008	Staphylococcus epidermidis	DEN107	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207212	5207212	Illumina Hiseq	3430578	343057800	31.4	100	100	100	100	35	121	SPAdes v3.7.1	110	2469742	2469742	0	0	207	22452	234898	64653	Meric et al., 2015	10.1093/gbe/evv066
271_ICE87	SAMN05207213	271	SRS1526009	Staphylococcus epidermidis	ICE87	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207213	5207213	Illumina Hiseq	5143144	514314400	32.6	100	100	100	100	35.8	181	SPAdes v3.7.1	103	2603490	2603490	0	0	200	25276	190508	91133	Meric et al., 2015	10.1093/gbe/evv066
272_DEN116	SAMN05207214	272	SRS1526010	Staphylococcus epidermidis	DEN116	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207214	5207214	Illumina Hiseq	5582608	558260800	33	100	100	100	100	35.4	197	SPAdes v3.7.1	100	2486403	2486403	0	0	222	24864	196963	67311	Meric et al., 2015	10.1093/gbe/evv066
273_ICE122	SAMN05207215	273	SRS1526011	Staphylococcus epidermidis	ICE122	ITQB	1998	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207215	5207215	Illumina Hiseq	4015796	401579600	33.2	100	100	100	100	35.3	141	SPAdes v3.7.1	114	2621150	2621150	0	0	203	22992	196990	70586	Meric et al., 2015	10.1093/gbe/evv066
274_MCO150	SAMN05207216	274	SRS1526012	Staphylococcus epidermidis	MCO150	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207216	5207216	Illumina Hiseq	3338608	333860800	32.8	100	100	100	100	35	118	SPAdes v3.7.1	120	2572952	2572952	0	0	207	21441	233503	75822	Meric et al., 2015	10.1093/gbe/evv066
275_COB20	SAMN05207217	275	SRS1526014	Staphylococcus epidermidis	COB20	ITQB	1997	Colombia	Urine	4.72 N 74.07 W	Homo sapiens	UTI	SAMN05207217	5207217	Illumina Hiseq	5066234	506623400	32.9	100	100	100	100	35.7	178	SPAdes v3.7.1	139	2671984	2671984	0	0	201	19222	190100	64803	Meric et al., 2015	10.1093/gbe/evv066
276_ICE102	SAMN05207218	276	SRS1526013	Staphylococcus epidermidis	ICE102	ITQB	1998	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207218	5207218	Illumina Hiseq	3104170	310417000	32.3	100	100	100	100	34.7	109	SPAdes v3.7.1	103	2448365	2448365	0	0	204	23770	239946	79444	Meric et al., 2015	10.1093/gbe/evv066
277_JAP271	SAMN05207219	277	SRS1526015	Staphylococcus epidermidis	JAP271	ITQB	1998	Japan	Urine	35.69 N 139.69 E	Homo sapiens	Commensal	SAMN05207219	5207219	Illumina Hiseq	3735200	373520000	33.1	100	100	100	100	34.4	132	SPAdes v3.7.1	84	2423951	2423951	0	0	323	28856	265245	75319	Meric et al., 2015	10.1093/gbe/evv066
278_DEN189	SAMN05207220	278	SRS1526018	Staphylococcus epidermidis	DEN189	ITQB	1998	Denmark	unknown	55.67 N 12.57 E	Homo sapiens	Unknown	SAMN05207220	5207220	Illumina Hiseq	5221424	522142400	32.6	100	100	100	100	35.5	184	SPAdes v3.7.1	94	2576365	2576365	0	0	207	27408	236080	81636	Meric et al., 2015	10.1093/gbe/evv066
279_HFA6162	SAMN05207221	279	SRS1526016	Staphylococcus epidermidis	HFA6162	ITQB	2000	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207221	5207221	Illumina Hiseq	3173286	317328600	31.5	100	100	100	100	35.2	112	SPAdes v3.7.1	121	2460698	2460698	0	0	312	20336	263636	82582	Meric et al., 2015	10.1093/gbe/evv066
280_BUG46	SAMN05207222	280	SRS1526019	Staphylococcus epidermidis	BUG46	ITQB	1997	Bulgaria	unknown	42.70 N 23.32 E	Homo sapiens	Infection	SAMN05207222	5207222	Illumina Hiseq	3558460	355846000	32.2	100	100	100	100	34.6	125	SPAdes v3.7.1	100	2445790	2445790	0	0	202	24457	234398	78587	Meric et al., 2015	10.1093/gbe/evv066
281_SEPI	SAMN03197475	281_SEPI	SRS746680	Staphylococcus epidermidis	281_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197475	3197475	Illumina Hiseq	3995798	399579800	32	100	100	100	100	35.3	141	SPAdes v3.7.1	88	2445430	2445430	0	0	206	27788	210369	75496	Roach et al., 2015	10.1371/journal.pgen.1005413
282_HFA6226	SAMN05207224	282	SRS1526021	Staphylococcus epidermidis	HFA6226	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207224	5207224	Illumina Hiseq	3113792	311379200	32.4	100	100	100	100	34.9	110	SPAdes v3.7.1	98	2471686	2471686	0	0	206	25221	234371	82911	Meric et al., 2015	10.1093/gbe/evv066
283_MEX60	SAMN05207225	283	SRS1526022	Staphylococcus epidermidis	MEX60	ITQB	1996	Mexico	Catheter	19.43 N 99.13 W	Homo sapiens	Catheter infection	SAMN05207225	5207225	Illumina Hiseq	3328790	332879000	32.2	100	100	100	100	34.7	117	SPAdes v3.7.1	89	2603896	2603896	0	0	261	29257	189833	82835	Meric et al., 2015	10.1093/gbe/evv066
284_MEX61	SAMN05207226	284	SRS1526023	Staphylococcus epidermidis	MEX61	ITQB	1996	Mexico	Blood	19.43 N 99.13 W	Homo sapiens	Bacteraemia	SAMN05207226	5207226	Illumina Hiseq	5517848	551784800	33	100	100	100	100	35.4	194	SPAdes v3.7.1	94	2607725	2607725	0	0	243	27741	189833	82962	Meric et al., 2015	10.1093/gbe/evv066
285_GRE34	SAMN05207227	285	SRS1526024	Staphylococcus epidermidis	GRE34	ITQB	1998	Greece	unknown	37.98 N 23.73 E	Homo sapiens	Unknown	SAMN05207227	5207227	Illumina Hiseq	3546060	354606000	32.4	100	100	100	100	34.4	125	SPAdes v3.7.1	138	2562651	2562651	0	0	207	18569	233280	69783	Meric et al., 2015	10.1093/gbe/evv066
286_HFA6391	SAMN05207228	286	SRS1526025	Staphylococcus epidermidis	HFA6391	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207228	5207228	Illumina Hiseq	3247838	324783800	32.5	100	100	100	100	34.9	114	SPAdes v3.7.1	137	2432244	2432244	0	0	202	17753	198073	79124	Meric et al., 2015	10.1093/gbe/evv066
287_CV45	SAMN05207229	287	SRS1526026	Staphylococcus epidermidis	CV45	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207229	5207229	Illumina Hiseq	2226036	222603600	32.4	100	100	100	100	35.1	78	SPAdes v3.7.1	105	2463433	2463433	0	0	314	23461	212171	81904	Meric et al., 2015	10.1093/gbe/evv066
288_GRE41	SAMN05207230	288	SRS1526028	Staphylococcus epidermidis	GRE41	ITQB	1998	Greece	unknown	37.98 N 23.73 E	Homo sapiens	Infection	SAMN05207230	5207230	Illumina Hiseq	3761646	376164600	31.9	100	100	100	100	35.1	132	SPAdes v3.7.1	101	2460811	2460811	0	0	233	24364	208178	82758	Meric et al., 2015	10.1093/gbe/evv066
289_DEN161	SAMN05207231	289	SRS1526029	Staphylococcus epidermidis	DEN161	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207231	5207231	Illumina Hiseq	4182898	418289800	32.6	100	100	100	100	35.3	147	SPAdes v3.7.1	88	2446471	2446471	0	0	233	27800	263657	82912	Meric et al., 2015	10.1093/gbe/evv066
290_TAW113	SAMN05207232	290	SRS1526030	Staphylococcus epidermidis	TAW113	ITQB	1997	Taiwan	Sputum	23.70 N 120.96 E	Homo sapiens	Pneumonia	SAMN05207232	5207232	Illumina Hiseq	4081892	408189200	32.1	100	100	100	100	35.3	144	SPAdes v3.7.1	107	2483732	2483732	0	0	200	23212	255850	89115	Meric et al., 2015	10.1093/gbe/evv066
291_URU23	SAMN05207233	291	SRS1526032	Staphylococcus epidermidis	URU23	ITQB	1997	Uruguay	Urine	34.90 S 56.16 W	Homo sapiens	UTI	SAMN05207233	5207233	Illumina Hiseq	2790718	279071800	32.5	100	100	100	100	34.1	98	SPAdes v3.7.1	165	2681553	2681553	0	0	211	16251	216123	64881	Meric et al., 2015	10.1093/gbe/evv066
292_HFA6286	SAMN05207234	292	SRS1526031	Staphylococcus epidermidis	HFA6286	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207234	5207234	Illumina Hiseq	3281338	328133800	32	100	100	100	100	34.8	116	SPAdes v3.7.1	115	2453956	2453956	0	0	216	21338	255952	82759	Meric et al., 2015	10.1093/gbe/evv066
294_HFA6096	SAMN05207236	294	SRS1526034	Staphylococcus epidermidis	HFA6096	ITQB	2000	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207236	5207236	Illumina Hiseq	2427574	242757400	31.7	100	100	100	100	34.7	85	SPAdes v3.7.1	87	2468644	2468644	0	0	207	28375	286501	87535	Meric et al., 2015	10.1093/gbe/evv066
295_619N1	SAMN05207237	295	SRS1526035	Staphylococcus epidermidis	619N1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207237	5207237	Illumina Hiseq	3746874	374687400	32.2	100	100	100	100	35.3	132	SPAdes v3.7.1	86	2463253	2463253	0	0	207	28642	286563	87576	Meric et al., 2015	10.1093/gbe/evv066
296_DEN62	SAMN05207238	296	SRS1526036	Staphylococcus epidermidis	DEN62	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207238	5207238	Illumina Hiseq	4406710	440671000	32.8	100	100	100	100	35.2	155	SPAdes v3.7.1	93	2565617	2565617	0	0	317	27587	632047	95692	Meric et al., 2015	10.1093/gbe/evv066
297_MEX37	SAMN05207239	297	SRS1526038	Staphylococcus epidermidis	MEX37	ITQB	1996	Mexico	CSF	19.43 N 99.13 W	Homo sapiens	CSF infection	SAMN05207239	5207239	Illumina Hiseq	3210102	321010200	32.3	100	100	100	100	34.8	113	SPAdes v3.7.1	77	2579977	2579977	0	0	320	33506	429393	106732	Meric et al., 2015	10.1093/gbe/evv066
298_JAP263	SAMN05207240	298	SRS1526039	Staphylococcus epidermidis	JAP263	ITQB	1998	Japan	Wound	35.69 N 139.69 E	Homo sapiens	Wound infection	SAMN05207240	5207240	Illumina Hiseq	3745388	374538800	32.7	100	100	100	100	35.1	132	SPAdes v3.7.1	112	2493079	2493079	0	0	200	22259	278993	95683	Meric et al., 2015	10.1093/gbe/evv066
299_HUR51	SAMN05207241	299	SRS1526040	Staphylococcus epidermidis	HUR51	ITQB	1997	Hungary	Wound	47.50 N 19.04 E	Homo sapiens	Wound infection	SAMN05207241	5207241	Illumina Hiseq	3987658	398765800	32.2	100	100	100	100	35.1	140	SPAdes v3.7.1	83	2431913	2431913	0	0	208	29300	254752	104257	Meric et al., 2015	10.1093/gbe/evv066
300_ITL34	SAMN05207242	300	SRS1526041	Staphylococcus epidermidis	ITL34	ITQB	1997	Italy	Wound	44.49 N 11.34 E	Homo sapiens	Wound infection	SAMN05207242	5207242	Illumina Hiseq	4515188	451518800	33.1	100	100	100	100	35.2	159	SPAdes v3.7.1	112	2607774	2607774	0	0	208	23283	210810	96076	Meric et al., 2015	10.1093/gbe/evv066
301_DEN110	SAMN05207243	301	SRS1526042	Staphylococcus epidermidis	DEN110	ITQB	1998	Denmark	unknown	55.67 N 12.57 E	Homo sapiens	Commensal	SAMN05207243	5207243	Illumina Hiseq	4242330	424233000	32.9	100	100	100	100	34.8	149	SPAdes v3.7.1	98	2523906	2523906	0	0	289	25754	231678	96409	Meric et al., 2015	10.1093/gbe/evv066
302_BUG37	SAMN05207244	302	SRS1526043	Staphylococcus epidermidis	BUG37	ITQB	1997	Bulgaria	Wound	42.70 N 23.32 E	Homo sapiens	Commensal	SAMN05207244	5207244	Illumina Hiseq	3918802	391880200	32	100	100	100	100	35	138	SPAdes v3.7.1	86	2516704	2516704	0	0	210	29264	453718	136686	Meric et al., 2015	10.1093/gbe/evv066
303_CV13	SAMN05207245	303	SRS1526044	Staphylococcus epidermidis	CV13	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207245	5207245	Illumina Hiseq	4668730	466873000	32.3	100	100	100	100	35.7	164	SPAdes v3.7.1	133	2439497	2439497	0	0	202	18342	381244	91782	Meric et al., 2015	10.1093/gbe/evv066
304_747N1	SAMN05207246	304	SRS1526045	Staphylococcus epidermidis	747N1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207246	5207246	Illumina Hiseq	5416922	541692200	32.6	100	100	100	100	35.5	191	SPAdes v3.7.1	67	2508575	2508575	0	0	218	37441	453433	102933	Meric et al., 2015	10.1093/gbe/evv066
305_CV28	SAMN05207247	305	SRS1526046	Staphylococcus epidermidis	CV28	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207247	5207247	Illumina Hiseq	3750542	375054200	31.5	100	100	100	100	35.2	132	SPAdes v3.7.1	178	2562113	2562113	0	0	202	14393	264078	79175	Meric et al., 2015	10.1093/gbe/evv066
306_59N	SAMN05207248	306	SRS1526047	Staphylococcus epidermidis	59N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207248	5207248	Illumina Hiseq	3413348	341334800	32.5	100	100	100	100	35.2	120	SPAdes v3.7.1	116	2502509	2502509	0	0	200	21573	263767	82130	Meric et al., 2015	10.1093/gbe/evv066
307_HFA6173B	SAMN05207249	307	SRS1526048	Staphylococcus epidermidis	HFA6173B	ITQB	2000	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207249	5207249	Illumina Hiseq	4775896	477589600	32.2	100	100	100	100	35.3	168	SPAdes v3.7.1	157	2599308	2599308	0	0	201	16556	398744	108148	Meric et al., 2015	10.1093/gbe/evv066
308_MEX35	SAMN05207250	308	SRS1526050	Staphylococcus epidermidis	MEX35	ITQB	1996	Mexico	Catheter	19.43 N 99.13 W	Homo sapiens	Catheter infection	SAMN05207250	5207250	Illumina Hiseq	5493610	549361000	32.6	100	100	100	100	35.6	193	SPAdes v3.7.1	107	2552783	2552783	0	0	205	23857	409686	111472	Meric et al., 2015	10.1093/gbe/evv066
309_ITL299	SAMN05207251	309	SRS1526051	Staphylococcus epidermidis	ITL299	ITQB	1997	Italy	Wound	44.49 N 11.34 E	Homo sapiens	Wound infection	SAMN05207251	5207251	Illumina Hiseq	3983808	398380800	32.6	100	100	100	100	34.9	140	SPAdes v3.7.1	97	2462743	2462743	0	0	206	25389	264792	82633	Meric et al., 2015	10.1093/gbe/evv066
310_GRE53	SAMN05207252	310	SRS1526052	Staphylococcus epidermidis	GRE53	ITQB	1998	Greece		37.98 N 23.73 E	Homo sapiens	Unknown	SAMN05207252	5207252	Illumina Hiseq	3245152	324515200	32.1	100	100	100	100	35.1	114	SPAdes v3.7.1	83	2533088	2533088	0	0	200	30519	255031	119591	Meric et al., 2015	10.1093/gbe/evv066
311_DEN69	SAMN05207253	311	SRS1526054	Staphylococcus epidermidis	DEN69	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207253	5207253	Illumina Hiseq	4053384	405338400	32.5	100	100	100	100	35	143	SPAdes v3.7.1	126	2476815	2476815	0	0	200	19657	224870	97019	Meric et al., 2015	10.1093/gbe/evv066
312_CV20	SAMN05207254	312	SRS1526053	Staphylococcus epidermidis	CV20	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207254	5207254	Illumina Hiseq	4978678	497867800	33	100	100	100	100	35.6	175	SPAdes v3.7.1	70	2488372	2488372	0	0	241	35548	213721	107267	Meric et al., 2015	10.1093/gbe/evv066
313_93N	SAMN05207255	313	SRS1526055	Staphylococcus epidermidis	93N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207255	5207255	Illumina Hiseq	4958062	495806200	31.8	100	100	100	100	35.7	175	SPAdes v3.7.1	77	2515945	2515945	0	0	206	32674	266126	140040	Meric et al., 2015	10.1093/gbe/evv066
314_1076NL	SAMN05207256	314	SRS1526056	Staphylococcus epidermidis	1076NL	ITQB	1998	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207256	5207256	Illumina Hiseq	5900436	590043600	32.7	100	100	100	100	35.9	208	SPAdes v3.7.1	102	2554577	2554577	0	0	202	25044	234206	77386	Meric et al., 2015	10.1093/gbe/evv066
315_148N	SAMN05207257	315	SRS1526057	Staphylococcus epidermidis	148N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207257	5207257	Illumina Hiseq	5321372	532137200	32.4	100	100	100	100	35.5	187	SPAdes v3.7.1	122	2569367	2569367	0	0	206	21060	220483	68694	Meric et al., 2015	10.1093/gbe/evv066
316_19N	SAMN05207258	316	SRS1526058	Staphylococcus epidermidis	19N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207258	5207258	Illumina Hiseq	5719816	571981600	32.8	100	100	100	100	35.7	201	SPAdes v3.7.1	98	2615913	2615913	0	0	200	26692	491026	118109	Meric et al., 2015	10.1093/gbe/evv066
317_612N1	SAMN05207259	317	SRS1526059	Staphylococcus epidermidis	612N1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207259	5207259	Illumina Hiseq	4934150	493415000	32.5	100	100	100	100	35.8	174	SPAdes v3.7.1	249	2491636	2491636	0	0	200	10006	198667	83604	Meric et al., 2015	10.1093/gbe/evv066
318_1243N	SAMN05207260	318	SRS1526061	Staphylococcus epidermidis	1243N	ITQB	1998	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207260	5207260	Illumina Hiseq	4380930	438093000	32.8	100	100	100	100	35.6	154	SPAdes v3.7.1	123	2528612	2528612	0	0	207	20557	245406	73296	Meric et al., 2015	10.1093/gbe/evv066
319_1290N	SAMN05207261	319	SRS1526062	Staphylococcus epidermidis	1290N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207261	5207261	Illumina Hiseq	5501480	550148000	32.8	100	100	100	100	35.8	194	SPAdes v3.7.1	72	2528565	2528565	0	0	211	35118	235260	90392	Meric et al., 2015	10.1093/gbe/evv066
320_1437N	SAMN05207262	320	SRS1526063	Staphylococcus epidermidis	1437N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207262	5207262	Illumina Hiseq	4324770	432477000	33.1	100	100	100	100	35.3	152	SPAdes v3.7.1	119	2508392	2508392	0	0	200	21078	357269	119279	Meric et al., 2015	10.1093/gbe/evv066
322_1340N	SAMN05207264	322	SRS1526065	Staphylococcus epidermidis	1340N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207264	5207264	Illumina Hiseq	4856848	485684800	32.8	100	100	100	100	35.7	171	SPAdes v3.7.1	93	2552082	2552082	0	0	205	27441	262286	100557	Meric et al., 2015	10.1093/gbe/evv066
323_603N	SAMN05207265	323	SRS1526066	Staphylococcus epidermidis	603N	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207265	5207265	Illumina Hiseq	5028808	502880800	32.7	100	100	100	100	35.6	177	SPAdes v3.7.1	94	2525767	2525767	0	0	200	26869	353528	139373	Meric et al., 2015	10.1093/gbe/evv066
324_56N	SAMN05207266	324	SRS1526067	Staphylococcus epidermidis	56N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207266	5207266	Illumina Hiseq	6292240	629224000	33.1	100	100	100	100	35.8	222	SPAdes v3.7.1	88	2564293	2564293	0	0	204	29139	389361	91211	Meric et al., 2015	10.1093/gbe/evv066
326_676NR1	SAMN05207268	326	SRS1526069	Staphylococcus epidermidis	676NR1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207268	5207268	Illumina Hiseq	3749890	374989000	32.4	100	100	100	100	35	132	SPAdes v3.7.1	63	2439824	2439824	0	0	312	38727	367253	148094	Meric et al., 2015	10.1093/gbe/evv066
327_790N	SAMN05207269	327	SRS1526070	Staphylococcus epidermidis	790N	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207269	5207269	Illumina Hiseq	3850804	385080400	32.8	100	100	100	100	35.1	136	SPAdes v3.7.1	123	2553713	2553713	0	0	204	20761	254501	114598	Meric et al., 2015	10.1093/gbe/evv066
328_803NLR2	SAMN05207270	328	SRS1526072	Staphylococcus epidermidis	803NLR2	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207270	5207270	Illumina Hiseq	4366260	436626000	33	100	100	100	100	35.6	154	SPAdes v3.7.1	110	2524777	2524777	0	0	312	22952	316590	84150	Meric et al., 2015	10.1093/gbe/evv066
329_94N2B	SAMN05207271	329	SRS1526073	Staphylococcus epidermidis	94N2B	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207271	5207271	Illumina Hiseq	4729942	472994200	32.9	100	100	100	100	35.6	167	SPAdes v3.7.1	114	2598846	2598846	0	0	200	22796	292451	100018	Meric et al., 2015	10.1093/gbe/evv066
330_619N2	SAMN05207272	330	SRS1526074	Staphylococcus epidermidis	619N2	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207272	5207272	Illumina Hiseq	4856064	485606400	32.5	100	100	100	100	35.5	171	SPAdes v3.7.1	93	2462435	2462435	0	0	207	26477	286563	82915	Meric et al., 2015	10.1093/gbe/evv066
364_SEPI	SAMN03197554	364_SEPI	SRS746762	Staphylococcus epidermidis	364_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197554	3197554	Illumina Hiseq	1081352	105480929	32.8	19	97	101	101	37.3	37	SPAdes v3.7.1	103	2605868	2605868	0	0	253	25299	177778	67582	Roach et al., 2015	10.1371/journal.pgen.1005413
365_SEPI	SAMN03197555	365_SEPI	SRS746763	Staphylococcus epidermidis	365_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197555	3197555	Illumina Hiseq	924624	90030093	32.5	19	97	101	101	37.2	32	SPAdes v3.7.1	83	2553532	2553532	0	0	324	30765	200927	81539	Roach et al., 2015	10.1371/journal.pgen.1005413
366_SEPI	SAMN03197556	366_SEPI	SRS746766	Staphylococcus epidermidis	366_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197556	3197556	Illumina Hiseq	977098	95212423	32.7	19	97	101	101	37.3	34	SPAdes v3.7.1	88	2611779	2611779	0	0	314	29679	150035	81995	Roach et al., 2015	10.1371/journal.pgen.1005413
393_SEPI	SAMN03197584	393_SEPI	SRS746792	Staphylococcus epidermidis	393_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197584	3197584	Illumina Hiseq	1167430	114996261	32.3	19	98	101	101	37.2	40	SPAdes v3.7.1	96	2456813	2456813	0	0	324	25591	213432	64534	Roach et al., 2015	10.1371/journal.pgen.1005413
422.rep1_SEPI	SAMN03197614	422.rep1_SEPI	SRS746823	Staphylococcus epidermidis	422.rep1_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197614	3197614	Illumina Hiseq	1025704	99307199	32.9	19	96	101	101	36.3	35	SPAdes v3.7.1	114	2517020	2517020	0	0	323	22079	177337	58513	Roach et al., 2015	10.1371/journal.pgen.1005413
450_SEPI	SAMN03197642	450_SEPI	SRS746849	Staphylococcus epidermidis	450_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197642	3197642	Illumina Hiseq	1031406	100080324	32.8	19	97	101	101	36.2	35	SPAdes v3.7.1	87	2593388	2593388	0	0	320	29809	196477	70545	Roach et al., 2015	10.1371/journal.pgen.1005413
458_SEPI	SAMN03197651	458_SEPI	SRS746861	Staphylococcus epidermidis	458_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197651	3197651	Illumina Hiseq	919458	88930220	32.9	19	96	101	101	36.1	31	SPAdes v3.7.1	73	2591822	2591822	0	0	320	35504	177628	82454	Roach et al., 2015	10.1371/journal.pgen.1005413
460_SEPI	SAMN03197654	460_SEPI	SRS746865	Staphylococcus epidermidis	460_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197654	3197654	Illumina Hiseq	1064412	102980455	33	19	96	101	101	36.2	36	SPAdes v3.7.1	98	2543213	2543213	0	0	321	25951	263719	82832	Roach et al., 2015	10.1371/journal.pgen.1005413
475_SEPI	SAMN03197667	475_SEPI	SRS746874	Staphylococcus epidermidis	475_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197667	3197667	Illumina Hiseq	904910	87648473	33.1	19	96	101	101	36.2	31	SPAdes v3.7.1	92	2494099	2494099	0	0	324	27109	286755	65596	Roach et al., 2015	10.1371/journal.pgen.1005413
535_SEPI	SAMN03197731	535_SEPI	SRS746937	Staphylococcus epidermidis	535_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197731	3197731	Illumina Hiseq	1068006	104501038	32.3	19	97	101	101	37.1	37	SPAdes v3.7.1	81	2593538	2593538	0	0	317	32018	177208	81585	Roach et al., 2015	10.1371/journal.pgen.1005413
568_SEPI	SAMN03197769	568_SEPI	SRS746977	Staphylococcus epidermidis	568_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197769	3197769	Illumina Hiseq	1097880	106151778	33	19	96	101	101	36	37	SPAdes v3.7.1	119	2564781	2564781	0	0	300	21552	208250	58536	Roach et al., 2015	10.1371/journal.pgen.1005413
597_SEPI	SAMN03197796	597_SEPI	SRS747003	Staphylococcus epidermidis	597_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197796	3197796	Illumina Hiseq	890156	87622424	32.2	19	98	101	101	37.1	31	SPAdes v3.7.1	66	2478903	2478903	0	0	331	37559	263594	80427	Roach et al., 2015	10.1371/journal.pgen.1005413
644_SEPI	SAMN03197845	644_SEPI	SRS747049	Staphylococcus epidermidis	644_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197845	3197845	Illumina Hiseq	872644	85727101	32.1	19	98	101	101	37.2	30	SPAdes v3.7.1	89	2474153	2474153	0	0	329	27799	234342	82858	Roach et al., 2015	10.1371/journal.pgen.1005413
652_SEPI	SAMN03197854	652_SEPI	SRS747060	Staphylococcus epidermidis	652_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197854	3197854	Illumina Hiseq	1108586	108899046	32.4	19	98	101	101	37.1	38	SPAdes v3.7.1	103	2554020	2554020	0	0	315	24796	254334	71828	Roach et al., 2015	10.1371/journal.pgen.1005413
678_SEPI	SAMN03197880	678_SEPI	SRS747088	Staphylococcus epidermidis	678_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197880	3197880	Illumina Hiseq	1005280	97275345	33.4	19	96	101	101	37	34	SPAdes v3.7.1	89	2712294	2712294	0	0	283	30475	196156	74569	Roach et al., 2015	10.1371/journal.pgen.1005413
73_SEPI	SAMN03197934	73_SEPI	SRS747142	Staphylococcus epidermidis	73_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03197934	3197934	Illumina Hiseq	1284976	121123287	32	19	94	101	101	36.4	43	SPAdes v3.7.1	65	2390144	2390144	0	0	209	36771	264428	87137	Roach et al., 2015	10.1371/journal.pgen.1005413
798_SEPI	SAMN03198010	798_SEPI	SRS747218	Staphylococcus epidermidis	798_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198010	3198010	Illumina Hiseq	1157344	113443023	32	19	98	101	101	37.4	40	SPAdes v3.7.1	83	2594518	2594518	0	0	323	31259	178008	67386	Roach et al., 2015	10.1371/journal.pgen.1005413
801_SEPI	SAMN03198015	801_SEPI	SRS747224	Staphylococcus epidermidis	801_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198015	3198015	Illumina Hiseq	1185566	113387790	32.1	19	95	101	101	35.8	40	SPAdes v3.7.1	81	2542422	2542422	0	0	315	31387	152208	81855	Roach et al., 2015	10.1371/journal.pgen.1005413
802_SEPI	SAMN03198016	802_SEPI	SRS747226	Staphylococcus epidermidis	802_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198016	3198016	Illumina Hiseq	1078034	103674485	32.1	19	96	101	101	35.6	37	SPAdes v3.7.1	83	2595349	2595349	0	0	322	31269	252391	81483	Roach et al., 2015	10.1371/journal.pgen.1005413
803_SEPI	SAMN03198017	803_SEPI	SRS747222	Staphylococcus epidermidis	803_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198017	3198017	Illumina Hiseq	944172	91066463	32.3	19	96	101	101	35.7	32	SPAdes v3.7.1	80	2594922	2594922	0	0	314	32436	196376	81363	Roach et al., 2015	10.1371/journal.pgen.1005413
849_SEPI	SAMN03198058	849_SEPI	SRS747265	Staphylococcus epidermidis	849_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198058	3198058	Illumina Hiseq	980938	93795388	32.4	19	95	101	101	35.7	33	SPAdes v3.7.1	41	2439674	2439674	0	0	397	59504	350554	96387	Roach et al., 2015	10.1371/journal.pgen.1005413
87_SEPI	SAMN03198076	87_SEPI	SRS747284	Staphylococcus epidermidis	87_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198076	3198076	Illumina Hiseq	1259178	119415846	31.9	19	94	101	101	35.8	42	SPAdes v3.7.1	84	2551659	2551659	0	0	315	30376	227342	70504	Roach et al., 2015	10.1371/journal.pgen.1005413
91_SEPI	SAMN03198117	91_SEPI	SRS747325	Staphylococcus epidermidis	91_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198117	3198117	Illumina Hiseq	1575102	146009620	31.9	19	92	101	101	36.6	51	SPAdes v3.7.1	81	2514742	2514742	0	0	202	31046	368432	89931	Roach et al., 2015	10.1371/journal.pgen.1005413
92_SEPI	SAMN03198134	92_SEPI	SRS747342	Staphylococcus epidermidis	92_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198134	3198134	Illumina Hiseq	1732152	162132545	31.9	19	93	101	101	36.2	57	SPAdes v3.7.1	78	2613605	2613605	0	0	202	33507	277602	82124	Roach et al., 2015	10.1371/journal.pgen.1005413
934_SEPI	SAMN03198153	934_SEPI	SRS747361	Staphylococcus epidermidis	934_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198153	3198153	Illumina Hiseq	2032668	176434667	33.3	8	86	101	101	34.3	62	SPAdes v3.7.1	100	2501950	2501950	0	0	323	25019	263748	65891	Roach et al., 2015	10.1371/journal.pgen.1005413

Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*
Supplementary Table 1 - Sheet E. SRA metadata

935_SEPI	SAMN03198154	935_SEPI	SRS747362	Staphylococcus epidermidis	935_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198154	3198154
936_SEPI	SAMN03198155	936_SEPI	SRS747364	Staphylococcus epidermidis	936_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198155	3198155
938_SEPI	SAMN03198157	938_SEPI	SRS747365	Staphylococcus epidermidis	938_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198157	3198157
939_SEPI	SAMN03198158	939_SEPI	SRS747366	Staphylococcus epidermidis	939_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198158	3198158
94_SEPI	SAMN03198159	94_SEPI	SRS747367	Staphylococcus epidermidis	94_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198159	3198159
946_SEPI	SAMN03198166	946_SEPI	SRS747377	Staphylococcus epidermidis	946_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198166	3198166
947_SEPI	SAMN03198167	947_SEPI	SRS747372	Staphylococcus epidermidis	947_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198167	3198167
961_SEPI	SAMN03198181	961_SEPI	SRS747388	Staphylococcus epidermidis	961_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198181	3198181
968_SHAE	SAMN03198188	968_SHAE	SRS747396	Staphylococcus epidermidis	968_SHAE	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198188	3198188
971_SEPI	SAMN03198191	971_SEPI	SRS747398	Staphylococcus epidermidis	971_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198191	3198191
972_SEPI	SAMN03198192	972_SEPI	SRS747400	Staphylococcus epidermidis	972_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198192	3198192
973_SEPI	SAMN03198193	973_SEPI	SRS747404	Staphylococcus epidermidis	973_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198193	3198193
978_SEPI	SAMN03198198	978_SEPI	SRS747410	Staphylococcus epidermidis	978_SEPI	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198198	3198198
997_SHAE	SAMN03198219	997_SHAE	SRS747428	Staphylococcus epidermidis	997_SHAE	UW clinical laboratory	2012-2013	USA WA	unknown	unknown	Homo sapiens	unknown	SAMN03198219	3198219
M0026	SAMN02325127	M0026	SRS486821	Staphylococcus epidermidis	M0026	-	15-Sep-03	USA Boston, MA	blood	-	Homo sapiens	Bacteraemia	SAMN02325127	2325127
UCLA-Sa-LR-13	SAMN02671415	UCLA-Sa-LR-13	SRS565652	Staphylococcus epidermidis	UCLA-Sa-LR-13	Romney Humphries - UCLA	Nov-07	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671415	2671415
UCLA-Sa-LR-14	SAMN02671416	UCLA-Sa-LR-14	SRS565653	Staphylococcus epidermidis	UCLA-Sa-LR-14	Romney Humphries - UCLA	Apr-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671416	2671416
UCLA-Sa-LR-15	SAMN02671417	UCLA-Sa-LR-15	SRS565654	Staphylococcus epidermidis	UCLA-Sa-LR-15	Romney Humphries - UCLA	Jun-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671417	2671417
UCLA-Sa-LR-17	SAMN02671418	UCLA-Sa-LR-17	SRS565655	Staphylococcus epidermidis	UCLA-Sa-LR-17	Romney Humphries - UCLA	Jun-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671418	2671418
UCLA-Sa-LR-18	SAMN02671419	UCLA-Sa-LR-18	SRS565656	Staphylococcus epidermidis	UCLA-Sa-LR-18	Romney Humphries - UCLA	Aug-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671419	2671419
UCLA-Sa-LR-19	SAMN02671420	UCLA-Sa-LR-19	SRS565659	Staphylococcus epidermidis	UCLA-Sa-LR-19	Romney Humphries - UCLA	Aug-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671420	2671420
UCLA-Sa-LR-20	SAMN02671421	UCLA-Sa-LR-20	SRS565660	Staphylococcus epidermidis	UCLA-Sa-LR-20	Romney Humphries - UCLA	Oct-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671421	2671421
UCLA-Sa-LR-21	SAMN02671422	UCLA-Sa-LR-21	SRS565661	Staphylococcus epidermidis	UCLA-Sa-LR-21	Romney Humphries - UCLA	Nov-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671422	2671422
UCLA-Sa-LR-22	SAMN02671423	UCLA-Sa-LR-22	SRS565662	Staphylococcus epidermidis	UCLA-Sa-LR-22	Romney Humphries - UCLA	Nov-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671423	2671423
UCLA-Sa-LR-23	SAMN02671424	UCLA-Sa-LR-23	SRS565663	Staphylococcus epidermidis	UCLA-Sa-LR-23	Romney Humphries - UCLA	Dec-08	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671424	2671424
UCLA-Sa-LR-25	SAMN02671425	UCLA-Sa-LR-25	SRS565664	Staphylococcus epidermidis	UCLA-Sa-LR-25	Romney Humphries - UCLA	Sep-09	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671425	2671425
UCLA-Sa-LR-27	SAMN02671427	UCLA-Sa-LR-27	SRS565666	Staphylococcus epidermidis	UCLA-Sa-LR-27	Romney Humphries - UCLA	Sep-09	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671427	2671427
UCLA-Sa-LR-28	SAMN02671428	UCLA-Sa-LR-28	SRS565667	Staphylococcus epidermidis	UCLA-Sa-LR-28	Romney Humphries - UCLA	Oct-09	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671428	2671428
UCLA-Sa-LR-3	SAMN02671410	UCLA-Sa-LR-03	SRS565634	Staphylococcus epidermidis	UCLA-Sa-LR-3	Romney Humphries - UCLA	Mar-11	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671410	2671410
UCLA-Sa-LR-4	SAMN02671411	UCLA-Sa-LR-04	SRS565637	Staphylococcus epidermidis	UCLA-Sa-LR-4	Romney Humphries - UCLA	Jun-11	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671411	2671411
UCLA-Sa-LR-5	SAMN02671412	UCLA-Sa-LR-05	SRS565640	Staphylococcus epidermidis	UCLA-Sa-LR-5	Romney Humphries - UCLA	Jul-11	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671412	2671412
UCLA-Sa-LR-6	SAMN02671413	UCLA-Sa-LR-06	SRS565641	Staphylococcus epidermidis	UCLA-Sa-LR-6	Romney Humphries - UCLA	Sep-11	Los Angeles, CA, USA	human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671413	2671413

935_SEPI	Illumina Hiseq	1792820	155693094	33.2	8	86	101	101	34.4	55	SPAdes v3.7.1	108	2504390	2504390	0	0	339	23188	213644	53727	Roach et al., 2015	10.1371/journal.pgen.1005413
936_SEPI	Illumina Hiseq	1848914	161029021	32.9	8	87	101	101	34.3	57	SPAdes v3.7.1	101	2707266	2707266	0	0	314	26804	189819	74589	Roach et al., 2015	10.1371/journal.pgen.1005413
938_SEPI	Illumina Hiseq	1383888	121237405	33	8	87	101	101	33.3	43	SPAdes v3.7.1	102	2491699	2491699	0	0	322	24428	199146	73844	Roach et al., 2015	10.1371/journal.pgen.1005413
939_SEPI	Illumina Hiseq	1676038	146244019	33	8	87	101	101	34.3	52	SPAdes v3.7.1	78	2545612	2545612	0	0	322	32636	195370	78557	Roach et al., 2015	10.1371/journal.pgen.1005413
94_SEPI	Illumina Hiseq	1327234	124993100	31.8	19	94	101	101	36.3	44	SPAdes v3.7.1	73	2507967	2507967	0	0	205	34355	301048	83394	Roach et al., 2015	10.1371/journal.pgen.1005413
946_SEPI	Illumina Hiseq	2008238	173782767	33	8	86	101	101	34.3	61	SPAdes v3.7.1	93	2478843	2478843	0	0	314	26654	263678	81511	Roach et al., 2015	10.1371/journal.pgen.1005413
947_SEPI	Illumina Hiseq	1862754	160833298	33	8	86	101	101	34.5	57	SPAdes v3.7.1	90	2479388	2479388	0	0	319	27548	263848	72154	Roach et al., 2015	10.1371/journal.pgen.1005413
961_SEPI	Illumina Hiseq	2034408	175872546	33	8	86	101	101	34.4	62	SPAdes v3.7.1	99	2612210	2612210	0	0	315	26385	149755	67564	Roach et al., 2015	10.1371/journal.pgen.1005413
968_SHAE	Illumina Hiseq	1823714	158373557	33.1	8	86	101	101	34.1	56	SPAdes v3.7.1	93	2593870	2593870	0	0	323	27891	208694	66733	Roach et al., 2015	10.1371/journal.pgen.1005413
971_SEPI	Illumina Hiseq	1827304	157894163	33	8	86	101	101	34.4	56	SPAdes v3.7.1	91	2442707	2442707	0	0	277	26842	262684	63408	Roach et al., 2015	10.1371/journal.pgen.1005413
972_SEPI	Illumina Hiseq	2419308	206673569	33.2	8	85	101	101	34.4	73	SPAdes v3.7.1	87	2468264	2468264	0	0	225	28370	263652	79628	Roach et al., 2015	10.1371/journal.pgen.1005413
973_SEPI	Illumina Hiseq	1579384	138318303	32.9	8	87	101	101	34.4	49	SPAdes v3.7.1	97	2727413	2727413	0	0	202	28117	196597	64598	Roach et al., 2015	10.1371/journal.pgen.1005413
978_SEPI	Illumina Hiseq	1902254	164816978	33.2	8	86	101	101	33.9	58	SPAdes v3.7.1	144	2344162	2344162	0	0	314	16278	234340	72487	Roach et al., 2015	10.1371/journal.pgen.1005413
997_SHAE	Illumina Hiseq	2423748	206613220	33.1	8	85	101	101	34.5	73	SPAdes v3.7.1	88	2523106	2523106	0	0	320	28671	149591	63398	Roach et al., 2015	10.1371/journal.pgen.1005413
M0026	Illumina Hiseq	5384482	543832682	33.3	101	101	101	101	35.7	192	SPAdes v3.7.1	123	2555077	2555077	0	0	200	20772	327259	95958	Unpublished	PRJEB2655
UCLA-Sa-LR-13	Illumina Hiseq	1E+07	1037913400	33.4	100	100	100	100	36.6	366	SPAdes v3.7.1	187	2553163	2553163	0	0	200	13653	254739	83071	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-14	Illumina Hiseq	9244600	924460000	33.9	100	100	100	100	36.7	326	SPAdes v3.7.1	168	2538652	2538652	0	0	201	15111	234775	83071	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-15	Illumina Hiseq	1E+07	1030452600	33.3	100	100	100	100	36.7	363	SPAdes v3.7.1	220	2771289	2771289	0	0	200	12596	253553	92523	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-17	Illumina Hiseq	1.1E+07	1066446000	33.5	100	100	100	100	36.8	376	SPAdes v3.7.1	224	2790991	2790991	0	0	203	12459	212261	93488	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-18	Illumina Hiseq	1.1E+07	1066270200	33.3	100	100	100	100	36.8	376	SPAdes v3.7.1	196	2782416	2782416	0	0	200	14196	239814	94973	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-19	Illumina Hiseq	8780378	878037800	33.4	100	100	100	100	36.7	309	SPAdes v3.7.1	106	2633443	2633443	0	0	214	24843	294582	78525	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-20	Illumina Hiseq	1E+07	1018152200	33.1	100	100	100	100	36.8	359	SPAdes v3.7.1	284	2924246	2924246	0	0	201	10296	196709	70431	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-21	Illumina Hiseq	1E+07	1029912800	33.5	100	100	100	100	36.7	363	SPAdes v3.7.1	184	2608644	2608644	0	0	201	14177	227396	72187	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-22	Illumina Hiseq	9778746	977874600	33.2	100	100	100	100	36.8	344	SPAdes v3.7.1	250	2782627	2782627	0	0	200	11130	212558	87652	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-23	Illumina Hiseq	8183090	818309000	33.6	100	100	100	100	36.7	288	SPAdes v3.7.1	216	2466065	2466065	0	0	202	11416	253911	82973	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-25	Illumina Hiseq	9551042	955104200	33.3	100	100	100	100	36.7	336	SPAdes v3.7.1	195	2801501	2801501	0	0	202	14366	234033	75562	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-27	Illumina Hiseq	1E+07	1014411200	33.5	100	100	100	100	36.7	357	SPAdes v3.7.1	211	2643007	2643007	0	0	202	12526	227434	72021	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-28	Illumina Hiseq	1.1E+07	1075238400	33.1	100	100	100	100	36.8	379	SPAdes v3.7.1	198	2633332	2633332	0	0	200	13299	234103	82992	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-3	Illumina Hiseq	9531114	953111400	33.3	100	100	100	100	36.8	336	SPAdes v3.7.1	203	2651249	2651249	0	0	201	13060	508038	76085	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-4	Illumina Hiseq	4427150	442715000	33.7	100	100	100	100	36.5	156	SPAdes v3.7.1	116	2619378	2619378	0	0	237	22580	234220	70379	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-5	Illumina Hiseq	8630226	863022600	33.6	100	100	100	100	36.8	304	SPAdes v3.7.1	108	2624507	2624507	0	0	214	24300	263996	70791	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-6	Illumina Hiseq	1E+07	1008935600	33.1	100	100	100	100	36.8	355	SPAdes v3.7.1	168	2759224	2759224	0	0	200	16423	253727	83080	Tewhey et al., 2014	10.1128/mBio.00894-14

APPENDIX B.

Chapter 4. Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification – Supplementary Tables

Isolate	Organism	in silico MLST	BioSample	BioProject	Assembly	Size (Mb)	GC%	Replicons	Scaffolds	Genes	Proteins	Level	RefSeq FTP	GenBank FTP	Sequencing reference	DOI
04-02981	*Staphylococcus aureus*	225	SAMN02603764	PRJNA34809	GCA_000025145.2	2.82145	32.90	chromosome:NC_017340.1/CP001844.2	1	2864	2746	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/025/145/GCF_000025145.1_ASM2514v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/025/145/GCA_000025145.2_ASM2514v1	Nubel et al., 2010	10.1371/journal.ppat.1000855
08-02119	*Staphylococcus aureus*	582	SAMN04939716	PRJNA321000	GCA_001656045.1	2.79689	32.90	chromosome:NZ_CP015645.1/CP015645.1	1	2830	2698	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/656/045/GCF_001656045.1_ASM165604v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/656/045/GCA_001656045.1_ASM165604v1	Weber et al., 2016	10.1128/genomeA.00716-16
08-02300	*Staphylococcus aureus*	7	SAMN04939717	PRJNA321000	GCA_001656075.1	2.74281	32.90	chromosome:NZ_CP015646.1/CP015646.1	1	2757	2625	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/656/075/GCF_001656075.1_ASM165607v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/656/075/GCA_001656075.1_ASM165607v1	Weber et al., 2016	10.1128/genomeA.00716-16
08BA02176	*Staphylococcus aureus*	398	SAMN02603722	PRJNA174226	GCA_000296595.1	2.78231	32.90	chromosome:NC_018608.1/CP003808.1	1	2768	2626	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/296/595/GCF_000296595.1_ASM29659v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/296/595/GCA_000296595.1_ASM29659v1	Golding et al., 2012	10.1128/JB.01680-12
11819-97	*Staphylococcus aureus*	80	SAMN02603886	PRJNA78269	GCA_000239235.1	2.86886	32.87	chromosome:NC_017351.1/CP003194.1; plasmid p11819-97:NC_017350.1/CP003193.1	2	2914	2791	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/239/235/GCF_000239235.1_ASM23923v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/239/235/GCA_000239235.1_ASM23923v1	Stegger et al., 2012	10.1128/JB.06653-11
144_S7	*Staphylococcus aureus*	772	SAMN03339012	PRJNA274731	GCA_000934245.1	2.73086	32.70	chromosome:CP010943.1	1	2760	2641	Chromosome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/934/245/GCA_000934245.1_ASM93424v1	Biswas et al., 2015	10.1128/genomeA.00836-15
1625.CO1	*Staphylococcus aureus*	8	SAMN04964640	PRJNA321054	GCA_001717725.1	2.93199	32.76	chromosome:CP016863.1; plasmid unnamed1:CP016862.1	2	3012	2934	Complete Genome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/717/725/GCA_001717725.1_ASM171772v1	Unpublished	PRJNA321054
1969.N	*Staphylococcus aureus*	8	SAMN04966149	PRJNA321120	GCA_001717705.1	2.92615	32.73	chromosome:CP016861.1; plasmid unnamed1:CP016860.1	2	2977	2898	Complete Genome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/717/705/GCA_001717705.1_ASM171770v1	Unpublished	PRJNA321120
1971.CO1	*Staphylococcus aureus*	-	SAMN04966145	PRJNA321116	GCA_001717685.1	2.95994	32.70	chromosome:CP016858.1; plasmid unnamed1:CP016857.1; plasmid unnamed2:CP016859.1	3	3097	3021	Complete Genome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/717/685/GCA_001717685.1_ASM171768v1	Unpublished	PRJNA321116
2148.CO1	*Staphylococcus aureus*	8	SAMN04966147	PRJNA321118	GCA_001717975.1	2.95276	32.73	chromosome:CP017094.1; plasmid p2148.CO1a:CP017095.1; plasmid p2148.CO1b:CP017096.1; plasmid p2148.CO1	4	3093	3013	Complete Genome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/717/975/GCA_001717975.1_ASM171797v1	Unpublished	PRJNA321118
2148.N	*Staphylococcus aureus*	-	SAMN04966148	PRJNA321119	GCA_001717665.1	2.74854	32.80	chromosome:CP016856.1	1	2834	2755	Complete Genome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/717/665/GCA_001717665.1_ASM171766v1	Unpublished	PRJNA321119
2395_USA500	*Staphylococcus aureus*	8	SAMN03290990	PRJNA240091	GCA_000746505.1	2.98805	32.86	chromosome:NZ_CP007499.1/CP007499.1; plasmid pUSA500:NZ_CP007500.1/CP007500.1	2	3097	2979	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/746/505/GCF_000746505.1_ASM74650v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/746/505/GCA_000746505.1_ASM74650v1	Benson et al., 2014	10.1111/mmi.12682
25b_MRSA	*Staphylococcus aureus*	8	SAMN03251895	PRJNA268517	GCA_000815205.1	2.87278	32.80	chromosome:NZ_CP010299.1/CP010299.1	1	2687	2598	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/815/205/GCF_000815205.1_ASM81520v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/815/205/GCA_000815205.1_ASM81520v1	Daum et al., 2015	10.1128/genomeA.00011-15
502A	*Staphylococcus aureus*	5	SAMN02690781	PRJNA240093	GCA_000597965.1	2.78769	32.85	chromosome:NZ_CP007454.1/CP007454.1; plasmid unnamed:NZ_CP007455.1/CP007455.1	2	2831	2707	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/597/965/GCF_000597965.1_ASM59796v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/597/965/GCA_000597965.1_ASM59796v1	Parker et al., 2014	10.1128/genomeA.00284-14
5118.N	*Staphylococcus aureus*	-	SAMN04966150	PRJNA321122	GCA_001717645.3	2.93013	32.74	chromosome:NZ_CP016855.1/CP016855.2; plasmid ; plasmid p5118.Nb:NZ_CP016854.1/CP016854.2	1	3073	2309	Chromosome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/717/645/GCF_001717645.1_ASM171764v3	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/717/645/GCA_001717645.3_ASM171764v3	Unpublished	PRJNA321122
55_2053	*Staphylococcus aureus*	30	SAMN00103091	PRJNA34891	GCA_000160335.2	2.77737	32.82	chromosome:NC_022113.1/CP002388.1; plasmid unnamed:NC_022126.1/CP002389.1	2	2796	2661	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/160/335/GCF_000160335.2_ASM16033v2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/160/335/GCA_000160335.2_ASM16033v2	Unpublished	PRJNA34891
6850	*Staphylococcus aureus*	50	SAMN02604264	PRJNA216988	GCA_000462955.1	2.73656	32.80	chromosome:NC_022222.1/CP006706.1	1	2736	2554	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/462/955/GCF_000462955.1_ASM46295v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/462/955/GCA_000462955.1_ASM46295v1	Fraunholz et al., 2013	10.1128/genomeA.00775-13
71193	*Staphylococcus aureus*	398	SAMN02603419	PRJNA66999	GCA_000258685.1	2.71500	32.90	chromosome:NC_017673.1/CP003045.1	1	2767	2598	Chromosome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/258/685/GCF_000258685.1_ASM25868v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/258/685/GCA_000258685.1_ASM25868v1	Uhlemann et al., 2012	10.1128/mBio.00027-12
71A_S11	*Staphylococcus aureus*	22	SAMN03339002	PRJNA274731	GCA_000934185.1	2.75643	32.70	chromosome:CP010940.1	1	2788	2662	Chromosome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/934/185/GCA_000934185.1_ASM93418v1	Biswas et al., 2015	10.1128/genomeA.00836-15
79_S10	*Staphylococcus aureus*	772	SAMN03339013	PRJNA274731	GCA_000934265.1	2.72652	32.70	chromosome:CP010944.1	1	2755	2626	Chromosome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/934/265/GCA_000934265.1_ASM93426v1	Biswas et al., 2015	10.1128/genomeA.00836-15
93b_S9	*Staphylococcus aureus*	121	SAMN03343952	PRJNA274731	GCA_000934925.1	2.78835	32.70	chromosome:CP010952.1	1	2831	2675	Chromosome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/934/925/GCA_000934925.1_ASM93492v1	Biswas et al., 2015	10.1128/genomeA.00836-15
ATCC_25923	*Staphylococcus aureus*	243	SAMN03009478	PRJNA259557	GCA_000756205.1	2.80634	32.88	chromosome:NZ_CP009361.1/CP009361.1; plasmid pS1945:NZ_CP009362.1/CP009362.1	2	2817	2676	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/756/205/GCF_000756205.1_ASM75620v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/756/205/GCA_000756205.1_ASM75620v1	Treangen et al., 2014	10.1128/genomeA.01110-14
AUS0325	*Staphylococcus aureus*	88	SAMEA4443152	PRJEB15428	GCA_900096745.1	2.77158	32.90	chromosome 1:NZ_LT615218.1/LT615218.1	1	2815	2665	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/900/096/745/GCF_900096745.1_AUS0325	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/900/096/745/GCA_900096745.1_AUS0325	Kpeli et al., 2017	10.7717/peerj.3047
BB155	*Staphylococcus aureus*	152	SAMEA3432862	PRJEB9500	GCA_900004855.1	2.77808	32.80	chromosome 1:NZ_LN854556.1/LN854556.1	1	2789	2632	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/900/004/855/GCF_900004855.1_BB155	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/900/004/855/GCA_900004855.1_BB155	Unpublished	PRJNA224116
Be62	*Staphylococcus aureus*	239	SAMN03765155	PRJNA286121	GCA_001515685.1	3.00124	32.90	chromosome:NZ_CP012013.1/CP012013.1; plasmid pBe62:NZ_CP012014.1/CP012014.1	2	3092	2940	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/515/685/GCF_001515685.1_ASM151568v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/515/685/GCA_001515685.1_ASM151568v1	Unpublished	PRJNA286121
Bmb9393	*Staphylococcus aureus*	239	SAMN02603524	PRJNA196937	GCA_000418345.1	2.98346	32.90	chromosome:NC_021670.1/CP005288.1; plasmid pBmb9393:NC_021657.1/CP005289.1	2	3068	2930	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/418/345/GCF_000418345.1_ASM41834v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/418/345/GCA_000418345.1_ASM41834v1	Costa et al., 2013	10.1128/genomeA.00576-13
CA-347	*Staphylococcus aureus*	45	SAMN02603909	PRJNA197422	GCA_000412775.1	2.87516	32.96	chromosome:NC_021554.1/CP006044.1; plasmid unnamed:NC_021552.1/CP006045.1	2	2913	2765	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/412/775/GCF_000412775.1_ASM41277v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/412/775/GCA_000412775.1_ASM41277v1	Stegger et al., 2013	10.1128/genomeA.00517-13
CA12	*Staphylococcus aureus*	8	SAMN02767090	PRJNA246373	GCA_001045795.2	2.86537	32.76	chromosome:NZ_CP007672.1/CP007672.1; plasmid pCA12:NZ_CP007673.1/CP007673.1	2	2932	2816	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/045/795/GCF_001045795.2_ASM104579v2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/045/795/GCA_001045795.2_ASM104579v2	Planet et al., 2015	10.1093/infdis/jiv320
CA15	*Staphylococcus aureus*	8	SAMN02767179	PRJNA246386	GCA_001021895.1	2.86428	32.76	chromosome:NZ_CP007674.1/CP007674.1; plasmid pCA15:NZ_CP007675.1/CP007675.1	2	2684	2578	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/021/895/GCF_001021895.1_ASM102189v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/021/895/GCA_001021895.1_ASM102189v1	Planet et al., 2015	10.1093/infdis/jiv320
Clinical_isolate	*Staphylococcus aureus*	8	SAMEA17757418	PRJEB18112	GCA_900129335.1	2.82187	32.75	chromosome I:NZ_LT671859.1/LT671859; plasmid II:NZ_LT671860.1/LT671860.1	-	2945	2790	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/900/129/335/GCF_900129335.1_PP_HGAG_QV30_2SC_T4	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/900/129/335/GCA_900129335.1_PP_HGAG_QV30_2SC_T4	Unpublished	PRJEB18112
CN1	*Staphylococcus aureus*	72	SAMN02603420	PRJNA162343	GCA_000463055.1	2.75707	32.79	chromosome:NC_022226.1/CP003979.1; plasmid unnamed:NC_022227.1/CP003980.1; plasmid unnamed2:NC_0222	3	2775	2650	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/463/055/GCF_000463055.1_ASM46305v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/463/055/GCA_000463055.1_ASM46305v1	Chen et al, 2013	10.1371/journal.pone.0072803
COL	*Staphylococcus aureus*	250	SAMN02603996	PRJNA238	GCA_000012045.1	2.81386	32.80	chromosome:NC_002951.2/CP000046.1; plasmid pT181:NC_006629.2/CP000045.2	2	2863	2767	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/012/045/GCF_000012045.1_ASM1204v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/012/045/GCA_000012045.1_ASM1204v1	Gill et al., 2005	10.1128/JB.187.7.2426-2438.2005
DAR4145	*Staphylococcus aureus*	772	SAMN03268345	PRJNA270689	GCA_000828035.1	2.86051	32.80	chromosome:NZ_CP010526.1/CP010526.1	1	2677	2579	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/828/035/GCF_000828035.1_ASM82803v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/828/035/GCA_000828035.1_ASM82803v1	Steinig et al, 2015	10.1186/s12864-015-1599-9
DSM_20231	*Staphylococcus aureus*	8	SAMN03651231	PRJNA283471	GCA_001027105.1	2.78256	32.88	chromosome:NZ_CP011526.1/CP011526.1; plasmid unnamed:NZ_CP011527.1/CP011527.1	2	2584	2488	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/027/105/GCF_001027105.1_ASM102710v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/027/105/GCA_001027105.1_ASM102710v1	Shiroma et al., 2015	10.1128/genomeA.00800-15
ECT-R_2	*Staphylococcus aureus*	5	SAMEA2271964	PRJEA52833	GCA_000253135.1	2.75905	32.84	chromosome:NC_017343.1/FR714927.1; plasmid pLUH01:NC_017346.1/FR714928.1; plasmid pLUH02:NC_017344.1	3	2776	2657	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/253/135/GCF_000253135.1_ASM25313v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/253/135/GCA_000253135.1_ASM25313v1	Lindqvist et al., 2012	10.1007/s10096-011-1286-y
ED133	*Staphylococcus aureus*	133	SAMN02604166	PRJNA41277	GCA_000210315.1	2.83248	32.90	chromosome:NC_017337.1/CP001996.1	1	2892	2740	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/210/315/GCF_000210315.1_ASM21031v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/210/315/GCA_000210315.1_ASM21031v1	Guinane et al., 2010	10.1093/gbe/evq031
ED98	*Staphylococcus aureus*	5	SAMN02604165	PRJNA39547	GCA_000024585.1	2.84754	32.77	chromosome:NC_013450.1/CP001781.1; plasmid pAVX:NC_013453.1/CP001784.1; plasmid pAVY:NC_013451.1/CP(	4	2904	2773	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/024/585/GCF_000024585.1_ASM2458v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/024/585/GCA_000024585.1_ASM2458v1	Lowder et al., 2009	10.1073/pnas.0909285106
FCFHV36	*Staphylococcus aureus*	105	SAMN03444404	PRJNA279236	GCA_000969225.1	2.84981	32.90	chromosome:NZ_CP011147.1/CP011147.1	1	2654	2571	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/969/225/GCF_000969225.1_ASM96922v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/969/225/GCA_000969225.1_ASM96922v1	McCulloch et al., 2015	10.1128/genomeA.00893-15
FDA209P	*Staphylococcus aureus*	464	SAMD00039082	PRJDB4160	GCA_001548295.1	2.80322	32.88	chromosome Unknown:NZ_AP014942.1/AP014942.1; plasmid pFDA209P:NZ_AP014943.1/AP014943.1	2	2835	2699	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/548/295/GCF_001548295.1_ASM154829v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/548/295/GCA_001548295.1_ASM154829v1	Singh et al., 2015	10.1128/genomeA.01343-15
FDAARGOS_159	*Staphylococcus aureus*	-	SAMN03996304	PRJNA231221	GCA_001558795.1	2.83917	32.83	chromosome:NZ_CP014064.1/CP014064.1; plasmid unnamed:NZ_CP014063.1/CP014063.1	2	2893	2759	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/558/795/GCF_001558795.1_ASM155879v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/558/795/GCA_001558795.1_ASM155879v1	Unpublished	PRJNA231221
FORC_001	*Staphylococcus aureus*	30	SAMN03140315	PRJNA259937	GCA_000772025.1	2.88602	32.80	chromosome:NZ_CP009554.1/CP009554.1	1	2915	2754	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/772/025/GCF_000772025.1_ASM77202v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/772/025/GCA_000772025.1_ASM77202v1	Unpublished	PRJNA259937
FORC_012	*Staphylococcus aureus*	72	SAMN03387935	PRJNA277068	GCA_001580495.1	2.81542	32.90	chromosome:NZ_CP010998.1/CP010998.1	1	2845	2718	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/580/495/GCF_001580495.1_ASM158049v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/580/495/GCA_001580495.1_ASM158049v1	Unpublished	PRJNA277068
RKI4	*Staphylococcus aureus*	27	SAMN03699895	PRJNA284222	GCA_001027045.1	2.74356	32.77	chromosome:NZ_CP011528.1/CP011528.1; plasmid unnamed:NZ_CP011529.1/CP011529.1	2	2551	2414	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/027/045/GCF_001027045.1_ASM102704v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/027/045/GCA_001027045.1_ASM102704v1	Stevens et al., 2015	10.1128/genomeA.00769-15
SA17_S6	*Staphylococcus aureus*	152	SAMN03339003	PRJNA274731	GCA_000934205.1	2.67218	32.70	chromosome:CP010941.1	1	2700	2536	Chromosome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/934/205/GCA_000934205.1_ASM93420v1	Biswas et al., 2015	10.1128/genomeA.00836-15
SA268	*Staphylococcus aureus*	59	SAMN02261725	PRJNA213166	GCA_000737615.1	2.83390	32.90	chromosome:NZ_CP006630.1/CP006630.1	1	2853	2708	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/737/615/GCF_000737615.1_ASM73761v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/737/615/GCA_000737615.1_ASM73761v1	Qu et al., 2014	10.1371/journal.pone.0089235
SA40	*Staphylococcus aureus*	59	SAMN02603110	PRJNA167373	GCA_000470865.1	2.72831	32.90	chromosome:NC_022443.1/CP003604.1	1	2698	2560	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/470/865/GCF_000470865.1_ASM47086v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/470/865/GCA_000470865.1_ASM47086v1	Chen et al., 2013	10.1371/journal.pone.0063210
SA40TW	*Staphylococcus aureus*	-	SAMN04263915	PRJNA302097	GCA_001880265.1	2.77185	32.90	chromosome:NZ_CP013182.1/CP013182.1	1	2872	2617	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/880/265/GCF_001880265.1_ASM188026v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/880/265/GCA_001880265.1_ASM188026v1	Unpublished	PRJNA302097
SA564	*Staphylococcus aureus*	5	SAMN03267711	PRJNA270620	GCA_001281145.1	2.81081	32.84	chromosome:NZ_CP010890.1/CP010890.1; plasmid pSA564:NZ_CP010891.1/CP010891.1	2	2842	2730	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/281/145/GCF_001281145.1_ASM128114v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/281/145/GCA_001281145.1_ASM128114v1	Giraud et al., 2015	10.1080/15476286.2015.1035505
SA957	*Staphylococcus aureus*	59	SAMN00996491	PRJNA167119	GCA_000470845.1	2.78954	32.90	chromosome:NC_022442.1/CP003603.1	1	2787	2650	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/470/845/GCF_000470845.1_ASM47084v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/470/845/GCA_000470845.1_ASM47084v1	Chen et al., 2013	10.1371/journal.pone.0063210
SJTUF_J27	*Staphylococcus aureus*	433	SAMN06200256	PRJNA353940	GCA_001956755.1	2.80476	32.90	chromosome:NZ_CP019117.1/CP019117.1	1	2907	2718	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/956/755/GCF_001956755.1_ASM195675v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/956/755/GCA_001956755.1_ASM195675v1	Unpublished	PRJNA224116
ST20130938	*Staphylococcus aureus*	15	SAMN04166246	PRJNA298722	GCA_001611405.1	2.74688	32.87	chromosome:NZ_CP012972.1/CP012972.1; plasmid pST20130938:NZ_CP012973.1/CP012973.1	2	2765	2634	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/611/405/GCF_001611405.1_ASM161140v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/611/405/GCA_001611405.1_ASM161140v1	Trouillet-Assant et al., 2016	10.1111/cmi.12582
ST20130940	*Staphylococcus aureus*	15	SAMN04166494	PRJNA298753	GCA_001611325.1	2.70036	32.87	chromosome:NZ_CP012979.1/CP012979.1; plasmid pST20130940:NZ_CP012980.1/CP012980.1	2	2695	2565	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/611/325/GCF_001611325.1_ASM161132v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/611/325/GCA_001611325.1_ASM161132v1	Trouillet-Assant et al., 2016	10.1111/cmi.12582
ST20130942	*Staphylococcus aureus*	25	SAMN04166544	PRJNA298756	GCA_001611365.1	2.76713	32.77	chromosome:NZ_CP012976.1/CP012976.1; plasmid pST20130942:NZ_CP012977.1/CP012977.1	2	2792	2663	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/611/365/GCF_001611365.1_ASM161136v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/611/365/GCA_001611365.1_ASM161136v1	Trouillet-Assant et al., 2016	10.1111/cmi.12582
ST228-10388	*Staphylococcus aureus*	228	SAMEA2272458	PRJEA71349	GCA_000382965.1	2.79069	32.89	chromosome:NC_020533.1/HE579065.1; plasmid pI4T8:NC_020534.1/HE579066.1	2	2820	2684	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/382/965/GCF_000382965.1_ASM38296v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/382/965/GCA_000382965.1_ASM38296v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-10497	*Staphylococcus aureus*	228	SAMEA2272299	PRJEA71351	GCA_000382985.1	2.79674	32.84	chromosome:NC_020566.1/HE579067.1; plasmid pI5S5:NC_020535.1/HE579068.1	2	2822	2681	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/382/985/GCF_000382985.1_ASM38298v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/382/985/GCA_000382985.1_ASM38298v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-15532	*Staphylococcus aureus*	228	SAMEA2272502	PRJEA71357	GCA_000383005.1	2.78309	32.87	chromosome:NC_020568.1/HE579073.1; plasmid pI8T7:NC_020539.1/HE579074.1	2	2807	2670	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/383/005/GCF_000383005.1_ASM38300v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/383/005/GCA_000383005.1_ASM38300v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-16035	*Staphylococcus aureus*	228	SAMEA3283121	PRJEA71343	GCA_000967325.1	2.79036	32.89	chromosome:NC_020529.1/HE579059.1; plasmid pI1T1:NC_020530.1/HE579060.1	2	2816	2684	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/967/325/GCF_000967325.1_ASM96732v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/967/325/GCA_000967325.1_ASM96732v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-16125	*Staphylococcus aureus*	228	SAMEA3283122	PRJEA71345	GCA_000967345.1	2.79037	32.89	chromosome:NC_020564.1/HE579061.1; plasmid pI2T2:NC_020531.1/HE579062.1	2	2816	2678	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/967/345/GCF_000967345.1_ASM96734v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/967/345/GCA_000967345.1_ASM96734v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-18341	*Staphylococcus aureus*	228	SAMEA3283122	PRJEA71347	GCA_000967365.1	2.79207	32.89	chromosome:NC_020532.1/HE579063.1; plasmid pI3T3:NC_020565.1/HE579064.1	2	2817	2676	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/967/365/GCF_000967365.1_ASM96736v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/967/365/GCA_000967365.1_ASM96736v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-18412	*Staphylococcus aureus*	228	SAMEA3283124	PRJEA71353	GCA_000967385.1	2.79217	32.89	chromosome:NC_020536.1/HE579069.1; plasmid pI6T6:NC_020567.1/HE579070.1	2	2817	2678	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/967/385/GCF_000967385.1_ASM96738v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/967/385/GCA_000967385.1_ASM96738v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
ST228-18583	*Staphylococcus aureus*	228	SAMEA3283125	PRJEA71355	GCA_000967405.1	2.78302	32.87	chromosome:NC_020537.1/HE579071.1; plasmid pI7S6:NC_020538.1/HE579072.1	2	2809	2669	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/967/405/GCF_000967405.1_ASM96740v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/967/405/GCA_000967405.1_ASM96740v1	Vogel et al., 2012	https://doi.org/10.1371/journal.pone.0038969
T0131	*Staphylococcus aureus*	239	SAMN02603905	PRJNA65323	GCA_000204665.1	2.91390	32.80	chromosome:NC_017347.1/CP002643.1	1	2972	2856	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/204/665/GCF_000204665.1_ASM20466v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/204/665/GCA_000204665.1_ASM20466v1	Li et al., 2011	10.1128/JB.05135-11
Tager_104	*Staphylococcus aureus*	49	SAMN02393830	PRJNA213989	GCA_000452385.2	2.82084	32.90	chromosome:NZ_CP012409.1/CP012409.1	1	2857	2636	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/452/385/GCF_000452385.2_ASM45238v2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/452/385/GCA_000452385.2_ASM45238v2	Davis et al., 2013	10.1128/genomeA.00706-13
TCH1516	*Staphylococcus aureus*	8	SAMN00253845	PRJNA19489	GCA_000017085.1	2.90308	32.77	chromosome:NC_010079.1/CP000730.1; plasmid pUSA01-HOU:NC_012417.1/CP001544.1; plasmid pUSA300HOUM	3	2978	2876	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/017/085/GCF_000017085.1_ASM1708v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/017/085/GCA_000017085.1_ASM1708v1	Highlander et al., 2007	10.1186/1471-2180-7-99
TCH60	*Staphylococcus aureus*	-	SAMN00002240	PRJNA31539	GCA_000159535.2	2.82717	32.86	chromosome:NC_017342.1/CP002110.1; plasmid unnamed:NC_017345.1/CP002111.1	2	2822	2655	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/159/535/GCF_000159535.2_ASM15953v2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/159/535/GCA_000159535.2_ASM15953v2	Unpublished	PRJNA31539
TMUS2126	*Staphylococcus aureus*	72	SAMD00020283	PRJDB3193	GCA_001549655.1	2.77016	32.80	chromosome Unknown:NZ_AP014652.1/AP014652.1	1	2789	2649	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/549/655/GCF_001549655.1_ASM154965v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/549/655/GCA_001549655.1_ASM154965v1	Yamaguchi et al., 2015	10.1128/AAC.05159-14
TW20	*Staphylococcus aureus*	239	SAMEA2272282	PRJEA36647	GCA_000027045.1	3.07581	32.80	chromosome:NC_017331.1/FN433596.1; plasmid pTW20_1:NC_017352.1/FN433597.1; plasmid pTW20_2:NC_01733	3	3163	3041	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/027/045/GCF_000027045.1_ASM2704v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/027/045/GCA_000027045.1_ASM2704v1	Holden et al., 2010	10.1128/JB.01255-09
S0385 (ST398)	*Staphylococcus aureus*	398	SAMEA2272644	PRJEA29427	GCA_000009585.1	2.88537	32.89	chromosome:NC_017333.1/AM990992.1; plasmid pS0385-1:NC_017334.1/AM990993.1; plasmid pS0385-2:NC_0173	4	2917	2751	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/009/585/GCF_000009585.1_ASM958v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/009/585/GCA_000009585.1_ASM958v1	Schijffelen et al., 2010	10.1186/1471-2164-11-376
UA-S391_USA300	*Staphylococcus aureus*	8	SAMN02777327	PRJNA246435	GCA_000695875.1	2.87292	32.80	chromosome:NZ_CP007690.1/CP007690.1	1	2941	2840	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/695/875/GCF_000695875.1_ASM69587v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/695/875/GCA_000695875.1_ASM69587v1	Sabirova et al., 2014	10.1128/genomeA.00610-14
UCI_28	*Staphylococcus aureus*	5	SAMN06046106	PRJNA354449	GCA_001975045.1	2.86258	32.85	chromosome:NZ_CP018768.1/CP018768.1; plasmid pUCI28:NZ_CP018769.1/CP018769.1	-	2976	2804	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/975/045/GCF_001975045.1_ASM197504v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/975/045/GCA_001975045.1_ASM197504v1	Hau et al., 2017	10.1128/genomeA.00099-17
UCI62	*Staphylococcus aureus*	5	SAMN06046111	PRJNA354450	GCA_001975005.1	2.92724	32.88	chromosome:NZ_CP018766.1/CP018766.1; plasmid pUCI62:NZ_CP018767.1/CP018767.1	-	3062	2980	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/975/005/GCF_001975005.1_ASM197500v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/975/005/GCA_001975005.1_ASM197500v1	Hau et al., 2017	10.1128/genomeA.00099-17
USA300_2014.C01	*Staphylococcus aureus*	8	SAMN03787328	PRJNA287576	GCA_001183705.1	2.94564	32.78	chromosome:NZ_CP012119.1/CP012119.1; plasmid unnamed:NZ_CP012118.1/CP012118.1	2	3042	2933	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/183/705/GCF_001183705.1_ASM118370v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/183/705/GCA_001183705.1_ASM118370v1	Johnson et al., 2015	10.1128/JCM.01771-15
USA300_FPR3757	*Staphylococcus aureus*	8	SAMN02604150	PRJNA16313	GCA_000013465.1	2.91747	32.74	chromosome:NC_007793.1/CP000255.1; plasmid pUSA01:NC_007790.1/CP000256.1; plasmid pUSA02:NC_007791.	4	2982	2887	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/013/465/GCF_000013465.1_ASM1346v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/013/465/GCA_000013465.1_ASM1346v1	Diep et al., 2006	10.1016/S0140-6736(06)68231-7
USA300_SUR1	*Staphylococcus aureus*	8	SAMN06238073	PRJNA260675	GCA_001956815.1	2.93497	32.77	chromosome:NZ_CP009423.1/CP009423.1; plasmid pSUR1A:NZ_CP009424.1/CP009424.1; plasmid pSUR1B:NZ_CF	3	3029	2930	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/956/815/GCF_001956815.1_ASM195681v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/956/815/GCA_001956815.1_ASM195681v1	Sabat et al., 2016	10.1038/srep41050
USA300-ISMMS1	*Staphylococcus aureus*	8	SAMN03081531	PRJNA237099	GCA_000568455.1	2.95120	32.77	chromosome:NZ_CP007176.1/CP007176.1; plasmid pUSA01-ISMMS:NZ_CP007177.1/CP007177.1; plasmid pUSA02	3	3038	2931	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/568/455/GCF_000568455.1_ASM56845v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/568/455/GCA_000568455.1_ASM56845v1	Altman et al., 2014	10.1111/ajt.12897
UTSW_MRSA_55	*Staphylococcus aureus*	8	SAMN04254471	PRJNA273427	GCA_001580515.1	3.03979	32.69	chromosome:NZ_CP013231.1/CP013231.1; plasmid UTSW55_1:NZ_CP013227.1/CP013227.1; plasmid UTSW55_2:N	5	3120	2949	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/580/515/GCF_001580515.1_ASM158051v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/580/515/GCA_001580515.1_ASM158051v1	Unpublished	PRJNA273427
V2200	*Staphylococcus aureus*	923	SAMN02743812	PRJNA246185	GCA_001046095.2	2.87437	32.86	chromosome:NZ_CP007657.1/CP007657.1; plasmid pV2200:NZ_CP007658.1/CP007658.1	2	2934	2816	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/046/095/GCF_001046095.2_ASM104609v2	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/046/095/GCA_001046095.2_ASM104609v2	Planet et al., 2015	10.1093/infdis/jiv320
V521	*Staphylococcus aureus*	239	SAMN04219577	PRJNA300829	GCA_001641025.1	3.11428	32.80	chromosome:NZ_CP013957.1/CP013957.1; plasmid pV521:NZ_CP013958.1/CP013958.1	2	3225	3057	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/641/025/GCF_001641025.1_ASM164102v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/641/025/GCA_001641025.1_ASM164102v1	Unpublished	PRJNA300829
V605	*Staphylococcus aureus*	-	SAMN04219581	PRJNA300829	GCA_001641045.1	3.13248	32.74	chromosome:CP013959.1; plasmid pV605:CP013960.1	2	3240	3102	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/641/045/GCF_001641045.1_ASM164104v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/641/045/GCA_001641045.1_ASM164104v1	Unpublished	PRJNA300829
VC40	*Staphylococcus aureus*	8	SAMN02603393	PRJNA66591	GCA_000245495.1	2.69257	32.80	chromosome:NC_016912.1/CP003033.1	1	2723	2611	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/245/495/GCF_000245495.1_ASM24549v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/245/495/GCA_000245495.1_ASM24549v1	Sass et al., 2012	10.1128/JB.06631-11
XN108	*Staphylococcus aureus*	239	SAMN02693431	PRJNA239544	GCA_000709475.1	3.05206	32.80	chromosome:CP007447.1	1	3064	2992	Complete Genome	-	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/709/475/GCA_000709475.1_ASM70947v1	Zhang et al., 2014	10.1128/genomeA.00449-14
XQ	*Staphylococcus aureus*	121	SAMN04253017	PRJNA301649	GCA_001444345.1	2.80359	32.80	chromosome:NZ_CP013137.1/CP013137.1	1	2862	2639	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/444/345/GCF_001444345.1_ASM144434v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/444/345/GCA_001444345.1_ASM144434v1	Unpublished	PRJNA301649
Z172	*Staphylococcus aureus*	239	SAMN02370325	PRJNA222409	GCA_000485885.1	3.01830	32.80	chromosome:NC_022604.1/CP006838.1; plasmid pZ172_1:NC_022610.1/CP006839.1; plasmid pZ172_2:NC_022605	3	3119	2993	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/485/885/GCF_000485885.1_ASM48588v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/485/885/GCA_000485885.1_ASM48588v1	Chen et al., 2013	10.1128/genomeA.01011-13
ZJ5499	*Staphylococcus aureus*	5	SAMN03742530	PRJNA285271	GCA_001640885.1	2.84809	32.80	chromosome:NZ_CP011685.1/CP011685.1	1	2897	2766	Complete Genome	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/640/885/GCF_001640885.1_ASM164088v1	ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/001/640/885/GCA_001640885.1_ASM164088v1	Unpublished	PRJNA285271

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 1 - Sheet A. *S. aureus* reference genomes

Strain	Organism	*in silico* MLST	Year isolation	Country	Institution location	Host	Clinical Presentation	Sample	NCBI Assembly	PacBio	Sequencing Reference	DOI
12228	*Staphylococcus epidermidis*	8	1955	United States	Unknown	Non-clinical	Not applicable	Not applicable	GCA_000007645.1	N	Zhang et al., 2003	10.1046/j.1365-2958.2003.03671.x
BPH0662	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	CNS infection	CSF	GCA_900086615.1	Y	Lee et al., 2016	10.1099/mgen.0.000077
PM221	*Staphylococcus epidermidis*	184	1999-2002	Finland	Unknown	Bovine	Mastitis	Mastitis	GCA_000751035.1	N	Savijoki et al., 2014	10.1021/pr500322d
RP62a	*Staphylococcus epidermidis*	10	1979-1980	United States	Memphis, Tennessee	Human	Bacteraemia	Blood culture	GCA_000011925.1	Y	Gill et al., 2005	10.1128/JB.187.7.2426-2438.2005
SEI	*Staphylococcus epidermidis*	-	Unknown	United States	Unknown	Non-clinical	Not applicable	Not applicable	GCA_000759555.1	N	Davenport et al., 2014	10.1128/genomeA.01059-14
1457	*Staphylococcus epidermidis*	86	1990	Gemany	Hamburg	Human	Bacteraemia	Blood culture (CVC)	GCA_002085695.1	Y	Galac et al., 2017	10.1128/genomeA.00450-17
14.1.R1	*Staphylococcus epidermidis*	-	2015	Denmark	Aarhus	Human	Colonising	Skin	GCA_001956655.2	Y	Lassen et al., 2017	10.1128/genomeA.00004-17

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 1 - Sheet B. *S. epidermidis* reference genomes

ISOLATE	*in silico* MLST	BIOSAMPLE	SAMPLE NAME	SRA	ORGANISM	Strain	Collected by	Collection Date	Geographic Location	Isolation Source	Latitude & Longitude	Host	Host Disease	Accession	ID	Sequencing Method	Reads	Yield	GC	MinLen	AvgLen	ModeLen	MaxLen	AvgQual	Depth	Assembler	Contigs	bp	ok	Ns	Gaps	Min	Avg	Max	N50	Reference	DOI
100_SEPI	2	SAMN03196961	100_SEPI	SRS746168	*Staphylococcus epidermidis*	100_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196961	3196961	Illumina Hiseq	1470304	137671202	31.8	19	93	101	101	36	48	SPAdes v3.7.1	78	2712016	2712016	0	0	201	34769	191611	73522	Roach et al., 2015	10.1371/journal.pgen.1005413
101_SEPI	2	SAMN03196971	101_SEPI	SRS746179	*Staphylococcus epidermidis*	101_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196971	3196971	Illumina Hiseq	1415016	134256554	31.9	19	94	101	101	35.3	47	SPAdes v3.7.1	100	2627628	2627628	0	0	324	26276	177746	64643	Roach et al., 2015	10.1371/journal.pgen.1005413
1013_SEPI	2	SAMN03196975	1013_SEPI	SRS746184	*Staphylococcus epidermidis*	1013_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196975	3196975	Illumina Hiseq	1457560	127076805	33.1	8	87	101	101	33.9	45	SPAdes v3.7.1	101	2606122	2606122	0	0	319	25803	206454	59071	Roach et al., 2015	10.1371/journal.pgen.1005413
102_SEPI	2	SAMN03196982	102_SEPI	SRS746192	*Staphylococcus epidermidis*	102_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196982	3196982	Illumina Hiseq	1523558	142081238	31.8	19	93	101	101	35.9	50	SPAdes v3.7.1	72	2704030	2704030	0	0	203	37555	208811	81893	Roach et al., 2015	10.1371/journal.pgen.1005413
1021.rep1_SEPI	2	SAMN03196984	1021.rep1_SEPI	SRS746191	*Staphylococcus epidermidis*	1021.rep1_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196984	3196984	Illumina Hiseq	1614092	137918480	33.1	8	85	101	101	34	49	SPAdes v3.7.1	105	2606186	2606186	0	0	335	24820	227697	59082	Roach et al., 2015	10.1371/journal.pgen.1005413
1022_SEPI	2	SAMN03196986	1022_SEPI	SRS746194	*Staphylococcus epidermidis*	1022_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196986	3196986	Illumina Hiseq	2256332	192910230	33	8	85	101	101	34.5	68	SPAdes v3.7.1	126	2611572	2611572	0	0	329	20726	206538	57923	Roach et al., 2015	10.1371/journal.pgen.1005413
1023_SEPI	2	SAMN03196987	1023_SEPI	SRS746195	*Staphylococcus epidermidis*	1023_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196987	3196987	Illumina Hiseq	1881180	162535076	32.9	8	86	101	101	34.4	57	SPAdes v3.7.1	100	2542191	2542191	0	0	320	25421	196625	71805	Roach et al., 2015	10.1371/journal.pgen.1005413
1026_SEPI	2	SAMN03196990	1026_SEPI	SRS746198	*Staphylococcus epidermidis*	1026_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03196990	3196990	Illumina Hiseq	1180544	103384001	32.9	8	87	101	101	34.6	36	SPAdes v3.7.1	77	2555301	2555301	0	0	316	33185	227103	71686	Roach et al., 2015	10.1371/journal.pgen.1005413
1040_SEPI	2	SAMN03197006	1040_SEPI	SRS746213	*Staphylococcus epidermidis*	1040_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197006	3197006	Illumina Hiseq	1896172	164176780	33	8	86	101	101	34.8	58	SPAdes v3.7.1	113	2611226	2611226	0	0	316	23108	177766	64674	Roach et al., 2015	10.1371/journal.pgen.1005413
1067_SEPI	101	SAMN03197029	1067_SEPI	SRS746237	*Staphylococcus epidermidis*	1067_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197029	3197029	Illumina Hiseq	1854226	159906388	33	8	86	101	101	34.1	56	SPAdes v3.7.1	97	2459827	2459827	0	0	314	25359	198471	81463	Roach et al., 2015	10.1371/journal.pgen.1005413
1088_SEPI	2	SAMN03197050	1088_SEPI	SRS746258	*Staphylococcus epidermidis*	1088_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197050	3197050	Illumina Hiseq	1645192	142966913	33	8	86	101	101	34.4	50	SPAdes v3.7.1	123	2566325	2566325	0	0	321	20864	208269	58577	Roach et al., 2015	10.1371/journal.pgen.1005413
1097_SEPI	5	SAMN03197059	1097_SEPI	SRS746267	*Staphylococcus epidermidis*	1097_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197059	3197059	Illumina Hiseq	1480548	129039475	33.1	8	87	101	101	34.1	45	SPAdes v3.7.1	87	2449985	2449985	0	0	338	28160	189678	76015	Roach et al., 2015	10.1371/journal.pgen.1005413
1115_SEPI	88	SAMN03197069	1115_SEPI	SRS746277	*Staphylococcus epidermidis*	1115_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197069	3197069	Illumina Hiseq	1794454	154746779	33.1	8	86	101	101	34.6	54	SPAdes v3.7.1	92	2442175	2442175	0	0	323	26545	157038	63100	Roach et al., 2015	10.1371/journal.pgen.1005413
1129_SEPI	2	SAMN03197083	1129_SEPI	SRS746291	*Staphylococcus epidermidis*	1129_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197083	3197083	Illumina Hiseq	979878	93457829	32.3	19	95	101	101	36.3	33	SPAdes v3.7.1	60	2511785	2511785	0	0	321	41863	196263	82543	Roach et al., 2015	10.1371/journal.pgen.1005413
1142_SEPI	69	SAMN03197098	1142_SEPI	SRS746305	*Staphylococcus epidermidis*	1142_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197098	3197098	Illumina Hiseq	899214	85910372	32.2	19	95	101	101	36.3	30	SPAdes v3.7.1	89	2489839	2489839	0	0	317	27975	233894	76908	Roach et al., 2015	10.1371/journal.pgen.1005413
1188_SEPI	5	SAMN03197147	1188_SEPI	SRS746355	*Staphylococcus epidermidis*	1188_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197147	3197147	Illumina Hiseq	1259430	119219979	32.2	19	94	101	101	36.2	42	SPAdes v3.7.1	70	2476525	2476525	0	0	328	35378	253806	82440	Roach et al., 2015	10.1371/journal.pgen.1005413
1190_SEPI	2	SAMN03197150	1190_SEPI	SRS746357	*Staphylococcus epidermidis*	1190_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197150	3197150	Illumina Hiseq	1149722	109541947	32	19	95	101	101	36.5	39	SPAdes v3.7.1	83	2747960	2747960	0	0	317	33107	189811	75077	Roach et al., 2015	10.1371/journal.pgen.1005413
1191_SEPI	83	SAMN03197151	1191_SEPI	SRS746359	*Staphylococcus epidermidis*	1191_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197151	3197151	Illumina Hiseq	1183788	112463290	32	19	95	101	101	36.3	40	SPAdes v3.7.1	78	2542915	2542915	0	0	335	32601	196685	75070	Roach et al., 2015	10.1371/journal.pgen.1005413
1226_SEPI	2	SAMN03197190	1226_SEPI	SRS746397	*Staphylococcus epidermidis*	1226_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197190	3197190	Illumina Hiseq	1111406	106006779	32.2	19	95	101	101	36.5	37	SPAdes v3.7.1	86	2523111	2523111	0	0	320	29338	177532	78593	Roach et al., 2015	10.1371/journal.pgen.1005413
1284_SEPI	184	SAMN03197255	1284_SEPI	SRS746463	*Staphylococcus epidermidis*	1284_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197255	3197255	Illumina Hiseq	979684	93539793	32.1	19	95	101	101	36.3	33	SPAdes v3.7.1	65	2466656	2466656	0	0	332	37948	268773	79380	Roach et al., 2015	10.1371/journal.pgen.1005413
1289_SEPI	17	SAMN03197260	1289_SEPI	SRS746467	*Staphylococcus epidermidis*	1289_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197260	3197260	Illumina Hiseq	1015442	96813478	32.2	19	95	101	101	36.5	34	SPAdes v3.7.1	67	2422096	2422096	0	0	208	36150	233757	75570	Roach et al., 2015	10.1371/journal.pgen.1005413
1295_SEPI	2	SAMN03197267	1295_SEPI	SRS746475	*Staphylococcus epidermidis*	1295_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197267	3197267	Illumina Hiseq	968766	92724819	32.1	19	95	101	101	36.5	33	SPAdes v3.7.1	80	2603330	2603330	0	0	318	32541	196342	73147	Roach et al., 2015	10.1371/journal.pgen.1005413
1296_SEPI	17	SAMN03197268	1296_SEPI	SRS746476	*Staphylococcus epidermidis*	1296_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197268	3197268	Illumina Hiseq	1017942	96839330	33.8	19	95	101	101	36.3	34	SPAdes v3.7.1	235	2495643	2495643	0	0	314	10619	233872	67393	Roach et al., 2015	10.1371/journal.pgen.1005413
1306.rep2_SEPI	17	SAMN03197281	1306.rep2_SEPI	SRS746488	*Staphylococcus epidermidis*	1306.rep2_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197281	3197281	Illumina Hiseq	999056	95076568	32.2	19	95	101	101	36.4	33	SPAdes v3.7.1	72	2425396	2425396	0	0	350	33686	252948	70327	Roach et al., 2015	10.1371/journal.pgen.1005413
248_DEN19	1	SAMN05207190	248	SRS1525994	*Staphylococcus epidermidis*	DEN19	ITQB	1997	Denmark	Unknown	55.67 N 12.57 E	Homo sapiens	Infection	SAMN05207190	5207190	Illumina Hiseq	3276956	327695600	31.3	100	100	100	100	34.9	115	SPAdes v3.7.1	92	2482742	2482742	0	0	224	26986	184839	70571	Meric et al., 2015	10.1093/gbe/evv066
249_ICE18	1	SAMN05207191	249	SRS1525993	*Staphylococcus epidermidis*	ICE18	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207191	5207191	Illumina Hiseq	4319056	431905600	32	100	100	100	100	35.8	152	SPAdes v3.7.1	185	2527316	2527316	0	0	201	13661	198502	57394	Meric et al., 2015	10.1093/gbe/evv066
250_ICE7	2	SAMN05207192	250	SRS1526005	*Staphylococcus epidermidis*	ICE7	ITQB	1997	Iceland	Blood	64.13 N 21.82 W	Homo sapiens	Bacteraemia	SAMN05207192	5207192	Illumina Hiseq	3172982	317298200	32.5	100	100	100	100	35.2	112	SPAdes v3.7.1	125	2703929	2703929	0	0	206	21631	177404	65973	Meric et al., 2015	10.1093/gbe/evv066
251_1347N	55	SAMN05207193	251	SRS1526017	*Staphylococcus epidermidis*	1347N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207193	5207193	Illumina Hiseq	2211012	221101200	32.3	100	100	100	100	33.8	78	SPAdes v3.7.1	99	2424659	2424659	0	0	313	24491	198492	81635	Meric et al., 2015	10.1093/gbe/evv066
252_DEN22	4	SAMN05207194	252	SRS1526027	*Staphylococcus epidermidis*	DEN22	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207194	5207194	Illumina Hiseq	3409886	340988600	32.3	100	100	100	100	35.1	120	SPAdes v3.7.1	116	2490903	2490903	0	0	233	21473	207847	58821	Meric et al., 2015	10.1093/gbe/evv066
253_ICE192	5	SAMN05207195	253	SRS1526037	*Staphylococcus epidermidis*	ICE192	ITQB	1998	Iceland	Blood	64.13 N 21.82 W	Homo sapiens	Bacteraemia	SAMN05207195	5207195	Illumina Hiseq	3569974	356997400	32.6	100	100	100	100	35.1	126	SPAdes v3.7.1	120	2456915	2456915	0	0	201	20474	263670	82257	Meric et al., 2015	10.1093/gbe/evv066
254_DEN178	5	SAMN05207196	254	SRS1526049	*Staphylococcus epidermidis*	DEN178	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207196	5207196	Illumina Hiseq	3671280	367128000	32.3	100	100	100	100	34.9	129	SPAdes v3.7.1	145	2637079	2637079	0	0	204	18186	263578	82907	Meric et al., 2015	10.1093/gbe/evv066
255_ICE9	6	SAMN05207197	255	SRS1526060	*Staphylococcus epidermidis*	ICE9	ITQB	1997	Iceland	Urine	64.13 N 21.82 W	Homo sapiens	UTI	SAMN05207197	5207197	Illumina Hiseq	5217288	521728800	32.9	100	100	100	100	35.7	184	SPAdes v3.7.1	98	2481963	2481963	0	0	218	25326	252334	78522	Meric et al., 2015	10.1093/gbe/evv066
256_ICE95	10	SAMN05207198	256	SRS1526071	*Staphylococcus epidermidis*	ICE95	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207198	5207198	Illumina Hiseq	4642176	464217600	32.8	100	100	100	100	35.6	163	SPAdes v3.7.1	100	2435923	2435923	0	0	204	24359	233824	82969	Meric et al., 2015	10.1093/gbe/evv066
257_DEN76	14	SAMN05207199	257	SRS1526075	*Staphylococcus epidermidis*	DEN76	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207199	5207199	Illumina Hiseq	4035442	403544200	32.1	100	100	100	100	35.1	142	SPAdes v3.7.1	106	2415933	2415933	0	0	316	22791	185124	78266	Meric et al., 2015	10.1093/gbe/evv066
259_ICE99	20	SAMN05207201	259	SRS1525996	*Staphylococcus epidermidis*	ICE99	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207201	5207201	Illumina Hiseq	4956620	495662000	32.6	100	100	100	100	35.3	175	SPAdes v3.7.1	96	2431352	2431352	0	0	202	25326	198589	72163	Meric et al., 2015	10.1093/gbe/evv066
260_DEN185	21	SAMN05207202	260	SRS1525997	*Staphylococcus epidermidis*	DEN185	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207202	5207202	Illumina Hiseq	5396584	539658400	32.7	100	100	100	100	35.9	190	SPAdes v3.7.1	141	2558445	2558445	0	0	201	18145	198505	70556	Meric et al., 2015	10.1093/gbe/evv066
261_DEN73	21	SAMN05207203	261	SRS1525998	*Staphylococcus epidermidis*	DEN73	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207203	5207203	Illumina Hiseq	4702852	470285200	32.8	100	100	100	100	35.5	166	SPAdes v3.7.1	265	2667187	2667187	0	0	200	10064	215774	67260	Meric et al., 2015	10.1093/gbe/evv066
262_DEN61	22	SAMN05207204	262	SRS1525999	*Staphylococcus epidermidis*	DEN61	ITQB	1997	Denmark	Wound	55.67 N 12.57 E	Homo sapiens	Commensal	SAMN05207204	5207204	Illumina Hiseq	2872812	287281200	32	100	100	100	100	34.8	101	SPAdes v3.7.1	91	2429777	2429777	0	0	314	26700	263666	81903	Meric et al., 2015	10.1093/gbe/evv066
263_HFA6228	22	SAMN05207205	263	SRS1526000	*Staphylococcus epidermidis*	HFA6228	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207205	5207205	Illumina Hiseq	3651130	365113000	32.8	100	100	100	100	35.1	129	SPAdes v3.7.1	114	2466138	2466138	0	0	202	21632	263664	82908	Meric et al., 2015	10.1093/gbe/evv066
264_AGT24	23	SAMN05207206	264	SRS1526001	*Staphylococcus epidermidis*	AGT24	ITQB	1997	Argentina	Blood	34.60 S 58.38 W	Homo sapiens	Bacteraemia	SAMN05207206	5207206	Illumina Hiseq	6120474	612047400	32.7	100	100	100	100	35.7	216	SPAdes v3.7.1	103	2441041	2441041	0	0	203	23699	234095	81914	Meric et al., 2015	10.1093/gbe/evv066
265_HUR105	23	SAMN05207207	265	SRS1526002	*Staphylococcus epidermidis*	HUR105	ITQB	1998	Hungary	Sputum	47.50 N 19.04 E	Homo sapiens	Pneumonia	SAMN05207207	5207207	Illumina Hiseq	3883156	388315600	32.5	100	100	100	100	35.1	137	SPAdes v3.7.1	126	2491932	2491932	0	0	201	19777	234400	75404	Meric et al., 2015	10.1093/gbe/evv066
266_ICE26	248	SAMN05207208	266	SRS1526003	*Staphylococcus epidermidis*	ICE26	ITQB	1997	Iceland	Blood	64.13 N 21.82 W	Homo sapiens	Bacteraemia	SAMN05207208	5207208	Illumina Hiseq	4093300	409330000	31.8	100	100	100	100	35.2	144	SPAdes v3.7.1	176	2722358	2722358	0	0	205	15467	195608	76698	Meric et al., 2015	10.1093/gbe/evv066
267_966N	35	SAMN05207209	267	SRS1526004	*Staphylococcus epidermidis*	966N	ITQB	1998	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207209	5207209	Illumina Hiseq	3148714	314871400	32.4	100	100	100	100	34.4	111	SPAdes v3.7.1	131	2503277	2503277	0	0	215	19108	263583	82913	Meric et al., 2015	10.1093/gbe/evv066
268_ICE21	36	SAMN05207210	268	SRS1526006	*Staphylococcus epidermidis*	ICE21	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207210	5207210	Illumina Hiseq	4008034	400803400	32.5	100	100	100	100	35.3	141	SPAdes v3.7.1	99	2597625	2597625	0	0	319	26238	196856	70540	Meric et al., 2015	10.1093/gbe/evv066
269_ICE24	38	SAMN05207211	269	SRS1526007	*Staphylococcus epidermidis*	ICE24	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207211	5207211	Illumina Hiseq	5002040	500204000	32.5	100	100	100	100	35.2	176	SPAdes v3.7.1	118	2477811	2477811	0	0	201	20998	207184	82854	Meric et al., 2015	10.1093/gbe/evv066
270_DEN107	40	SAMN05207212	270	SRS1526008	*Staphylococcus epidermidis*	DEN107	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207212	5207212	Illumina Hiseq	3430578	343057800	31.4	100	100	100	100	35	121	SPAdes v3.7.1	110	2469742	2469742	0	0	207	22452	234898	64653	Meric et al., 2015	10.1093/gbe/evv066
271_ICE87	40	SAMN05207213	271	SRS1526009	*Staphylococcus epidermidis*	ICE87	ITQB	1997	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207213	5207213	Illumina Hiseq	5143144	514314400	32.6	100	100	100	100	35.8	181	SPAdes v3.7.1	103	2603490	2603490	0	0	200	25276	190508	91133	Meric et al., 2015	10.1093/gbe/evv066
272_DEN116	215	SAMN05207214	272	SRS1526010	*Staphylococcus epidermidis*	DEN116	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207214	5207214	Illumina Hiseq	5582608	558260800	33	100	100	100	100	35.4	197	SPAdes v3.7.1	100	2486403	2486403	0	0	222	24864	196963	67311	Meric et al., 2015	10.1093/gbe/evv066
273_ICE122	215	SAMN05207215	273	SRS1526011	*Staphylococcus epidermidis*	ICE122	ITQB	1998	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Commensal	SAMN05207215	5207215	Illumina Hiseq	4015796	401579600	33.2	100	100	100	100	35.3	141	SPAdes v3.7.1	114	2621150	2621150	0	0	203	22992	196990	70586	Meric et al., 2015	10.1093/gbe/evv066
274_MCO150	46	SAMN05207216	274	SRS1526012	*Staphylococcus epidermidis*	MCO150	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207216	5207216	Illumina Hiseq	3338608	333860800	32.8	100	100	100	100	35	118	SPAdes v3.7.1	120	2572952	2572952	0	0	207	21441	233503	75822	Meric et al., 2015	10.1093/gbe/evv066
275_COB20	21	SAMN05207217	275	SRS1526014	*Staphylococcus epidermidis*	COB20	ITQB	1997	Colombia	Urine	4.72 N 74.07 W	Homo sapiens	UTI	SAMN05207217	5207217	Illumina Hiseq	5066234	506623400	32.9	100	100	100	100	35.7	178	SPAdes v3.7.1	139	2671984	2671984	0	0	201	19222	190100	64803	Meric et al., 2015	10.1093/gbe/evv066
276_ICE102	51	SAMN05207218	276	SRS1526013	*Staphylococcus epidermidis*	ICE102	ITQB	1998	Iceland	Wound	64.13 N 21.82 W	Homo sapiens	Wound infection	SAMN05207218	5207218	Illumina Hiseq	3104170	310417000	32.3	100	100	100	100	34.7	109	SPAdes v3.7.1	103	2448365	2448365	0	0	204	23770	239946	79444	Meric et al., 2015	10.1093/gbe/evv066
277_JAP271	2#	SAMN05207219	277	SRS1526015	*Staphylococcus epidermidis*	JAP271	ITQB	1998	Japan	Urine	35.69 N 139.69 E	Homo sapiens	Commensal	SAMN05207219	5207219	Illumina Hiseq	3735200	373520000	33.1	100	100	100	100	34.4	132	SPAdes v3.7.1	84	2423951	2423951	0	0	323	28856	265245	75319	Meric et al., 2015	10.1093/gbe/evv066
278_DEN189	57	SAMN05207220	278	SRS1526018	*Staphylococcus epidermidis*	DEN189	ITQB	1998	Denmark	Unknown	55.67 N 12.57 E	Homo sapiens	Unknown	SAMN05207220	5207220	Illumina Hiseq	5221424	522142400	32.6	100	100	100	100	35.5	184	SPAdes v3.7.1	94	2576365	2576365	0	0	207	27408	236080	81636	Meric et al., 2015	10.1093/gbe/evv066
279_HFA6162	2	SAMN05207221	279	SRS1526016	*Staphylococcus epidermidis*	HFA6162	ITQB	2000	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207221	5207221	Illumina Hiseq	3173286	317328600	31.5	100	100	100	100	35.2	112	SPAdes v3.7.1	121	2460698	2460698	0	0	312	20336	263636	82582	Meric et al., 2015	10.1093/gbe/evv066
280_BUG46	189	SAMN05207222	280	SRS1526019	*Staphylococcus epidermidis*	BUG46	ITQB	1997	Bulgaria	Unknown	42.70 N 23.32 E	Homo sapiens	Infection	SAMN05207222	5207222	Illumina Hiseq	3558460	355846000	32.2	100	100	100	100	34.6	125	SPAdes v3.7.1	100	2445790	2445790	0	0	202	24457	234398	78587	Meric et al., 2015	10.1093/gbe/evv066
281_SEPI	59	SAMN03197475	281_SEPI	SRS746680	*Staphylococcus epidermidis*	281_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	unknown	Homo sapiens	Unknown	SAMN03197475	3197475	Illumina Hiseq	3995798	399579800	32	100	100	100	100	35.3	141	SPAdes v3.7.1	88	2445430	2445430	0	0	206	27788	210369	75496	Roach et al., 2015	10.1371/journal.pgen.1005413
282_HFA6226	60	SAMN05207224	282	SRS1526021	*Staphylococcus epidermidis*	HFA6226	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207224	5207224	Illumina Hiseq	3113792	311379200	32.4	100	100	100	100	34.9	110	SPAdes v3.7.1	98	2471686	2471686	0	0	206	25221	234371	82911	Meric et al., 2015	10.1093/gbe/evv066
283_MEX60	61	SAMN05207225	283	SRS1526022	*Staphylococcus epidermidis*	MEX60	ITQB	1996	Mexico	Catheter	19.43 N 99.13 W	Homo sapiens	Catheter infection	SAMN05207225	5207225	Illumina Hiseq	3328790	332879000	32.2	100	100	100	100	34.7	117	SPAdes v3.7.1	89	2603896	2603896	0	0	261	29257	189833	82835	Meric et al., 2015	10.1093/gbe/evv066
284_MEX61	61	SAMN05207226	284	SRS1526023	*Staphylococcus epidermidis*	MEX61	ITQB	1996	Mexico	Blood	19.43 N 99.13 W	Homo sapiens	Bacteraemia	SAMN05207226	5207226	Illumina Hiseq	5517848	551784800	33	100	100	100	100	35.4	194	SPAdes v3.7.1	94	2607725	2607725	0	0	243	27741	189833	82962	Meric et al., 2015	10.1093/gbe/evv066
285_GRE34	69	SAMN05207227	285	SRS1526024	*Staphylococcus epidermidis*	GRE34	ITQB	1998	Greece	Unknown	37.98 N 23.73 E	Homo sapiens	Unknown	SAMN05207227	5207227	Illumina Hiseq	3546060	354606000	32.4	100	100	100	100	34.4	125	SPAdes v3.7.1	138	2562651	2562651	0	0	207	18569	233280	69783	Meric et al., 2015	10.1093/gbe/evv066
286_HFA6391	73	SAMN05207228	286	SRS1526025	*Staphylococcus epidermidis*	HFA6391	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207228	5207228	Illumina Hiseq	3247838	324783800	32.5	100	100	100	100	34.9	114	SPAdes v3.7.1	137	2432244	2432244	0	0	202	17753	198073	79124	Meric et al., 2015	10.1093/gbe/evv066
287_CV45	23	SAMN05207229	287	SRS1526026	*Staphylococcus epidermidis*	CV45	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207229	5207229	Illumina Hiseq	2226036	222603600	32.4	100	100	100	100	35.1	78	SPAdes v3.7.1	105	2463433	2463433	0	0	314	23461	212171	81904	Meric et al., 2015	10.1093/gbe/evv066
288_GRE41	83	SAMN05207230	288	SRS1526028	*Staphylococcus epidermidis*	GRE41	ITQB	1998	Greece	Unknown	37.98 N 23.73 E	Homo sapiens	Infection	SAMN05207230	5207230	Illumina Hiseq	3761646	376164600	31.9	100	100	100	100	35.1	132	SPAdes v3.7.1	101	2460811	2460811	0	0	233	24364	208178	82758	Meric et al., 2015	10.1093/gbe/evv066
289_DEN161	35	SAMN05207231	289	SRS1526029	*Staphylococcus epidermidis*	DEN161	ITQB	1998	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207231	5207231	Illumina Hiseq	4182698	418289800	32.6	100	100	100	100	35.3	147	SPAdes v3.7.1	88	2446471	2446471	0	0	233	27800	263657	82912	Meric et al., 2015	10.1093/gbe/evv066
290_TAW113	85	SAMN05207232	290	SRS1526030	*Staphylococcus epidermidis*	TAW113	ITQB	1997	Taiwan	Sputum	23.70 N 120.96 E	Homo sapiens	Pneumonia	SAMN05207232	5207232	Illumina Hiseq	4081892	408189200	32.1	100	100	100	100	34.1	144	SPAdes v3.7.1	107	2483732	2483732	0	0	200	23212	255850	89115	Meric et al., 2015	10.1093/gbe/evv066
291_URU23	86	SAMN05207233	291	SRS1526032	*Staphylococcus epidermidis*	URU23	ITQB	1997	Uruguay	Urine	34.90 S 56.16 W	Homo sapiens	UTI	SAMN05207233	5207233	Illumina Hiseq	2790718	279071800	32.5	100	100	100	100	34.8	98	SPAdes v3.7.1	165	2681553	2681553	0	0	211	16251	216123	64881	Meric et al., 2015	10.1093/gbe/evv066
292_HFA6286	88	SAMN05207234	292	SRS1526031	*Staphylococcus epidermidis*	HFA6286	ITQB	2001	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207234	5207234	Illumina Hiseq	3281338	328133800	32	100	100	100	100	34.7	116	SPAdes v3.7.1	115	2453956	2453956	0	0	216	21338	255952	82759	Meric et al., 2015	10.1093/gbe/evv066
294_HFA6096	184	SAMN05207236	294	SRS1526034	*Staphylococcus epidermidis*	HFA6096	ITQB	2000	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207236	5207236	Illumina Hiseq	2427574	242757400	31.7	100	100	100	100	35.3	85	SPAdes v3.7.1	87	2468644	2468644	0	0	207	28375	286501	87535	Meric et al., 2015	10.1093/gbe/evv066
295_619N1	184	SAMN05207237	295	SRS1526035	*Staphylococcus epidermidis*	619N1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207237	5207237	Illumina Hiseq	3746874	374687400	32.2	100	100	100	100	35.2	132	SPAdes v3.7.1	86	2463253	2463253	0	0	207	28642	286563	87576	Meric et al., 2015	10.1093/gbe/evv066
296_DEN62	230	SAMN05207238	296	SRS1526036	*Staphylococcus epidermidis*	DEN62	ITQB	1997	Denmark	Blood	55.67 N 12.57 E	Homo sapiens	Bacteraemia	SAMN05207238	5207238	Illumina Hiseq	4406710	440671000	32.8	100	100	100	100	34.8	155	SPAdes v3.7.1	93	2565617	2565617	0	0	317	27587	632047	95692	Meric et al., 2015	10.1093/gbe/evv066
297_MEX37	71	SAMN05207239	297	SRS1526038	*Staphylococcus epidermidis*	MEX37	ITQB	1996	Mexico	CSF	19.43 N 99.13 W	Homo sapiens	CSF infection	SAMN05207239	5207239	Illumina Hiseq	3210102	321010200	32.3	100	100	100	100	35.1	113	SPAdes v3.7.1	77	2579977	2579977	0	0	320	33506	429393	106732	Meric et al., 2015	10.1093/gbe/evv066
298_JAP263	33	SAMN05207240	298	SRS1526039	*Staphylococcus epidermidis*	JAP263	ITQB	1998	Japan	Wound	35.69 N 139.69 E	Homo sapiens	Wound infection	SAMN05207240	5207240	Illumina Hiseq	3745388	374538800	32.7	100	100	100	100	35.1	132	SPAdes v3.7.1	112	2493079	2493079	0	0	200	22259	278993	95683	Meric et al., 2015	10.1093/gbe/evv066
299_HUR51	47	SAMN05207241	299	SRS1526040	*Staphylococcus epidermidis*	HUR51	ITQB	1997	Hungary	Wound	47.50 N 19.04 E	Homo sapiens	Wound infection	SAMN05207241	5207241	Illumina Hiseq	3987658	398765800	32.2	100	100	100	100	35.1	140	SPAdes v3.7.1	83	2431913	2431913	0	0	208	29300	254752	104257	Meric et al., 2015	10.1093/gbe/evv066
300_ITL34	66	SAMN05207242	300	SRS1526041	*Staphylococcus epidermidis*	ITL34	ITQB	1997	Italy	Wound	44.49 N 11.34 E	Homo sapiens	Wound infection	SAMN05207242	5207242	Illumina Hiseq	4515188	451518800	33.1	100	100	100	100	35.2	159	SPAdes v3.7.1	112	2607774	2607774	0	0	208	23283	210810	96076	Meric et al., 2015	10.1093/gbe/evv066
301_DEN110	66	SAMN05207243	301	SRS1526042	*Staphylococcus epidermidis*	DEN110	ITQB	1998	Denmark	Unknown	55.67 N 12.57 E	Homo sapiens	Commensal	SAMN05207243	5207243	Illumina Hiseq	4242330	424233000	32.9	100	100	100	100	34.8	149	SPAdes v3.7.1	98	2523906	2523906	0	0	289	25754	231678	96409	Meric et al., 2015	10.1093/gbe/evv066
303_CV13	369	SAMN05207245	303	SRS1526044	Staphylococcus epidermidis	CV13	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207245	5207245	Illumina Hiseq	4668730	466873000	32.3	100	100	100	100	35.7	164	SPAdes v3.7.1	133	2439497	2439497	0	0	202	18342	381244	91782	Meric et al., 2015	10.1093/gbe/evv066
306_59N	212	SAMN05207248	306	SRS1526047	*Staphylococcus epidermidis*	59N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207248	5207248	Illumina Hiseq	3413348	341334800	32.5	100	100	100	100	35.2	120	SPAdes v3.7.1	116	2502509	2502509	0	0	200	21573	263767	82130	Meric et al., 2015	10.1093/gbe/evv066
307_HFA6173B	37	SAMN05207249	307	SRS1526048	*Staphylococcus epidermidis*	HFA6173B	ITQB	2000	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207249	5207249	Illumina Hiseq	4775896	477589600	32.2	100	100	100	100	35.3	168	SPAdes v3.7.1	157	2599308	2599308	0	0	201	16556	398744	108148	Meric et al., 2015	10.1093/gbe/evv066
308_MEX35	559	SAMN05207250	308	SRS1526050	*Staphylococcus epidermidis*	MEX35	ITQB	1996	Mexico	Catheter	19.43 N 99.13 W	Homo sapiens	Catheter infection	SAMN05207250	5207250	Illumina Hiseq	5493610	549361000	32.6	100	100	100	100	35.6	193	SPAdes v3.7.1	107	2552783	2552783	0	0	205	23857	409686	111472	Meric et al., 2015	10.1093/gbe/evv066
309_ITL299	32	SAMN05207251	309	SRS1526051	*Staphylococcus epidermidis*	ITL299	ITQB	1997	Italy	Wound	44.49 N 11.34 E	Homo sapiens	Wound infection	SAMN05207251	5207251	Illumina Hiseq	3983808	398380800	32.6	100	100	100	100	34.9	140	SPAdes v3.7.1	97	2462743	2462743	0	0	206	25389	264792	82633	Meric et al., 2015	10.1093/gbe/evv066
310_GRE53	39	SAMN05207252	310	SRS1526052	*Staphylococcus epidermidis*	GRE53	ITQB	1998	Greece	Unknown	37.98 N 23.73 E	Homo sapiens	Unknown	SAMN05207252	5207252	Illumina Hiseq	3245152	324515200	32.1	100	100	100	100	35.1	114	SPAdes v3.7.1	83	2533088	2533088	0	0	200	30519	255031	119591	Meric et al., 2015	10.1093/gbe/evv066
313_93N	329	SAMN05207255	313	SRS1526055	*Staphylococcus epidermidis*	93N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207255	5207255	Illumina Hiseq	4958062	495806200	31.8	100	100	100	100	35.7	175	SPAdes v3.7.1	77	2515945	2515945	0	0	206	32674	266126	140040	Meric et al., 2015	10.1093/gbe/evv066
314_1076NL	307	SAMN05207256	314	SRS1526056	*Staphylococcus epidermidis*	1076NL	ITQB	1998	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207256	5207256	Illumina Hiseq	5900436	590043600	32.7	100	100	100	100	35.9	208	SPAdes v3.7.1	102	2554577	2554577	0	0	202	25044	234206	77386	Meric et al., 2015	10.1093/gbe/evv066
315_148N	390	SAMN05207257	315	SRS1526057	*Staphylococcus epidermidis*	148N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207257	5207257	Illumina Hiseq	5321372	532137200	32.8	100	100	100	100	35.5	187	SPAdes v3.7.1	122	2569367	2569367	0	0	206	21060	220483	68694	Meric et al., 2015	10.1093/gbe/evv066
316_19N	393	SAMN05207258	316	SRS1526058	*Staphylococcus epidermidis*	19N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207258	5207258	Illumina Hiseq	5719816	571981600	32.8	100	100	100	100	35.7	201	SPAdes v3.7.1	98	2615913	2615913	0	0	200	26692	491026	118109	Meric et al., 2015	10.1093/gbe/evv066
318_1243N	433	SAMN05207260	318	SRS1526061	Staphylococcus epidermidis	1243N	ITQB	1998	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207260	5207260	Illumina Hiseq	4380930	438093000	33.1	100	100	100	100	35.6	154	SPAdes v3.7.1	123	2528612	2528612	0	0	207	20557	245406	73296	Meric et al., 2015	10.1093/gbe/evv066
320_1437N	400	SAMN05207262	320	SRS1526063	*Staphylococcus epidermidis*	1437N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207262	5207262	Illumina Hiseq	4324770	432477000	32.8	100	100	100	100	35.3	152	SPAdes v3.7.1	119	2508392	2508392	0	0	200	21078	357269	119279	Meric et al., 2015	10.1093/gbe/evv066
322_1340N	402	SAMN05207264	322	SRS1526065	*Staphylococcus epidermidis*	1340N	ITQB	1999	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207264	5207264	Illumina Hiseq	4856848	485684800	32.8	100	100	100	100	35.7	177	SPAdes v3.7.1	93	2552082	2552082	0	0	205	27441	262286	100557	Meric et al., 2015	10.1093/gbe/evv066
323_603N	402	SAMN05207265	323	SRS1526066	*Staphylococcus epidermidis*	603N	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207265	5207265	Illumina Hiseq	5028808	502880800	32.7	100	100	100	100	35.6	177	SPAdes v3.7.1	94	2525767	2525767	0	0	200	26869	353528	139373	Meric et al., 2015	10.1093/gbe/evv066
324_56N	402	SAMN05207266	324	SRS1526067	*Staphylococcus epidermidis*	56N	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207266	5207266	Illumina Hiseq	6292240	629224000	33.1	100	100	100	100	35.8	222	SPAdes v3.7.1	88	2564293	2564293	0	0	204	29139	389361	91211	Meric et al., 2015	10.1093/gbe/evv066
326_676NR1	405	SAMN05207268	326	SRS1526069	*Staphylococcus epidermidis*	676NR1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207268	5207268	Illumina Hiseq	3749890	374989000	32.4	100	100	100	100	35	132	SPAdes v3.7.1	63	2439824	2439824	0	0	312	38727	367253	148094	Meric et al., 2015	10.1093/gbe/evv066
327_790N	406	SAMN05207269	327	SRS1526070	*Staphylococcus epidermidis*	790N	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207269	5207269	Illumina Hiseq	3850804	385080400	32.8	100	100	100	100	35.1	136	SPAdes v3.7.1	123	2553713	2553713	0	0	204	20761	254501	114598	Meric et al., 2015	10.1093/gbe/evv066
328_803NLR2	595	SAMN05207270	328	SRS1526072	*Staphylococcus epidermidis*	803NLR2	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207270	5207270	Illumina Hiseq	4366260	436626000	33	100	100	100	100	35.6	154	SPAdes v3.7.1	110	2524777	2524777	0	0	312	22952	316590	84150	Meric et al., 2015	10.1093/gbe/evv066
329_94N2B	328	SAMN05207271	329	SRS1526073	*Staphylococcus epidermidis*	94N2B	ITQB	1996	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207271	5207271	Illumina Hiseq	4729942	472994200	32.5	100	100	100	100	35.6	167	SPAdes v3.7.1	114	2598846	2598846	0	0	200	22796	292451	100018	Meric et al., 2015	10.1093/gbe/evv066
330_619N2	184	SAMN05207272	330	SRS1526074	*Staphylococcus epidermidis*	619N2	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207272	5207272	Illumina Hiseq	4856064	485606400	32.5	100	100	100	100	35.5	171	SPAdes v3.7.1	93	2462435	2462435	0	0	207	26477	286563	82915	Meric et al., 2015	10.1093/gbe/evv066
364_SEPI	2	SAMN03197554	364_SEPI	SRS746762	*Staphylococcus epidermidis*	364_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197554	3197554	Illumina Hiseq	1081352	105480929	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	103	2605868	2605868	0	0	253	25299	177778	67582	Roach et al., 2015	10.1371/journal.pgen.1005413
365_SEPI	2	SAMN03197555	365_SEPI	SRS746763	*Staphylococcus epidermidis*	365_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197555	3197555	Illumina Hiseq	924624	90030093	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	83	2553532	2553532	0	0	324	30765	200927	81539	Roach et al., 2015	10.1371/journal.pgen.1005413
366_SEPI	2	SAMN03197556	366_SEPI	SRS746766	*Staphylococcus epidermidis*	366_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197556	3197556	Illumina Hiseq	977098	95212423	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	88	2611779	2611779	0	0	314	29679	150035	81995	Roach et al., 2015	10.1371/journal.pgen.1005413
393_SEPI	210	SAMN03197584	393_SEPI	SRS746792	*Staphylococcus epidermidis*	393_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197584	3197584	Illumina Hiseq	1167430	114996261	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	96	2456813	2456813	0	0	324	25591	213432	64534	Roach et al., 2015	10.1371/journal.pgen.1005413
422.rep1_SEPI	2	SAMN03197614	422.rep1_SEPI	SRS746823	*Staphylococcus epidermidis*	422.rep1_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197614	3197614	Illumina Hiseq	1025704	99307199	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	114	2517020	2517020	0	0	320	22079	177337	58513	Roach et al., 2015	10.1371/journal.pgen.1005413
450_SEPI	2	SAMN03197642	450_SEPI	SRS746849	*Staphylococcus epidermidis*	450_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197642	3197642	Illumina Hiseq	1031406	100080324	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	87	2593388	2593388	0	0	320	29809	196477	70545	Roach et al., 2015	10.1371/journal.pgen.1005413
458_SEPI	2	SAMN03197651	458_SEPI	SRS746861	*Staphylococcus epidermidis*	458_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197651	3197651	Illumina Hiseq	919458	88930220	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	73	2591822	2591822	0	0	321	35504	177628	82454	Roach et al., 2015	10.1371/journal.pgen.1005413
460_SEPI	16	SAMN03197654	460_SEPI	SRS746865	*Staphylococcus epidermidis*	460_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197654	3197654	Illumina Hiseq	1064412	102980455	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	98	2543213	2543213	0	0	324	25951	263719	82832	Roach et al., 2015	10.1371/journal.pgen.1005413
475_SEPI	5	SAMN03197667	475_SEPI	SRS746874	*Staphylococcus epidermidis*	475_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197667	3197667	Illumina Hiseq	904910	87648473	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	92	2494099	2494099	0	0	317	27109	286755	65596	Roach et al., 2015	10.1371/journal.pgen.1005413
535_SEPI	2	SAMN03197731	535_SEPI	SRS746937	*Staphylococcus epidermidis*	535_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197731	3197731	Illumina Hiseq	1068006	104501038	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	81	2593538	2593538	0	0	300	32018	177208	81585	Roach et al., 2015	10.1371/journal.pgen.1005413
568_SEPI	2	SAMN03197769	568_SEPI	SRS746977	*Staphylococcus epidermidis*	568_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197769	3197769	Illumina Hiseq	1097880	106151778	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	119	2564781	2564781	0	0	331	21552	208250	58536	Roach et al., 2015	10.1371/journal.pgen.1005413
597_SEPI	73	SAMN03197796	597_SEPI	SRS747003	*Staphylococcus epidermidis*	597_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197796	3197796	Illumina Hiseq	890156	86722424	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	66	2478903	2478903	0	0	329	37559	263594	80427	Roach et al., 2015	10.1371/journal.pgen.1005413
644_SEPI	60	SAMN03197845	644_SEPI	SRS747049	*Staphylococcus epidermidis*	644_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197845	3197845	Illumina Hiseq	872644	85727101	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	89	2474153	2474153	0	0	315	27799	234342	82858	Roach et al., 2015	10.1371/journal.pgen.1005413
652_SEPI	5	SAMN03197854	652_SEPI	SRS747060	*Staphylococcus epidermidis*	652_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197854	3197854	Illumina Hiseq	1108586	108899046	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	103	2554020	2554020	0	0	283	24796	254334	71828	Roach et al., 2015	10.1371/journal.pgen.1005413
678_SEPI	2	SAMN03197880	678_SEPI	SRS747088	*Staphylococcus epidermidis*	678_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197880	3197880	Illumina Hiseq	1005280	97275345	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	89	2712294	2712294	0	0	209	30475	196156	74569	Roach et al., 2015	10.1371/journal.pgen.1005413
73_SEPI	16	SAMN03197934	73_SEPI	SRS747142	*Staphylococcus epidermidis*	73_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03197934	3197934	Illumina Hiseq	1284976	121123287	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	65	2390144	2390144	0	0	323	36771	264428	87137	Roach et al., 2015	10.1371/journal.pgen.1005413
798_SEPI	2	SAMN03198010	798_SEPI	SRS747218	*Staphylococcus epidermidis*	798_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198010	3198010	Illumina Hiseq	1157344	113443023	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	83	2594518	2594518	0	0	315	31259	178008	67386	Roach et al., 2015	10.1371/journal.pgen.1005413
801_SEPI	2	SAMN03198015	801_SEPI	SRS747224	*Staphylococcus epidermidis*	801_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198015	3198015	Illumina Hiseq	1185566	[illegible]	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	81	2542422	2542422	0	0	322	31387	152208	81855	Roach et al., 2015	10.1371/journal.pgen.1005413
802_SEPI	2	SAMN03198016	802_SEPI	SRS747226	*Staphylococcus epidermidis*	802_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198016	3198016	Illumina Hiseq	1078034	103674485	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	83	2595349	2595349	0	0	314	31269	252391	81483	Roach et al., 2015	10.1371/journal.pgen.1005413
803_SEPI	2	SAMN03198017	803_SEPI	SRS747222	*Staphylococcus epidermidis*	803_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198017	3198017	Illumina Hiseq	944172	91066463	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	80	2594922	2594922	0	0	[illegible]	32436	196376	81363	Roach et al., 2015	10.1371/journal.pgen.1005413
849_SEPI	7	SAMN03198058	849_SEPI	SRS747265	*Staphylococcus epidermidis*	849_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198058	3198058	Illumina Hiseq	980938	93795388	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	41	2439674	2439674	0	0	[illegible]	59504	350554	96387	Roach et al., 2015	10.1371/journal.pgen.1005413
87_SEPI	2	SAMN03198076	87_SEPI	SRS747284	*Staphylococcus epidermidis*	87_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198076	3198076	Illumina Hiseq	1259178	119415846	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	84	2551659	2551659	0	0	[illegible]	30376	227342	70504	Roach et al., 2015	10.1371/journal.pgen.1005413
91_SEPI	69	SAMN03198117	91_SEPI	SRS747325	*Staphylococcus epidermidis*	91_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198117	3198117	Illumina Hiseq	1575102	146009620	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	81	2514742	2514742	0	0	315	31046	368432	89931	Roach et al., 2015	10.1371/journal.pgen.1005413
92_SEPI	2	SAMN03198134	92_SEPI	SRS747342	*Staphylococcus epidermidis*	92_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198134	3198134	Illumina Hiseq	1732152	162132545	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	78	2613605	2613605	0	0	202	33507	277602	82124	Roach et al., 2015	10.1371/journal.pgen.1005413
934_SEPI	5	SAMN03198153	934_SEPI	SRS747361	*Staphylococcus epidermidis*	934_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198153	3198153	Illumina Hiseq	2032668	176434667	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	100	2501950	2501950	0	0	202	25019	263748	65891	Roach et al., 2015	10.1371/journal.pgen.1005413
935_SEPI	5	SAMN03198154	935_SEPI	SRS747362	*Staphylococcus epidermidis*	935_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198154	3198154	Illumina Hiseq	1792820	155693094	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	108	2504390	2504390	0	0	323	23188	213644	53727	Roach et al., 2015	10.1371/journal.pgen.1005413
936_SEPI	2	SAMN03198155	936_SEPI	SRS747364	*Staphylococcus epidermidis*	936_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198155	3198155	Illumina Hiseq	1848914	161092021	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	101	2707266	2707266	0	0	339	26804	189819	74589	Roach et al., 2015	10.1371/journal.pgen.1005413
938_SEPI	59	SAMN03198157	938_SEPI	SRS747365	*Staphylococcus epidermidis*	938_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198157	3198157	Illumina Hiseq	1383888	121237405	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	102	2491699	2491699	0	0	314	24428	199146	73844	Roach et al., 2015	10.1371/journal.pgen.1005413
939_SEPI	2	SAMN03198158	939_SEPI	SRS747366	*Staphylococcus epidermidis*	939_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198158	3198158	Illumina Hiseq	1676038	146244019	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	78	2545612	2545612	0	0	322	32636	195370	78557	Roach et al., 2015	10.1371/journal.pgen.1005413
94_SEPI	69	SAMN03198159	94_SEPI	SRS747367	*Staphylococcus epidermidis*	94_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198159	3198159	Illumina Hiseq	1327234	[illegible]	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	73	2507967	2507967	0	0	322	34355	301048	83394	Roach et al., 2015	10.1371/journal.pgen.1005413
946_SEPI	22	SAMN03198166	946_SEPI	SRS747377	*Staphylococcus epidermidis*	946_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198166	3198166	Illumina Hiseq	2008238	173782767	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	93	2478843	2478843	0	0	205	26654	263678	81511	Roach et al., 2015	10.1371/journal.pgen.1005413
947_SEPI	22	SAMN03198167	947_SEPI	SRS747372	*Staphylococcus epidermidis*	947_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198167	3198167	Illumina Hiseq	1862754	160833298	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	90	2479388	2479388	0	0	314	27548	263848	72154	Roach et al., 2015	10.1371/journal.pgen.1005413
961_SEPI	2	SAMN03198181	961_SEPI	SRS747388	*Staphylococcus epidermidis*	961_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198181	3198181	Illumina Hiseq	2034408	175872546	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	99	2612210	2612210	0	0	319	26385	149755	67564	Roach et al., 2015	10.1371/journal.pgen.1005413
968_SHAE	2	SAMN03198188	968_SHAE	SRS747396	*Staphylococcus epidermidis*	968_SHAE	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198188	3198188	Illumina Hiseq	1823714	158373557	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	93	2593870	2593870	0	0	315	27891	208694	66733	Roach et al., 2015	10.1371/journal.pgen.1005413
971_SEPI	520	SAMN03198191	971_SEPI	SRS747398	*Staphylococcus epidermidis*	971_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198191	3198191	Illumina Hiseq	1827304	157894163	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	91	2442707	2442707	0	0	323	26842	262684	63408	Roach et al., 2015	10.1371/journal.pgen.1005413
972_SEPI	5	SAMN03198192	972_SEPI	SRS747400	*Staphylococcus epidermidis*	972_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198192	3198192	Illumina Hiseq	2419308	206673569	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	87	2468264	2468264	0	0	277	28370	263652	79628	Roach et al., 2015	10.1371/journal.pgen.1005413
973_SEPI	2	SAMN03198193	973_SEPI	SRS747404	*Staphylococcus epidermidis*	973_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198193	3198193	Illumina Hiseq	1579384	138318303	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	97	2727413	2727413	0	0	225	28117	196597	64598	Roach et al., 2015	10.1371/journal.pgen.1005413
978_SEPI	218	SAMN03198198	978_SEPI	SRS747410	*Staphylococcus epidermidis*	978_SEPI	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198198	3198198	Illumina Hiseq	1902254	164816978	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	144	2344162	2344162	0	0	202	16278	234340	72487	Roach et al., 2015	10.1371/journal.pgen.1005413
997_SHAE	83	SAMN03198219	997_SHAE	SRS747428	*Staphylococcus epidermidis*	997_SHAE	UW clinical laboratory	2012-2013	USA WA	Unknown	Unknown	Homo sapiens	Unknown	SAMN03198219	3198219	Illumina Hiseq	2423748	206613220	[illegible]	[illegible]	[illegible]	101	101	[illegible]	[illegible]	SPAdes v3.7.1	88	2523106	2523106	0	0	314	28671	149591	63398	Roach et al., 2015	10.1371/journal.pgen.1005413
305_CV28	44	SAMN05207247	305	SRS1526046	*Staphylococcus epidermidis*	CV28	ITQB	1997	Cape Verde	Nasal swab	15.12 N 23.61 W	Homo sapiens	Commensal	SAMN05207247	5207247	Illumina Hiseq	3750542	375054200	31.5	100	100	100	100	35.2	132	SPAdes v3.7.1	178	2562113	2562113	0	0	320	14393	264078	79175	Meric et al., 2015	10.1093/gbe/evv066
317_612N1	556	SAMN05207259	317	SRS1526059	*Staphylococcus epidermidis*	612N1	ITQB	1997	Portugal	Nasal swab	38.72 N 9.14 W	Homo sapiens	Commensal	SAMN05207259	5207259	Illumina Hiseq	4934150	493415000	32.5	100	100	100	100	35.8	174	SPAdes v3.7.1	249	2491636	2491636	0	0	202	10006	198667	83604	Meric et al., 2015	10.1093/gbe/evv066
DRS013951	2	SAMD00003128	DRS013951	DRS013951	*Staphylococcus epidermidis*	DRS013951	Gifu University School of Medicine	-	Japan	Unknown	Unknown	Homo sapiens	Unknown	SAMD00003128	2730774	Illumina Hiseq	31766932	3176693200	33.7	101	101	101	101	37.6	1119	SPAdes v3.7.1	133	2735612	2735612	0	0	200	20568	357317	128945	Unpublished	PRJDB1866
M0026	5	SAMN02325127	M0026	SRS486821	*Staphylococcus epidermidis*	M0026	-	15-Sep-03	USA Boston, MA	Blood	Unknown	Homo sapiens	Bacteraemia	SAMN02325127	2325127	Illumina Hiseq	5384482	543832682	33.3	100	100	101	101	35.7	192	SPAdes v3.7.1	123	2555077	2555077	0	0	200	20772	327259	95958	Feldgarden et al., 2014	PRJEB2655
UCLA-Sa-LR-13	23	SAMN02671415	UCLA-Sa-LR-13	SRS565652	*Staphylococcus epidermidis*	UCLA-Sa-LR-13	Romney Humphries - UCLA	Nov-07	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671415	2671415	Illumina Hiseq	10379134	1037913400	33.4	100	100	100	100	36.6	366	SPAdes v3.7.1	187	2553163	2553163	0	0	200	13653	254739	83071	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-14	186	SAMN02671416	UCLA-Sa-LR-14	SRS565653	*Staphylococcus epidermidis*	UCLA-Sa-LR-14	Romney Humphries - UCLA	Apr-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671416	2671416	Illumina Hiseq	9244600	924460000	33.9	100	100	100	100	36.7	326	SPAdes v3.7.1	168	2538652	2538652	0	0	201	15111	234775	83071	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-15	185	SAMN02671417	UCLA-Sa-LR-15	SRS565654	*Staphylococcus epidermidis*	UCLA-Sa-LR-15	Romney Humphries - UCLA	Jun-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671417	2671417	Illumina Hiseq	10304526	1030452600	33.5	100	100	100	100	36.7	363	SPAdes v3.7.1	220	2771289	2771289	0	0	200	12596	253553	92523	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-17	186	SAMN02671418	UCLA-Sa-LR-17	SRS565655	*Staphylococcus epidermidis*	UCLA-Sa-LR-17	Romney Humphries - UCLA	Jun-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671418	2671418	Illumina Hiseq	10664460	1066446000	33.5	100	100	100	100	36.8	376	SPAdes v3.7.1	224	2790991	2790991	0	0	203	12459	212261	93488	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-18	23	SAMN02671419	UCLA-Sa-LR-18	SRS565656	*Staphylococcus epidermidis*	UCLA-Sa-LR-18	Romney Humphries - UCLA	Aug-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671419	2671419	Illumina Hiseq	10662702	1066270200	33.3	100	100	100	100	36.8	376	SPAdes v3.7.1	196	2782416	2782416	0	0	200	14196	239814	94973	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-19	23	SAMN02671420	UCLA-Sa-LR-19	SRS565659	*Staphylococcus epidermidis*	UCLA-Sa-LR-19	Romney Humphries - UCLA	Aug-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671420	2671420	Illumina Hiseq	8780378	878037800	33.4	100	100	100	100	36.7	309	SPAdes v3.7.1	106	2633443	2633443	0	0	214	24843	294582	78525	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-20	23	SAMN02671421	UCLA-Sa-LR-20	SRS565660	*Staphylococcus epidermidis*	UCLA-Sa-LR-20	Romney Humphries - UCLA	Oct-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671421	2671421	Illumina Hiseq	10181522	1018152200	33.1	100	100	100	100	36.8	359	SPAdes v3.7.1	284	2924246	2924246	0	0	201	10296	196709	70431	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-21	186	SAMN02671422	UCLA-Sa-LR-21	SRS565661	*Staphylococcus epidermidis*	UCLA-Sa-LR-21	Romney Humphries - UCLA	Nov-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671422	2671422	Illumina Hiseq	10299128	1029912800	33.5	100	100	100	100	36.8	363	SPAdes v3.7.1	184	2608644	2608644	0	0	201	14177	227396	72187	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-22	23	SAMN02671423	UCLA-Sa-LR-22	SRS565662	*Staphylococcus epidermidis*	UCLA-Sa-LR-22	Romney Humphries - UCLA	Nov-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671423	2671423	Illumina Hiseq	9778746	977874600	33.2	100	100	100	100	36.7	344	SPAdes v3.7.1	250	2782627	2782627	0	0	200	11130	212558	87652	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-23	186	SAMN02671424	UCLA-Sa-LR-23	SRS565663	*Staphylococcus epidermidis*	UCLA-Sa-LR-23	Romney Humphries - UCLA	Dec-08	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671424	2671424	Illumina Hiseq	8183090	818309000	33.6	100	100	100	100	36.7	288	SPAdes v3.7.1	216	2466065	2466065	0	0	202	11416	253911	82973	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-25	186	SAMN02671425	UCLA-Sa-LR-25	SRS565664	*Staphylococcus epidermidis*	UCLA-Sa-LR-25	Romney Humphries - UCLA	Sep-09	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671425	2671425	Illumina Hiseq	9551042	955104200	33.3	100	100	100	100	36.7	336	SPAdes v3.7.1	195	2801501	2801501	0	0	202	14366	234033	75562	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-27	186	SAMN02671427	UCLA-Sa-LR-27	SRS565666	*Staphylococcus epidermidis*	UCLA-Sa-LR-27	Romney Humphries - UCLA	Sep-09	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671427	2671427	Illumina Hiseq	10144112	1014411200	33.5	100	100	100	100	36.8	357	SPAdes v3.7.1	211	2643007	2643007	0	0	202	12526	227434	72021	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-28	23	SAMN02671428	UCLA-Sa-LR-28	SRS565667	*Staphylococcus epidermidis*	UCLA-Sa-LR-28	Romney Humphries - UCLA	Oct-09	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671428	2671428	Illumina Hiseq	10752384	1075238400	33.1	100	100	100	100	36.8	379	SPAdes v3.7.1	198	2633332	2633332	0	0	200	13299	234103	82992	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-3	5	SAMN02671410	UCLA-Sa-LR-03	SRS565634	*Staphylococcus epidermidis*	UCLA-Sa-LR-3	Romney Humphries - UCLA	Mar-11	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671410	2671410	Illumina Hiseq	9531114	953111400	33.3	100	100	100	100	36.8	336	SPAdes v3.7.1	203	2651249	2651249	0	0	201	13060	508038	76085	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-4	5	SAMN02671411	UCLA-Sa-LR-04	SRS565637	*Staphylococcus epidermidis*	UCLA-Sa-LR-4	Romney Humphries - UCLA	Jun-11	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671411	2671411	Illumina Hiseq	4427150	442715000	33.7	100	100	100	100	36.5	156	SPAdes v3.7.1	116	2619378	2619378	0	0	237	22580	234220	70379	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-5	23	SAMN02671412	UCLA-Sa-LR-05	SRS565640	*Staphylococcus epidermidis*	UCLA-Sa-LR-5	Romney Humphries - UCLA	Jul-11	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671412	2671412	Illumina Hiseq	8630226	863022600	33.6	100	100	100	100	36.8	304	SPAdes v3.7.1	108	2624507	2624507	0	0	214	24300	263996	70791	Tewhey et al., 2014	10.1128/mBio.00894-14
UCLA-Sa-LR-6	23	SAMN02671413	UCLA-Sa-LR-06	SRS565641	*Staphylococcus epidermidis*	UCLA-Sa-LR-6	Romney Humphries - UCLA	Sep-11	Los Angeles, CA, USA	Human blood	34.0664 118.4461	Homo sapiens	Bacteraemia	SAMN02671413	2671413	Illumina Hiseq	10089356	1008935600	33.1	100	100	100	100	36.8	355	SPAdes v3.7.1	168	2759224	2759224	0	0	200	16423	253727	83080	Tewhey et al., 2014	10.1128/mBio.00894-14

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 1 - Sheet C. *S. epidermidis* SRA metadata

Strain	Organism	*in silico* MLST	Year	Country	Institution location	Host	Clinical Presentation	Sample	Sample unique name	Uploaded to	Study accession	Sample accession	Sample ID	Sequencer	Reads	Yield	GC	MinLen	AvgLen	MaxLen	ModeLen	Phred	AvgQual	Depth	Assembler	No	bp	ok	Ns	Gaps	Min	Avg	Max	N50	Reference	DOI	Comments
BPH0666	*Staphylococcus epidermidis*	23	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0666	ENA	PRJEB12090	ERS1019850	Se_BPH0666	Illumina NextSeq	1938512	287905754	34.8	35	148	151	151	33	32.8	101x	SPAdes v3.7.1	73	2441668	2E+06	0	0	505	33447	312071	76264	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0667	*Staphylococcus epidermidis*	59	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0667	ENA	PRJEB12090	ERS1019851	Se_BPH0667	Illumina MiSeq	687032	150574538	34.6	35	219	301	214	33	36.0	53x	SPAdes v3.7.1	74	2460192	2E+06	0	0	504	33245	145633	62948	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0668	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0668	ENA	PRJEB12090	ERS1019852	Se_BPH0668	Illumina NextSeq	2259914	335766013	34.3	35	148	151	151	33	32.8	118x	SPAdes v3.7.1	69	2775172	3E+06	0	0	504	40219	201713	86785	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0669	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0669	ENA	PRJEB12090	ERS1019853	Se_BPH0669	Illumina MiSeq	881676	167656908	35.1	35	190	301	214	33	36.2	59x	SPAdes v3.7.1	104	2471246	2E+06	0	0	500	23761	122723	43256	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0670	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0670	ENA	PRJEB12090	ERS1019854	Se_BPH0670	Illumina MiSeq	1360360	230371010	34.8	35	169	301	214	33	36.3	81x	SPAdes v3.7.1	154	2750775	3E+06	0	0	504	17862	118098	36556	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0671	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0671	ENA	PRJEB12090	ERS1019855	Se_BPH0671	Illumina MiSeq	1303138	226314287	34.9	35	173	301	214	33	35.9	79x	SPAdes v3.7.1	102	2598090	3E+06	0	0	500	25471	181009	58113	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0672	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0672	ENA	PRJEB12090	ERS1019856	Se_BPH0672	Illumina MiSeq	1357862	232796526	34.5	35	171	301	215	33	36.1	82x	SPAdes v3.7.1	76	2738935	3E+06	0	0	504	36038	216358	80611	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0673	*Staphylococcus epidermidis*	59	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0673	ENA	PRJEB12090	ERS1019857	Se_BPH0673	Illumina MiSeq	1831908	312764296	35.1	35	170	301	215	33	36.1	110x	SPAdes v3.7.1	45	2450384	2E+06	0	0	504	54452	267079	125369	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0674	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0674	ENA	PRJEB12090	ERS1019858	Se_BPH0674	Illumina MiSeq	1982404	338574803	34.9	35	170	301	215	33	36.1	119x	SPAdes v3.7.1	65	2748661	3E+06	0	0	504	42287	227261	80331	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0675	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0675	ENA	PRJEB12090	ERS1019859	Se_BPH0675	Illumina MiSeq	1948848	325153308	34.9	35	166	301	215	33	35.8	114x	SPAdes v3.7.1	77	2740571	3E+06	0	0	504	35591	225669	80568	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0676	*Staphylococcus epidermidis*	2	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0676	ENA	PRJEB12090	ERS1019860	Se_BPH0676	Illumina MiSeq	857140	158310082	34.6	35	184	301	214	33	35.6	55x	SPAdes v3.7.1	96	2598181	3E+06	0	0	504	27064	139697	71191	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0677	*Staphylococcus epidermidis*	225	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0677	ENA	PRJEB12090	ERS1019862	Se_BPH0677	Illumina MiSeq	1348260	250299890	34.5	35	185	301	214	33	36.3	88x	SPAdes v3.7.1	78	2509146	3E+06	0	0	505	32168	234495	59637	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0678	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0678	ENA	PRJEB12090	ERS1019863	Se_BPH0678	Illumina MiSeq	3458424	782725288	33.9	35	226	251	251	33	37.7	274x	SPAdes v3.7.1	88	2814869	3E+06	0	0	504	31987	221409	85738	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0679	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0679	ENA	PRJEB12090	ERS1019864	Se_BPH0679	Illumina MiSeq	1029724	226791381	34.6	35	220	301	214	33	35.7	79x	SPAdes v3.7.1	78	2729763	3E+06	0	0	504	34996	198700	71333	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0681	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0681	ENA	PRJEB12090	ERS1019865	Se_BPH0681	Illumina MiSeq	1894640	345475614	34.5	35	182	301	214	33	36.3	121x	SPAdes v3.7.1	53	2549438	3E+06	0	0	504	48102	204822	130064	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0684	*Staphylococcus epidermidis*	487	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0684	ENA	PRJEB12090	ERS1019866	Se_BPH0684	Illumina MiSeq	1576776	297376064	34.7	35	188	301	215	33	36.2	104x	SPAdes v3.7.1	46	2446950	2E+06	0	0	504	53194	245809	144699	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0685	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0685	ENA	PRJEB12090	ERS1019867	Se_BPH0685	Illumina MiSeq	1827142	350101274	34.7	35	191	301	215	33	36.2	123x	SPAdes v3.7.1	79	2786873	3E+06	0	0	504	35276	190528	80431	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0686	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0686	ENA	PRJEB12090	ERS1019868	Se_BPH0686	Illumina MiSeq	1875284	327923113	34.8	35	174	301	215	33	36.1	115x	SPAdes v3.7.1	66	2770154	3E+06	0	0	504	41972	216360	88492	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0687	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0687	ENA	PRJEB12090	ERS1019869	Se_BPH0687	Illumina MiSeq	1792360	349923301	34.6	35	195	301	215	33	35.9	123x	SPAdes v3.7.1	79	2793740	3E+06	0	0	504	35363	221409	81415	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0689	*Staphylococcus epidermidis*	59	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0689	ENA	PRJEB12090	ERS1019870	Se_BPH0689	Illumina MiSeq	1228646	220677703	34.5	35	179	301	214	33	36.1	77x	SPAdes v3.7.1	48	2443029	2E+06	0	0	504	50896	211285	96507	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0690	*Staphylococcus epidermidis*	7	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0690	ENA	PRJEB12090	ERS1019871	Se_BPH0690	Illumina MiSeq	978916	165994266	34.8	35	169	301	214	33	36.2	58x	SPAdes v3.7.1	56	2340119	2E+06	0	0	505	41787	193052	66652	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0691	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0691	ENA	PRJEB12090	ERS1019872	Se_BPH0691	Illumina MiSeq	4190182	807890652	33.5	35	192	251	251	33	37.9	283x	SPAdes v3.7.1	62	2740484	3E+06	0	0	504	44201	216459	89085	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0692	*Staphylococcus epidermidis*	2	2013	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0692	ENA	PRJEB12090	ERS1019873	Se_BPH0692	Illumina MiSeq	2062758	489552869	34.5	35	237	301	301	33	35.7	171x	SPAdes v3.7.1	65	2740301	3E+06	0	0	504	42158	207199	87844	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0693	*Staphylococcus epidermidis*	188	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0693	ENA	PRJEB12090	ERS1019874	Se_BPH0693	Illumina MiSeq	2015628	476501974	34.5	35	236	301	301	33	35.6	167x	SPAdes v3.7.1	48	2601839	3E+06	0	0	504	54204	308939	127464	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0694	*Staphylococcus epidermidis*	2	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0694	ENA	PRJEB12090	ERS1019875	Se_BPH0694	Illumina NextSeq	2243354	330745904	34.6	35	147	151	151	33	33.1	116x	SPAdes v3.7.1	86	2743179	3E+06	0	0	504	31897	207299	73528	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0695	*Staphylococcus epidermidis*	23	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0695	ENA	PRJEB12090	ERS1019876	Se_BPH0695	Illumina MiSeq	824284	167801131	35.1	35	203	251	215	33	35.2	59x	SPAdes v3.7.1	135	2471534	2E+06	0	0	505	18307	104169	38063	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0696	*Staphylococcus epidermidis*	2	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0696	ENA	PRJEB12090	ERS1019877	Se_BPH0696	Illumina MiSeq	3064148	710138518	34.5	35	231	251	251	33	37.7	249x	SPAdes v3.7.1	63	2739504	3E+06	0	0	572	43484	226625	89085	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0699	*Staphylococcus epidermidis*	210	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0699	ENA	PRJEB12090	ERS1019879	Se_BPH0699	Illumina MiSeq	1288656	235013488	34.5	35	182	301	215	33	35.7	82x	SPAdes v3.7.1	57	2460843	2E+06	0	0	505	43172	210466	102523	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0700	*Staphylococcus epidermidis*	86	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0700	ENA	PRJEB12090	ERS1019880	Se_BPH0700	Illumina MiSeq	1604442	287276663	34.4	35	179	301	215	33	34.7	101x	SPAdes v3.7.1	64	2465908	2E+06	0	0	504	38529	194127	75251	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0701	*Staphylococcus epidermidis*	89	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0701	ENA	PRJEB12090	ERS1019881	Se_BPH0701	Illumina MiSeq	1021294	177204659	34.4	35	173	301	214	33	36.3	62x	SPAdes v3.7.1	74	2454303	2E+06	0	0	504	33166	244635	57667	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0702	*Staphylococcus epidermidis*	2	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0702	ENA	PRJEB12090	ERS1019882	Se_BPH0702	Illumina MiSeq	944810	159209892	34.4	35	168	301	214	33	36.0	56x	SPAdes v3.7.1	122	2733030	3E+06	0	0	504	22401	182736	42781	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0703	*Staphylococcus epidermidis*	23	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0703	ENA	PRJEB12090	ERS1019883	Se_BPH0703	Illumina MiSeq	2704480	638861619	34.5	35	236	251	251	33	37.4	224x	SPAdes v3.7.1	172	2557035	3E+06	0	0	622	14866	311814	132476	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0705	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0705	ENA	PRJEB12090	ERS1019885	Se_BPH0705	Illumina MiSeq	1294582	272511125	34.9	35	210	301	215	33	35.7	95x	SPAdes v3.7.1	62	2593608	3E+06	0	0	504	41832	185179	82986	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0706	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0706	ENA	PRJEB12090	ERS1019886	Se_BPH0706	Illumina MiSeq	1519164	282116923	34.8	35	185	301	215	33	36.0	99x	SPAdes v3.7.1	65	2717009	3E+06	0	0	504	41800	220691	94308	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0707	*Staphylococcus epidermidis*	46	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0707	ENA	PRJEB12090	ERS1019887	Se_BPH0707	Illumina MiSeq	1787056	346131507	34.6	35	193	301	215	33	36.0	121x	SPAdes v3.7.1	47	2472202	2E+06	0	0	501	52600	213503	105951	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0708	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0708	ENA	PRJEB12090	ERS1019888	Se_BPH0708	Illumina MiSeq	1323952	250872727	34.4	35	189	301	215	33	35.9	88x	SPAdes v3.7.1	49	2662546	3E+06	0	0	504	54337	287867	97277	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0709	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0709	ENA	PRJEB12090	ERS1019889	Se_BPH0709	Illumina NextSeq	2137046	319190030	33.7	25	149	151	151	33	32.7	112x	SPAdes v3.7.1	60	2732976	3E+06	0	0	504	45549	271176	87844	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0710	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0710	ENA	PRJEB12090	ERS1019890	Se_BPH0710	Illumina NextSeq	2238186	332079426	34.5	35	148	151	151	33	32.9	116x	SPAdes v3.7.1	63	2654541	3E+06	0	0	504	42135	365465	83092	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0712	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0712	ENA	PRJEB12090	ERS1019892	Se_BPH0712	Illumina NextSeq	2120058	314060912	33.9	35	148	151	151	33	33.1	110x	SPAdes v3.7.1	61	2734147	3E+06	0	0	504	44822	226625	87844	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0713	*Staphylococcus epidermidis*	23	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0713	ENA	PRJEB12090	ERS1019893	Se_BPH0713	Illumina MiSeq	2650490	489056589	35.0	35	184	301	215	33	36.0	171x	SPAdes v3.7.1	69	2485264	2E+06	0	0	505	36018	271087	127939	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0714	*Staphylococcus epidermidis*	2	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0714	ENA	PRJEB12090	ERS1019894	Se_BPH0714	Illumina MiSeq	1358422	222084982	34.5	35	163	301	215	22	35.9	78x	SPAdes v3.7.1	84	2740680	3E+06	0	0	500	32627	226105	66141	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0715	*Staphylococcus epidermidis*	23	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0715	ENA	PRJEB12090	ERS1019895	Se_BPH0715	Illumina MiSeq	2305354	403723544	34.7	35	175	301	215	33	36.0	141x	SPAdes v3.7.1	66	2476034	2E+06	0	0	505	37515	186066	93798	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0716	*Staphylococcus epidermidis*	2	2009	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0716	ENA	PRJEB12090	ERS1019896	Se_BPH0716	Illumina MiSeq	2137038	495111059	34.7	35	231	301	301	33	35.6	173x	SPAdes v3.7.1	61	2586852	3E+06	0	0	504	42407	204822	85009	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0717	*Staphylococcus epidermidis*	2	2009	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0717	ENA	PRJEB12090	ERS1019897	Se_BPH0717	Illumina MiSeq	848962	196879102	34.5	35	231	301	215	33	34.9	69x	SPAdes v3.7.1	63	2593819	3E+06	0	0	504	41171	198812	83091	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0718	*Staphylococcus epidermidis*	2	2009	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0718	ENA	PRJEB12090	ERS1019898	Se_BPH0718	Illumina MiSeq	1189744	213814783	34.0	35	179	301	215	33	35.5	75x	SPAdes v3.7.1	83	2745492	3E+06	0	0	500	33078	197645	62203	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0719	*Staphylococcus epidermidis*	2	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0719	ENA	PRJEB12090	ERS1019899	Se_BPH0719	Illumina NextSeq	1258832	185676332	31.7	35	147	151	151	33	32.6	65x	SPAdes v3.7.1	49	2574274	3E+06	0	0	504	52536	521025	149749	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0720	*Staphylococcus epidermidis*	2	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0720	ENA	PRJEB12090	ERS1019900	Se_BPH0720	Illumina MiSeq	1387394	266850272	34.4	35	192	301	214	33	36.3	93x	SPAdes v3.7.1	85	2738524	3E+06	0	0	500	32217	183843	80012	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0721	*Staphylococcus epidermidis*	210	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0721	ENA	PRJEB12090	ERS1019901	Se_BPH0721	Illumina MiSeq	1281838	250504008	34.4	35	195	301	214	33	36.1	88x	SPAdes v3.7.1	41	2398618	2E+06	0	0	505	58502	213976	99445	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0722	*Staphylococcus epidermidis*	2	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0722	ENA	PRJEB12090	ERS1019902	Se_BPH0722	Illumina MiSeq	929496	179486597	34.6	35	193	301	214	22	35.9	63x	SPAdes v3.7.1	121	2563756	3E+06	0	0	504	21188	95536	43280	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0724	*Staphylococcus epidermidis*	2	2009	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0724	ENA	PRJEB12090	ERS1019904	Se_BPH0724	Illumina MiSeq	1924104	421912231	34.8	35	203	301	215	33	36.1	148x	SPAdes v3.7.1	56	2558707	3E+06	0	0	504	45691	264885	120988	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0726	*Staphylococcus epidermidis*	59	2009	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0726	ENA	PRJEB12090	ERS1019905	Se_BPH0726	Illumina MiSeq	1243748	245606935	34.7	35	197	301	215	33	35.8	86x	SPAdes v3.7.1	100	2482849	2E+06	0	0	505	24828	327470	102523	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0727	*Staphylococcus epidermidis*	2	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0727	ENA	PRJEB12090	ERS1019906	Se_BPH0727	Illumina MiSeq	999748	203410081	34.5	35	203	301	214	33	35.5	71x	SPAdes v3.7.1	98	2739374	3E+06	0	0	538	27952	183829	52025	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0728	*Staphylococcus epidermidis*	2	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0728	ENA	PRJEB12090	ERS1019907	Se_BPH0728	Illumina MiSeq	811344	168381690	34.4	35	207	301	214	33	35.7	59x	SPAdes v3.7.1	119	2723567	3E+06	0	0	504	22887	209918	41360	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0729	*Staphylococcus epidermidis*	86	2010	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0729	ENA	PRJEB12090	ERS1019908	Se_BPH0729	Illumina MiSeq	781414	156409916	34.9	35	200	301	214	33	36.2	55x	SPAdes v3.7.1	115	2456937	2E+06	0	0	504	21364	79576	39853	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0730	*Staphylococcus epidermidis*	2	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0730	ENA	PRJEB12090	ERS1019909	Se_BPH0730	Illumina MiSeq	751644	151753781	34.7	35	201	301	214	33	36.1	53x	SPAdes v3.7.1	159	2542154	3E+06	0	0	504	15988	97664	33434	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0731	*Staphylococcus epidermidis*	2	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0731	ENA	PRJEB12090	ERS1019910	Se_BPH0731	Illumina MiSeq	1406286	281543899	34.3	35	200	301	215	33	36.3	99x	SPAdes v3.7.1	63	2731907	3E+06	0	0	504	43363	226149	87844	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0732	*Staphylococcus epidermidis*	2	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0732	ENA	PRJEB12090	ERS1019911	Se_BPH0732	Illumina MiSeq	1355242	267030244	34.6	35	197	301	215	33	36.1	94x	SPAdes v3.7.1	39	2531475	3E+06	0	0	504	64909	283819	136301	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0733	*Staphylococcus epidermidis*	2	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0733	ENA	PRJEB12090	ERS1019912	Se_BPH0733	Illumina MiSeq	1965162	368816353	34.4	35	187	301	215	33	36.2	129x	SPAdes v3.7.1	76	2787111	3E+06	0	0	504	36672	221409	87733	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0734	*Staphylococcus epidermidis*	59	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0734	ENA	PRJEB12090	ERS1019913	Se_BPH0734	Illumina MiSeq	1471548	301489643	34.8	35	204	301	215	33	36.0	106x	SPAdes v3.7.1	37	2422377	2E+06	0	0	504	65469	292413	157876	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0735	*Staphylococcus epidermidis*	2	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0735	ENA	PRJEB12090	ERS1019914	Se_BPH0735	Illumina MiSeq	702656	145802756	34.8	35	207	301	214	33	36.0	51x	SPAdes v3.7.1	161	2701420	3E+06	0	0	506	16779	175263	31488	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0737	*Staphylococcus epidermidis*	225	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0737	ENA	PRJEB12090	ERS1019917	Se_BPH0737	Illumina NextSeq	1258832	185676332	31.7	35	147	151	151	33	32.6	65x	SPAdes v3.7.1	74	2511073	3E+06	0	0	502	33933	211032	82569	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0738	*Staphylococcus epidermidis*	59	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0738	ENA	PRJEB12090	ERS1019918	Se_BPH0738	Illumina NextSeq	2300312	339766212	34.1	35	147	151	151	33	33.2	119x	SPAdes v3.7.1	29	2429410	2E+06	0	0	504	83772	487209	211391	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0739	*Staphylococcus epidermidis*	7	2012	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Se_BPH0739	ENA	PRJEB12090	ERS1019919	Se_BPH0739	Illumina MiSeq	2399586	475805579	34.5	35	198	301	215	33	36.1	167x	SPAdes v3.7.1	54	2425519	2E+06	0	0	505	44917	659150	122119	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0743 (UK01)	*Staphylococcus epidermidis*	2	2015	United Kingdon	East, England	Human	Bacteraemia	Blood culture	Se_BPH0743	NCBI SRA	PRJNA470752	SAMN09103953	Se_BPH0743	Illumina NextSeq	3049548	450748227	33.8	30	147	151	151	33	31.6	158x	SPAdes v3.7.1	55	2568312	3E+06	0	0	504	46696	239817	116445	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK01" in Lee et al., 2018
BPH0744 (UK02)	*Staphylococcus epidermidis*	2	2015	United Kingdon	S East, England	Human	CNS infection	CSF	Se_BPH0744	NCBI SRA	PRJNA470752	SAMN09103954	Se_BPH0744	Illumina NextSeq	2346856	346171912	33.6	30	147	151	151	33	31.5	121x	SPAdes v3.7.1	58	2729891	3E+06	0	0	504	47067	278416	81312	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK02" in Lee et al., 2018
BPH0745 (UK03)	*Staphylococcus epidermidis*	2	2015	United Kingdon	E Midlands, England	Human	Osteomyelitis	Spinal tissue	Se_BPH0745	NCBI SRA	PRJNA470752	SAMN09103955	Se_BPH0745	Illumina NextSeq	2426780	358990904	33.8	30	147	151	151	33	31.6	126x	SPAdes v3.7.1	60	2710723	3E+06	0	0	504	45178	441996	80013	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK03" in Lee et al., 2018
BPH0746 (UK04)	*Staphylococcus epidermidis*	87	2016	United Kingdon	Yorkshire & Humber England	Human	Bacteraemia	Blood culture	Se_BPH0746	NCBI SRA	PRJNA470752	SAMN09103956	Se_BPH0746	Illumina NextSeq	2487722	368090416	33.6	30	147	151	151	33	31.4	129x	SPAdes v3.7.1	65	2453752	2E+06	0	0	505	37750	307416	83082	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK04" in Lee et al., 2018
BPH0747 (IRL01)	*Staphylococcus epidermidis*	2	2016	Republic of Ireland	Republic of Ireland	Human	Bacteraemia	Blood culture	Se_BPH0747	NCBI SRA	PRJNA470752	SAMN09103952	Se_BPH0747	Illumina NextSeq	2271514	336487373	33.5	30	148	151	151	33	31.4	118x	SPAdes v3.7.1	48	2695604	3E+06	0	0	504	56158	713465	122086	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "IRL01" in Lee et al., 2018
BPH0748 (UK05)	*Staphylococcus epidermidis*	2	2016	United Kingdon	London, England	Human	Bacteraemia	CVP tip	Se_BPH0748	NCBI SRA	PRJNA470752	SAMN09103957	Se_BPH0748	Illumina NextSeq	2493554	371211646	33.8	30	148	151	151	33	31.4	130x	SPAdes v3.7.1	63	2726974	3E+06	0	0	504	43285	271177	78842	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK05" in Lee et al., 2018
BPH0749 (UK06)	*Staphylococcus epidermidis*	2	2016	United Kingdon	S West, England	Human	Septic arthritis	Hip tissue	Se_BPH0749	NCBI SRA	PRJNA470752	SAMN09103958	Se_BPH0749	Illumina NextSeq	2490668	364628898	33.9	30	146	151	151	33	31.8	128x	SPAdes v3.7.1	66	2728502	3E+06	0	0	504	41340	239150	80449	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK06" in Lee et al., 2018
BPH0750 (UK07)	*Staphylococcus epidermidis*	2	2016	United Kingdon	S East, England	Human	Septic arthritis	Knee fluid	Se_BPH0750	NCBI SRA	PRJNA470752	SAMN09103959	Se_BPH0750	Illumina NextSeq	2290592	339805541	33	30	148	151	151	33	31.5	119x	SPAdes v3.7.1	68	2695610	3E+06	0	0	743	39641	189764	80195	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK07" in Lee et al., 2018
BPH0751 (UK08)	*Staphylococcus epidermidis*	2	2016	United Kingdon	N Ireland	Human	Osteomyelitis	Femoral tissue	Se_BPH0751	NCBI SRA	PRJNA470752	SAMN09103960	Se_BPH0751	Illumina NextSeq	2580912	382363574	33.7	30	148	151	151	33	31.6	134x	SPAdes v3.7.1	60	2669105	3E+06	0	0	504	44485	258721	87844	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK08" in Lee et al., 2018
BPH0752 (UK09)	*Staphylococcus epidermidis*	2	2016	United Kingdon	East, England	Human	Bacteraemia	Blood culture	Se_BPH0752	NCBI SRA	PRJNA470752	SAMN09103961	Se_BPH0752	Illumina NextSeq	2226326	329683200	33.8	30	148	151	151	33	31.3	115x	SPAdes v3.7.1	69	2720313	3E+06	0	0	504	39424	206743	86643	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK09" in Lee et al., 2018
BPH0753 (UK10)	*Staphylococcus epidermidis*	2	2016	United Kingdon	S East, England	Human	Osteomyelitis	Femoral tissue	Se_BPH0753	NCBI SRA	PRJNA470752	SAMN09103962	Se_BPH0753	Illumina NextSeq	2291640	341310543	33.5	30	148	151	151	33	31.4	120x	SPAdes v3.7.1	57	2681833	3E+06	0	0	504	47049	278239	87843	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK10" in Lee et al., 2018
BPH0754 (UK11)	*Staphylococcus epidermidis*	2	2016	United Kingdon	E Midlands, England	Human	Bacteraemia	Line tip	Se_BPH0754	NCBI SRA	PRJNA470752	SAMN09103963	Se_BPH0754	Illumina NextSeq	2676064	395191323	33.7	30	147	151	151	33	31.7	138x	SPAdes v3.7.1	60	2692275	3E+06	0	0	504	44871	271124	87892	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK11" in Lee et al., 2018
BPH0755 (UK12)	*Staphylococcus epidermidis*	2	2016	United Kingdon	S East, England	Human	Septic arthritis	Hip tissue	Se_BPH0755	NCBI SRA	PRJNA470752	SAMN09103964	Se_BPH0755	Illumina NextSeq	2297576	340782858	33.6	30	148	151	151	33	31.7	119x	SPAdes v3.7.1	54	2682944	3E+06	0	0	504	49684	278415	96186	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as "UK12" in Lee et al., 2018

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 1 - Sheet D. Lee *et al.*, 2018 *S. epidermidis* isolates

Isolate	*in silico* MLST	BioSample	BioProject	Assembly	Organism	Strain	Collected by	Collection Date	Geographic Location	Isolation Source	Latitude & Longitude	Host	Host Disease	Sequencing Method	Depth	Assembler	Contigs	bp	ok	Ns	Gaps	Min	Avg	Max	N50	Sequencing Reference	DOI	Methylation Reference	DOI
NIH04008	2	SAMN00993294	PRJNA62395	GCA_000276085.1	*Staphylococcus epidermidis*	NIH04008	NIH Intramural Sequencing Centre	2004	USA	Blood	Unknown	Human	Unknown	454 GS FLX	16x	Newbler v. 2.3	130	2737591	2737587	4	0	232	21058	248427	55960	Conlan et al., 2012	10.1186/gb-2012-13-7-r64	Costa et al., 2017	10.1128/JB.00271-17
NIH051475	89	SAMN00993392	PRJNA86755	GCA_000275965.1	*Staphylococcus epidermidis*	NIH051475	NIH Intramural Sequencing Centre	2005	USA	Joint	Unknown	Human	Unknown	454 GS FLX	76x	Newbler v. 2.5.3	91	2508563	2508563	0	0	238	27566	430957	82215	Conlan et al., 2012	10.1186/gb-2012-13-7-r64	Costa et al., 2017	10.1128/JB.00271-17
VCU036	4	SAMN00138235	PRJNA53719	GCA_000697905.1	*Staphylococcus epidermidis*	VCU036	J. Craig Venter Institute	Unknown	USA	Unknown	Unknown	Unknown	Unknown	454; Illumina	87x	Newbler v. 2.6	68	2425380	2425380	0	0	621	35667	194283	66785	Unpublished	PRJNA53719	Costa et al., 2017	10.1128/JB.00271-17

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 1 - Sheet E. Costa *et al.*, 2017 *S. epidermidis* isolates

Representative strain HsdS	Motif	Partner motif	Number of isolates	MLST	HsdS NCBI protein accession	aa length	REBASE	Strain genome reference	Strain genome reference DOI	Methylation reference	DOI
Newman_UoM_MS1	AGGNNNNNGAT	ATCNNNNNCCT	60	ST5 (ST225, ST105), ST239, ST8 (ST250, ST254, 923)	WP_000072584.1	403	S.SauNewORF398P	Monk et al., 2017	10.1038/ncomms14403	Monk et al., 2017	10.1038/ncomms14403
Newman_UoM_MS2	CCAYNNNNNNTGT	ACANNNNNNRTGG	42	ST151, ST1, ST8 (ST250, ST254, 923)	WP_000072576.1	399	S.SauNewORF1709P	Monk et al., 2017	10.1038/ncomms14403	Monk et al., 2017	10.1038/ncomms14403
MW2_MS1	CCAYNNNNNTTAA	TTAANNNNNNRTGG	3	ST1	WP_000072566.1	419	S.SauMW2I	Baba et al., 2002	10.1016/S0140-6736(02)08713-5	Monk et al., 2015	10.1128/mBio.00308-15
N315_MS2	CCAYNNNNNNGTA	TACNNNNNNRTGG	30	ST5 (ST225, ST105), ST228	WP_000072627.1	409	S.SauN315II	Kuroda et al., 2001	10.1016/S0140-6736(00)04403-2	Cooper et al., 2017	10.1093/nar/gkx067
FDAARGOS_159_MS1	CCAYNNNNNGAT	ATCNNNNNRTGG	10	ST5, ST228, ST25	WP_000072629.1	407	S.Sau159II	PRJNA231221	GCA_001568795.2	PRJNA231221	S.Sau159II
NCTC13435_MS3	TCTANNNNNNRTTC	GAAYNNNNNNTAGA	4	ST25, ST72	WP_000072557.1	406	S.Sau1343ORF2165P	PRJEB6403	GCA_001457495.1	Cooper et al., 2017	10.1093/nar/gkx067
NCTC13435_MS2	GACNNNNNNTTYG	CRAANNNNNNGTC	3	ST72	WP_000072638.1	402	S.Sau1343ORF1751P	PRJEB6403	GCA_001457495.1	Cooper et al., 2017	10.1093/nar/gkx067
DAR4145_MS1	CTANNNNNNNTAG	CTANNNNNNNTAG	6	ST772, ST72	WP_000072592.1	401	S.Sau772ORF391P	Steinig et al., 2015	10.1186/s12864-015-1599-9	Steinig et al., 2015	10.1186/s12864-015-1599-9
DAR4145_MS2	GAAGNNNNNNTTRG	CYAANNNNNNCTTC	4	ST772, ST27	WP_000072555.1	421	S.Sau772ORF1908P	Steinig et al., 2015	10.1186/s12864-015-1599-9	Steinig et al., 2015	10.1186/s12864-015-1599-9
ST20130938_MS1	predict?--RTGA		3	ST582, ST15	WP_000066763.1	398	S.Sau938ORF3659P	Trouillet-Assant et al., 2016	10.1111/cmi.12582		
ST20130938_MS2	predictGGHA--?--TTYG		3	ST582, ST15	WP_000072556.1	411	S.Sau938ORF3653P	Trouillet-Assant et al., 2016	10.1111/cmi.12582		
CN_MS1	GARANNNNNNRTGT	ACAYNNNNNNTYTC	5	ST7, ST72	WP_000072573.1	398	S.SauCN1ORF415P	Chen et al., 2013	10.1371/journal.pone.0072803	Cooper et al., 2017	10.1093/nar/gkx067
CN_MS2	GGANNNNNNNTGC	GCANNNNNNNTCC	5	ST50, ST72	WP_000072558.1	372	S.SauCN1ORF1757P	Chen et al., 2013	10.1371/journal.pone.0072803	Cooper et al., 2017	10.1093/nar/gkx067
AUS0325_MS2	GAGNNNNNNTCG	CGANNNNNNNCTC	2	ST1093, ST88	WP_089992008.1	412	S.SauS25ORFBP	Kpeli et al., 2017	10.7717/peerj.3047	Kpeli et al., 2017	10.7717/peerj.3047
HO_5096_0412_MS1	AGGNNNNNNNTGAR	YTCANNNNNNCCT	3	ST22	WP_000072565.1	419	S.Sau5096I	Holden et al., 2013	10.1101/gr.147710.112	Cooper et al., 2017	10.1093/nar/gkx067
HO_5096_0412_MS2 truncated	predictnotfunctional		3	ST22	WP_000323907.1	156	S.Sau5096ORF1724P	Holden et al., 2013	10.1101/gr.147710.112	Cooper et al., 2017	10.1093/nar/gkx067
MRSA252_MS1	GWAGNNNNNGAT	ATCNNNNNCTWC	8	ST30 (ST36, ST243), ST433	WP_000072632.1	410	S.SauMRSII	Holden et al., 2004	10.1073/pnas.0402521101	Monk et al., 2015	10.1128/mBio.00308-15
MRSA252_MS2	GGANNNNNNNTCG	CGANNNNNNNTCC	10	ST30 (ST36, ST243), ST433	WP_000072622.1	384	S.SauMRSI	Holden et al., 2004	10.1073/pnas.0402521101	Monk et al., 2015	10.1128/mBio.00308-15
BB155_MS1	predictAGG--?		2	ST152	WP_054190421.1	417	S.Sau155ORF3220P	PRJEB6500	GCA_900004855.1		
Tager_104_MS2	predict?--RTTC		3	ST152, ST50, ST49	WP_000072571.1	406	S.Sau104ORF2433P	Davis et al., 2016	10.1186/s12864-016-2433-8		
S0385 (ST398_MS1)	ACCNNNNNRTGA	TCAYNNNNNGGT	8	ST398	WP_000072568.1	392	S.Sau5TORF469P	Schijffelen et al., 2010	10.1186/1471-2164-11-376	Cooper et al., 2017	10.1093/nar/gkx067
CA-347_MS1	GWAGNNNNNNTAAA	TTTANNNNNNCTWC	2	ST45	WP_000072579.1	419	S.Sau347I	Stegger et al., 2013	10.1128/genomeA.00517-13	Cooper et al., 2017	10.1093/nar/gkx067
XQ_MS1	GGANNNNNNCCT	AGGNNNNNNTCC	2	ST121	WP_058008355.1	386	S.SauXQORF13815P	PRJNA301649	GCA_001444345.1	PRJNA231221	S.SauNRS153II
XQ_MS2	GACNNNNNNTAYG	CRTANNNNNNGTC	2	ST121	WP_058008121.1	396	S.SauXQORF677P	PRJNA301649	GCA_001444345.1	PRJNA231221	S.SauNRS153I
SA_40_S	GGANNNNNNRTGT	ACAYNNNNNNTC	6	ST39 (ST338)	WP_000072559.1	369	S.SauSA40ORF570P	Chen et al., 2013	10.1371/journal.pone.0063210	Cooper et al., 2017	10.1093/nar/gkx067
JKD6159_MS1	CAGNNNNNNTTC	GAANNNNNNCTG	1	ST93	WP_000072616.1	411	S.SauJKDI	Chua et al., 2010	10.1128/JB.00878-10	Monk et al., 2015	10.1128/mBio.00308-15
JKD6159_MS2	GGHANNNNNNNTCG	CGANNNNNNNTDCC	1	ST93	WP_000072554.1	412	S.SauJKDII	Chua et al., 2010	10.1128/JB.00878-10	Monk et al., 2015	10.1128/mBio.00308-15
RF122_MS1	unknown		1	ST151	WP_000072567.1	410	S.SauRF122ORF265P	Herron et al., 2002	10.1128/AAL70.7.3978-3981.2002		
RF122_MS2 truncated	unknown		1	ST151	WP_070007671.1 (truncated at aa 203)	409 (full length)	S.SauRF122ORF384P	Herron et al., 2002	10.1128/AAL70.7.3978-3981.2002		
ED133_MS1	CAGNNNNNNRTGA	TCAYNNNNNCTG	1	ST133	WP_000072617.1	397	S.Sau133ORF451P	Guinane et al., 2010	10.1093/gbe/evq031	Cooper et al., 2017	10.1093/nar/gkx067
ED133_MS2	GGANNNNNNNTTRG	CYAANNNNNNTCC	1	ST133	WP_000072562.1	392	S.Sau133ORF1794P	Guinane et al., 2010	10.1093/gbe/evq031	Cooper et al., 2017	10.1093/nar/gkx067
LGA251_MS1	GWAGNNNNNRTGA	TCAYNNNNNCTWC	1	ST425	WP_000072635.1	400	S.Sau251I	Garcia-Alvarez et al., 2011	10.1016/S1473-3099(11)70126-8	Cooper et al., 2017	10.1093/nar/gkx067
LGA251_MS2 truncated	not expressed		1	ST425	WP_044122248.1 (truncated at aa 249)	404 (full length)	S.Sau251ORF16900P	Garcia-Alvarez et al., 2011	10.1016/S1473-3099(11)70126-8	Cooper et al., 2017	10.1093/nar/gkx067
Tager_104_S1	predictGGA--?-RTGA		1	ST49	WP_000072560.1	366	S.Sau104ORF1102P	Davis et al., 2016	10.1186/s12864-016-2433-8		
JS395_MS1	predictCAG-?		1	ST1093	WP_000072352.1 (truncated at aa 8)	416 (full length)	S.Sau395ORF9335P	Lasen et al., 2017	10.1093/ac/dlw044		
FDA209P_MS1	CCAYNNNNNNRTC	GAYNNNNNNRTGG	1	ST464	WP_000072586.1	412	S.Sau209ORF368P	Singh et al., 2015	10.1128/genomeA.01343-15	PRJDB4160	S.SauCOI791ORFAP
FDA209P_MS2	predictCCAY--?TTYG		1	ST464	WP_047210362.1	414	S.Sau209ORF1697P	Singh et al., 2015	10.1128/genomeA.01343-15		
RKH_MS1	TCTANNNNNNTTAA	TTAANNNNNNTAGA	1	ST27	WP_000072580.1	408	S.SauRKHORF8860P	Stevens et al., 2015	10.1128/genomeA.00769-15	PRJNA243331	S.Sau18750II
08-02300_MS2	predict?--RTTC		1	ST7	WP_000072588.1	409		Weber et al., 2016	10.1128/genomeA.00716-16		
AUS0325_MS1	ACCNNNNNRTGT	ACAYNNNNNGGT	1	ST88	WP_000072613.1	395	S.Sau325ORFCP	Kpeli et al., 2017	10.7717/peerj.3047	Kpeli et al., 2017	10.7717/peerj.3047
IMPORTED											
JKD6159_MS3R	GAAGNNNNNTAC	GTANNNNNCTTC	7	ST93, ST425, ST1093, ST22, ST464	WP_000394004.1	410	S.SauJKDIII	Chua et al., 2010	10.1128/JB.00878-10	Monk et al., 2015	10.1128/mBio.00308-15
BPH0736_MS3R	GATNNNNCTTA	TAAGNNNNATC	4	ST59 (ST338), ST5	WP_000456234.1	381	unknown	This study		This study	
12228_RtruvdS3	GAANNNNNNNCTTA	TAAGNNNNNNTTC	3	ST5, ST8,	WP_016310129.1	400	S.SauRPORF2472P	Zhang et al., 2003	10.1046/j.1365-2958.2003.03671.x		
CA-347_MS2	unknown		2	ST45	WP_000809140.1	435	unknown	Stegger et al., 2013	10.1128/genomeA.00517-13		
DAR4145_MS3R	TTACNNNNNNTAC	GTANNNNNGTAA	3	ST772	WP_000394606.1	385	S.Sau772ORF50P	Steinig et al., 2015	10.1186/s12864-015-1599-9	Steinig et al., 2015	10.1186/s12864-015-1599-9
RMV8897_RMS2	unknown		1	ST398	WP_060585130.1	425	S.Sau389ORF296P	Bosch et al., 2016	10.1128/AEM.00773-16		
MSSA476_MS3R	unknown		2	ST1	WP_000085808.1	436	S.SauMSSII	Holden et al., 2004	10.1073/pnas.0402521101		
HUV05_RMS3 *plasmid	unknown		1	ST8	WP_048520805.1	408	S.SauHUV05ORF13980P	Planet et al., 2015	10.1093/infdis/jiv320		
ALREADY REPRESENTED IN TABLE											
MW2_MS2	CCAYNNNNNNTGT	ACANNNNNNRTGG		ST151, ST1, ST8 (ST250, ST254, 923)	WP_000072576.1	399	S.SauMW2II	Baba et al., 2002	10.1016/S0140-6736(02)08713-5	Cooper et al., 2017	10.1093/nar/gkx067
TCH1516_MS2	CCAYNNNNNNTGT	ACANNNNNNRTGG		ST151, ST1, ST8 (ST250, ST254, 923)	WP_000072576.1	399	S.SauTCHI	Highlander et al., 2007	10.1186/1471-2180-7-99	PRJNA19489	S.SauTCHI
TCH1516_MS1	AGGNNNNNNGAT	ATCNNNNNCCT		ST5 (ST225, ST105), ST239, ST8 (ST250, ST254, 923)	WP_000072584.1	403	S.SauTCHII	Highlander et al., 2007	10.1186/1471-2180-7-99	PRJNA19489	S.SauTCHII
NZAK3_MS1	AGGNNNNNNGAT	ATCNNNNNCCT		ST5 (ST225, ST105), ST239, ST8 (ST250, ST254, 923)	WP_000072584.1	403	S.SauNZAK3ORFBP	Baines et al., 2016	10.1128/AAC.03020-15	Baines et al., 2016	10.1128/AAC.03020-15
N315_MS1	AGGNNNNNNGAT	ATCNNNNNCCT		ST5 (ST225, ST105), ST239, ST8 (ST250, ST254, 923)	WP_000072584.1	403	S.SauN315I	Kuroda et al., 2001	10.1016/S0140-6736(00)04403-2	Cooper et al., 2017	10.1093/nar/gkx067
FDAARGOS_159_MS2	CCAYNNNNNNTGA	TACNNNNNNRTGG		ST5 (ST225, ST105), ST228	WP_000072627.1	409	S.Sau159I	PRJNA231221	GCA_001568795.2	PRJNA231221	S.Sau159I
LGA251_MS1	GAAGNNNNNTAC	GTANNNNNCTTC		ST93, ST425, ST1093, ST22, ST464	WP_000394004.1	410	S.Sau251II	Garcia-Alvarez et al., 2011	10.1016/S1473-3099(11)70126-8	Cooper et al., 2017	10.1093/nar/gkx067
ATCC_25923_MS1	GWAGNNNNNGAT	ATCNNNNNCTWC		ST30 (ST36, ST243), ST433	WP_000072632.1	384	S.Sau2592ORF1940P	Treangen et al., 2014	10.1128/genomeA.01110-14	Treangen et al., 2014	10.1128/genomeA.01110-14
ATCC_25923_MS2	GGANNNNNNNTCG	CGANNNNNNNTCC		ST30 (ST36, ST243), ST433	WP_000072622.1	417	S.Sau2592ORF9435P	Treangen et al., 2014	10.1128/genomeA.01110-14	Treangen et al., 2014	10.1128/genomeA.01110-14
NCTC13435_MS1	not expressed			ST772, ST72	WP_060540312.1	401	S.Sau1343ORF394P	PRJEB6403	GCA_001457495.1	Cooper et al., 2017	10.1093/nar/gkx067

Methylome determined by PacBio sequencing
Methylome determined by DNA cleavage with purified enzyme

PS187 (draft genome)											
PS187_MS1	predictCAG-?			ST395 plus ST1093	WP_000072352.1 (truncated at aa 8)	416 (full length)	S.Sau395ORF9335P	Winstel et al., 2013	10.1038/ncomms3345		
PS187_MS2	predictGAGNNNNNNTCG	predictCGANNNNNNCTC		ST395 plus ST1093	WP_000072574.1	411	S.Sau395ORF1940P	Winstel et al., 2013	10.1038/ncomms3345		

Full amino acid translation of all 48 S. aureus HsdS variants can be downloaded from Figshare DOI:10.26188/5cb01b089ab2

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 2 - Sheet A. *S. aureus* HsdS

Strain	Subunit	NCBI protein accession	aa length	Identity	Positive	REBASE	Comments
Sa_Newman_UoM	HsdR	WP_000331347.1	929	100%	100%	SauNewORF139P	
Sa_Newman_UoM	HsdM	WP_000028628.1	518	100%	100%	M.SauNewORF398P	

Representative strain HsdS	Motif	Partner motif	Number of isolates	STs with HsdS	HsdS NCBI protein accession	aa length	REBASE	Interacting HsdM	HsdM NCBI protein accession	Genome reference	Genome reference DOI	Methylation reference	Methylation reference DOI	Comments
BPH0662-S1	GAGNNNNNAAT	ATTNNNNCTC	43	2	WP_002594637.1	406	S.SepBPH0662I	BPH0662-M1	WP_002904638.1	Lee et al., 2016	10.1099/mgen.0.000077	Lee et al., 2016	10.1099/mgen.0.000077	
BPH0662-S2	CAGNNNNATC	GATTNNNNCTG	52	2, 23, 35	WP_002594701.1	424	S.SepBPH0662II	BPH0662-M2	WP_002904700.1	Lee et al., 2016	10.1099/mgen.0.000077	Lee et al., 2016	10.1099/mgen.0.000077	
BPH0736-S	GATNNNNCTTA	TAAGNNNNNATC	18	2, 16,210	WP_000498234.1	433	unknown	BPH0662-M2	WP_002904700.1	This study		This study		
RP62a-S	GAGNNNNNNNTAC	GTANNNNNNNCTC	9	10, 23, 40, 71, 230	WP_002489618.1	388	S.SepRP62aI?;472P	BPH0662-M1	WP_002904638.1	Gill et al., 2005	10.1128/JB.187.7.2426-2438.2005	This study		
12228-S	GAANNNNNCTTA	TAAGNNNNNNTTC	64	2, 5, 8, 6, 22, 23, 59, 185	WP_001931029.1	400	S.SepECRF48P	BPH0662-M1	WP_002904638.1	Zhang et al., 2003	10.1046/j.1365-2958.2003.03671.x	Costa et al., 2017	10.1128/JB.00271-17	All examples of this system are incomplete ; not functional unless BPH0662-M1 variant of HsdM is present elsewhere in genome
BPH0710-S	predict not functional		2	2	WP_064984491.1(1st 81 aa truncated)	336	unknown	BPH0710-M	WP_064984489.1 (D293N substitution)	Lee et al., 2018	10.1038/s41564-018-0230-7			Full *hsdMSR*, but prediction 4unctional due to truncation of 1st 81 aa in BPH0710 HsdS
BPH0722-S	GAAYNNNNTCC	GCANNNNNRTTC	13	5, 21, 32, 46, 83, 210	WP_002489391.1	418	unknown	BPH0662-M2	WP_002904700.1	This study		This study		
1497-S	predict not functional		4	86	WP_079178799.1 (L1M substitution)	236	S.Sep1497ORF326P	unknown		Galac et al., 2017	10.1128/genomeA.00450-17	Galac et al., 2017	10.1128/genomeA.00450-17	All 4 genomes have incomplete system (*hsdR* and *hsdS* both truncated, no *hsdM*)
BPH0711-S	GGANNNNNNTAG	CTANNNNNNTCC	2	59	WP_002437522.1	400	unknown	BPH0662-M2	WP_002904700.1	This study		This study		
BPH0704-S	CYTANNNNNNCGT	ACGNNNNNNTRRG	1	358	WP_061544233.1	412	unknown	BPH0662-M1	WP_002904638.1	This study		This study		
BPH0697-S	ACANNNNNGTG	CACNNNNNTGT	4	4, 22, 89	WP_002505893.1	416	unknown	BPH0697-M	WP_002905892.1	This study		This study		
BPH0747-S	CNACNNNNRTA	TKATNNNNGTNG	1	2	WP_130481761.1	427	unknown	BPH0662-M2	WP_002904700.1	This study		This study		
14.1.R1-S	unknown			-	WP_102841642.1	397	S.SepR1ORF114?P	unknown		Lassen et al., 2017	10.1128/genomeA.00004-17	Lassen et al., 2017	10.1128/genomeA.00004-17	Incomplete system: complete *hsdS* (half same as 12228S), truncated *hsdR* and no *hsdM*
SRA														
248_DEN19-S	predict		1	1	novel	397	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			
271_ICE87-S	predict		1	40	novel	411	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			
288_GRE41-S	unknown		3	83	WP_049366366.1	398	unknown	BPH0662-M2	WP_002904700.1	Mericel at., 2015	10.1093/gbe/evv066			
290_TAW13-S	unknown		1	85	novel	402	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			
298_IAP60-S1	unknown		1	33	novel	376	unknown	unknown		Mericel at., 2015	10.1093/gbe/evv066			
298_IAP60-S2	predict		1	33	novel	413	unknown	298_IAP60-M	WP_056936150.1 (*10 aa substitutions)	Mericel at., 2015	10.1093/gbe/evv066			*10 aa substitutions: N106K, E154D, I159L, M163I, G167A, S174G, M176L, D282N, K459E, D461A
300_ITL34-S	predict		3	57, 66	WP_021298801.1(S299P substitution)	389	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			
307_HRA17-S	unknown		1	37	novel	403	unknown	BPH0662-M2	WP_002904700.1	Mericel at., 2015	10.1093/gbe/evv066			
308_MEX3-S truncated	predict not functional		2	328, 559	WP_080035763.1	174	unknown	unknown		Mericel at., 2015	10.1093/gbe/evv066			
313_93N-S1	unknown		1	329	WP_104992793.1	416	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			
313_93N-S2 truncated	predict not functional		1	329	novel	309	unknown	unknown		Mericel at., 2015	10.1093/gbe/evv066			
315_14I-N-S	unknown		1	390	WP_107639500.1 (*11 aa substitutions)	443	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			*11 aa substitutions: K28E, I 56V, E59K, E71K, K174R, K175T, E178A, I193V, D201N, Y386F, V434I
332_134ON-S1	unknown		2	402	novel	310	unknown	BPH0662-M2	WP_002904700.1	Mericel at., 2015	10.1093/gbe/evv066			
332_134ON-S2	unknown		2	402	novel	412	unknown	332_134ONM	WP_053025623.1 (*6 aa substitutions)	Mericel at., 2015	10.1093/gbe/evv066			*6 aa substitutions: K13Q, A53V, E103K, T146N, V329I, N493N
327_79GN-S	predict 7-ATC		1	406	WP_038032270.1	423	unknown	BPH0662-M2	WP_002904700.1	Mericel at., 2015	10.1093/gbe/evv066			2nd half same as BPH0756-S (TAAGNNNNNATC) & BPH0662-S2 (CAGNNNN**ATC**)
328_80ILR2-S	unknown		1	595	WP_002490392.1	406	unknown	BPH0662-M1	WP_002904638.1	Mericel at., 2015	10.1093/gbe/evv066			
972_SEP-S	unknown		4	5, 6, 20	WP_049387567.1	416	unknown	BPH0662-M2	WP_002904700.1	Roach et al., 2015	10.1371/journal.pgen.1005413			
UCLA-14	unknown		7	2, 186	WP_002504281.1	431	unknown	BPH0662-M2	WP_002904700.1	Tewhey et al., 2014	10.1128/mBio.00894-14			
Replicates of HsdS														
NIH04008-S3	GAANNNNNNCTTA	TAAGNNNNNNTTC			WP_001931029.1	400	S.Sep408ORF127P	BPH0662-M1	WP_002904638.1	Conlan et al., 2012	10.1186/gb-2012-13-7-r64	Costa et al., 2017	10.1128/JB.00271-17	same as 12228-S
NIH51675-S	ACANNNNNGTG	CACNNNNNTGT			WP_002505893.1	416	S.Sep51475I	BPH0697-M	WP_002905892.1	Conlan et al., 2012	10.1186/gb-2012-13-7-r64	Costa et al., 2017	10.1128/JB.00271-17	same as BPH0697; ST 89
VCU036-S	ACANNNNNGTG	CACNNNNNTGT			WP_002505893.1	416	S.Sep36I	BPH0697-M	WP_002905892.1	PRJNA63719	GCA_000697505.1	Costa et al., 2017	10.1128/JB.00271-17	same as BPH0697; **in silico ST4 NOT ST89**

Full amino acid translation of all 31 S. epidermidis HsdS variants can be downloaded from Figshare DOI: 10.26186/5c01a#f23105
Full amino acid translation of all 6 S. epidermidis HsdM variants can be downloaded from Figshare DOI: 10.26186/5c019a696f05

***S. epidermidis* HsdR subunit variants**

Strain	Subunit	NCBI protein accession	aa length	Identity	Positive	N isolates w complete	REBASE
BPH0662	BPH0662-R1	WP_002504640.1	930	100%	100%	64	SepiBPH0662IP
BPH0662	BPH0662-R2	WP_002504702.1	1039	100%	100%	107	SepiBPH0662IIP
BPH0710	BPH0710-R	WP_064584495.1	1009	100%	100%	10	unknown
298_JAP63	298_JAP63-R	WP_053025625.1 (*4 aa substitutions)	930	926/930 99%	928/930 99%	1	unknown
322_1340N	322_1340N-R (1 of 2)	WP_002511775.1 (*16 aa substitutions)	932	916/932 98%	927/932 99%	1	unknown

***S. epidermidis* HsdM subunit variants**

Strain	Subunit	NCBI protein accession	aa length	Identity	Positive	N isolates w complete	REBASE
BPH0662	BPH0662-M1	WP_002504638.1	518	100%	100%	61	M.SepiBPH0662I
BPH0662	BPH0662-M2	WP_002504700.1	504	100%	100%	99	M.SepiBPH0662II
BPH0710	BPH0710-M	WP_064584489.1 (D293N substitution)	595	594/595 99%	100%	10	unknown
BPH0697	BPH0697-M	WP_002505892.1	504	100%	100%	5	M.Sep51475I
298_JAP263	298_JAP263-M	WP_056936150.1 (*10 aa substitutions)	518	508/518 98%	514/518 99%	1	unknown
322_1340N	322_1340N-M (1 of 2)	WP_053025623.1 (*6 aa subtitutions)	518	512/518 99%	516/518 99%	1	unknown

Full amino acid translation of all 5 S. epidermidids HsdR variants can be downloaded from Figshare DOI: 10.26188/5cb01b55b4411
Full amino acid translation of all 6 S. epidermidids HsdM variants can be downloaded from Figshare DOI: 10.26188/5cb019b506f53

STRAIN	*in silico* MLST	Year	Country	Institution location	Host	Clinical Presentation	Sample	Reference	DOI	Comments
BPH0662	2	2012	Australia	Melbourne, Victoria	Human	CNS infection	CSF	Lee et al., 2016	10.1099/mgen.0.000077	
BPH0697	89	2007	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0704	368	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0711	59	2008	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0723	5	2009	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0736	2	2011	Australia	Melbourne, Victoria	Human	Bacteraemia	Blood culture	Lee et al., 2018	10.1038/s41564-018-0230-7	
BPH0747 (IRL01)	2	2016	Republic of Ireland	Republic of Ireland	Human	Bacteraemia	Blood culture	Lee et al., 2018	10.1038/s41564-018-0230-7	Isolate referred to as IRL01 in Lee et al., 2018
RP62a_UoM	10	1979-1980	United States	Memphis, Tennessee	Human	Bacteraemia	Blood culture	Gill et al., 2005	10.1128/JB.187.7.2426-2438.2005	PacBio sequencing of RP62a reference to obtain methylome

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 3 - Sheet A. Clinical metadata

Strain	Organism	BioProject	BioSample (NCBI)	BioSample (ENA)	Assembly accession	Assembly method	Sequencing techonology	Replicon	Size (bp)	GC %	Scaffolds	Number coding genes	Level
BPH0662	Staphylococcus epidermidis	PRJEB13975	SAMEA3982825	ERS1153935	GCA_900086615.1	HGAP2	PacBio RS with Illumina correction	BPH0662	2,793,003	32.0	1	2653	Complete genome
								pBPH0662-01	45,807	30.0	1	40	Complete genome
								pBPH0662-02	2,366	31.1	1	2	Complete genome
								pBPH0662-03	13,569	28.6	1	14	Complete genome
BPH0697	Staphylococcus epidermidis	PRJEB35032	SAMEA3712729	ERS1019878	GCA_902509485.1	HGAP3	PacBio RSII with Illumina correction	BPH0697	2,480,158	32.1	1	2254	Complete genome
								pBPH0697-01	62,421	29.9	1	77	Complete genome
								pBPH0697-02	4,615	31.9	1	6	Complete genome
BPH0704	Staphylococcus epidermidis	PRJEB35032	SAMEA3712735	ERS1019884	GCA_902509495.1	HGAP3	PacBio RSII with Illumina correction	BPH0704	2,526,011	32.1	1	2309	Complete genome
								pBPH0704-01	15,030	29.5	1	13	Complete genome
BPH0711	Staphylococcus epidermidis	PRJEB35032	SAMEA3712742	ERS1019891	GCA_902509535.1	HGAP3	PacBio RSII with Illumina correction	BPH0711	2,529,109	32.3	1	2338	Complete genome
								pBPH0711-01	16,590	28.8	1	19	Complete genome
								pBPH0711-02	16,034	29.1	1	16	Complete genome
BPH0723	Staphylococcus epidermidis	PRJEB35032	SAMEA3712754	ERS1019903	GCA_902509515.1	HGAP3	PacBio RSII with Illumina correction	BPH0723	2,556,537	32.3	1	2334	Complete genome
								pBPH0723-01	39,876	31.3	1	45	Complete genome
								pBPH0723-02	2,241	30.3	1	2	Complete genome
BPH0736	Staphylococcus epidermidis	PRJEB35032	SAMEA3712766	ERS1019915	GCA_902509505.1	HGAP3	PacBio RSII with Illumina correction	BPH0736	2,533,804	32.1	1	2327	Complete genome
								pBPH0736-01	29,558	29.6	1	31	Complete genome
								pBPH0736-02	17,639	31.6	1	18	Complete genome
BPH0747 (IRL01)	Staphylococcus epidermidis	PRJNA532483	SAMN09103952	NA	CP045648 (chromosome) & CP045647 (plasmid)	HGAP3	PacBio RSII with Illumina correction	BPH0747	2,737,464	31.0	1	2591	Complete genome
								pBPH0747-01	13,568	28.6	1	14	Complete genome
RP62a_UoM	Staphylococcus epidermidis	PRJEB35032	SAMEA3712772	ERS1019921	GCA_902509525.1	HGAP3	PacBio RSII with Illumina correction	RP62a_UoM	2,619,158	32.2	1	2409	Complete genome
								pRP62a_UoM-01	27,310	31.9	1	33	Complete genome

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 3 - Sheet B. Genome accession

Strain	Organism	Motif	% Motifs Detected	# Motifs Detected	# Motifs in Genome	Mean Modification QV	Mean Motif Coverage
BPH0662	Staphylococcus epidermidis	G**A**GNNNNNAAT	99.87	1582	1584	110.70	75.90
		ATTNNNNNCTC	99.56	1577	1584	105.10	76.10
		C**A**GNNNNATC	100.00	1319	1319	125.40	76.40
		G**A**TNNNNCTG	97.80	1290	1319	104.30	76.20
BPH0697	Staphylococcus epidermidis	AC**A**NNNNNGTG	100.00	759	759	212.33	161.79
		C**A**CNNNNNTGT	99.60	756	759	198.27	162.26
BPH0704	Staphylococcus epidermidis	CAGR**A**G	99.03	1737	1754	133.48	99.14
		CYY**A**NNNNNNCGT	98.48	453	460	128.89	102.81
		ACGNNNNNNTRRG	98.04	451	460	132.24	101.70
BPH0711	Staphylococcus epidermidis	CT**A**NNNNNNTCC	98.83	422	427	260.11	205.98
		GG**A**NNNNNNTAG	96.49	412	427	244.45	206.21
BPH0723	Staphylococcus epidermidis	GGA**A**C	99.35	2602	2619	339.46	240.36
		GA**A**YNNNNNTGC	98.88	354	358	310.69	234.54
		GC**A**HNNNNRTTC	97.34	293	301	288.42	240.60
BPH0736	Staphylococcus epidermidis	G**A**TNNNNCTTA	98.47	385	391	101.63	61.75
		TA**A**GNNNNATC	98.47	385	391	101.39	58.75
BPH0747 (IRL01)	Staphylococcus epidermidis	TA**A**YNNNNGTNG	99.21	875	882	198.52	146.51
		CN**A**CNNNNRTTA	98.30	867	882	194.57	151.17
RP62a_UoM	Staphylococcus epidermidis	G**A**GNNNNNNNTAC	98.64	651	660	227.08	170.82
		GT**A**NNNNNNNCTC	98.64	651	660	211.30	169.64

Strain	CRISPR-Cas	Start	End	Cas	CRISPR DR sequence	DR length	Number of CRISPR DR	Number of CRISPR spacers	CRISPR spacer sequence	CRISPR spacer target
BPH0662	None									
BPH0697	None									
BPH0704	Yes	69068	70091	Type IIC	GTTTTAGTACTCTGTAATTTTAGGTATAAGGTATAT	36	16	15	1. CAAAAGTTTTTCCAAATGATGGGCACAAC	Phage tail protein
									2. ATTGATTCAGTTTGTAGCACTTTTCACTAC	Unknown
									3. TATTAAAGATTTGGTTTACTATCGCTATGT	Hypothetical protein, hit on *Staphylococcus* phage IME1323_01
									4. ACTTATCGCTGCATGAACAGCACCCATAA	Unknown
									5. TTGGACTGGTTATCATGTTTAATCCATTAG	Phage DNA packaging protein & head tail adaptor protein (overlaping coding regions)
									6. AAGGCGAAACAGATCATGACAAACTAGGCA	Unknown
									7. **CATCTTACCTTGCTATTATGACGGTAAACA**	Unknown
									8. TATCTTTTATGTTGGAACCAAATTCAACTG	Phage tail tape measure protein
									9. TTGTAAAAGCTAAAAATGAGGAAGAACTGT	Unknown
									10. CAACCGAAATAGAACAAGCTATAAAAAATA	Unknown
									11. GAAAAATTCATTCACTCAATGGGAAATCAA	Hypothetical protein, hit on *Staphylococcus* phage phiSa119
									12. GATAAGACGTTTACTCATAACAGAAACAGC	Unknown
									13. TCTTGCCTCTTTGAACAGACCAGAATACAC	Unknown
									14. TGTTTATTCAGTTTTGTGATTTATATAATA	Unknown
									15. AAATCGCTCATGCGCATCACTACACAGAGG	Unknown
BPH0711	None									
BPH0723	Yes	92786	93615	Type IIIA	GATCGATAACTATCCCGAAGAACAGGGGATGAGAAC	36	12	11	1. AGCTAATATTGTTTAAATCGTCAATTCCTGCATGTT	*ermC* [23S rRNA (adenine(2058)-N(6))-methyltransferase Erm(C)]
									2. TTGTACCGTGTATATAACTTGCAAGATCTGAAGATA	*glucose 1-dehydrogenase*
									3. TTTAACAAGTTAAAAATGTATGCAAAAGTTAAAAAGA	*rep* [plasmid rolling cicle replication initiator protein]
									4. TAGTGAGGGGATTTTTTAGGTGTACTTAAATTTGTCAT	Unknown
									5. CTAACTTTTTCGCTAAATATTTTCTCACAACCAAAAT	*res* [resolvase]
									6. CATATCTCTTTGATCATTCATTTGTTTATCACTTGC	Hypothetical protein, all hits on Staphylococcal plasmids
									7. TTCTAGTTACGTTGTCAATAGTTTTTTTTAAATTTC	Unknown
									8. ATTAGGTGTTAAATCAATAATTTCTGAATCATCA	*tra* [transfer gene]
									9. TCCAGTTTCTTGTATTTCTTTGCTAAATAAACCACC	*pre* [plasmid recombination enzyme]
									10. GATAAATATCTATAATTAACAATTTTCTTATTGAA	Hits on non-coding region of *S. epidermidis* ACME V mobile element
BPH0736	None								11. TCCAACTTACTATTTCCTTCTTGAAAAGTAAACTTCA	Hits on non-coding region of *S. epidermidis* ACME IV & V mobile elements
BPH0747 (IRL01)	None									
RP62a_UoM	Yes	98464	98712	Type IIIA	GGATCGATACCCACCCCGAAGAAAAGGGGACGAGAAC	37	4	3	1. ACGTATGCCGAAGTATATAAATCATCAGTACAAA	*oriT* nickase
									2. TAGTAATAATTGTCATTTGCATACGTTACATCGA	*Staphylococcus* phage PH15
									3. TAGTACGGTCGTGAACATTTTTTCTTGATTCTC	Unknown

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 3 - Sheet D. CRISPR

STRAIN	PEN	OXA	CFX	COT	GEN	CIP	ERY	CLI	FUS	RIF	TEI	VAN	DAP	TET	LZD
BPH0662	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S
BPH0697	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S
BPH0704	R	R	R	S	R	S	R	S	S	S	S	S	S	S	S
BPH0711	R	R	R	S	R	S	S	S	S	S	S	S	S	R	S
BPH0723	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S
BPH0736	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S
BPH0747 (IRL01)	R	R	R	R	R	R	R	R	R	R	S	S	S	I*	R
RP62a_UoM	R	R	R	R	R	S	R	R	S	S	S	S	S	S	S

LEGEND	
S	Susceptible by both CLSI and EUCAST 2017 breakpoints
R	Resistant by both CLSI and EUCAST 2017 breakpoints
I*	Intermediate by CLSI but resistant by EUCAST

STRAIN	NUMBER	AAC(6')-Ie-APH(2'')-Ia	ANT(4')-Ib	APH(3')-IIIa	aad(6)	ErmA	ErmC	mphC	msrA	PC1_beta-lactamase_(blaZ)	mecA	mecI	mecR1	dfrC	dfrG	fusB	SAT-4	Staphylococcus_intermedius_chloramphenicol_acetyltransferase	mupA	arlR	mgrA	norA	qacA	tet(K)
BPH0662	14	Y	Y	.	.	.	Y	.	.	Y(2)	Y	Y	Y	Y+I	.	Y	.	.	Y	Y	Y	Y	Y	.
BPH0697	9	Y(2)	.	.	.	.	.	.	.	Y(2)	Y	.	I	Y(2)	.	.	.	Y	.	Y	Y	Y	.	.
BPH0704	12	Y+I	.	.	.	.	.	Y	Y	Y	Y	Y	Y	Y	.	.	.	.	.	Y	Y	Y	Y	.
BPH0711	11	Y	.	.	.	.	.	.	.	Y	Y	.	I	Y	Y	.	.	.	.	Y	Y	Y	Y	Y
BPH0723	10	Y	.	.	.	.	Y	.	.	Y	Y	.	I	Y(2)	.	.	.	.	.	Y	Y	Y	Y	.
BPH0736	10	.	.	.	.	.	.	Y	Y	Y	Y	.	I	Y(2)	.	.	.	.	.	Y	Y	Y	Y	.
BPH0747 (IRL01)	11	Y	Y	.	.	.	.	.	.	Y(2)	Y	Y	Y	Y+I	.	.	.	.	.	Y	Y	Y	Y	.
RP62a_UoM	13	Y	.	Y	Y	Y(3)	.	.	.	Y(2)	Y	Y	Y	Y(2)	.	.	Y	.	.	Y	Y	Y	.	.

LEGEND

Y	Complete gene
Y(2)	Number in brackets denotes number of complete copies of gene
I	Incomplete gene
Y+I	Complete plus incomplete copy of gene

Mining the methylome reveals extensive diversity in *Staphylococcus epidermidis* restriction modification
Supplementary Table 3 - Sheet F. Resistome

STRAIN	CHL 10	TET 5	ERY 30	KAN 50
BPH0662	S	S	R	R
BPH0697	R	S	S	R
BPH0704	S	S	R	R
BPH0711	S	R	S	R
BPH0723	S	S	R	R
BPH0736	S	S	R	S
BPH0747 (IRL01)	R	S	S	R
RP62a_UoM	S	S	R	R
	(10 - 30 µg/ml)	(5 - 10 µg/ml)	(30 - 100 µg/ml)	(10 - 100 µg/ml)

Mining the methylome reveals extensive diversity in Staphylococcus epidermidis restriction modification
Supplementary Table 3 - Sheet G. Plasmid selection markers

Strain	Organism	Year	Country	Institution location	Sample	Sample unique name	Uploaded to	BioProject	BioSample	Sequencing technology	Reference	DOI	Comments
Ec_Se736I	*Escherichia coli*	2016	Australia	Melbourne, Victoria	Laboratory derived mutant	Ec_Se736I	SRA	PRJNA532483	SAMN13051824	PacBio RSII	This study		E. coli DC10B with S. epidermidis BPH0736 hsdMS integrated between essQ and cspB
Ec_Se662I	*Escherichia coli*	2014	Australia	Melbourne, Victoria	Laboratory derived mutant	DC10B-MS1	ENA	PRJEB13975	SAMEA3982823	PacBio RS	Lee et al., 2016	10.1099/mgen.0.000077	E. coli DC10B with S. epidermidis BPH0662 hsdMS1 integrated between ybbD and ylbG. Previously named DC10B-MS1 in Lee et al.,2016.
Ec_Se662II	*Escherichia coli*	2014	Australia	Melbourne, Victoria	Laboratory derived mutant	DC10B-MS2	ENA	PRJEB13975	SAMEA3982824	PacBio RS	Lee et al., 2016	10.1099/mgen.0.000077	E. coli DC10B with S. epidermidis BPH0662 hsdMS2 integrated between essQ and cspB. Previously named DC10B-MS2 in Lee et al., 2016.

Mining the methylome reveals extensive diversity in Staphylococcus epidermidis restriction modification
Supplementary Table 5 - Sheet A. *E. coli* PacBio sequencing accession

Strain	Organism	Motif	% Motifs Detected	# Motifs Detected	# Motifs in Genome	Mean Modification QV	Mean Motif Coverage
Ec_Se736I	*E. coli*	G**A**TC	98.42	37985	38594	144.11	110.40
		TA**A**GNNNNATC	100.00	422	422	155.96	107.14
		G**A**TDNNNCTTA	97.33	328	337	142.63	109.21
		G**A**TCNNNCTTA	100.00	85	85	147.57	108.12
Ec_Se662I	*E. coli*	G**A**TC	98.36	37954	38588	59.70	33.70
		G**A**GNNNNNAAT	90.45	1573	1739	55.80	33.20
		ATTNNNNNCTC	89.65	1559	1739	54.60	32.70
Ec_Se662II	*E. coli*	G**A**TC	99.52	38405	38592	70.40	40.40
		C**A**GNNNNATC	99.41	4185	4210	70.80	39.70
		G**A**TDNNNCTG	98.18	3183	3242	68.40	40.20
		G**A**TCNNNCTG	99.90	967	968	60.70	39.80

Mining the methylome reveals extensive diversity in Staphylococcus epidermidis restriction modification
Supplementary Table 5 - Sheet B. *E. coli* PacBio methylation data

STRAIN	Organism	Year	Country	Institution location	Sample	Sample unique name	Uploaded to	BioProject	BioSample	Sequencer	Reads	Yield	GC	MinLen	AvgLen	MaxLen	ModeLen	Phred	AvgQual	Depth	Reference	DOI	Comments
BPH0662ΔhsdS1	*Staphylococcus epidermidis*	2016	Australia	Melbourne, Victoria	Laboratory derived mutant	Se_BPH0662_delHsdS1	SRA	PRJNA532483	SAMN13051818	NextSeq	2711494	409656146	34.1	35	148	151	151	33	32.3	141	This study		S. epidermidis BPH0662 mutant with hsdS1 deletion
BPH0662ΔhsdS2	*Staphylococcus epidermidis*	2016	Australia	Melbourne, Victoria	Laboratory derived mutant	Se_BPH0662_delHsdS2	SRA	PRJNA532483	SAMN13051819	NextSeq	1675974	248380865	34.1	35	148	151	151	33	32.0	87	This study		S. epidermidis BPH0662 mutant with hsdS2 deletion
BPH0662ΔhsdS1ΔhsdS2	*Staphylococcus epidermidis*	2016	Australia	Melbourne, Victoria	Laboratory derived mutant	Se_BPH0662_delHsdS1_hsdS2	SRA	PRJNA532483	SAMN13051820	NextSeq	2121870	312653900	34.2	35	147	151	151	33	33.1	110	This study		S. epidermidis BPH0662 mutant with hsdS1 and hsdS2 deletions
BPH0736Δ*hsdS*	*Staphylococcus epidermidis*	2016	Australia	Melbourne, Victoria	Laboratory derived mutant	Se_BPH0736_delHsdS	SRA	PRJNA532483	SAMN13051821	Miseq	2312798	174036941	34.5	35	75	76	76	33	36.8	66	This study		S. epidermidis BPH0736 mutant with hsdS deletion
RP62AΔ*hsdS*	*Staphylococcus epidermidis*	2016	Australia	Melbourne, Victoria	Laboratory derived mutant	Se_RP62a_del-hsdS	SRA	PRJNA532483	SAMN13051822	Miseq	1780888	264434192	34.3	35	148	151	151	33	32.0	101	This study		S. epidermidis RP62a mutant with hsdSdeletion
Ec_SeM62-II	*Escherichia coli*	2014	Australia	Melbourne, Victoria	Laboratory derived mutant	Ec_SeM62-II	SRA	PRJNA532483	SAMN13051825	Miseq	1623178	447778980	52.5	35	275	301	301	33	30.1	96	Lee et al., 2018	10.1038/s41564-018-0230-7	E. coli DC10B with S. epidermidis BPH0662 hsdMS1 integrated between ybiD and ybiQ, and BPH0662 hsdMS2 between essQ and cspB
Ec_SeRP62aI	*Escherichia coli*	2011	Ireland	Dublin, Ireland	Laboratory derived mutant	Ec_SeRP62aI	SRA	PRJNA532483	SAMN13051826	NextSeq	4317852	643201247	50.9	35	148	151	151	33	31.6	137	This study		E. coli DC10B with S. epidermidis RP62a hsdMS integrated between gidB and atpI

Mining the methylome reveals extensive diversity in Staphylococcus epidermidis restriction modification
Supplementary Table 5 - Sheet C. *E. coli* Illumina sequencing accession