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Strategies of adaptation of *Staphylococcus epidermidis* to hospital and community: amplification and diversification of SCC*mec*

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Objectives: *Staphylococcus epidermidis* is a harmless commensal, but it can become a human pathogen, mainly in the hospital environment. In order to clarify strategies used by these bacteria to adapt to the hospital environment, we compared the population structure and staphylococcal cassette chromosome *mec* (SCC*mec*) content of *S. epidermidis* from the community and hospital.

Methods: *S. epidermidis* were collected from nasal swabs of both healthy military draftees (192 isolates) and patients (94 isolates) recovered in the same time period and geographical region. *S. epidermidis* were characterized by PFGE, multilocus sequence typing and SCC*mec* typing.

Results: Clonal complex 5 was predominant in the hospital (100%) and the community (58%), but some clonal types were specific to each environment and others were found in both (C/H clones). The methicillin-resistant *S. epidermidis* (MRSE) colonization rate in the community was very low (7%) when compared with the hospital (30%; $P<0.05$). Community-associated MRSE carried mostly SCC*mec* IV and V [Simpson's index of diversity (SID)=57.52%; 95% CI 38.35–76.69], whereas hospital-associated MRSE carried 17 SCC*mec* structures (SID=82.67%; 95% CI 77.38–87.96). Isolates of the same PFGE type had a much higher number of different SCC*mec* types when collected in the hospital than in the community.

Conclusions: Our data suggest that the *S. epidermidis* population is composed of hospital-associated clonal types, community-associated clonal types and types that are able to survive in both environments. Moreover, adaptation to the hospital environment in *S. epidermidis* appears to promote an increase in the frequency and diversification of SCC*mec*.

Keywords: population structure, evolution, methicillin resistance, mobile genetic elements, MGEs, recombination

Introduction

Staphylococcus epidermidis is one of the main colonizers of the human skin, but can turn into a pathogen if the cutaneous barrier is broken or the host is compromised.[1] *S. epidermidis* is one of the most important pathogens in the hospital environment, being responsible for 40%–90% of infections associated with indwelling devices.[2]

The success of *S. epidermidis* as a pathogen is mainly linked to its capacity to form biofilm, a multistep process involving several genetic determinants (*atlE*, *aap* and *ica* operon).[1] In addition, *S. epidermidis* has the ability to accumulate multiple antibiotic resistance determinants.[1,2] In particular, the frequency of methicillin-resistant *S. epidermidis* (MRSE) can reach 80% in hospitals worldwide,[3] although is considerably lower (30%–40%) in countries like Denmark and Iceland, where methicillin-resistant *Staphylococcus aureus* (MRSA) frequency is <1%.[4]

Methicillin resistance is conferred by the *mecA* gene, which is carried in a family of mobile genetic elements called staphylococcal cassette chromosomes (SCCs).[5] In *S. aureus*, SCCs insert in a unique specific chromosomal site (*orfX*), have characteristic inverted and direct repeats and contain chromosome cassette recombinases (*ccr*) that are responsible for SCC mobility. SCC*mec* is composed of the *mec* complex, containing *mecA* and its regulators, and the *ccr* complex, containing one (*ccrC*)[6] or two (*ccrAB*) recombinases.[5] Up until now 11 major types of SCC*mec* (I–XI)[7–10] and 8 subtypes of SCC*mec* IV[7,11] have been described in *S. aureus*. The few data available suggest that in *S. epidermidis*, SCC*mec* is also inserted in *orfX*[12,13] and has a structure similar to that described for *S. aureus*.[12,14] However, several studies have demonstrated that there is a large pool of

uncharacterized SCC*mec* types among *S. epidermidis* and other coagulase-negative staphylococci.[15,16] Besides SCC*mec*, other SCC elements have been described in staphylococci that transport genes important for survival and virulence.[17–19]

The population structure of *S. epidermidis* in the hospital environment (1996–2001) was shown to be composed of a major and highly diverse genetic lineage disseminated worldwide [clonal complex (CC) 2] and several minor CCs.[20] In this population, SCC*mec* IV[14,16] was found to be the most frequent type, but other SCC*mec* types[15,16] and a high number of novel cassettes have been identified as well.[15,16] Nosocomial *S. epidermidis* were described to carry several *ccr* alleles, which were suggested to correspond to the acquisition of multiple SCCs in tandem.[13] It was also shown that these isolates had a higher estimated rate of recombination/mutation (2.5:1) and a higher frequency of SCC*mec* acquisition than *S. aureus*.[20] Additionally, nosocomial isolates, particularly those belonging to CC2, were found to be associated with the presence of the *ica* operon[21] and the arginine catabolic mobile genetic element (ACME I.02),[18] considered a virulence factor in the community-associated MRSA (CA-MRSA) strain USA300.

Much less is known regarding the epidemiology of community-associated *S. epidermidis* (CA-SE). The few studies available describe frequencies of nasal colonization with CA-MRSE of 20% among children[22] and military personnel.[23] The molecular characterization of CA-SE showed a high genetic diversity, as illustrated by the high number of types of PFGE found; however, dissemination of *S. epidermidis* epidemic strains was also observed to occur in this setting.[23] Regarding SCC*mec* distribution, the few studies available showed that SCC*mec* type IVa was the most prevalent among CA-MRSE, but other types were also found.[22,24] However, the population structure of *S. epidermidis* in the community and hospital and the frequency of antibiotic resistance genes have never been compared before.

In the study described here, we addressed this question by comparing *S. epidermidis* from the hospital and the community, collected from the same ecological niche, same time period and geographical location, in terms of genetic background and SCC*mec* content.

Methods

Study population

A total of 1483 Air Force draftees from different regions in Portugal and attending Centro de Formação da Ota (Lisbon, Portugal) were swabbed in the anterior nares. This study was conducted in four consecutive years (1996–99). Each draftee filled in a questionnaire assessing demographic data and the presence of risk factors for carriage of antibiotic-resistant staphylococci (namely, recent antibiotic consumption, reason for antibiotic prescription and contact with animals) and recent contact with the hospital (specifically, recent emergency department attendance and previous hospitalization or surgery) (the questionnaire is available as Supplementary data at *JAC* Online). A group of 1160 draftees did not take any antibiotics and had no contact with the hospital in the 3 months prior to sampling and were considered as being 'healthy draftees'.

In addition, 253 patients attending the medicine (160 patients) and orthopaedic (93 patients) services of Hospital da Força Aérea (Lisbon, Portugal) were swabbed in the anterior nares during two sampling periods (2000 and 2001). This hospital is a private hospital with only 86 beds and presents particular characteristics: it does not contain an emergency department, the surgeries are all scheduled, the average period of hospital stay is 11 days and the prevalence of MRSA is extremely low: 3% in medicine and 0.9% in orthopaedics (I. Santos-Sanches, FCT/UNL, personal communication).

Ethics statement

The nasal screening of patients from Hospital da Força Aérea (Lisbon, Portugal) was performed with approval from the local medical Ethics Committee. Written informed consent was not required for the screening of patients from Hospital da Força Aérea, according to Ethics Committee-approved guidelines. The screening of draftees attending Centro de Formação da Ota (Lisbon, Portugal) was performed with written informed consent and approval was obtained from all the necessary military authorities. Patient records were de-identified and analysed anonymously and the strains, not human subjects, were studied.

Isolation of bacteria

The swabs obtained from the 1160 healthy draftees were streaked onto mannitol salt agar (MSA; Difco, BBL, Becton Dickinson, Franklin Lakes, New Jersey, USA) and incubated for 24 h at 37°C. Bacterial isolates were further tested for coagulase production using the Staphytec Plus assay (Oxoid, Cambridge, UK). A total of 736 healthy draftees were colonized with coagulase-negative staphylococci (CoNS), of whom 199 were selected for further study to include the highest diversity in terms of geographical origin, gender and smoking habits. A total of 170/199 draftees were colonized with *S. epidermidis*, from whom 192 *S. epidermidis* isolates were collected (some draftees were colonized with more than one strain). These 192 *S. epidermidis* isolates were considered as having a community origin (CA-SE) and were included in this study.

Additionally, *Staphylococcus* were isolated from the nasal swabs obtained from 253 patients by growth on MSA as previously described.[25] The presence of the coagulase enzyme was assessed for all staphylococcal isolates by the Staphytec Plus assay (Oxoid). A total of 94 hospital patients out of 253 were colonized with *S. epidermidis*. These isolates were considered as being associated with the hospital [hospital-associated *S. epidermidis* (HA-SE)].

Species identification

S. epidermidis strains were identified by internal transcribed spacer PCR (ITS-PCR).[26]

mecA detection

The presence of the *mecA* gene was detected by PCR amplification for all isolates.[13] *S. epidermidis* isolates carrying *mecA* were considered as MRSE and those lacking *mecA* were considered as methicillin-susceptible *S. epidermidis* (MSSE), regardless of the oxacillin susceptibility results obtained.

S. epidermidis nasal colonization rate in the community

The *S. epidermidis* colonization rate in the community analysed in this study was estimated by calculating: (i) the number of draftees colonized with *S. epidermidis* as a percentage of the total number of draftees selected for study who were colonized with CoNS (170/199=0.854) (85.4%); and (ii) the number of draftees colonized with *S. epidermidis* among the total number of healthy draftees colonized with CoNS (736), assuming that the rate is the same as obtained in (i) (0.854×736=628 draftees). The number obtained in (ii) as a percentage of the total number of healthy draftees screened (1160) was considered the CA-SE colonization rate (628/1160×100=54.1%). The same

approach was applied to estimate the CA-MRSE nasal colonization rate (see the sections Study population and Isolation of bacteria).

Antimicrobial susceptibility testing

Susceptibility to penicillin, oxacillin, erythromycin, clindamycin, vancomycin, tetracycline and ciprofloxacin (Oxoid) was tested according to the guidelines of the CLSI.[27]

DNA preparation

Agarose discs for PFGE and DNA for PCR were prepared as described previously.[4,13,28]

PFGE

The SmaI DNA restriction fragments were separated by PFGE[28] and the resulting patterns were analysed using the BioNumerics software (version 4.61 of Applied Maths, Saint-Martens-Latem, Belgium) with previously optimized settings for *S. epidermidis*.[16]

Multilocus sequence typing (MLST)

MLST was performed as suggested by Thomas *et al.*[29] for one isolate of each PFGE type in the case of CA-SE isolates and for one isolate of each of the most representative PFGE types (containing more than four isolates) in the case of HA-SE. The MLST data were analysed using the goeBURST algorithm (http://goeBURST.phyloviz.net).

SCC*mec* typing

The SCC*mec* type was determined by the combination of the class of *mec* complex and the type of *ccr* complex as previously suggested.[7] In the detection of *ccr* genes, the following strains were used as positive controls: *S. aureus* COL (*ccrAB1*),[30] *S. epidermidis* RP62A (*ccrAB2*),[12] *S. aureus* ANS46 (*ccrAB3*),[30] *S. epidermidis* ATCC 12228 (*ccrAB4*)[17] and *S. aureus* WIS (*ccrC*).[31] To determine the class of *mec* complex, the strains *S. aureus* N315 (*mec* complex A),[6] COL (*mec* complex B)[32] and WIS (*mec* complex C)[31] were used as controls.

The subtype of SCC*mec* IV was determined by multiplex PCR as described by Milheiriço *et al.*[11] SCC*mec* was considered non-typeable when either *mec* complex or *ccr* complex, or both, were non-typeable by the methods used or when the isolate carried more than one *ccr* type. SCC*mec* was considered to be new if a new combination of *mec* complex and *ccr* complex was found.

Clonal type definition

S. epidermidis clonal types were defined by the association of PFGE type and SCC*mec* type for MRSE as previously proposed[16] and by PFGE type alone for MSSE.[16]

Statistical analysis

The degree of genetic diversity was assessed by Simpson's index of diversity (SID), using a 95% CI.[33] In this analysis, each PFGE type or SCC*mec* subtype was considered a 'type' or a 'species'. The statistical significance of differences between proportions was evaluated by the χ^2 test using a 95% CI.

Results

Frequency of nasal colonization of *S. epidermidis* and MRSE in the community

Out of 199 draftees selected, 170 were colonized with at least one *S. epidermidis* isolate ($n=192$ isolates), which corresponds to a colonization rate of *S. epidermidis* among healthy people of 54%. However, we found a low number of MRSE (18 isolates carrying *mecA*) in the community, which corresponds to a colonization rate of 7%. Moreover, we observed that the rates of resistance to non-β-lactam antibiotics among this population were also low: 33% were resistant to erythromycin, 18% to clindamycin and 9% to tetracycline. In addition, only 19% (36 out of 192 isolates) of CA-SE isolates, both MRSE (17 isolates) and MSSE (19 isolates), were resistant to three or more classes of antimicrobial agents and as many as 17% of the isolates were susceptible to all antimicrobials. Resistance to penicillin was high and reached 72%.

In contrast, in the hospital we found a much higher number of MRSE (75 out of 94 isolates), which corresponds to a nasal colonization rate of 30% ($P<0.05$). This rate is comparable to the ones obtained in countries with a low frequency of MRSA.[4]

Population structure of *S. epidermidis* isolated in the community

A total of 50 PFGE types were identified among the 192 CA-SE isolates studied, which corresponds to a high level of genetic diversity (SID=94.71%; 95% CI 92.94–96.48). A major PFGE type (PFGE type 10) accounted for 17% of the isolates, 45% belonged to 11 minor PFGE types and 38% were sporadic (43 different types).

A total of 53 CA-SE isolates were analysed by MLST and 40 different sequence types (STs) were found. ST184 was the most prevalent ST (five isolates), followed by ST59 (three isolates) and ST402 (two isolates). The remaining STs were detected in single isolates only, with the great majority of the isolates (72%, 38) being new.

The application of the algorithm goeBURST to MLST data obtained in this study and the data available online (www.mlst.net) allowed the identification of a change in the ancestor of the major CC from ST2 to ST5. This occurred as a result of the increase in the number of isolates belonging to ST5 and of single-locus variants of ST5 in the *S. epidermidis* MLST database. Consequently, the CC previously known as CC2 is now called CC5. This CC is now composed of 27 subgroup founders, including ST2 (see Figure 1). Thirty-one out of the 53 isolates (58%) analysed in this study belonged to the major clonal lineage (CC5) (see Figure 1). In addition, two isolates belonged to CC171, one isolate was related to CC19 and another one to CC212. The remaining 18 isolates (34%) were singletons.

In comparison with CA-SE, HA-SE isolates were more clonal. Twenty PFGE types were determined among the 94 nosocomial isolates analysed (SID=81.79%; 95% CI 76.57–87.01) and 70% of the isolates belonged to three major PFGE types: 10 (33%), 12 (25%) and 11 (12%). Each of the remaining 17 PFGE types corresponded to <5% of the entire collection. The analysis of 16 representative isolates (one of each major PFGE type) by MLST showed that they all belonged to previously described STs and to the major CC, CC5.

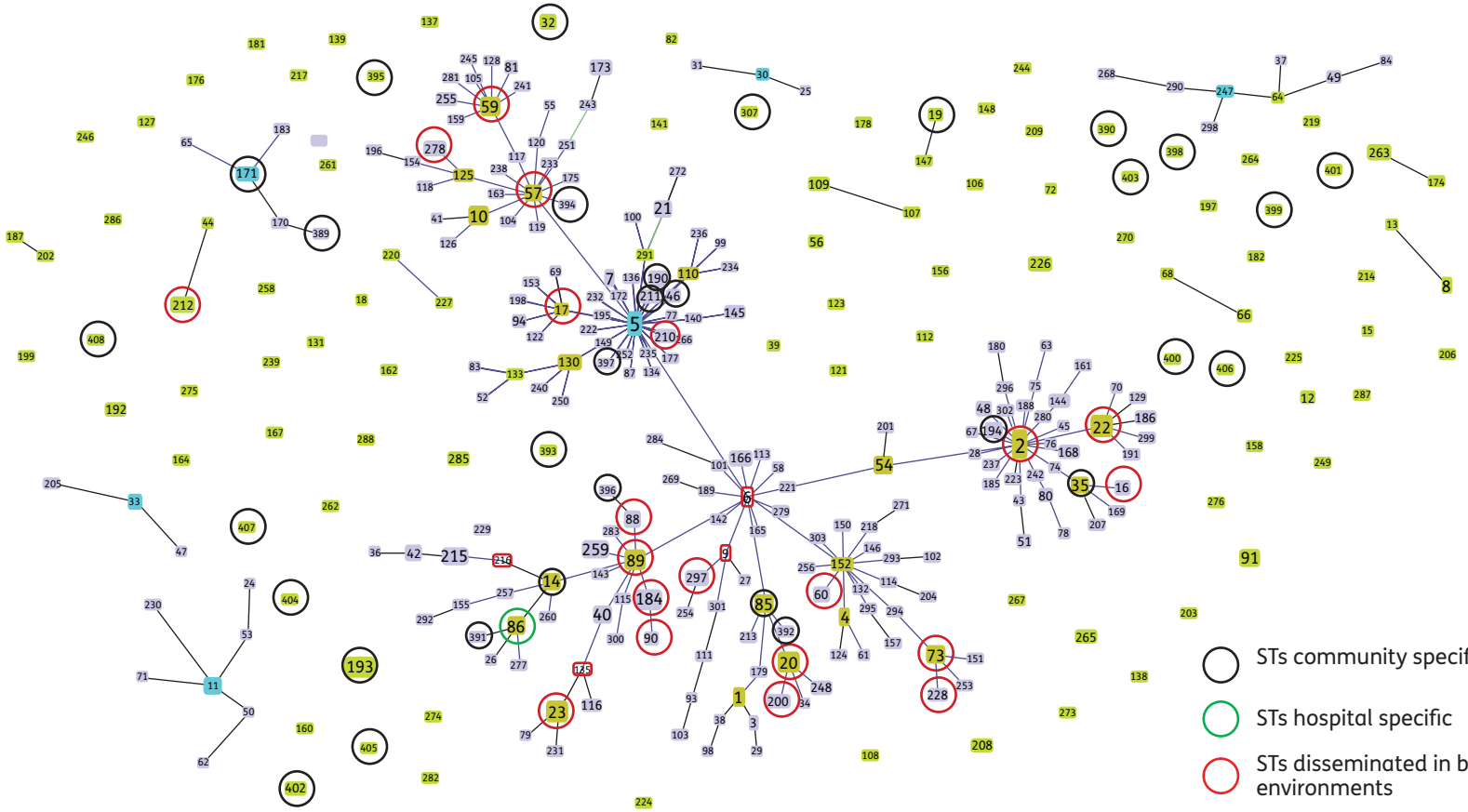

Figure 1. Analysis of MLST data with goeBURST. The most prevalent STs are represented by a bigger font. Cyan STs indicate probable ancestors (group founders) and green STs constitute subgroup founders. Blue STs correspond to STs that share the same background (CC). Black circles indicate STs that were found exclusively in the community. Green circles indicate STs exclusively associated with the hospital setting. Red circles indicate STs that were found in both environments.

Table 1. Molecular characterization of *S. epidermidis* isolates that belong to PFGE types identified both in the community and the hospital

PFGE pattern (no. of isolates)	STs	CCs	SCC*mec* types (no. of isolates)	
			community	hospital
10 (66)	16, 22	5	4&5A (1)	2&5A, VIII, 5A, NTA, 5B,2&5C1, 3&5C1, II, IVa, IVc, IVg, 2&5NT (25)
	2, 17, 60, 210, 212	5, 212	MSSE (34)	MSSE (6)
12 (32)	20, 22, 194	5	IVg (1)	2&4&5A, 5A, NTA, 2NT, II, IVg (21)
	73, 228	5	MSSE (8)	MSSE (2)
16 (15)	184, 90	5	MSSE (13)	MSSE (2)
11 (13)	23	5	MSSE (2)	IVa, IVc, IVd, IVg, IVnt (11)
9 (12)	59	5	IVa, IVnt (6)	5A, IVa (3)
	88	5	MSSE (2)	MSSE (1)
24 (7)	57	5	MSSE (6)	IVa (1)
13 (5)	20	5	5D (1)	IVa (1)
	—	—	MSSE (3)	—
30 (5)	—	—	—	3&5A (2)
	278	5	MSSE (2)	MSSE (1)
8 (4)	297	5	IVa (1)	IVa (1)
	—	—	MSSE (2)	—
31 (3)	89	5	MSSE (2)	MSSE (1)
4 (2)	57	5	MSSE (1)	MSSE (1)
32 (2)	200	5	MSSE (1)	MSSE (1)

Table 2. Comparison of CA-SE and HA-SE

Feature	CA-SE	HA-SE	Statistical validation
MRSE nasal colonization	7%	30%	$P<0.05$
Frequency of C/H clones	35%	85%	$P<0.05$
Multiple *ccr*	7%	17%	$P<0.05$
SCC*mec* types	IVa, IVc, IVg, IVh, IVnt, V, 5D, NT1	IVa, IVc, IVd, IVg, II, VIII, 5A, 5B, NT2–NT10	—
Diversity in SCC*mec* types[a] (SID)	57%, 95% CI 38.35–76.69	83%, 95% CI 77.38–87.96	95% CIs do not overlap
Diversity in genetic backgrounds (SID)	95%, 95% CI 92.94–96.48	82%, 95% CI 76.57–87.01	95% CIs do not overlap

[a]Considering SCC*mec* subtypes.

When we compared the population structures of CA- and HA-SE we found some PFGE types that were hospital specific (class H: 8 different types) or community specific (class C: 37 different types). Notably, we also found that the most prevalent genetic lineage in both environments was CC5 (Table 1) and that isolates within this lineage belonging to specific STs and PFGE types were frequently sampled in the hospital and community (class C/H).

The clones identified as C/H were more frequently found in the hospital (85%) than in the community (35%) and had particular genetic features that may be related to their capacity to survive in both environments. Specifically, all the isolates (except one) belonging to C/H clones were from CC5 and contained a high number of different SCC*mec* types when collected in the hospital environment (see PFGE types 10 and 12 in Table 1). In addition, they carried ACME with ClaI-*arcCB* type 4 or 6 (43%) (similar to the ACME I from USA300) and the *ica* genes (50%) (data not shown).

Genetic diversity of SCCmec among CA-MRSE

Of the 18 CA-MRSE isolates, 16 carried either SCC*mec* IV (11 isolates) or SCC*mec* V (5 isolates), 1 carried a new combination of *mec* complex and *ccr* type (5D) and 1 was non-typeable [4&5A (NT1)] (Table 2). Of the 11 isolates carrying SCC*mec* type IV, 6 carried subtype IVa and 2 carried subtype IVc. Subtypes IVg, IVh and a non-subtypeable variant were each identified in single isolates. Overall, the genetic diversity of SCC*mec*, considering the SCC*mec* subtypes, in the community environment was relatively low (SID=57.52%; 95% CI 38.35–76.69).

A much higher genetic diversity in SCC*mec* was observed among hospital isolates (SID=82.67%; 95% CI 77.38–87.96). Of the 75 HA-MRSE isolates studied, 25 (33%) carried SCC*mec* IV and 23 (31%) carried non-typeable structures (NT2–NT10) (Table 2). In addition, 12 of the isolates (16%) carried new associations between the class of *mec* complex and the *ccr* type (Table 2). Of the remaining isolates, 13 (17%) carried SCC*mec* II and 2 (3%) SCC*mec* VIII. The most prevalent subtype of

SCC*mec* IV was subtype IVa (11 isolates), followed by SCC*mec* IVc (6 isolates), SCC*mec* IVg (3 isolates) and SCC*mec* IVd (1 isolate). We also identified four isolates with SCC*mec* IV not subtypeable by the methods used.

Evidence for the role of the hospital environment in SCCmec amplification and diversification

We observed that SCC*mec* frequency and genetic diversity in the hospital were significantly higher than in the community (Table 2). In order to understand in detail the impact of the hospital environment on SCC*mec* diversification and amplification, we compared the SCC*mec* content of isolates with the same PFGE type collected either in the community or in the hospital (C/H). Interestingly, we observed that in several cases CA- and HA-SE with the same PFGE type differed significantly in the SCC*mec* content. In particular, isolates belonging to PFGE type 10 collected in the community environment carried the SCC*mec* type with *mec* complex class A associated with *ccrC* and *ccrAB4* only, whereas isolates with PFGE 10 collected in the hospital carried as many as 12 different SCC*mec* structures (see Table 1). Similarly, isolates of PFGE type 12 that originated in the community carried only SCC*mec* type IVg, whereas isolates from the hospital carried six different SCC*mec* structures. The same type of result was obtained when we compared CA-MSSE and HA-MRSE with exactly the same PFGE type. For example, isolates with PFGE type 11 collected in the community were all MSSE, whereas isolates collected in the hospital were all MRSE and carried five different SCC*mec* IV subtypes (Table 1). Similar observations were made for isolates belonging to PFGE types 24 and 30.

Overall, we observed a positive correlation between MRSE sample size and the number of different SCC*mec* types in the hospital environment, but the same was not observed in the community (Table 1). Moreover, we observed that multiple *ccr*s were present at higher frequency among hospital isolates when compared with community isolates (HA, 17%; CA, 7%; $P < 0.05$). Overall, the results suggest that this may represent new acquisitions of SCC*mec*/SCC, but the selection of isolates followed by recombination at the SCC*mec* level occurring in the hospital environment cannot be disregarded.

Interestingly, SCC*mec* types II, IVd and VIII, as well as new (5A and 5B) and non-typeable (2&4&5A, 3&5A, 2&5C1, 3&5C1, 2&5A, NTA, IVnt, 2NT and 2&5NT) SCC*mec* structures, were detected among hospital-associated isolates only, suggesting that these SCC*mec* types most probably were acquired and/or assembled in the hospital environment.

The two sets of isolates analysed in this study are not exactly contemporary (CA isolates were collected between 1996 and 1999 and HA isolates were collected in 2000–01). In order to exclude the hypothesis that time could be a factor influencing the results obtained, we analysed SCC*mec* diversity in isolates from the community and hospital in 2 year time blocks. The results obtained from block to block were comparable to those obtained for the entire time period, suggesting that time should not be a factor contributing to the differences observed between the two environments.

Overall, the results obtained suggest that the hospital environment promotes SCC*mec* diversification and amplification, either by SCC*mec* acquisition or by selection of MRSE strains.

Discussion

In the present work we compared the population structures and the frequency and diversity of SCC*mec* in *S. epidermidis* collected in two different environments from the same geographical region and comparable time periods. The molecular characterization of such collections showed that *S. epidermidis* strategies to adapt to hospital and community environments involved the divergent adaptation or selection of specific genetic backgrounds and SCC*mec* elements.

We found a low nasal colonization rate with MRSE (7%) in healthy Portuguese draftees. Other studies concerning different healthy populations have found a higher MRSE colonization rate, such as among military personnel (20%)[23] and children attending day care centres.[22] However, these two populations present risk behaviours for MRSE dissemination and colonization, such as frequent physical contact and higher antibiotic consumption, which are not observed in the population under study here, which consisted of unrelated healthy young individuals.

In addition, we found a low frequency of multiresistance to antibiotics (19%) among isolates that originated in the community. This scenario contrasts sharply with what was observed in the hospital environment, where the MRSE colonization rates obtained were higher (30%; $P < 0.05$) and multiresistance is frequent.[1,4] As a whole, these results suggest that CA-SE are probably not functioning as the primary reservoirs of SCC*mec* and determinants of antibiotic resistance to other, more pathogenic species, such as *S. aureus*. Nevertheless, we cannot exclude the hypothesis that certain CA-MRSE, when introduced into a hospital, may become amplified due to selective pressure and become reservoirs of SCC*mec* for *S. aureus*.

The difference observed in the frequencies of SCC*mec* and multiple *ccr* in the community and hospital suggests in addition that specific physiological conditions during infection and stresses imposed by the hospital environment can promote SCC*mec* excision/acquisition and dissemination in the *S. epidermidis* hospital population. This hypothesis is further sustained in our study by the existence of isolates with the same PFGE type that either lack or contain several different SCC*mec* types, depending on whether they were isolated in the community or hospital. Additionally, the discovery by others of *S. epidermidis* subpopulations with spontaneous deletion of the *mecA locus* in isolates from persistent infection[34] and the finding that β-lactams and vancomycin up-regulate *ccrA* expression[35] further support the hypothesis that the hospital environment may promote SCC*mec* excision and transfer. Likewise, it is possible that SCC*mec* transfer is promoted during biofilm formation, which is the most important virulence property of *S. epidermidis*, as was previously proved for other mobile genetic elements.[36] However, we cannot exclude the hypothesis that the higher frequency of SCC*mec* observed in the hospital resulted from the occurrence of selection of MRSE strains that originated either in the community or in the hospital.

Besides promoting SCC*mec* acquisition, the hospital environment appears to contribute to the generation of genetic diversity in the SCC*mec* elements. This hypothesis is supported by the very high number of SCC*mec* structures with new combinations of classes of *mec* complex and *ccr* types and non-typeable SCC*mec* found in nosocomial isolates belonging to a single PFGE type (PFGE type 10 or 12), when compared with isolates

of the same PFGE type that originated in the community. Previous studies analysing HA-MRSE also described a high number of new SCC*mec* structures.[13,15] In this study, however, only by comparing hospital and community isolates was it possible to envisage that the genetic diversity previously observed in SCC*mec* should be mostly created by factors associated with the hospital environment. One of the factors that probably contribute to this diversity is the recombination between SCC elements that might occur in the same strain upon multiple SCC acquisitions. Also, the increased expression of *ccr* genes after antimicrobial exposure, as previously observed,[35] may increase the opportunities for recombination between several excised SCC elements. Moreover, we should not disregard the fact that in the hospital a large reservoir of SCC*mec* types exist in other coagulase-negative species, and this may be contributing to the overall genetic diversity observed in *S. epidermidis*. The high number of different SCC*mec* types present in *S. epidermidis*, together with those present in other CoNS, build up a large reservoir of new SCC*mec* types for *S. aureus*. The acquisition of an additional SCC*mec* type (type IV) by *S. aureus* at the beginning of the 1990s could have resulted from the acquisition of this element from this highly diverse pool of SCC*mec*.

Although genetic diversity appears to be greater among hospital isolates than among community isolates, we cannot rule out the possibility that if a higher number of MRSE were to be collected in the community, greater diversity in terms of SCC*mec* would also be found. The analysis of SCC*mec* structure under stress conditions promoted in the hospital environment, such as subinhibitory concentrations of antibiotics, would probably contribute to the clarification of this question.

The comparison of *S. epidermidis* in the hospital and community showed that both populations had a high level of genetic diversity, but the community population was more diverse than the hospital population. This indicates that certain *S. epidermidis* clones are probably more adapted to hospital-associated stresses and spread easily in these surroundings. In spite of the high number of different PFGE types and STs in the two collections analysed, the great majority of the isolates from both community and hospital belonged to a single CC: CC5 (formerly CC2). This CC has been previously described as the most prevalent CC in the nosocomial population of *S. epidermidis*,[20] being characterized by a high level of genetic diversity, an increased recombination/mutation rate and a high number of acquisitions of SCC*mec* elements.[20] The fact that this lineage was identified in this study also as predominant in the community suggests it is well adapted to the host and that it also has the capacity to adapt to environments with distinct characteristics. In spite of belonging to CC5, the CA-SE and HA-SE isolates presented specific PFGE types and STs according to their origin, suggesting divergent evolution.

Moreover, among CC5 we also identified clones with the capacity to survive in both environments. These clones appear to adapt to different environments by modulating the acquisition of SCC*mec* and their level of genetic diversity. Interestingly, the great majority of the isolates belonging to these clones carried SCC*mec* type IV, which probably confers advantages and has no fitness cost in either environment. The fact that these isolates can survive in the community and hospital provides them with a higher capacity for dissemination and the accumulation of relevant genetic traits for survival in both settings. Indeed, it is possible that these clones are the ones responsible for shuffling genetic traits between the community and hospital, namely ACME and SCC*mec* IV. In some ways these clones appear to be similar to USA300 and EMRSA-15, which carry SCC*mec* IV and have gained the ability to survive in both community and hospital environments.[37–39]

The collection analysed in this study is not contemporary, but otherwise reflects the *S. epidermidis* epidemiology in a certain period of time (10–15 years ago) in a specific location. Since the time this study was performed some alterations in *S. aureus* epidemiology have occurred, such as the emergence of CA-MRSA as an epidemic, and additional changes might have also occurred in the epidemiology of *S. epidermidis*. Nonetheless, studies in which more recent *S. epidermidis* isolates were analysed continue to report ST2 as the most frequent ST in hospitals in several different geographical locations and SCC*mec* as very diverse in the hospital environment.[40–42] These observations suggest that the conclusions drawn for the isolates analysed in our study probably still hold at present.

Our data demonstrate for the first time the role of the hospital environment in the selection of some genetic backgrounds and in the diversification and acquisition and/or selection of SCC*mec* in *S. epidermidis*. Moreover, our data enabled us to identify a class of clones that are able to move between hospital and community. It will be critical to take these features of *S. epidermidis* epidemiology into consideration in any infection control programme directed to *S. epidermidis* and in evolutionary studies regarding SCC*mec*.

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Transparency declarations

None to declare.

Supplementary data

The questionnaire is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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