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Genetic Diversity of Arginine Catabolic Mobile Element in Staphylococcus epidermidis

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Abstract

Background: The methicillin-resistant Staphylococcus aureus clone USA300 contains a novel mobile genetic element, arginine catabolic mobile element (ACME), that contributes to its enhanced capacity to grow and survive within the host. Although ACME appears to have been transferred into USA300 from S. epidermidis, the genetic diversity of ACME in the latter species remains poorly characterized.

Methodology/Principal Findings: To assess the prevalence and genetic diversity of ACME, 127 geographically diverse S. epidermidis isolates representing 86 different multilocus sequence types (STs) were characterized. ACME was found in 51% (65/127) of S. epidermidis isolates. The vast majority (57/65) of ACME-containing isolates belonged to the predominant S. epidermidis clonal complex CC2. ACME was often found in association with different allotypes of staphylococcal chromosome cassette mec (SCCmec) which also encodes the recombinase function that facilitates mobilization ACME from the S. epidermidis chromosome. Restriction fragment length polymorphism, PCR scanning and DNA sequencing allowed for identification of 39 distinct ACME genetic variants that differ from one another in gene content, thereby revealing a hitherto uncharacterized genetic diversity within ACME. All but one ACME variants were represented by a single S. epidermidis isolate; the singular variant, termed ACME-I.02, was found in 27 isolates, all of which belonged to the CC2 lineage. An evolutionary model constructed based on the eBURST algorithm revealed that ACME-I.02 was acquired at least on 15 different occasions by strains belonging to the CC2 lineage.

Conclusions/Significance: ACME-I.02 in diverse S. epidermidis isolates were nearly identical in sequence to the prototypical ACME found in USA300 MRSA clone, providing further evidence for interspecies transfer of ACME from S. epidermidis into USA300.

Introduction

Staphylococcus epidermidis is a ubiquitous commensal of the human skin and mucosal surfaces and a major cause of indwelling medical device infections. This organism is notorious for its capacity to accumulate antibiotic resistance determinants and to produce biofilm, making infections caused by this opportunistic pathogen particularly difficult to treat. The large gene pool of antibiotic resistance and virulence determinants in S. epidermidis is shared with other more pathogenic species such as S. aureus. In particular, multidrug-resistant conjugative plasmids and staphylococcal chromosome cassette mec (SCCmec) elements conferring β-lactam resistance are transferred frequently between S. epidermidis and S. aureus, enabling rapid evolution and adaptation against antibiotic selection pressure [1,2,3].

Many S. epidermidis strains also carry the arginine catabolic mobile element (ACME), a novel genomic island that may contribute to enhanced capacity of this species to colonize the human skin and mucosal surfaces [4]. The horizontal transfer of ACME from S. epidermidis to S. aureus is thought to be central in the evolution of the highly transmissible community-associated methicillin resistant S. aureus (CA-MRSA) clone USA300 [4]. In USA300, ACME is integrated at the argX site downstream of SCCmec, and is flanked by repeat sequences typical of SCCmec.
cassettes [4,5]. Mobilization of ACME is believed to be mediated by the cassette chromosome recombinases (ccrAB) encoded by SCC element [5,6]. This element has been found also in diverse S. aureus genetic backgrounds, suggesting frequent horizontal dissemination [4,5,6,7]. ACME contains two gene clusters (arc encoding a secondary arginine deiminase system and opp-3 encoding an ABC transporter) that are homologs of virulence determinants in other bacterial species [5]. However, ACME was shown not to contribute to the severity of necrotizing pneumonia or skin abscesses using rat infection models [9], a finding consistent with the presence of this element among various commensal Staphylococcus species [4,9]. Using highly sensitive in vivo competition assays, ACME was shown to confer bacterial survival advantage in a rabbit bacteremia model and a mouse gastrointestinal colonization model [5,10]. Taken together, the data suggest that ACME may play a role in bacterial transmission among susceptible hosts by contributing to bacterial growth and survival.

Although ACME was found in diverse S. epidermidis [1], the genetic diversity of ACME among S. epidermidis has not yet been characterized. It is also not clear the extent to which S. epidermidis serves as a reservoir of ACME for horizontal transfer to related pathogenic species such as S. aureus. We report herein an analysis of 127 genotypically diverse isolates of S. epidermidis from worldwide sources. The results show extensive genetic variations within ACME. ACME allotypes are often found in association with diverse allotypes of SCCmec. The most prevalent allotype of ACME among S. epidermidis is nearly identical to prototypical ACME found in USA300, suggesting that ACME transfers from S. epidermidis into the epidemic USA300 clone.

Materials and Methods

Bacterial Isolates

A representative collection of S. epidermidis strains was selected to include isolates as diverse as possible, in terms of genetic background, geographic source and temporal origins. The study collection comprised 127 S. epidermidis strains, 93 methicillin-resistant S. epidermidis (MRSE) and 34 methicillin-susceptible S. epidermidis (MSSE), isolated between 1996 and 2005 in 18 different countries: Argentina (3), Bulgaria (3), Cape Verde (8), China (1), Colombia (2), Denmark (29), Greece (4), Hungary (2), Iceland (20), Italy (2), Japan (1), Mexico (3), Poland (2), Portugal (8), Spain (1), Taiwan (2), Uruguay (1) and USA (33). The collection selected included 79 isolates from human carriage, 40 from disease and 8 isolates for which no information on the clinical origin was available.

Restriction Fragment Length Polymorphism (RFLP) Analysis of Arginine Deiminase (arc) Gene Cluster

Chromosomal DNA of S. epidermidis strains was digested with ClaI and the resulting fragments were separated by a electrophoresis in a 1% agarose gel in 1x Tris-acetate-EDTA buffer at 30 volts for 17 h. DNA fragments were transferred by vacuum blotting to nitrocellulose membranes as previously described [11]. Hybridization with DNA probes for a region encompassing arcCB were performed with ECL direct prime labelling and detection using PCR-based assays using the primer pairs AIPS.27-AIPS.28 and AIPS.45-AIPS.46 (opp3), as previously described [3]. The arcA and opp-3 genes are surrogate markers of the arc gene cluster encoding for an arginine deiminase system and opp-3 encoding for an ABC transporter system, respectively. S. epidermidis isolates containing arcA and opp-3 clusters were further characterized by a PCR-based scanning/tiling method that allows for identification of variations in ACME gene content and gene synteny, as previously described [5]. This method is based on 31 individual PCR reactions using distinct primer pairs designed to generate 1–2 kb PCR fragments that overlap with one another at both ends for complete scanning coverage of the prototype ACME found in USA300. PCR scan patterns were classified into three ACME allotypes: (1) ACME-I contains both the arc and opp-3 gene clusters; (2) ACME-II contains arc but not opp-3; and (3) ACME-III contains opp-3 but not arc. Distinct PCR scan patterns within each ACME allotype are given subtype designations (e.g. ACME-I.02).

SCCmec Typing

S. epidermidis isolates were characterized for the two central elements of the staphylococcal cassette chromosome mec (SCCmec), namely, the ccr complex encoding for recombinases and the mec complex encoding for broad spectrum β-lactam resistance. The multiplex PCR strategy, M-PCR 1, was used to identify the 5 types of ccr gene complex, and M-PCR 2 to identify class A to class C mec complex, as previously described [12].

Ccr-mediated Excision of ACME

A tetracycline-selectable temperature-sensitive plasmid, pSR2, containing the ccrAB2 gene complex, was electroporated into S. epidermidis strain 1457. Se1457(pSR2) was passaged for three days in tryptic soy broth (TSB) supplemented with 10 μg/ml of tetracycline at 30 °C. Growth at the non-permissive temperature of 42°C in antibiotic-free TSB resulted in loss of pSR2 in the excision mutants. Individual colonies were screened for excision and loss of ACME by assaying for the loss of the arcA gene. Confirmation of ACME excision was performed by Southern hybridization of Smal DNA restriction fragments after pulse-field gel electrophoresis with the probe encompassing arcA and arcB as described above [13]. In vitro growth rate of S. epidermidis 1457 and its isogenic ACME-excision mutant was determined in tryptic soy broth as measured by OD600.

MLST and eBURST

Multilocus sequence typing (MLST) was performed, based on the sequencing of internal fragments of seven housekeeping genes, and using the revised scheme described by Thomas et al. [14]. The most likely patterns of evolutionary descent in the collection were assessed using the eBURST algorithm (http://eburst.mlst.net), using previously validated parameters [15]. Clonal complexes were represented by the abbreviation CC, and singletons were represented by the abbreviation S. CC2 was subdivided into clusters I and II, and cluster II was further separated into subclusters as previously described [15,16].

Construction of Evolutionary Models and Estimation of Independent ACME Acquisitions

An evolutionary model illustrating the number of ACME acquisitions was constructed, based on the evolutionary relation-
ships as defined by eBURST and ACME typing as defined by the PCR scanning strategy (see above). The number of independent ACME acquisitions was estimated based on the following assumptions: (i) there is a low probability of ACME excision, (ii) there is a low probability that the exact same mutation occurs twice; and (iii) for ACME acquisition/excision to occur, a gene coding for a recombinase (ccrAB or ccrC) must be present in the chromosome. The number of acquisition of SCC elements (SCCmec and SCC non-mec) was estimated using the same methods.

Statistical Analysis

Two-sided chi-square test statistics were used for between group comparisons (Stata, version 9, College Station, Texas).

Results

Distribution of ACME and SCCmec among S. epidermidis Lineages

Of the 127 S. epidermidis isolates selected to represent the broad genetic and geographic diversity of the species, 52% (65/127) contained either the ACME-encoded arcA and/or opp-3 gene clusters. Presence of arcA and/or opp-3 gene clusters did not correlate with isolates recovered from infection sites or colonization sites. Using a revised MLST scheme [14,16], 86 distinct sequence types (ST) were identified among the 127 isolates (Figure 1 and Table 1). The majority of the STs (50 of 86) were closely related and clustered into a single clonal complex, CC2. Of note, 65% (57/88) of isolates belonging to CC2 contained ACME-encoded arcA or opp-3, whereas only 21% (8/39) of isolates belonging to non-CC2 clonal complexes contained these genes (P<0.001).

Among MRSE isolates, 58% (54/93) contained SCCmec type I through V, and 42% (39/93) non-typeable SCCmec elements (Table 1). MRSE contained 1 to 3 different ccr gene complexes that could potentially mobilize SCCmec and ACME. There is no association between carriage of ACME and carriage of different SCCmec allotypes. Among MSSSE isolates, 29% (10/34) contained SCC-like elements containing ccr genes but lacking the mecA gene. In all, 81% (103/127) of S. epidermidis isolates carried ccr genes.

Excision of ACME by CcrAB in S. epidermidis

Horizontal transfer of ACME in S. aureus is mediated by SCC-encoded cassette recombinases (ccr), which catalyze the site-specific recombination between repeat sequences flanking the element and an attB site within orfX [5]. To test whether SCC-encoded ccr could mobilize ACME in S. epidermidis, we provided in trans ccrAB2 via plasmid pSR2 in a clinical isolate S. epidermidis 1457 and assayed for excision of ACME by pulsed-field gel electrophoresis of Smal-digested chromosomal DNA and hybridization with ACME-specific arcCB probe (Figure 2). A 240-kb Smal fragment hybridized with the

Figure 1. Application of eBURST algorithm to MLST data for the collection of 127 S. epidermidis isolates. Each ST is represented by a filled circle. Blue and yellow circles represent STs that are group and sub-group founders, respectively. CC comprised the groups of connected STs, considering that STs have at least 6 alleles in common with at least another ST inside a CC. doi:10.1371/journal.pone.0007722.g001
| Strain  | Country     | Methicillin resistance | CC | MLST | ACME PCR Scan | Clal-arcC/B pattern | SCCmec typing |
|---------|-------------|------------------------|----|------|--------------|--------------------|--------------|
| DEN112  | Denmark     | +                      | 2-I| ST2  | ACME-I.02    | 4                  | III          |
| ICE091  | Iceland     | +                      | 2-I| ST2  | ACME-I.02    | 4                  | III          |
| DEN049  | Denmark     | +                      | 2-I| ST2  | ACME-neg     | NH                 | III          |
| DEN102  | Denmark     | +                      | 2-I| ST2  | ACME-neg     | NH                 | III          |
| DEN121  | Denmark     | +                      | 2-I| ST2  | ACME-neg     | NH                 | III          |
| ICE027  | Iceland     | +                      | 2-I| ST2  | ACME-neg     | NH                 | III          |
| DEN167  | Denmark     | +                      | 2-I| ST2  | ACME-neg     | NH                 | IV           |
| ICE146  | Iceland     | +                      | 2-I| ST2  | ACME-neg     | NH                 | IV           |
| ICE181  | Iceland     | +                      | 2-I| ST2  | ACME-neg     | NH                 | IV           |
| DEN071  | Denmark     | +                      | 2-I| ST2  | ACME-I.02    | 4                  | A/ccrAB3,ccrAB4,ccrC |
| ICE050  | Iceland     | +                      | 2-I| ST2  | ACME-neg     | NH                 | A/ccrAB3,ccrAB4,ccrC |
| ICE124  | Iceland     | +                      | 2-I| ST2  | ACME-neg     | NH                 | A/ccrAB3,ccrAB4,ccrC |
| BD0917  | USA         | +                      | 2-I| ST2  | ACME-neg     | .                  | NT/ccrAB3,ccrC |
| BD0909  | USA         | –                      | 2-I| ST2  | ACME-neg     | .                  | mecA-neg/ccrAB2 |
| BD0942  | USA         | +                      | 2-I| ST16 | ACME-I.02    | 8                  | III          |
| BD0944  | USA         | +                      | 2-I| ST16 | ACME-I.02    | .                  | NT/ccrAB2    |
| BD0907  | USA         | –                      | 2-I| ST16 | ACME-I.02    | .                  | mecA-neg/ccrneg |
| BD0926  | USA         | –                      | 2-I| ST16 | ACME-I.02    | .                  | mecA-neg/ccrneg |
| BD0969  | USA         | –                      | 2-I| ST16 | ACME-I.02    | .                  | mecA-neg/ccrneg |
| BD0905  | USA         | +                      | 2-I| ST16 | ACME-I.02    | .                  | NT/ccrAB2,ccrC |
| DEN061  | Denmark     | +                      | 2-I| ST22 | ACME-I.02    | 8                  | III          |
| ICE019  | Iceland     | +                      | 2-I| ST22 | ACME-I.02    | 4                  | A/ccrAB2,ccrAB4,ccrC |
| ICE037  | Iceland     | +                      | 2-I| ST22 | ACME-I.02    | 4                  | A/ccrAB4,ccrC |
| HFA6014 | Portugal    | +                      | 2-I| ST35 | ACME-I.02    | 4                  | A/ccrC       |
| DEN004  | Denmark     | +                      | 2-I| ST45 | ACME-I.02    | 4                  | IV           |
| DEN087  | Denmark     | +                      | 2-I| ST48 | ACME-I.02    | 4                  | C/ccrAB2,ccrAB4 |
| BD0972  | USA         | –                      | 2-I| ST54 | ACME-I.02    | .                  | mecA-neg/ccrneg |
| DEN109  | Denmark     | +                      | 2-I| ST54 | ACME-I.02    | 3                  | III          |
| AGT18   | Argentina   | +                      | 2-I| ST63 | ACME-I.02    | 4                  | C/ccrAB2     |
| DEN055  | Denmark     | +                      | 2-I| ST70 | ACME-I.02    | 4                  | A/ccrAB4     |
| PLN131  | Poland      | +                      | 2-I| ST75 | ACME-I.02    | 4                  | III          |
| AGT17   | Argentina   | +                      | 2-I| ST78 | ACME-I.02    | 4                  | III          |
| ICE076  | Iceland     | +                      | 2-I| ST80 | ACME-I.02    | 4                  | III          |
| DEN139  | Denmark     | +                      | 2-I| ST54 | ACME-I.02    | 6                  | IV           |
| ICE175  | Iceland     | +                      | 2-I| ST43 | ACME-neg     | NH                 | A/ccrAB3,ccrAB4,ccrC |
| COB20   | Colombia    | +                      | 2-I| ST51 | ACME-neg     | NH                 | IV           |
| DEN036  | Denmark     | +                      | 2-I| ST67 | ACME-neg     | NH                 | III          |
| ESP43   | Spain       | +                      | 2-I| ST74 | ACME-neg     | NH                 | A/ccrAB3,ccrAB4,ccrC |
| GRE28   | Greece      | +                      | 2-I| ST76 | ACME-neg     | NH                 | A/ccrAB1,ccrAB2,ccrAB3 |
| BD0904  | USA         | +                      | 2-II| STS  | ACME-I.02    | A/ccrC          |
| ICE192  | Iceland     | +                      | 2-II| STS  | ACME-I.07    | 1                  | IV           |
| DEN002  | Denmark     | +                      | 2-II| STS  | ACME-I.07    | 6                  | C/ccrAB2,ccrC |
| BD0902  | USA         | +                      | 2-II| STS  | ACME-I.13    | NT/ccrAB2        |
| BD0922  | USA         | –                      | 2-II| STS  | ACME-neg     | mecA-neg/ccrneg   |
| ICE099  | Iceland     | +                      | 2-II| ST6  | ACME-I.08    | 0                  | A/ccrAB2,ccrAB3 |
| DEN076  | Denmark     | –                      | 2-II| ST14 | ACME-I.02    | 4                  | mecA-neg/ccrneg |
| HFA6181 | Portugal    | –                      | 2-II| ST17 | ACME-I.02    | 4                  | mecA-neg/ccrneg |
| CV27    | Cape Verde  | –                      | 2-II| ST20 | ACME-I.05    | 3                  | B/ccrAB2,ccrC |
| ICE026  | Iceland     | +                      | 2-II| ST34 | ACME-I.18    | 6                  | IV           |
| Strain  | Country   | Methicillin resistance¹ | CC² | MLST³ | ACME PCR Scan⁴ | Clal-arcC/B pattern⁵ | SCCmec typing⁶ |
|---------|-----------|------------------------|-----|-------|----------------|----------------------|----------------|
| ICE087  | Iceland   | +                      | 2-II| ST40  | (1-1-2-1-3-1-1) | ACME-I.02            | 8              | A/ccrAB4,ccrC  |
| DEN046  | Denmark   | –                      | 2-II| ST40  | (1-1-2-1-3-1-1) | ACME-I.04            | 4              | mecA-neg/ccr-neg |
| MCO150  | Mexico     | +                      | 2-II| ST46  | (1-1-2-1-2-1-7) | ACME-II.11           | 2              | IV             |
| HFA6162A| Portugal   | –                      | 2-II| ST57  | (1-1-1-2-1-1)   | ACME-I.06            | 3              | mecA-neg/ccrAB4 |
| CH35    | China      | +                      | 2-II| ST59  | (2-1-1-1-2-1-1) | ACME-II.04           | 3              | B/ccrAB2,ccrAB4 |
| TAW060  | Taiwan     | –                      | 2-II| ST59  | (2-1-1-1-2-1-1) | ACME-II.12           | 2              | mecA-neg/ccrAB4 |
| BD0912  | USA        | –                      | 2-II| ST59  | (2-1-1-1-2-1-1) | ACME-neg             | .              | mecA-neg/ccr-neg |
| BD0950  | USA        | +                      | 2-II| ST59  | (2-1-1-1-2-1-1) | ACME-neg             | .              | II             |
| GRE34   | Greece     | +                      | 2-II| ST69  | (1-18-6-2-2-1-1) | ACME-I.10            | 6              | IV             |
| BUG43   | Bulgaria   | +                      | 2-II| ST77  | (23-1-1-2-2-1-1) | ACME-II.08           | 6              | C/ccrAB2,ccrC  |
| DEN077  | Denmark    | +                      | 2-II| ST81  | (2-17-1-1-2-1-1) | ACME-II.09           | 2              | B/ccrAB2,ccrAB4 |
| BD0943  | USA        | +                      | 2-II| ST85  | (1-1-1-2-1-1-1) | ACME-II.19           | .              | V              |
| TAW113  | Taiwan     | –                      | 2-II| ST85  | (1-1-2-2-2-1-1) | ACME-II.13           | 3              | mecA-neg/ccrAB2 |
| BD0937  | USA        | +                      | 2-II| ST86  | (2-2-1-1-1-1-1) | ACME-I.11            | .              | II             |
| URU23   | Uruguay    | +                      | 2-II| ST86  | (2-2-1-1-1-1-1) | ACME-I.12            | 6              | NT/ccrAB2,ccrAB4 |
| HUR50   | Hungary    | +                      | 2-II| ST10  | (1-1-1-6-2-1-1) | ACME-I.14            | .              | mecA-neg/ccr-neg |
| BD0929  | USA        | –                      | 2-II| ST10  | (1-1-1-6-2-1-1) | ACME-I.15            | .              | mecA-neg/ccrAB2 |
| BD0936  | USA        | +                      | 2-II| ST148 | (1-1-1-2-1-1-1) | ACME-I.02            | .              | B/ccrC         |
| BD0915  | USA        | +                      | 2-II| ST149 | (1-1-1-2-2-1-10) | ACME-I.15            | .              | NT/ccrAB2      |
| BD0931  | USA        | –                      | 2-II| ST150 | (1-1-2-6-2-5-1) | ACME-II.16           | .              | mecA-neg/ccrAB2 |
| BD0964  | USA        | –                      | 2-II| ST152 | (1-1-2-6-2-1-1) | ACME-II.20           | .              | mecA-neg/ccrAB2 |
| BD0935  | USA        | –                      | 2-II| ST152 | (1-1-2-6-2-1-1) | ACME-neg             | .              | mecA-neg/ccr-neg |
| BD0965  | USA        | –                      | 2-II| ST153 | (2-1-6-2-2-1-1) | ACME-II.21           | .              | mecA-neg/ccrAB2 |
| BD0946  | USA        | –                      | 2-II| ST154 | (1-2-1-1-1-1-1) | ACME-III.01          | .              | mecA-neg/ccr-neg |
| BD0908  | USA        | –                      | 2-II| ST157 | (1-23-3-6-2-1-1) | ACME-I.09            | .              | mecA-neg/ccr-neg |
| BD0934  | USA        | +                      | 2-II| ST159 | (2-23-1-2-1-1)  | ACME-II.18           | .              | NT/ccrAB2      |
| DEN101  | Denmark    | +                      | 2-II| ST89  | (1-2-1-1-2-1-1) | ACME-III.02          | NH             | IV             |
| ICE120  | Iceland    | +                      | 2-II| ST89  | (1-2-1-1-2-1-1) | ACME-III.03          | NH             | IV             |
| BD0948  | USA        | +                      | 2-II| ST89  | (1-2-1-1-2-1-1) | ACME-neg             | .              | IV             |
| BD0971  | USA        | +                      | 2-II| ST89  | (1-2-1-1-2-1-1) | ACME-neg             | .              | IV             |
| DEN022  | Denmark    | +                      | 2-II| ST4   | (1-1-6-6-2-1-1) | ACME-neg             | NH             | IV             |
| DEN028  | Denmark    | +                      | 2-II| ST10  | (1-1-1-1-3-1-1) | ACME-neg             | NH             | IV             |
| ICE095  | Iceland    | +                      | 2-II| ST10  | (1-1-1-1-3-1-1) | ACME-neg             | NH             | IV             |
| DEN132  | Denmark    | +                      | 2-II| ST10  | (1-1-1-1-3-1-1) | ACME-neg             | NH             | B/ccrAB2,ccrAB4 |
| CV47    | Cape Verde | +                      | 2-II| ST41  | (1-1-1-1-3-1-1) | ACME-neg             | NH             | IV             |
| BUG46   | Bulgaria   | +                      | 2-II| ST58  | (1-1-2-2-1-3-1) | ACME-neg             | NH             | IV             |
| MEX060  | Mexico     | +                      | 2-II| ST61  | (2-1-6-2-1-1)   | ACME-neg             | NH             | NT/ccrAB2      |
| HFA6286 | Portugal   | –                      | 2-II| ST88  | (1-1-2-1-2-1-7) | ACME-neg             | NH             | mecA-neg/ccr-neg |
| DEN019  | Denmark    | +                      | 1   | ST1   | (1-2-2-1-1-10)  | ACME-neg             | NH             | IV             |
| ICE024  | Iceland    | +                      | 1   | ST38  | (2-2-5-1-1-10)  | ACME-neg             | NH             | IV             |
| GRE41   | Greece     | +                      | 1   | ST83  | (1-2-1-2-1-1-10)| ACME-neg             | NH             | IV             |
| DEN052  | Denmark    | +                      | 11  | ST11  | (3-1-5-5-3-4-11) | ACME-neg             | NH             | IV             |
| DEN148  | Denmark    | +                      | 11  | ST50  | (3-1-5-5-3-7-7-4)| ACME-neg             | NH             | IV             |
| CV59    | Cape Verde | +                      | 11  | ST53  | (3-1-5-5-11-4-11)| ACME-neg             | NH             | IV             |
| CV11    | Cape Verde | +                      | 11  | ST62  | (3-2-1-5-5-3-4-4)| ACME-neg             | NH             | NT/ccrAB2,ccrAB4 |
| MEX037  | Mexico     | +                      | 11  | ST71  | (3-1-5-5-3-1-11)| ACME-neg             | NH             | II             |
| DEN185  | Denmark    | +                      | 21  | ST21  | (2-1-12-1-1-1)  | ACME-I.03            | 4              | IV             |
| ICE102  | Iceland    | +                      | 21  | ST52  | (2-2-1-2-1-1-1) | ACME-I.16            | 4              | IV             |
The carriage of ACME does not engender a biological fitness cost [5].

Table 1. Cont.

| Strain | Country | Methicillin resistance | CC | MLST | ACME PCR Scan | Clal-arcC/B pattern | SCCmec typing |
|--------|---------|------------------------|----|------|--------------|---------------------|--------------|
| AGT24  | Argentina | +                       | 23 | ST23 | ACME-neg     | NH                   | III          |
| CV45   | Cape Verde | +                       | 23 | ST79 | ACME-neg     | NH                   | IV           |
| COB17  | Colombia  | -                       | 33 | ST33 | ACME-neg     | NH                   | mecA-neg/ccr-neg |
| JAP263 | Japan     | +                       | 33 | ST33 | ACME-neg     | NH                   | C/NT         |
| HUR51  | Hungary   | +                       | 34 | ST47 | ACME-neg     | NH                   | B/ccrAB2     |
| ICE021 | Iceland   | +                       | 42 | ST36 | ACME-neg     | NH                   | I            |
| DEN116 | Denmark   | +                       | 42 | ST42 | ACME-neg     | NH                   | A/ccrAB1     |
| ICE159 | Iceland   | +                       | 42 | ST42 | ACME-neg     | NH                   | B/ccrAB1,ccrC |
| HFA6173B | Portugal | +                       | 49 | ST37 | ACME-neg     | NH                   | IV           |
| DEN094 | Denmark   | +                       | 49 | ST49 | ACME-neg     | NH                   | IV           |
| MEX035 | Mexico    | +                       | 49 | ST49 | ACME-neg     | NH                   | IV           |
| PLN064 | Poland    | +                       | 49 | ST64 | ACME-neg     | NH                   | NT/ccrAB2    |
| DEN176 | Denmark   | +                       | 49 | ST84 | ACME-neg     | NH                   | IV           |
| ITL034 | Italy     | +                       | 66 | ST66 | ACME-neg     | NH                   | IV           |
| DEN110 | Denmark   | +                       | 66 | ST68 | ACME-neg     | NH                   | IV           |
| BDO932 | USA       | -                       | 5  | ST151| ACME-II.17   | mecA-neg/ccr-neg     |             |
| BDO920 | USA       | -                       | 5  | ST155| ACME-neg     | mecA-neg/ccr-neg     |             |
| BDO933 | USA       | -                       | 5  | ST156| ACME-neg     | mecA-neg/ccr-neg     |             |
| BDO910 | USA       | -                       | 5  | ST158| ACME-II.14   | mecA-neg/ccr-neg     |             |
| BUG37  | Bulgaria  | -                       | 5  | ST19 | ACME-neg     | mecA-neg/ccr-neg     |             |
| ITL299 | Italy     | -                       | 5  | ST32 | ACME-II.10   | mecA-neg/ccr-neg     |             |
| GRE53  | Greece    | +                       | 5  | ST39 | ACME-III.04  | NH                   | C/ccrAB2     |
| CV28   | Cape Verde| -                       | 5  | ST44 | ACME-II.06   | 3                     | mecA-neg/ccr-neg |
| HFA6226| Portugal  | -                       | 5  | ST60 | ACME-neg     | mecA-neg/ccr-neg     |             |
| CV13   | Cape Verde| +                       | 5  | ST65 | ACME-neg     | NT/ccrAB2,ccrAB4     |             |
| CV20   | Cape Verde| +                       | 5  | ST72 | ACME-I.05    | 3                     | IV           |
| HFA6391| Portugal  | -                       | 5  | ST73 | ACME-neg     | mecA-neg/ccr-neg     |             |
| MCO151 | Mexico    | +                       | 5  | ST82 | ACME-neg     | NH                   | IV           |
| HFA6096| Portugal  | -                       | 5  | ST90 | ACME-neg     | mecA-neg/ccrC        |             |

1 “+” methicillin-resistant *S. epidermidis; “−”, methicillin-susceptible *S. epidermidis.
2 CC, clonal complex, as previously defined by eBURST analysis [15,16]; 5, singleton; 6MLST, multilocus sequence typing [14]; 7-loci allelic profile listed in parenthesis (arc-opp-arc-aroE-gtr-mutS-pyrR-tpiA-yqiL).
3 ACME PCR scan method [5] defines genetic variants within 3 ACME allotypes: ACME-I contains arc and opp-3 gene clusters; ACME-II contains arc but not opp-3; and ACME-III contains opp-3 but not arc. ACME-neg is negative for both arc and opp-3. Amplicons from the ACME PCR scan for 5 underlined ACME-I/02 variants were used for DNA sequencing and construction of 25-kb contigs. 4NH, no hybridization with arcCB probe; Clal-arcC banding patterns shown in Figure 3; 5SCCmec typing to identify class A, B, C, and other non-typeable (NT) mec gene complex, and 5 types of ccr gene complex, ccrAB1, ccrAB2, ccrAB3, ccrAB4, ccrC. SCCmec type I contains B/ccrAB1; type II A/ccrAB2; type III A/ccrAB3; type IV B/ccrAB2; and type V C/ccrC.

Table 1. Cont.

| Strain | Country | Methicillin resistance | CC | MLST | ACME PCR Scan | Clal-arcC/B pattern | SCCmec typing |
|--------|---------|------------------------|----|------|--------------|---------------------|--------------|

arcCB probe in the *S. epidermidis* 1457 parental strain, whereas a corresponding 180-kb fragment did not hybridize with the *arcCB* probe in the ACME-excision mutant. This corresponds to a deletion of approximately 60-kb DNA fragment, which probably contains another SCC-like element mobilizable by CcrAB in addition to ACME (typically 30-kb in size). This is reminiscent of a CcrAB-mediated mobilization of 55-kb of DNA encompassing both SCCmec and ACME in *S. aureus* clone USA300 [5]. There was no difference in the in vitro growth rate of *S. epidermidis* 1457 and its ACME excision mutant, confirming the finding in *S. aureus* that carriage of ACME does not engender a biological fitness cost [5].

Genetic Diversity of ACME in *S. epidermidis*

For initial characterization of the genetic diversity of ACME found in *S. epidermidis* isolates, chromosomal DNA was digested with a frequent-cutting restriction enzyme *ClaI* and screened for restriction fragment length polymorphisms near the *arc* gene cluster by hybridization with a probe encompassing the ACME-encoded *arcC* and *arcB* genes. We selected 39 (64%) isolates positive for ACME-encoded *arcC* for this analysis. Since a single restriction site for *ClaI* is observed within *arcB* fragment (inside *arcC*), the hybridization band patterns obtained typically contained two bands, a constant 1.2 kb band, corresponding to a *ClaI* site upstream of this gene (Figure 3), and the other band varying in size between 7 and 9 kb, corresponding to a variable *ClaI* site upstream of this gene (Figure 3). A total of seven different *ClaI-arcCB* DNA restriction band patterns were identified among the 39 *S. epidermidis* strains carrying ACME (Table 1). Of these, *ClaI-arcCB* pattern-6 was the most common (n = 19), followed by pattern-4 (n = 8), pattern-7 (n = 6), patterns-2 and -5 (n = 2 each) and patterns-1 and -3 (n = 1 each).
To further characterize genetic diversity of ACME among the *S. epidermidis* isolates, we used a PCR-based scanning method for amplification of 30 overlapping segments of 1–2 kb in length spanning to the entire archetypal ACME found in USA300; this method allows for a comprehensive assessment of gene content, gene synteny and other structural features of ACME [5]. Among the 65 isolates containing either ACME-encoded arcA or opp-3 genes, 39 distinct PCR scan patterns or variants were identified (Table 1). Of these, 66% (43/65) were classified as ACME-I allotype because they contained both arc and opp-3 gene clusters. Only a single subtype of ACME-I, designated ACME-I variant 02 (abbreviated ACME-I.02), was found in 42% (27/65) of the isolates; the remaining ACME-I subtypes (i.e. subtypes ACME-I.03 to ACME-I.18) were represented by one isolate each. Additionally, there were 18 distinct PCR scan patterns represented by one isolate each that were classified as ACME-II (containing arc but not opp-3 gene cluster) and 4 patterns that were classified as ACME-III (containing opp-3 but not arc gene cluster).

ACME-I.02 was found in *S. epidermidis* isolates recovered from diverse locations, including Argentina, Denmark, Iceland, Hungary, Portugal, Poland and United States. Sequencing of the amplicons that resulted from the PCR scan of five ACME-I.02-positive *S. epidermidis* isolates from different countries yielded a 24,605-bp contig encompassing both the arc and opp-3 gene clusters (Table 1). This ACME-I.02 contig from diverse *S. epidermidis* differed from the archetypal ACME type I variant 01 (abbreviated ACME-I.01) found in USA300 in only 11 nucleotides that corresponded to the open reading frames SUSA300_0048 to SUSA300_0077 (GenBank accession number NC007793). From the eleven variant sites found, 10 were single nucleotide polymorphisms (6 non-synonymous mutations, 3 synonymous mutations, 1 mutation in non-coding region), and one site involved an inframe insertion/deletion of a 6-bp within a transposase-encoding sequence (SUSA300_0060). Altogether, these results showed that the prevalent ACME-I.02 type in *S. epidermidis* is nearly identical to the ACME-I.01 found in USA300, indicating a recent common origin.

**Estimated Frequency of Horizontal Acquisition of ACME-I.02 in the CC2 Lineage**

All 27 *S. epidermidis* isolates containing ACME-I.02 belonged to the prevalent CC2 lineage (Table 1). ACME-I.02 was distributed unevenly between the two clusters that comprised CC2: cluster I of CC2 (abbreviated CC2-I) contains 21 (78%) isolates, and cluster II of CC2 (CC2-II) contains 6 (22%) isolates. To estimate the
number of independent horizontal acquisitions of ACME-I.02 within CC2, an evolutionary model was constructed based on the genetic relationships revealed by the eBURST when applied to MLST data (see Figure 4 and Table 1). According to the model proposed, ACME-I.02 was estimated to have been acquired at least on 15 different occasions by strains belonging to CC2 lineage, suggesting frequent mobility of ACME-I.02 within but not beyond this S. epidermidis lineage.

Discussion

In the present study we found that 52% (65/127) of S. epidermidis isolates representing the broad genetic and geographic diversity of the species contained one of three ACME allotypes. There were extensive genetic diversity found in ACME islands of S. epidermidis, with 39 distinct variants identified by a PCR-based scanning method. Only one of these variants was represented by more than one isolate in the S. epidermidis population; this variant, ACME-I.02, contained both the arc and opp-3 gene clusters. All the other variants of ACME are likely to derive from the ancestral ACME-I.02 variant. ACME-I.02 was found in 21% (27/127) of the isolates recovered from seven countries. Importantly, a 24-kb DNA fragment of ACME-I.02 in five S. epidermidis isolates was virtually identical to a homologous contig of the ACME-I.01 variant found in USA300, suggesting the interspecies transfer of ACME from S. epidermidis into USA300. A similar observation was made for the interspecies transfer of SCCmec type IV from S. epidermidis strains to S. aureus, indicating that S. epidermidis provides a reservoir for genetic exchange with S. aureus [3].

The observation that the nearly identical ACME-I.01 and ACME-I.02 variants are prevalent among the most widely disseminated lineages of S. aureus (i.e., USA300) and S. epidermidis (i.e., CC2) suggests that these specific ACME allotypes may confer a particularly high biological fitness advantage. Several lines of evidence suggest that this fitness advantage is not associated to a higher capacity of causing disease. The high prevalence of ACME among Staphylococcus species that are common commensals of the human skin, e.g., S. epidermidis, S. capitis and S. haemolyticus [4,9], together with the fact that ACME was not found specifically associated with disease-causing isolates when compared to.
colazoning isolates of *S. epidermidis* and *S. haemolyticus* [9], suggest that this element is unlikely to contribute to the capacity of coagulase-negative staphylococci to cause disease in humans. Moreover, ACME was found not to contribute to the capacity of USA300 to cause skin abscess and necrotizing pneumonia in rat infection models [8]. An often overlooked feature of bacterial pathogenicity is the capacity to grow and survive within the host, thereby allowing for enhanced transmission. In this regard, ACME was shown to contribute to the growth and survival of USA300 in the rabbit and in the gastrointestinal tract of the mouse [5,10]. Furthermore, USA300 was found to be frequently recovered from axilla, inguinal, perineum and rectum [17,18], which are not common sites of colonization for *S. aureus*. These body sites are usually colonized ubiquitously by *S. epidermidis*, *S. capitis* and *S. haemolyticus*, which exhibit a high frequency of ACME carriage [19,20]. The acquisition of ACME by *S. aureus* might have allowed for the expansion of its typical colonization niches, providing new opportunities for transmission and dissemination. Altogether, these findings point to a potential role of ACME in conferring a fitness advantage for colonization and transmission rather than an enhanced capacity for infection.

These data also provide evidence for extensive intraspecies transfer of ACME, SCCmec, and other SCC elements among *S. epidermidis*, perhaps owing to the fact that 81% of the *S. epidermidis* population carry ccr gene complexes (Table 1 and Figure 1). Particularly, a high rate of intraspecies transfer of ACME-I.02 variant within the CC2 lineage was observed, which could be explained not only by the multiple ccr gene complexes frequently carried by these strains, but also to an enhanced capacity to accommodate multiple mobile elements, including ACME, SCCmec and other SCC elements, within the orfX [13]. This together with a high rate of recombination events, previously observed to occur frequently within CC2 lineage [15], may allow for the generation of the extensive genetic diversity among ACME islands afforded by strains belonging to this clonal lineage.

Although the role of *S. epidermidis* species as reservoir and donor of virulence and antibiotic resistance determinants to *S. aureus* is becoming unequivocal, the circumstances that favor the transfer of SCC elements between these two species is not completely understood and should be the focus of future studies. The understanding of the mechanisms and physiological conditions in which such transfer occur would provide us with fundamental tools to help to prevent the emergence of epidemic MRSA strains such as USA300.

**Author Contributions**

Conceived and designed the experiments: HiL GFS BAD. Performed the experiments: MM JH JL KIW KAK BAD. Analyzed the data: MM HeIL JH PS JL KIW KAK GFS BAD. Contributed reagents/materials/analysis tools: MM HeIL FPR HFC PS MO GFS BAD. Wrote the paper: MM HeIL BAD.

**References**

1. Jaffe HW, Sweeney HM, Nathan C, Weinstein RA, Kabins SA, et al. (1980) Identity and interspecific transfer of gentamicin-resistance plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis*. J Infect Dis 141: 736–747.
2. Wieders GL, Vriend MR, Brisse S, de Graaf-Malterberg LA, Troebstra A, et al. (2001) In-vivo transfer of meca DNA to *Staphylococcus aureus* [corrected]. Lancet 357: 1674–1675.
3. Würlinghoff H, Rosato AE, Enright MC, Noto M, Craig W, et al. (2003) Related Clones Containing SCCmec Type IV Predominate among Clinically Significant *Staphylococcus epidermidis* Isolates. Antimicrob Agents Chemother 47: 3574–3579.
4. Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, et al. (2006) Complete genome sequence of *S.*USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet 367: 731–739.
5. Diep BA, Stone GG, Basuino I, Graber CJ, Miller A, et al. (2008) The arginine catabolic mobile element and *staphylococcus chromosomal cassette mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 197: 1525–1530.
6. Goring RV, McDougall LK, Fosheim GE, Bonnstetter KK, Wolter DJ, et al. (2007) Epidemiologic Distribution of the Arginine Catabolic Mobile Element (ACME) Among Selected Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Isolates. J Clin Microbiol. 45:1153-1157.
7. Ellington MJ, Yearwood L, Ganner M, East C, Kearns AM (2008) Distribution of the ACME-arcA gene among methicillin-resistant *Staphylococcus aureus* from England and Wales. J Antimicrob Chemother 61: 73-77.
8. Montgomery CP, Boyle-Vavra S, Daum RS (2009) The Arginine Catabolic Mobile Element (ACME) is not associated with enhanced virulence in experimental invasive disease caused by the community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) genetic background USA300. Infect Immun. 77: 3622-3631.
9. Pi B, Yu M, Chen Y, Yu Y, Li L (2009) Distribution of the ACME-arcA gene among methicillin-resistant *Staphylococcus haemolyticus* and identification of a novel ccrX-like allele in CA-MRSA-positive isolates. J Med Microbiol 58: 731–736.
10. Kelley KE, Weidner ML, Diep BA, Lee JC (2008) Gastrointestinal colonization by *Staphylococcus aureus* Modes for Wall Teichoic Acids and Arginine Catabolic Mobile Element. International Symposium on Staphylococci and Staphyloccal Infections, Cairns, Australia [http://wwwwissi2008com/abstract/284asp last accessed 05-29-2009].
11. de Lencastre H, Couto I, Santos I, Melo-Cristino J, Torres-Perreira A, et al. (1994) Methicillin-resistant *Staphylococcus aureus* disease in a Portuguese hospital: characterization of clonal types by a combination of DNA typing methods. J Infect Dis 170: 64–73.
12. Kondo Y, Ito T, Ma XX, Watanabe S, Kriegsbrich BN, et al. (2007) Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. Antimicrob Agents Chemother 51: 264–274.
13. Miragaya M, Couto I, de Lencastre H (2005) Genetic diversity among methicillin-resistant *Staphylococcus epidermidis* (MRSE). Microb Drug Resist 11: 83–93.
14. Thomas JC, Vargas MR, Miragaya M, Peacock SJ, Archer GL, et al. (2007) Improved multiplex sequence typing scheme for *Staphylococcus epidermidis*. J Clin Microbiol 45: 616–619.
15. Miragaya M, Thomas JC, Couto I, Enright MC, de Lencastre H (2007) Inferring a population structure for *Staphylococcus epidermidis* from multilocus sequence typing data. J Bacteriol 189: 2540–2552.
16. Miragaya M, Carriço JA, Thomas JC, Couto I, Enright MC, et al. (2008) Comparison of molecular typing methods for characterization of *Staphylococcus epidermidis* epidemic proposal for clone definition. J Clin Microbiol 46: 118–129.
17. Miller LG, Diep BA (2008) Clinical practice: colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis 46: 752–760.
18. Wener KM, Gold HS, Wong M, Venkataraman L, Mayer KH, et al. (2006) High prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization in an urban outpatient population. Abstr 44th Ann Meet Infect Dis Soc Am Infectious Disease Society of America, Alexandria, VA p.118.
19. Noble WC (1981) Microbiology of Human Skin. London: Lloyd-Luke;ISBN 0-443-07324-150-3.
20. Lina G, Etienne J, Vandenesch F (2000) Biology and pathogenicity of *Staphylococcus aureus*. Roles for Wall Teichoic Acids and Arginine Catabolic Mobile Element. In: Rood (ed), Gram-positive pathogens ASM Press, Washington, DC: p. 450–462.