Comparative genomics of *Staphylococcus epidermidis* from prosthetic-joint infections and nares highlights genetic traits associated with antimicrobial resistance, not virulence

Emeli Månsson[1,2], Thor Bech Johannesen[3], Åsa Nilsdotter-Augustinsson[4,5], Bo Söderquist[1]† and Marc Stegger[1,3,*,†]

Abstract

There is increased awareness of the worldwide spread of specific epidemic multidrug-resistant (MDR) lineages of the human commensal *Staphylococcus epidermidis*. Here, using bioinformatic analyses accounting for population structure, we determined genomic traits (genes, SNPs and *k*-mers) that distinguish *S. epidermidis* causing prosthetic-joint infections (PJIs) from commensal isolates from nares, by analysing whole-genome sequencing data from *S. epidermidis* from PJIs prospectively collected over 10 years in Sweden, and contemporary *S. epidermidis* from the nares of patients scheduled for arthroplasty surgery. Previously suggested virulence determinants and the presence of genes and mutations linked to antimicrobial resistance (AMR) were also investigated. Publicly available *S. epidermidis* sequences were used for international extrapolation and validation of findings. Our data show that *S. epidermidis* causing PJIs differed from nasal isolates not by virulence but by traits associated with resistance to compounds used in prevention of PJIs: β-lactams, aminoglycosides and chlorhexidine. Almost a quarter of the PJI isolates did not belong to any of the previously described major nosocomial lineages, but the AMR-related traits were also over-represented in these isolates, as well as in international *S. epidermidis* isolates originating from PJIs. Genes previously associated with virulence in *S. epidermidis* were over-represented in individual lineages, but failed to reach statistical significance when adjusted for population structure. Our findings suggest that the current strategies for prevention of PJIs select for nosocomial MDR *S. epidermidis* lineages that have arisen from horizontal gene transfer of AMR-related traits into multiple genetic backgrounds.

DATA SUMMARY

Supporting data are provided in the supplementary data files.

INTRODUCTION

Due to an ageing population, improved access to surgery and increased prevalence of osteoarthritis and obesity, the number of total joint replacements performed annually is projected to continue to increase worldwide [1–4]. Consequently, the number of patients affected by a prosthetic-joint infection (PJI), the most feared complication of joint replacement surgery, will also increase [5]. Micro-organisms causing PJIs are generally thought to originate from the patient's microbiota [6]. *Staphylococcus epidermidis*, a ubiquitous skin commensal, was recently demonstrated to be the most frequent species in hip and knee PJIs, representing up to 33% of these infections [7]. *S. epidermidis* is recognized as an opportunistic pathogen [8] and global spread of hospital-adapted, multidrug-resistant

(MDR) *S. epidermidis* (MDRSE) predominated by sequence types (STs) 2, 5 and 23 causing clinical infections is evident [9]. These hospital-adapted MDRSE were recently demonstrated to also harbour biocide-resistance genes to a high extent [10]. Previous studies have indicated an association of the *ica* locus (associated with biofilm formation), SCC*mec* (staphylococcal cassette chromosome *mec*) and the insertion sequence IS*256* (associated with regulation of biofilm formation and genes encoding aminoglycoside resistance [11, 12]) with invasive *S. epidermidis* isolates, but the discriminatory capacity has been questioned [13]. As for clinical infections with *S. epidermidis* in general, molecular epidemiological data on *S. epidermidis* from PJIs are indicative of a predominance of MDRSE [14, 15], in contrast to the diversity of *S. epidermidis* strains present in the human microbiota [16].

PJIs are associated with increased mortality, and patients affected by PJIs suffer from pain, loss of independence and reduced quality of life [17–20]. This, and that treatment costs for PJIs are significant and projected to increase [21, 22], points to the importance of optimizing preventive measures for this routine orthopaedic surgical procedure. Current preventive measures include preoperative whole-body wash with chlorhexidine gluconate-containing soap, optimization of the operative environment and antimicrobial prophylaxis, both systemic (β-lactams) and local (antibiotic-loaded bone cement) [23].

We aimed to describe the molecular epidemiology of *S. epidermidis* obtained from hip and knee PJIs, and to determine what genomic traits differentiate these PJI isolates from nasal isolates, to understand the underlying factors explaining the success of the dominating MDRSE lineages in PJIs.

METHODS

Study design

In this molecular epidemiology study, we included *S. epidermidis* isolates from hip and knee PJIs between 2007 and 2016 in two administrative regions in central Sweden [referred to as A (Örebro) and B (Östergötland)], each served by a single microbiology department. Clinical isolates from PJIs were frozen and stored as per clinical routine. Isolates from PJIs were compared with contemporary *S. epidermidis* isolates from nares of patients with planned prosthetic-joint surgery 2 weeks (median) prior to hospitalization collected between 2012 and 2017. Nasal isolates were collected within four administrative regions in central Sweden [Örebro (A), Östergötland (B), Värmland (C) and Västmanland (D)] together comprising 45000 km^2, with a combined population of 1.4 million inhabitants (Fig. S1, available in the online version of this article).

Bacterial isolates

Clinical isolates with anonymized serial numbers were provided from the clinical microbiology departments together with information about patient sex, age and joint from the microbiology request form. Isolates from

Impact Statement

The global spread of multidrug-resistant *Staphylococcus epidermidis* (MDRSE) that cause hospital-associated infections poses a threat to treatment outcomes in patients with infections such as prosthetic-joint infection (PJI). Insights into *S. epidermidis* pathogenomics are needed to understand the success of MDRSE lineages in order to, by extension, describe targets for infection control measures including prophylaxis procedures. Attempts to define virulence determinants of *S. epidermidis* have so far mainly been based on targeted searches for previously suggested markers. Here, we used state-of-the-art genome-wide association analyses to compare a unique population-based collection of ~300 *S. epidermidis* isolates in total from PJIs and contemporary nasal isolates. We show that *S. epidermidis* causing PJIs differ from nasal *S. epidermidis* not by acquisition nor carriage of virulence determinants, but by harbouring genetic traits associated with antimicrobial resistance and decreased susceptibility to biocides. Using the available genomes of >800 international *S. epidermidis*, including >100 from PJIs, we demonstrate these findings extend internationally. With this combined data, our study highlights that the current preventive strategies in arthroplasty surgery, preoperative chlorhexidine bathing and antimicrobial prophylaxis with β-lactam (systemically) and aminoglycoside (locally in bone fixation cement), specifically select for MDRSE that have been associated with treatment failure.

PJIs were included if a minimum of two tissue cultures obtained during debridement or revision surgery for suspected PJI grew *S. epidermidis* with identical antibiograms, according to Swedish guidelines for bone and joint infections (www.infektion.net/). Only one isolate per patient during the 10 year period was included. Pure clinical isolates were preserved at −80 °C as per clinical routine in preservation medium: trypticase soy broth (BD Diagnostic Systems), supplemented with 0.3% yeast extract (BD Diagnostic Systems) and 29% horse serum (Håtunalab).

Nasal swabs were plated on Müller–Hilton II agar (3.8%, w/v) plates (BD Diagnostic Systems) incubated overnight at 36 °C in an aerobic atmosphere. A single colony, verified as *S. epidermidis* by matrix-assisted laser desorption/ionization-time of flight MS (MALDI-TOF MS) (Microflex LT; Bruker Daltonik) using Biotyper 3.1, was randomly selected for further analysis.

Written informed consent for nasal sampling was obtained from patients under a protocol approved by the Regional Ethical Review Board of Uppsala, Sweden (Dnr 2012/092, Dnr 2016/151/2). Ethical approval for working with bacterial isolates from humans is not applicable under Swedish law.

Table 1. Demographics of participants contributing *S. epidermidis* isolates

Data for the features are given as numbers, with percentages in parentheses.

Feature	PJI (*n*=139)	Nares (*n*=150)
Arthroplasty		
Total hip arthroplasty	103 (74%)	95* (63%)
Total knee arthroplasty	36 (26%)	53* (35%)
NA	–	2 (1%)
Gender		
Female	59 (42%)	75 (50%)
Male	80 (58%)	75 (50%)
Age (years)		
≤50	4 (3%)	6 (4%)
51–60	10 (7%)	16 (11%)
61–70	30 (22%)	27 (18%)
71–80	54 (39%)	33 (22%)
>80	41 (30%)	6 (4%)
NA	–	62 (41%)
Median [IQR]	75.0 [68–81]	69.3 [60–75]
Administrative region†		
A	55 (40%)	84 (56%)
B	84 (60%)	18 (12%)
C	–	29 (19%)
D	–	19 (13%)

IQR, Interquartile range; NA, not available.
*Planned arthroplasty.
†Administrative regions (all adjacent) are: A, Örebro; B, Östergötland; C, Värmland; D, Västmanland (see Fig. S1).

Antibiotic-susceptibility testing (AST)

AST was performed by disc diffusion test according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org) for the PJI isolates. The antibiotics tested were cefoxitin (30 µg), fusidic acid (10 µg), clindamycin (2 µg), erythromycin (15 µg), gentamicin (10 µg), rifampicin (5 µg), trimethoprim–sulfamethoxazole (25 µg) and norfloxacin (10 µg) (all discs from Oxoid). The clinical breakpoints were according to EUCAST recommendations (v9.0).

Genome analyses

An overview of the genome analyses performed in this study is presented in Fig. S2.

Genome sequencing

DNA from all 289 *S. epidermidis* was purified using a QIAsymphony DSP virus/pathogen kit (Qiagen) after incubation overnight in 36 °C on blood agar plates [3.9% Columbia blood agar base (Oxoid), supplemented with 6% defibrinated horse blood]. Extracted DNA was quantified using a Qubit (Invitrogen), followed by library preparation using a Nextera XT DNA library prep kit (Illumina), using the manufacturer's protocol. The libraries were sequenced on the NextSeq 550 platform (Illumina) using a 300-cycle kit to obtain paired-end 150 bp reads. The generated sequencing data (Table S1) were subjected to quality control using bifrost (https://github.com/ssi-dk/bifrost) to ensure adequate sequencing depth of all isolates and a check for contamination using Kraken v1.0 [24] prior to assembly using SPAdes v3.11.1 [25] using default settings.

SNP detection and phylogenies

We used SNPs identified in the core genome to infer relatedness of the *S. epidermidis* isolates. To detect SNPs with association with PJI and to infer phylogenetic relationships between isolates, we identified SNPs within the core genome by aligning the raw sequencing data against the *S. epidermidis* ATCC 12228 reference chromosome [26] (GenBank accession no. CP022247) using NASP v1.0.0 [27] by using BWA-MEM v0.7.12 [28] and SNPs were called using GATK [29]. If either a minimum coverage of 10 was not met or the nucleotide variant was present in <90% of the base calls per individual isolates, the respective position was excluded across the collection to retain only high-quality variant calling (Table S2).

To relate the diversity of the Swedish *S. epidermidis* isolates to circulating international isolates, sequence data from two recently published papers on (i) the emergence and population structure in relation to resistant *S. epidermidis* lineages (*n*=224) [9] and (ii) disease-associated genotypes of *S. epidermidis* (*n*=411) [30] were included. Furthermore, genome data on all additional sequence data from *S. epidermidis* of human origin present in the National Center for Biotechnology Information Reference Sequence Database (https://www.ncbi.nlm.nih.gov/refseq/) was obtained on March 13 2019 with metadata on country of isolation (*n*=237) for a total of 872 genomes of international origin after removal of isolates with low sequencing depth or signs of contamination using NASP v1.0.0 [27] (Table S3). If available, Illumina FASTQ sequence files were used instead of assembled genomes for depiction of the overall population diversity of *S. epidermidis* and for the ST2- and ST5-lineage-specific analyses. For identification and purging of recombinant regions, Gubbins [31] was used with default settings on the SNP-only alignments obtained from NASP.

Phylogenetic reconstruction was carried out using different approaches due to limitations in calculations on the larger dataset. For the complete set of Swedish and international human isolates of *S. epidermidis* (*n*=1161), the phylogeny was obtained with the approximately maximum-likelihood implementation in FastTree v2.1.10 [32] using the generalized time-reversible (GTR) substitution model, as well as
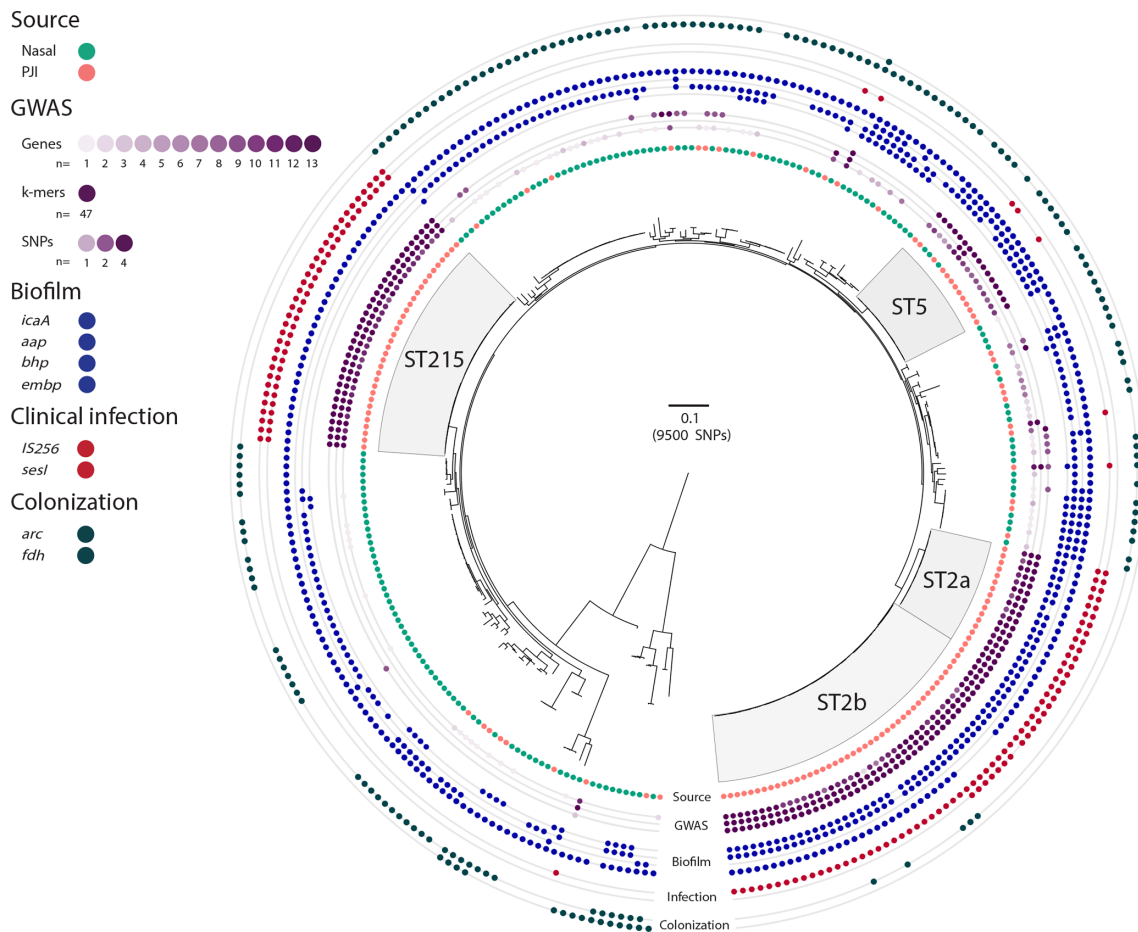
Fig. 1. Genomic traits and phylogenetic relatedness of *S. epidermidis* from PJIs and nasal microbiota. *S. epidermidis* isolates from PJIs (*n*=139; light red) and *S. epidermidis* isolates from nasal swabs from patients scheduled for arthroplasty (*n*=150; green). Major PJI lineages (ST2a, ST2b, ST5 and ST215) are coloured in grey. GWAS results, all linked to invasiveness, are presented in purple (colour intensity represents numbers of PJI-associated genes, *k*-mers and SNPs present). A gene is shown as absent in an isolate only if no allelic variant of the gene could be found. Genes associated with biofilm formation are presented in blue, from the centre and out: *icaA*, *aap*, *bhp*, *embp*. Genes previously associated with clinical infections are presented in dark red, from the centre and out: IS*256*, *sesI*. Genes previously associated with colonization are presented in dark green, from the centre and out: ACME-*arcA*, *fdh*. The midpoint-rooted maximum-likelihood phylogeny was based on a SNP matrix purged for recombination with 95 245 SNPs. Bar, substitutions per site.

nearest-neighbour interchanges (NNI) and subtree-prune-regraft (SPR) rearrangements for tree optimization. Phylogenies for the ST2-clade (*n*=353), the ST5-clade (*n*=93) and the Sweden-only collection (*n*=289) were obtained with RAxML v8.2.1 [33] using the GTRCAT model and 100 replicates for bootstrapping. Visualization and annotation of the phylogenies was performed in iTOL v4.3 [34] (https://itol.embl.de) with subsequent editing in Adobe Illustrator.

Accessory genome

The accessory genome contains genes that in large define the virulence and resistance phenotypes. To detect significant associations between genomic traits and PJIs in the Swedish collection, we initially determined gene content of the population by annotating genome assemblies using Prokka v1.12 [35] with default settings and comparing annotated genomes using Roary v3.11.2 [36] using a

minimum percentage identity for BLASTP of 90. This combination provides an excellent overview of the core- and accessory-gene content of a population, but is naturally limited by its dependency on the quality of the genomic assemblies. This leads to the occurrence of spurious genes caused by incidental contig terminations in the estimated accessory genome and, more importantly, inaccuracies in predicting the presence of genes found in or near regions that are consistently hard to assemble, such as the repeat regions associated with IS elements. Since these kinds of regions are often of particular interest, we found it crucial to apply an additional level in our approach to assess gene presence/absence across the population. Using Mykrobe Predictor v0.1.3 [37], we first tested each isolate against a panel of genes consisting of the complete accessory genome identified by Roary and defined genes as present if they had a coverage of at least 90% and a median sequencing depth

Table 2. Diversity and phylogenetic clustering of *S. epidermidis* isolates from PJIs and from nares

Data for the features are given as numbers, with percentages in parentheses.

Feature	PJI (*n*=139)	Nares (*n*=150)
No. of STs	36	64
1-DI (95% CI)	0.798 (0.742–0.855)	0.964 (0.950–0.978)
No. of isolates (%) per phylogenetic lineage:		
PJI		
ST2a	16 (12%)	0
ST2b	43 (31%)	0
ST5	13 (9%)	3 (2%)
ST215	34 (25%)	0
Nasal		
ST73	0	18 (12%)
ST218	2 (1%)	21 (14%)
Not within a defined lineage	31 (22%)	108 (72%)

of at least five. Additionally, to remove spurious genes from the presence/absence data, Roary-defined gene clusters were combined into a single gene if they had a pairwise sequence similarity >99% and a high level of concordance in their presence/absence across the population (Jaccard similarity >0.95); e.g. two (or more) gene clusters were taken to represent the same gene if they were highly identical and appeared in the same isolates across the collection according to the Mykrobe results. *k*-mer presence/absence data was obtained using *pyseer* v1.0.0 [38] to count *k*-mers in the assembled genomes. The analysis of *k*-mer presence/absence naturally has some overlap with the analyses of core-genome SNPs and accessory-gene content, but it also expands on these by examining genetic variation outside the core genome. Of note, we removed highly prevalent and very rare genetic traits (genes/SNPs/*k*-mers) with less than 5% or higher than 95% prevalence across the study collection for subsequent analyses.

Since a few specific lineages are dominant among the PJI isolates, we found it crucial to apply a genome-wide association study (GWAS) approach that takes into account population structure in order to identify genetic traits that are associated with PJI rather than just these specific lineages. We used treeWAS v1.0 [39] on gene, SNP and *k*-mer presence/absence data, using recombination-pruned phylogeny as outlined above as basis for lineage correction. A *P* value of 0.05 after Bonferroni adjustment for multiple testing was used as the threshold for significant associations based on the subsequent and terminal scores calculated by treeWAS. The presence of genes identified with this approach in

international PJI isolates was determined using Mykrobe on the raw sequencing data provided from these studies.

Virulence determinants

In addition, we examined the presence of previously described virulence determinants across the Swedish collection. The investigated genes are associated with biofilm formation (*aap* [40], *bhp* [40], *embp* [40], *icaA* [40, 41]), pathogenicity (*sesI* [42, 43], IS*256* [11, 41]) and commensalism (*fdh* [44], ACME-*arcA* [45]). The following reference sequences were used to query the assembled genomes using BLASTN searches in Biomatters Geneious Prime v2019.1 using >90% sequence similarity and coverage to identify hits: *aap* (CP000029.1, position 2461683–2454490), *bhp* (CP000029.1, position 2442370–2449578), *embp* (AY101364.1), *icaA* (U43366.1, position 761–1999), *sesI* (CP000029.1, position 1693235–1693840), *fdh* (NZ_AKGZ01000029, position 10249–13200), ACME-*arcA* (AB817064, position 4224–5459). Due to the repetitive nature of *aap* and *bhp* and the associated assembly problems, 801 nt and 2000 nt from the non-repetitive regions spanning positions 900–1701 and 1–2000 of the genes, respectively, were used as input. The IS*256* gene (CP018629.1, positions 533639–534811) is, due to the high plasticity of its surrounding genetic content, highly difficult to assemble properly and, thus, detection of IS*256* was done using reference mapping using Mykrobe as outlined above.

Resistance determinants

Resistance mechanisms are generally divided into acquired genes through horizontal gene transfer or mediated by mutations. Thus, we screened for acquired antimicrobial resistance (AMR) genes; we used the curated database used by ResFinder v3.1 [46] obtained on 21/12/2018 to search against gene matches using ABRicate v0.7 (https://github.com/tseemann/abricate) on the assembled genomes. Gene presence was determined based on a >80% hit length and >90% sequence identity. We further investigated point mutations associated with rifampicin [9, 47, 48], fusidic acid [49], co-trimoxazole [50], linezolid [9], daptomycin [51, 52] and quinolone resistance [53–55], as well as increased tolerance towards chlorhexidine [56], see Table S4 for positions included in analyses. In addition, the reference sequence CP022250, positions 4218–2674, was used to query the assembled genomes for presence of *qacA/B*. All analyses were performed using BLASTN in Geneious Prime v2019.1 with subsequent translation to amino acids of individual alignments. The term multidrug resistance is defined in this study as the identified presence of genes and/or point mutations associated with resistance towards three of the following antimicrobial categories: meticillin, fusidic acid, macrolide–lincosamide, rifampicin, aminoglycosides, fluoroquinolones and folate pathway inhibitors.

To understand the diversity of the mobile *mecA*-encoding element SCC*mec*, we used SCC*mec*Finder v1.2 [57] for initial typing. Elements were classified to known types including composites when previously described and acknowledged
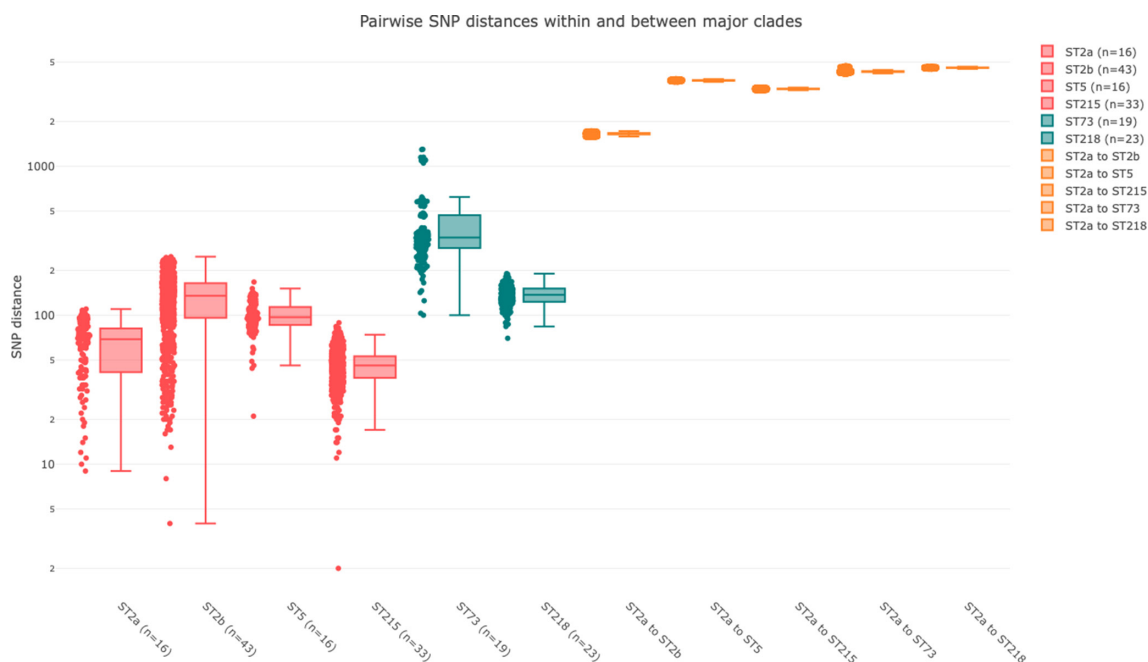
Fig. 2. Intra-lineage and inter-lineage diversity among major *S. epidermidis* lineages. Intra-lineage pairwise SNP distances of the major lineages among the Swedish PJI isolates (ST2a, ST2b, ST5 and ST215; light red) and nasal isolates (ST73 and ST218; green) after purging for recombination. Inter-lineages pairwise SNP distances between the ST2a lineage and the ST2b, ST5, ST215, ST73 and ST218 lineages are depicted to the right in orange.

by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements [58] (IWG-SCC; www.sccmec.org), whereas other types were presented as composites. If divergent results were obtained using both the BLAST- and the *k*-mer-based approaches, the elements in question were manually investigated for *mec* and *ccr* gene complexes for validation.

Statistical methods

Data management and analysis were performed using R v3.5.1 (https://www.R-project.org). All scripts for analysis are available at GitHub (https://github.com/thej-ssi/S_epidermidis_PJI). We used 1-Simpsons diversity index (DI) to compare ST diversity between PJI isolates and colonizing isolates. For the treeWAS analyses, any trait with $P < 0.05$ after correction for multiple testing using Bonferroni was considered significant. SPSS v25 (IBM) was used to test associations between gene of interest and origin of isolates (binary logistic regression and Fisher's exact test), PJI lineage and administrative region of isolation (chi-square), and statistically significant differences in frequency of treeWAS genes between PJI isolates obtained within this study and international PJI sequences (chi-square).

Data availability

The sequence reads, as well as the gene sequences, for the two hypothetical genes identified using GWAS are available from the European Nucleotide Archive under BioProject ID PRJEB32707 (Table S5).

RESULTS

In total, 11 556 primary hip and 9 770 primary knee prosthetic-joint surgeries were performed in the two administrative regions during the 10 year period (data from national registries: http://myknee.se, http://shpr.se). During this period, 139 patients with *S. epidermidis* PJIs were identified. Nasal isolates were retrieved from 150 individuals scheduled for arthroplasty. Demographics are presented in Tables 1 and S6.

Based on a conserved core genome of 1.7 Mbp (68.5% of the reference chromosome), the population structure of all 289 isolates showed four major PJI clusters, hereafter referred to as the ST2a (*n*=16), ST2b (*n*=43), ST5 (*n*=13) and ST215 (*n*=34) lineages (Fig. 1, Table 2). The ST2a and ST2b lineages represented two distinct lineages of ST2 found to be primarily distinguished by a single recombination event spanning ~74 kb with approximately 2800 SNPs. Isolates within each PJI lineage were closely related within lineages (Fig. 2) after removal of putative recombinant regions compared to the diversity observed among the few major commensal lineages. The remaining isolates (*n*=33, 24%) obtained from PJIs represented 21 STs scattered across the phylogeny. The proportion of isolates within each PJI lineage varied between year of isolation and region (Table S7, Fig. S3), but there was no trend noted over time nor any statistically significant association between region and lineages, nor any major regional clustering of isolates. In comparison, nasal isolates were highly diverse (1-DI 0.96 vs 0.79; Table 2), although two distinct lineages accounting for more than 10 isolates

Table 3. Results of population structure-adjusted GWAS; genes, SNPs and *k*-mers more prevalent in *S. epidermidis* isolated from PJIs compared to isolates from nares

Gene/SNP/*k*-mer	Association with resistance	MGE	PJI (*n*=139)	Nares (*n*=150)	*P* value*
Genes					
mecA	β-Lactam	SCC*mec*	113 (81%)	8 (5%)	<0.05
mvaS		SCC*mec*	108 (78%)	8 (5%)	<0.05
maoC		SCC*mec*	113 (81%)	8 (5%)	<0.05
ugpQ		SCC*mec*	112 (81%)	8 (5%)	<0.05
group_3224		SCC*mec*	98 (71%)	3 (2%)	<0.05
aac(6′)-aph(2″)	Aminoglycoside	Transposon	99 (71%)	1 (1%)	<0.05
group_4693		Transposon	99 (71%)	1 (1%)	<0.05
IS*256*	Aminoglycoside	IS element	99 (71%)	1 (1%)	<0.05
ermC	MLS	Plasmid	76 (55%)	1 (1%)	<0.05
repL		Plasmid	76 (55%)	1 (1%)	<0.05
qacA	Antiseptics	Plasmid	97 (70%)	14 (9%)	<0.05
qacR	Antiseptics	Plasmid	99 (71%)	14 (9%)	<0.05
group_596	Unknown	Undetermined	104 (75%)	1 (1%)	<0.05
SNPs					
parB::G24A	Quinolone		113 (81%)	16 (11%)	<0.05
parB::G577A	Quinolone		113 (81%)	16 (11%)	<0.05
grlA::C239A	Quinolone		111 (80%)	4 (3%)	<0.05
gyrA::C251T	Quinolone		112 (81%)	4 (3%)	<0.05
***k*-mers (*n*=47)**					
IS*256*	Aminoglycoside	Transposon	99 (71%)	1 (1%)	<0.05

MGE, Mobile genetic element; MLS, macrolide, lincosamide and streptogramin.
*After Bonferroni correction using treeWAS.

[ST73 (*n*=18, 12%) and ST218 (*n*=21, 14%)] were observed among nasal isolates. Three nasal isolates clustered within the four PJI lineages, all within the ST5 lineage.

We performed a GWAS to investigate whether *S. epidermidis* from PJIs harbour specific genetic features that could be anticipated to be important in the pathogenesis of PJI. We identified 13 genes, 4 SNPs and 47 *k*-mers, of which all were significantly associated with PJI origin (Figs 1 and S4–S6, Tables 3 and S8–S10). Most of the genes (12/13, 92%) encoded proteins involved in AMR or decreased susceptibility to biocides (meticillin, aminoglycosides, erythromycin and chlorhexidine), or were present on mobile genetic elements harbouring these genes (Table 3). Three out of the four core SNPs were non-synonymous: *gyrA*::S84F/Y, *parB*::A193T and *parC*::S80F/Y. Of these, the changes in *gyrA* and *parC* (*grlA*) had been reported elsewhere to imply reduced susceptibility to fluoroquinolones in *S. epidermidis* [53]. All *k*-mers were associated with IS*256*, an insertion sequence that confers genomic plasticity and has been described as being associated with regulation of biofilm formation and antimicrobial-resistance genes in *S. epidermidis* [11, 12].

We then complemented the genome-wide analyses with a targeted search for acquired AMR genes and point mutations associated with AMR across the collection. PJI isolates harboured genes and/or point mutations associated with AMR to a significantly greater extent than did nasal isolates (Tables S4 and S11–S13). In particular, we noted high rates of genotypic resistance to β-lactam antibiotics and aminoglycosides used in systemic and local prophylaxis, respectively, in arthroplasty surgery [meticillin, odds ratio (OR) 77.1, 95% confidence interval (CI) 33.6–177.0; and aminoglycosides, OR 196.7, 95% CI 46.4–833.7; Table S11]. In addition, the efflux pump-encoding *qacA/B* gene that causes decreased susceptibility to quaternary ammonium compounds such as chlorhexidine, used for preoperative skin disinfection in arthroplasty, was identified in 71% (99/139) of PJI isolates compared to 9% (14/150) of nasal isolates (OR 22.3, 95% CI 11.7–42.6) (Tables S14 and S15). Furthermore, recently
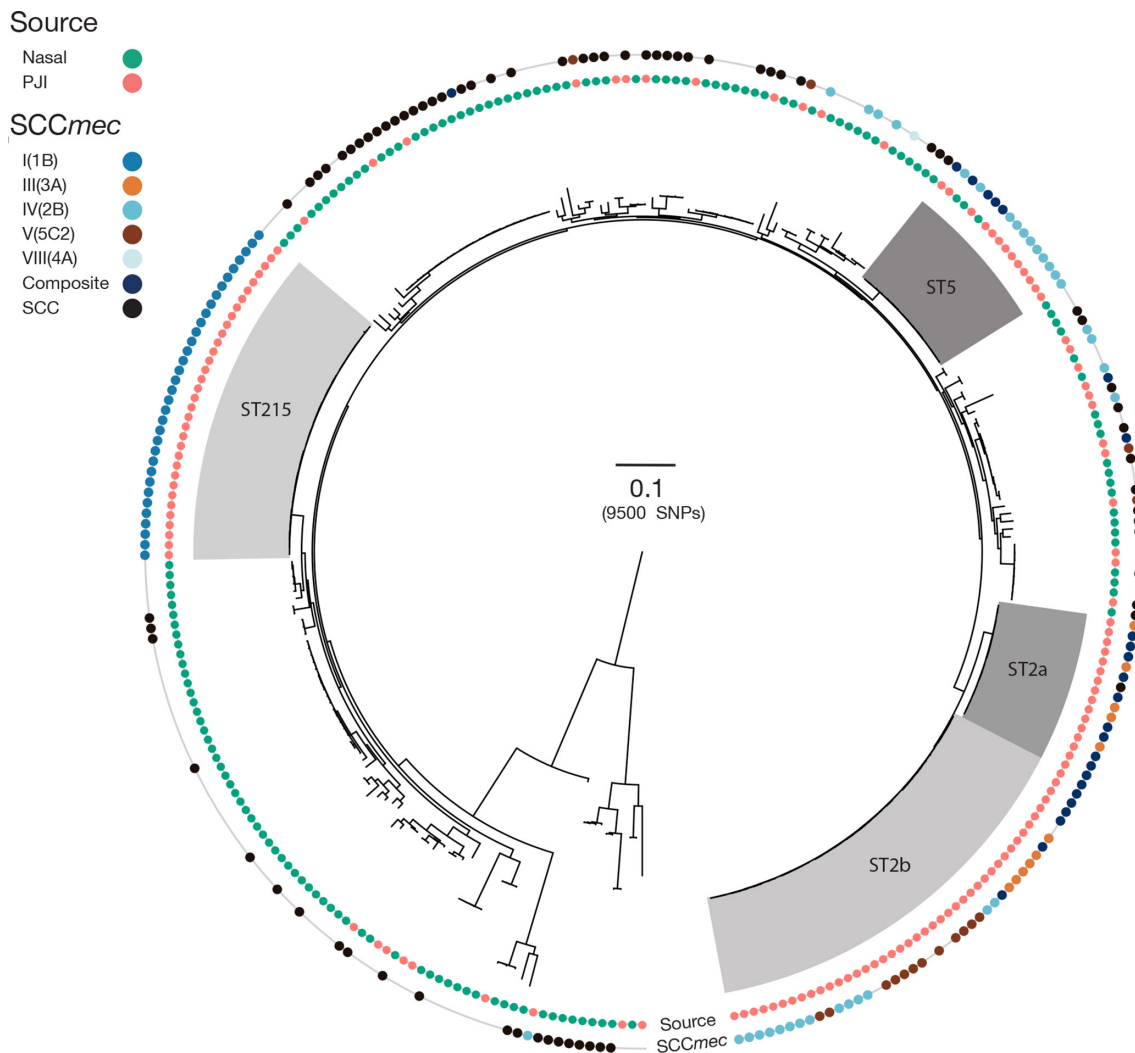
Fig. 3. SCC*mec* and phylogenetic relationships. *S. epidermidis* isolates from PJIs (*n*=139; light red) and *S. epidermidis* isolates from nasal swabs from patients with planned prosthetic-joint surgery (*n*=150; green). Major PJI lineages (ST2a, ST2b, ST5 and ST215) are coloured in grey. The presence and types of SCC are presented in the outermost circle: I(1B) (medium blue), III(3A) (orange), IV(2B) (turquoise), V(5C2) (brown), VIII(4A) (light blue), composite (dark blue) and SCC (i.e. *mecA* negative) (black). Isolates with no dot in the outermost circle are *mecA* (and SCC) negative. The midpoint-rooted phylogeny was based on 95 245 SNPs. Bar, substitutions per site.

described point mutations within *qacA/B* associated with increased tolerance of chlorhexidine [56] were identified in isolates in the ST2a and ST2b lineages (Table S4).

Higher rates of genetic resistance markers to compounds used for treatment of PJIs were noted among PJI isolates compared to nasal isolates: rifampicin 30/139 (22%) versus 0/150 (*P*<0.0001), fluoroquinolones 114/139 (82%) versus 16/150 (11%) (OR 38, 95% CI 19.4–75.0), and macrolide–lincosamide 87/139 (63%) versus 22/150 (15%) (OR 9.7, 95% CI 5.5–17.2) (Table S11). We found that rates of genotypic resistance differed between the major PJI lineages (Table S12). The largest difference was noted for rifampicin; all ST2a-isolates were classified as genotypic resistant due to specific dual mutations in *rpoB*::D471E/I527M, whereas additional mutations in *rpoB* linked to rifampicin resistance

were exclusive to other major PJI lineages (Table S4). No point mutations associated with daptomycin or linezolid resistance were identified among the 289 isolates (Table S4).

Overall, 115/139 (83%) PJI isolates and 7/150 (5%) nasal isolates were classified as MDR based on the presence of antimicrobial genes and/or point mutations associated with resistance (Table S4). Most PJI isolates classified as MDR based on genomic analyses were also classified as MDR based on phenotypic AST (113/115, 98%) (Tables S16–S18).

Our analysis of the staphylococcal cassette chromosome (SCC) element (Fig. 3, Table S19) indicated a high degree of plasticity due to the high prevalence of unrelated composite elements nested in lineages with non-composite elements, and display its frequent acquisition into the *S. epidermidis*

Table 4. Distribution of genes previously associated with biofilm formation, clinical infections and colonization in *S. epidermidis* from PJIs (presented by major lineages) and *S. epidermidis* from nares

Data for the genes are given as numbers, with percentages in parentheses.

Gene	PJI (*n*=139)					Nares (*n*=150)
	ST2a (*n*=16)	ST2b (*n*=43)	ST5 (*n*=13)	ST215 (*n*=34)	Other STs (*n*=33)	
Biofilm formation						
icaA	16 (100%)	42 (98%)	0	0	8 (24%)	36 (24%)
aap/sesF	15 (94%)	42 (98%)	12 (92%)	0	23 (70%)	104 (69%)
bhp/sesD	0	0	13 (100%)	0	10 (30%)	27 (18%)
embp	16 (100%)	37 (86%)	13 (100%)	34 (100%)	33 (100%)	149 (99%)
Clinical infections						
IS256	16 (100%)	43 (100%)	2 (15%)	34 (100%)	5 (15%)	1 (1%)
sesI	16 (100%)	13 (30%)	0	34 (100%)	0	0
Colonization						
fdh	0	0	0	0	2 (6%)	14 (9%)
ACME-*arcA*	0	5 (12%)	12 (92%)	0	24 (73%)	114 (76%)

population. Overall, 67% (194/289) of all isolates were found to carry part of this element as defined by detection of *ccr* recombinase genes. Among these, *mecA* was frequently absent (63/71; 89%) in nasal isolates, whereas SCC*mec* composite elements were abundant among PJI isolates. ST215 exclusively carried type I(1B); ST5 harboured type IV(2B) or composite variants thereof; and ST2a/b carried types III(3A), IV(2B) or V(5C2), where composite variants of IV(2B) were frequent (Table S19).

We found no association of biofilm-associated genes (*icaA*, *aap*, *bhp*, *embp*), a previously suggested marker for clinical infections (*sesI*) or previously suggested markers of commensalism (*fdh* and ACME-*arcA*) with PJI origin using a GWAS accounting for population structure, although they were over-represented in individual PJI lineages (Fig. 1, Tables 4, S20 and S21).

When we analysed the relatedness of the Swedish isolates with international *S. epidermidis* of human origin (*n*=872), the PJI isolates in this study did not constitute monophyletic groups but rather several subgroups within international epidemic ST2a/ST2b and ST5 lineages (Fig. 4). A third ST2 lineage, here denominated ST2c, was evident among international sequences. To validate our GWAS findings, we determined the presence of the 13 genes identified by GWAS among the international *S. epidermidis* with metadata indicating PJI origin (*n*=122, 14%). Within this international PJI collection, the four genes associated with the *mec* gene complex (*mecA*, *mvaS*, *maoC* and *ugpQ*) were as prevalent as in the PJI isolates sequenced within this study, while the proportion of isolates harbouring genes related to aminoglycoside and macrolide–lincosamide resistance and reduced susceptibility

to chlorhexidine was less predominant, but still significantly higher compared to nasal isolates of this study (Table S22).

DISCUSSION

In this study of the molecular epidemiology of *S. epidermidis* in PJIs, we showed that global MDRSE lineages ST2 and ST5, and the regional ST215, dominate. However, importantly, almost a quarter of the PJI isolates did not belong to any of the previously described major nosocomial lineages of which one third were MDR. To understand the underlying genomic traits important in the pathogenesis of PJI, we included nasal *S. epidermidis* isolates from individuals scheduled for arthroplasty surgery for comparison and performed GWAS analyses adjusting for lineage effects to look for shared characteristics between PJI isolates, rather than (confounding) genomic traits only associated with major lineages. We found that *S. epidermidis* causing PJIs including those outside the major known lineages differed from nasal isolates not by pathogenicity factors, but by presence of genomic traits associated with decreased susceptibility to compounds used for prevention of PJIs: chlorhexidine, β-lactams and aminoglycosides. These traits were also found to be highly prevalent in *S. epidermidis* from PJIs internationally.

In this study, increased rates of resistance were noted also for compounds that generally are not used for prophylaxis in arthroplasty (macrolides–lincosamides and fluoroquinolones). We interpret this finding as indicative of an adaptation to the hospital environment of lineages of various genetic backgrounds, suggesting that patients may acquire these strains in their microbiota during hospitalization for arthroplasty (Fig. 5). The current prophylaxis regimens including
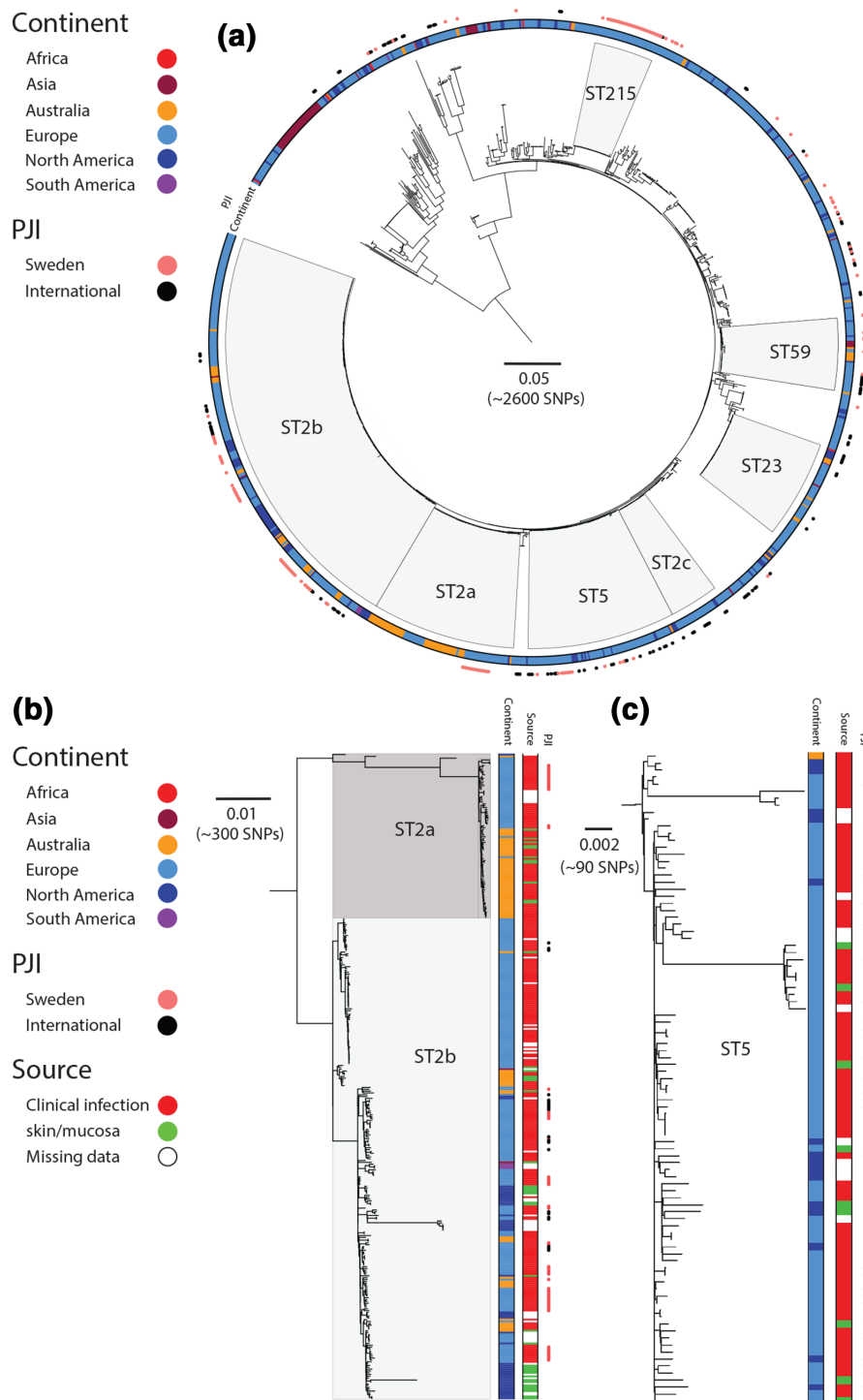
Fig. 4. Population structure of international and Swedish *S. epidermidis*. (a) Population structure of publicly available *S. epidermidis* sequences (*n*=872) and *S. epidermidis* sequenced in this study (*n*=289). Major PJI lineages (ST2a, ST2b, ST5 and ST215) are coloured in grey, as are ST2c, ST23 and ST59. Innermost circle: continent of isolation. Outermost circle: PJI isolates from this study (red), international PJI sequences (black). The midpoint-rooted phylogeny was based on 52 690 SNPs. Bar, substitutions per site. (b) Population structure of ST2a and ST2b [publicly available sequences and *S. epidermidis* sequenced in this study (*n*=353)]. The continent of isolation is coloured as in (a). Isolate sources are coloured in red (clinical infections), green (skin/mucosa) and white (missing data). PJI isolates from this study are indicated in light red and international PJI isolates in black. The phylogeny was based on 29 843 SNPs. Bar, substitutions per site. (c) Population structure of ST5 [publicly available sequences and *S. epidermidis* sequenced in this study (*n*=93)]. Continent of isolation, isolate source and PJI isolates are coloured as in (b). The phylogeny was based on 44 284 SNPs. Bar, substitutions per site.
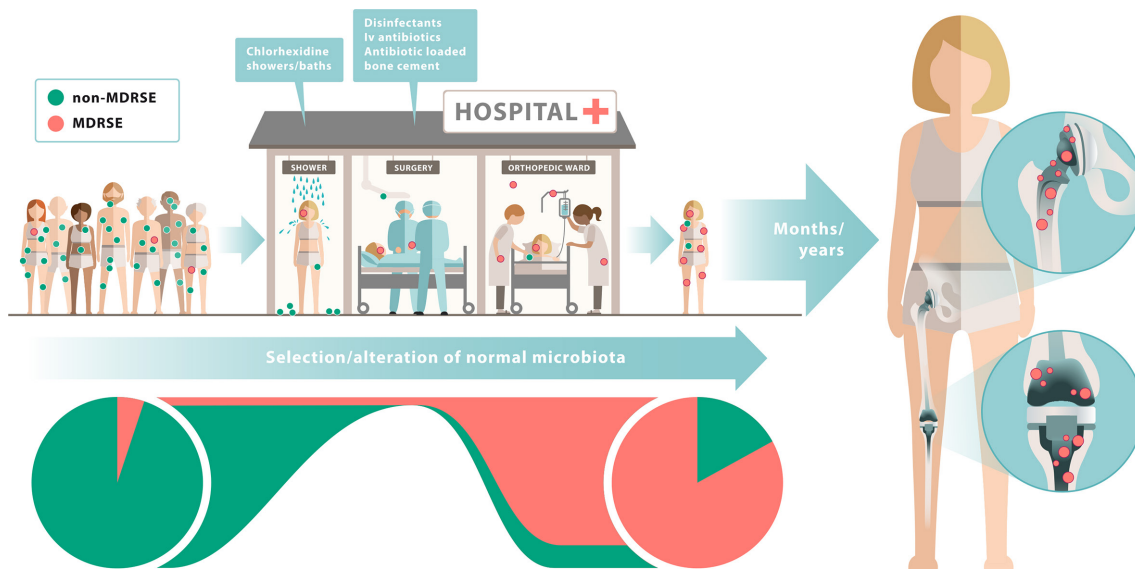
Fig. 5. Interpretation of findings; selection by prophylaxis regimens and/or recolonization with MDRSE during hospitalization. Hip and knee PJIs are dominated by MDRSE that are selected from normal flora and/or acquired during hospitalization. Current prophylaxis regimens are efficient in reducing the number of colonizing non-MDRSE before arthroplasty, but during hospitalization patients may acquire MDRSE. Iv, Intravenous.

chlorhexidine showers/bathing may select for these lineages by reducing the abundance of non-MDRSE in the microbiota and thereby facilitating recolonization with hospital-adapted MDRSE that subsequently may gain access to the prosthetic device. However, given the low incidence of *S. epidermidis* PJIs, it is also possible that MDRSE PJIs stem from a subset of patients who harbour MDRSE in their microbiota in low abundance prior to hospitalization, and that these are selected for by the current prophylaxis regimens (Fig. 3). Independent of whether acquisition of MDRSE lineages takes places during hospitalization or whether MDRSE lineages are selected from the normal microbiota, the high prevalence of the shared AMR traits between genetically diverse PJI isolates is worrisome, as treatment failure of *S. epidermidis* orthopaedic-device-related infections has been linked to prevalence of *qacA/B*, genes associated with SCC*mec* (*ccrA* and *ccrB*) and phenotypic aminoglycoside resistance [15].

Recent work on *S. epidermidis* has raised awareness of the impact of the global spread of specific clinical MDRSE lineages [9]. Combining our isolates with available international data highlighted significant overlap between international isolates and Swedish isolates belonging to ST2a, ST2b and ST5. Detailed analyses revealed multiple, locally circulating, clones in Sweden, originating from separate introductions of these lineages (Fig. 4). However, our data also support regional differences. ST215 is prevalent in our collection, but there are only three isolates (all from Nordic countries) within the extensive international collection. The ST215 lineage has previously been reported to circulate in hospital environments and cause clinical infections, even though it lacks the *icaA* gene and rarely forms biofilm *in vitro* [14, 59]. On the contrary, neither the international MDRSE lineage ST23 nor

a previously unreported ST2c lineage, predominantly of European origin (Fig. 4), was found in our collection.

The PJI isolates outside the four major *S. epidermidis* lineages in this study (*n*=33) were found to carry a varying number of GWAS-identified traits (Fig. 1). We found three ST59 MDRSE isolates, all harbouring the *mec* gene complex, and clustering with international ST59 isolates including PJI isolates. But there were also a number of isolates that were susceptible and did not harbour any of the traits identified with GWAS, which may be explained by inappropriate adherence to the prophylaxis regimen and/or host factors. However, the overall prevalence of GWAS traits was higher in this group compared to nasal isolates. Multiple models of *S. epidermidis* infection have been suggested, with recent findings supporting the importance of horizontal gene transfer into different genetic backgrounds [30]. Our data generally support this model, as 12 out of 13 genes associated with PJI origin were found on mobile genetic elements introduced into multiple genetic backgrounds. Likewise, the diversity of SCC elements points to frequent horizontal gene transfer. At the same time, mutations within the core genome (associated with fluoroquinolone resistance) were also found in the GWAS, implying that vertical transmission of antimicrobial traits also contributes to the overall high rates of AMR.

In general, virulence determinants such as *icaA* and *sesI* were found to be lineage dependent. This could be explained either by each of the dominating lineages having evolved different pathogenic traits important for their ability to cause PJI, which would not be identified be the GWAS approach, or by all *S. epidermidis* being able to cause PJIs, but *S. epidermidis* with AMR traits predominating in PJIs as they are able to

resist the prophylactic measures. Multidrug resistance was common among PJI isolates in this study. More specifically, genes encoding meticillin, aminoglycoside and macrolide–lincosamide resistance were more prevalent among Swedish PJI isolates compared to international *S. epidermidis* from PJIs. This was somewhat surprising, given the generally low rates of AMR in our setting, but could indicate high adherence to prophylaxis guidelines, thereby reducing the number of susceptible *S. epidermidis* isolates in PJIs. This is supported by the limited number of *S. epidermidis* PJI cases during the study period. A time factor could also contribute to the observed difference in AMR between international and Swedish isolates, as the international PJI isolates were sampled during 2000–2015 and the Swedish isolates during 2007–2017. Even though we found high rates of multidrug resistance in our collection, most PJI isolates in this study were susceptible to rifampicin, and rifampicin resistance, thus, was not a reliable marker for clinical isolates in Sweden, in contrast to recent international findings [9]. Device impregnation with rifampicin is uncommon in our setting, which could contribute to the lower rate of rifampicin resistance.

This study has limitations. First, the nasal isolates were not collected from the patients who subsequently were affected with PJIs. However, to include only nasal isolates from patients with PJIs would have required sampling of ~21000 patients before hospitalization over the 10 year period. We believe that the patients scheduled for arthroplasty surgery contributing nasal isolates were representative of the population at risk for PJI, although information regarding prior hospitalization was not available. Second, we included a single nasal *S. epidermidis* isolate per sample for comparison and no isolates originating in skin over hip or knee. Furthermore, this study was designed to identify genetic traits associated with PJIs and we had no access to clinical outcome data nor data on what specific prophylaxis the individual patients received at the time of primary surgery. However, preoperative showers with chlorhexidine gluconate-containing soap and antimicrobial systemic prophylaxis were mandatory in both regions when the clinical isolates were collected, and based on data from national registries gentamicin-loaded bone cement is used in approximately 60% of primary total hip arthroplasty and in more than 90% of primary total knee arthroplasty in Sweden.

This study also has several strengths. To our knowledge, this is the largest and most comprehensive investigation of the molecular epidemiology of *S. epidermidis* in PJIs. This study is not based on convenient or selective sampling or potentially biased publicly available data, but represents all prospectively collected isolates, exclusively from hip and knee PJIs, over a 10 year period in a well-defined region of Sweden with the same definition of PJI throughout the study period. We drew conclusions from high-quality sequence data and used only curated international data to extrapolate our findings.

The clinical implication of this study is dependent on when *S. epidermidis* gains access to the prosthetic device. If this takes place predominantly during surgery, improved measures to reduce the rate of translocation with MDRSE are warranted,

as are clinical studies of antimicrobial prophylaxis with agents that are efficient against MDRSE, both systemic and local. However, if *S. epidermidis* are also able to access the joint during the first days after surgery, before the wound is healed, it will be important to try to preserve the normal microbiota in order to hinder (re-)colonization with hospital-associated *S. epidermidis* lineages, with the intention to reduce the relative proportion of difficult-to-treat MDRSE PJIs. Thus, we are currently at a crossroads where we can accept the current low rate of what is to the individual a devastating complication or consider changing the antimicrobial prophylaxis, knowing that this is likely to result in further selection of AMR in hospital-adapted MDRSE lineages.

In conclusion, genomic analyses identified the parallel acquisition of specific combined traits that has given rise to highly distinct, globally successful MDRSE lineages causing nosocomial infections including PJIs. *S. epidermidis* causing PJIs differed from nasal isolates not by pathogenicity factors but by harbouring traits associated with resistance to compounds used in prevention of PJIs: β-lactams, aminoglycosides and chlorhexidine.

References
1. **Kurtz S**, **Ong K**, **Lau E**, **Mowat F**, **Halpern M**. Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. *J Bone Joint Surg Am* 2007;89:780–785.
2. **Inacio MCS**, **Graves SE**, **Pratt NL**, **Roughead EE**, **Nemes S**. Increase in total joint arthroplasty projected from 2014 to 2046 in Australia: a conservative local model with international implications. *Clin Orthop Relat Res* 2017;475:2130–2137.

3. **GBD 2016 Disease and Injury Incidence and Prevalence Collaborators**. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2017;390:1211–1259.

4. **Niu NN, Collins JE, Thornhill TS, Alcantara Abreu L, Ghazinouri R** *et al*. Pre-operative status and quality of life following total joint replacement in a developing country: a prospective pilot study. *Open Orthop J* 2011;5:307–314.

5. **Kurtz SM, Lau E, Schmier J, Ong KL, Zhao K** *et al*. Infection burden for hip and knee arthroplasty in the United States. *J Arthroplasty* 2008;23:984–991.

6. **Wenzel RP**. Surgical site infections and the microbiome: an updated perspective. *Infect Control Hosp Epidemiol* 2019;40:590–596.

7. **Flurin L, Greenwood-Quaintance KE, Patel R**. Microbiology of polymicrobial prosthetic joint infection. *Diagn Microbiol Infect Dis* 2019;94:255–259.

8. **Becker K, Heilmann C, Peters G**. Coagulase-negative staphylococci. *Clin Microbiol Rev* 2014;27:870–926.

9. **Lee JYH, Monk IR, Gonçalves da Silva A, Seemann T, Chua KYL** *et al*. Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. *Nat Microbiol* 2018;3:1175–1185.

10. **Zamudio R, Oggioni MR, Gould IM, Hijazi K**. Time for biocide stewardship? *Nat Microbiol* 2019;4:732–733.

11. **Kozitskaya S, Cho S-H, Dietrich K, Marre R, Naber K** *et al*. The bacterial insertion sequence element IS256 occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: association with biofilm formation and resistance to aminoglycosides. *Infect Immun* 2004;72:1210–1215.

12. **Schoenfelder SMK, Lange C, Eckart M, Hennig S, Kozytska S** *et al*. Success through diversity - how *Staphylococcus epidermidis* establishes as a nosocomial pathogen. *Int J Med Microbiol* 2010;300:380–386.

13. **Heilmann C, Ziebuhr W, Becker K**. Are coagulase-negative staphylococci virulent? *Clin Microbiol Infect* 2019;25:1071–1080.

14. **Hellmark B, Söderquist B, Unemo M, Nilsdotter-Augustinsson Åsa**, . Comparison of *Staphylococcus epidermidis* isolated from prosthetic joint infections and commensal isolates in regard to antibiotic susceptibility, *agr* type, biofilm production, and epidemiology. *Int J Med Microbiol* 2013;303:32–39.

15. **Post V, Harris LG, Morgenstern M, Mageiros L, Hitchings MD** *et al*. Comparative genomics study of *Staphylococcus epidermidis* isolates from orthopedic-device-related infections correlated with patient outcome. *J Clin Microbiol* 2017;55:3089–3103.

16. **Oh J, Byrd AL, Deming C, Conlan S, NISC Comparative Sequencing Program**, *et al*. Biogeography and individuality shape function in the human skin metagenome. *Nature* 2014;514:59–64.

17. **Moore AJ, Blom AW, Whitehouse MR, Gooberman-Hill R**. Deep prosthetic joint infection: a qualitative study of the impact on patients and their experiences of revision surgery. *BMJ Open* 2015;5:e009495.

18. **Cahill JL, Shadbolt B, Scarvell JM, Smith PN**. Quality of life after infection in total joint replacement. *J Orthop Surg* 2008;16:58–65.

19. **Zmistowski B, Karam JA, Durinka JB, Casper DS, Parvizi J**. Periprosthetic joint infection increases the risk of one-year mortality. *J Bone Joint Surg Am* 2013;95:2177–2184.

20. **Gundtoft PH, Pedersen AB, Varnum C, Overgaard S**. Increased mortality after prosthetic joint infection in primary THA. *Clin Orthop Relat Res* 2017;475:2623–2631.

21. **Puhto T, Puhto A-P, Vielma M, Syrjälä H**. Infection triples the cost of a primary joint arthroplasty. *Infect Dis* 2019;51:348–355.

22. **Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J**. Economic burden of periprosthetic joint infection in the United States. *J Arthroplasty* 2012;27:61–65.

23. **Kapadia BH, Berg RA, Daley JA, Fritz J, Bhave A** *et al*. Periprosthetic joint infection. *Lancet* 2016;387:386–394.

24. **Wood DE, Salzberg SL**. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biol* 2014;15:R46.

25. **Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M** *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.

26. **MacLea KS, Trachtenberg AM**. Complete genome sequence of *Staphylococcus epidermidis* ATCC 12228 chromosome and plasmids, generated by long-read sequencing. *Genome Announc* 2017;5:e00954-17.

27. **Sahl JW, Lemmer D, Travis J, Schupp JM, Gillece JD** *et al*. NASP: an accurate, rapid method for the identification of SNPs in WGS datasets that supports flexible input and output formats. *Microb Genom* 2016;2:e000074.

28. **Li H, Durbin R**. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–1760.

29. **McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K** *et al*. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;20:1297–1303.

30. **Méric G, Mageiros L, Pensar J, Laabei M, Yahara K** *et al*. Disease-associated genotypes of the commensal skin bacterium *Staphylococcus epidermidis*. *Nat Commun* 2018;9:5034.

31. **Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA** *et al*. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res* 2015;43:e15.

32. **Price MN, Dehal PS, Arkin AP**. FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One* 2010;5:e9490.

33. **Stamatakis A**. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.

34. **Letunic I, Bork P**. Interactive tree of life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2019;47:W256–W259.

35. **Seemann T**. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.

36. **Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S** *et al*. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015;31:3691–3693.

37. **Bradley P, Gordon NC, Walker TM, Dunn L, Heys S** *et al*. Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*. *Nat Commun* 2015;6:10063.

38. **Lees JA, Galardini M, Bentley SD, Weiser JN, Corander J**. pyseer: a comprehensive tool for microbial pangenome-wide association studies. *Bioinformatics* 2018;34:4310–4312.

39. **Collins C, Didelot X**. A phylogenetic method to perform genome-wide association studies in microbes that accounts for population structure and recombination. *PLoS Comput Biol* 2018;14:e1005958.

40. **Otto M**. Molecular basis of *Staphylococcus epidermidis* infections. *Semin Immunopathol* 2012;34:201–214.

41. **Rohde H, Kalitzky M, Kröger N, Scherpe S, Horstkotte MA** *et al*. Detection of virulence-associated genes not useful for discriminating between invasive and commensal *Staphylococcus epidermidis* strains from a bone marrow transplant unit. *J Clin Microbiol* 2004;42:5614–5619.

42. **Söderquist B, Andersson M, Nilsson M, Nilsdotter-Augustinsson Å, Persson L** *et al*. *Staphylococcus epidermidis* surface protein I (*SesI*): a marker of the invasive capacity of *S. epidermidis*? *J Med Microbiol* 2009;58:1395–1397.

43. **Qi X, Jin Y, Duan J, Hao Z, Wang S** *et al*. SesI may be associated with the invasiveness of *Staphylococcus epidermidis*. *Front Microbiol* 2017;8:2574.

44. **Conlan S, Mijares LA, Becker J, Blakesley RW, NISC Comparative Sequencing Program**, *et al*. *Staphylococcus epidermidis* pan-genome sequence analysis reveals diversity of skin commensal and hospital infection-associated isolates. *Genome Biol* 2012;13:R64.

45. **Hellmark B, Berglund C, Nilsdotter-Augustinsson A, Unemo M, Söderquist B**. Staphylococcal cassette chromosome *mec* (SCC*mec*)

and arginine catabolic mobile element (ACME) in *Staphylococcus epidermidis* isolated from prosthetic joint infections. *Eur J Clin Microbiol Infect Dis* 2013;32:691–697.

46. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012;67:2640–2644.

47. Wi YM, Greenwood-Quaintance KE, Brinkman CL, Lee JYH, Howden BP *et al*. Rifampicin resistance in *Staphylococcus epidermidis*: molecular characterisation and fitness cost of *rpoB* mutations. *Int J Antimicrob Agents* 2018;51:670–677.

48. Hellmark B, Unemo M, Nilsdotter-Augustinsson A, Söderquist B. Antibiotic susceptibility among *Staphylococcus epidermidis* isolated from prosthetic joint infections with special focus on rifampicin and variability of the *rpoB* gene. *Clin Microbiol Infect* 2009;15:238–244.

49. Castanheira M, Watters AA, Mendes RE, Farrell DJ, Jones RN. Occurrence and molecular characterization of fusidic acid resistance mechanisms among *Staphylococcus* spp. from European countries (2008). *J Antimicrob Chemother* 2010;65:1353–1358.

50. Dale GE, Broger C, D'Arcy A, Hartman PG, DeHoogt R *et al*. A single amino acid substitution in *Staphylococcus aureus* dihydrofolate reductase determines trimethoprim resistance. *J Mol Biol* 1997;266:23–30.

51. Yang S-J, Mishra NN, Rubio A, Bayer AS. Causal role of single nucleotide polymorphisms within the *mprF* gene of *Staphylococcus aureus* in daptomycin resistance. *Antimicrob Agents Chemother* 2013;57:5658–5664.

52. Jiang J-H, Dexter C, Cameron DR, Monk IR, Baines SL. Evolution of daptomycin resistance in coagulase-negative staphylococci involves mutations of the essential two-component regulator WalKR. *Antimicrob Agents Chemother* 2019;63:e01926-18.

53. Yamada M, Yoshida J, Hatou S, Yoshida T, Minagawa Y. Mutations in the quinolone resistance determining region in *Staphylococcus epidermidis* recovered from conjunctiva and their association with susceptibility to various fluoroquinolones. *Br J Ophthalmol* 2008;92:848–851.

54. Trong HN, Prunier A-L, Leclercq R. Hypermutable and fluoroquinolone-resistant clinical isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2005;49:2098–2101.

55. Hooper DC. Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin Infect Dis* 2000;31 (Suppl. 2):S24–S28.

56. Addetia A, Greninger AL, Adler A, Yuan S, Makhsous N. A novel, widespread *qacA* allele results in reduced chlorhexidine susceptibility in *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 2019;63:e02607-18.

57. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB. SCC*mec*Finder, a web-based tool for typing of staphylococcal cassette chromosome *mec* in *Staphylococcus aureus* using whole-genome sequence data. *mSphere* 2018;3:e00612-17.

58. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* 2009;53:4961–4967.

59. Widerström M, Wiström J, Edebro H, Marklund E, Backman M *et al*. Colonization of patients, healthcare workers, and the environment with healthcare-associated *Staphylococcus epidermidis* genotypes in an intensive care unit: a prospective observational cohort study. *BMC Infect Dis* 2016;16:743.