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High prevalence of the arginine catabolic mobile element in carriage isolates of methicillin-resistant *Staphylococcus epidermidis*

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Background: The arginine catabolic mobile element (ACME) associated with staphylococcal cassette chromosome *mec* (SCC*mec*) in the USA300 clone of community-acquired methicillin-resistant *Staphylococcus aureus* enhances its fitness and ability to colonize the host. *Staphylococcus epidermidis* may act as a reservoir of ACME for *S. aureus*. We assessed the diffusion of ACME in methicillin-resistant *S. epidermidis* (MRSE) isolates colonizing outpatients.

Methods: Seventy-eight MRSE strains isolated in outpatients from five countries were characterized by multilocus sequence typing (MLST) and SCC*mec* typing and screened for the *arcA* and *opp3AB* markers of ACME. ACME-*arcA* and ACME-*opp3AB* were sequenced. ACME type I from MRSE and USA300 were compared by long-range PCR (LR-PCR).

Results: Fifty-three (67.9%) MRSE strains carried an ACME element, including 19 (24.4%), 32 (41.0%) and 2 (2.6%) with ACME type I (*arcA*+/*opp3AB*+), II (*arcA*+/*opp3AB*−) and III (*arcA*−/*opp3AB*+), respectively. The prevalence of ACME did not differ between clonal complex 2 (42/60 strains) and other sequence types (11/18 strains, $P=0.7$), with MLST data suggesting frequent intraspecies acquisition. ACME-*arcA* sequences were highly conserved, whereas ACME-*opp3AB* displayed 11 distinct allotypes. ACME was found in 14/29, 9/11 and 30/37 strains with type IV, type V and non-typeable SCC*mec*, respectively ($P=0.01$). ACME was more frequently associated with *ccrC* than with *ccrAB2* (82.4% versus 60.0%, $P=0.048$). LR-PCR indicated structural homologies of ACME I between MRSE and USA300.

Conclusions: ACME is widely disseminated in MRSE strains colonizing outpatients and may contribute to their spread in a community environment with low antibiotic exposure, as suggested for USA300.

Keywords: community-acquired methicillin-resistant *Staphylococcus aureus*, USA300, horizontal transfer, cassette chromosome recombinase, *ccr*, *orfX*, eBurst, multilocus sequence typing (MLST)

Introduction

The arginine catabolic mobile element (ACME) is a novel class of staphylococcal genetic island that was first described in the epidemic USA300 clone of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).[1,2] ACME was subsequently reported in isolates from other *S. aureus* lineages and several coagulase-negative staphylococci (CoNS) species, namely, *Staphylococcus haemolyticus*, *Staphylococcus capitis* and *Staphylococcus epidermidis*.[1,3–8] Three ACME allotypes are currently described. Type I contains both the *arcR/A/D/B/C* gene cluster, differing from the native chromosomal *arc* cluster of staphylococci and encoding a complete, additional arginine deiminase pathway, and the *opp3A–E* cluster, a putative oligopeptide permease operon. Type II carries the *arc* locus but not *opp3*, while type III contains *opp3* without *arc*.[1,7]

S. epidermidis is a major component of the normal human skin and mucosal staphylococcal flora, including the nasal microbiota.[9,10] The precise functions of ACME-encoded genes have not yet been investigated in this species. However, the archetypal ACME variant I-0.1 carried by USA300 improves both its fitness and its ability to colonize the skin and the mucosal

surfaces,[1,3,7,11] thereby appearing as a significant determinant of its successful spread.[12] ACME may provide *S. epidermidis* with a similar selective advantage in terms of host colonization and transmission capacities.[1,7] Besides, recent data suggest that ACME I has been transferred from *S. epidermidis* to the USA300 lineage of CA-MRSA, indicating that this species probably acts as a reservoir of ACME for *S. aureus*.[7]

S. epidermidis is increasingly recognized as a causative pathogen of various community-acquired diseases, such as native valve endocarditis and late-onset infections of indwelling medical devices.[13–15] In this context, the current dissemination of methicillin-resistant strains of *S. epidermidis* (MRSE) in the community elicits significant concerns.[16–19] ACME shares common features with staphylococcal cassette chromosome *mec* (SCC*mec*), a class of mobile genetic elements carrying *mecA*, the determinant of methicillin resistance in staphylococci. Both are flanked by homologous inverted- and direct-repeat (IR/DR) sequences that recombine with the *attB* site of *orfX*, resulting in their integration into this chromosomal open reading frame, and both are mobilized by the SCC-encoded cassette chromosome recombinases (*ccr*),[1,3,7] suggesting that horizontal transfers of ACME and SCC*mec* are linked. To date, the dissemination of ACME within the *S. epidermidis* species has scarcely been assessed,[7] and has not been specifically investigated in MRSE strains spreading in the community. Thus, it remains unknown whether ACME contributes to their dissemination in an environment with relatively low antibiotic exposure when compared with the hospital setting, as proposed for USA300.[12] In this study we report the distribution of ACME allotypes in MRSE strains prospectively isolated from outpatients as part of a multinational survey of methicillin-resistant CoNS dissemination outside the hospital setting.[17,19]

Methods

MRSE strains

Seventy-eight non-duplicate carriage strains of MRSE from five countries (Algeria, $n=22$; Cambodia, $n=24$; France, $n=15$; Mali, $n=9$; and Moldova, $n=8$) were included in this study. These strains were randomly selected from a collection of MRSE strains prospectively collected between March 2005 and October 2006 by nasal swabs in adult outpatients on admission to either emergency departments (Algeria, Cambodia, Mali, Moldova) or an orthopaedic surgery ward (France) within 8 h following hospital admission. Procedures of isolation, epidemiological data and a description of SCC*mec* diversity concerning this collection of MRSE have been reported elsewhere.[17,19]

SCCmec typing

ccr (*ccrAB1* to *ccrAB4*, and *ccrC*) and *mec* (classes A, B and C) gene complexes were typed by multiplex PCR,[20,21] enabling the characterization of SCC*mec* elements as type I/1B (i.e. *ccrAB1*-Class B *mec*), type II/2A, type III/3A, type IV/2B, type V/5C2, type VI/4B and type VIII/4A, according to the current nomenclature used for MRSA.[20,21] MRSA strains COL/SCC*mec* type I, BK2464/SCC*mec* type II, ANS46c/SCC*mec* type III, USA300-FPR3757/SCC*mec* type IV(a), WCH100/SCC*mec* type V and HDE288/SCC*mec* type VI were used as reference. SCC*mec* IV were subtyped as IVa, IVb, IVc, IVd and non-subtypeable (IVnst) by multiplex PCR.[20] Non-typeable (NT) SCC*mec* were defined by the absence of typeable *ccr* allotype or an undescribed *ccr-mec* combination.

Multilocus sequence typing (MLST) and eBURST

MLST was performed by sequencing internal regions of seven housekeeping genes (*arcC*, *aroE*, *gtr*, *mutS*, *pyr*, *tpi* and *yqiL*).[22] Sequence types (STs) were determined using the MLST database and characterized as singletons or members of a clonal complex (CC) by the eBURST algorithm (accessible at http://eburst.mlst.net). Numbers for new alleles and STs reported here were assigned by the *S. epidermidis* MLST database curator.

Screening and typing of ACME

ACME-*arcA* and ACME-*opp3AB* genes were used as markers of the ACME-*arc* cluster and the ACME-*opp3* cluster, respectively.[3] PCR screening was performed using primers arcA-F/arcA-R for ACME-*arcA* and primers AIPS45/AIPS46 for ACME-*opp3AB* (Table 1). CA-MRSA strain USA300-FPR3757 was used as positive control for both PCRs. Amplicons were revealed after migration in 1.7% agar Tris-acetate EDTA 0.5× (TAE 0.5×) using SybrSafe (Invitrogen, Cergy-Pontoise, France) as a double-strand DNA marker and 1 kb+ (Invitrogen) as a DNA size ladder. ACME

Table 1. Primers and reference sequences used for LR-PCR amplification of ACME type I in MRSE

Primers and corresponding regions	Reference	Constructed on	5′-3′ sequence	Reference sequences		
				strain[a]	start position, nt	length, bp
LR-PCR 1 (overlapping the ACME-*arc* locus)						
SC36366U	this study	*arcB*	ACATTCCACCTAAAACACGAGC	USA300-FPR3757	70537	3533
arcA-F	this study	*arcA*	GAGCCAGAAGTACGCGAG	USA300-FPR3757	74111	
LR-PCR 2 (between ACME-*arc* and ACME-*opp3*)						
arcA-R	this study	*arcA*	CACGTAACTTGCTAGAACGAG	USA300-FPR3757	73388	9095
AIPS46	3	*opp3B*	GAAGATTGGCAGCACAAAGTG	USA300-FPR3757	82483	
LR-PCR 3 (between ACME-*opp3* and the ACME chromosome junction)						
AIPS45	3	*opp3A*	GCAAATCTGTAAATGGTCTGTT	USA300-FPR3757	81301	—
SE130-360	19	*SE130*	GATTGTTTTATTTAGCGGCGAGC	ATCC 12228	126481	

[a]GenBank accession numbers of MRSA strain USA300-FPR3757 and MSSE strain ATCC 12228 are CP000255 and AE015929, respectively.

were typed as type I (i.e. containing both the *arc* and the *opp3* gene cluster), type II (*arc* without *opp3*) and type III (*opp3* without *arc*).[7]

Sequencing of ACME-arcA and ACME-opp3AB

ACME-*arcA* (671 bp) and ACME-*opp3AB* (1183 bp) were sequenced in all positive strains using the primers used for PCR. Two additional internal primers designed on the *opp3AB* gene sequenced in USA300-FPR3757 (GenBank accession number CP000255, locus SAUSA300_0074) and designated opp3-I1 (5′-TGGGTTGGACATGCACTYACGGG-3′) and opp3-I2 (5′-CCCGTRAGTGCATGTCCAACCCA-3′) were used for *opp3AB* sequencing. DNA sequences were obtained with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The BioEdit Biological Sequence Editor 5.0.6 software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) was used for alignment and comparison with reference sequences (indicated as strain/GenBank accession number/5′-3′ nt position) of ACME-*arcA* (MRSA USA300-FPR3757/CP000255/73397-74067 and MSSE ATCC 12228/AE015929/102520-103190), chromosomal *arcA* [MRSA USA300-FPR3757/CP000255/2781998-2782668, MRSA COL/CP000046/2718736-2719406, MRSA Mu50/BA000017/2789601-2790271, MRSA N315/BA000018/2725887-2726557, methicillin-susceptible *S. epidermidis* (MSSE) ATCC 12228/AE015929/2270393-2271063 and MRSE RP62A/CP000029/2277897-2278567], and ACME-*opp3AB* (MRSA USA300-FPR3757/CP000255/81369-82475). Phylogenic analysis was carried out using a neighbour-joining algorithm (Kimura-2 Parameter Distance Estimation) as implemented by the MEGA v4.0 software.[23]

Structural analysis of ACME type I by long-range PCR (LR-PCR)

ACME type I was compared by LR-PCR with the one described in MRSA strain USA300-FPR3757 in terms of orientation and size of the ACME-*arc* locus (LR-PCR 1), the region between ACME-*arc* and ACME-*opp3* (LR-PCR 2) and the region between ACME-*opp3* and the ACME chromosome junction (LR-PCR 3). *SE130*, the open reading frame located immediately downstream of *orfX* on the *S. epidermidis* chromosome,[24] was used as the left anchor for LR-PCR 3. Primers and reference sequences are listed in Table 1. All LR-PCRs were performed by using the GeneAmp® XL PCR Kit (Applied Biosystems, Foster City, CA, USA), with an initial denaturation step (94°C, 4 min), 10 cycles of denaturation (94°C, 15 s), annealing (55°C, 30 s) and extension (68°C, 7 min), followed by 25 cycles of denaturation (94°C, 15 s), annealing (55°C, 30 s) and extension (68°C, 7–10 min, with a 7 s increment per cycle), and a final extension step (68°C, 10 min). Amplicons were analysed after migration in 1% agar TAE 0.5× using SybrSafe as a double-strand DNA marker and 1 kb+ (Invitrogen) as a DNA size ladder. USA300-FPR3757 was used as positive control for LR-PCR 1 and 2. No reference strain of *S. epidermidis* carrying ACME type I was available to serve as a positive control for LR-PCR 3.

Statistical analysis

Prevalences of ACME-*arcA* and ACME-*opp3AB* among MRSE strains were compared on the basis of geographical origin, MLST data (CC2 versus other lineages), SCC*mec* types and *ccr* allotypes by the χ^2 test using Epi-Info v3.2.2 software (CDC, Atlanta, GA, USA). A P value <0.05 was considered significant.

Results

MLST data

MLST analysis of the 78 MRSE strains identified 35 distinct STs, including 18 new ones [Figure 1 and Figure S1 (available as Supplementary data at *JAC* Online)]. ST59 ($n=9$ strains), ST5 ($n=7$), ST193 ($n=6$), ST57 ($n=5$), ST89 ($n=5$) and ST2 ($n=4$) were the most common STs. Twenty-six STs, accounting for 60 strains (76.9%), were part of CC2. Three STs (ST66, ST187 and ST202), each accounting for one strain, were defined as part of duplets, groups of two STs that differ by only one of the seven MLST loci and not included in other CCs. The remaining six STs were singletons ($n=15$), defined as STs differing at two or more alleles from every other ST form in the *S. epidermidis* MLST database. CC2 included strains from the five countries. Conversely, a few singletons were identified only in strains from a single country, e.g. ST192 and ST193 for Cambodia, and ST226 for Mali.

ACME-arcA and ACME-opp3AB screening

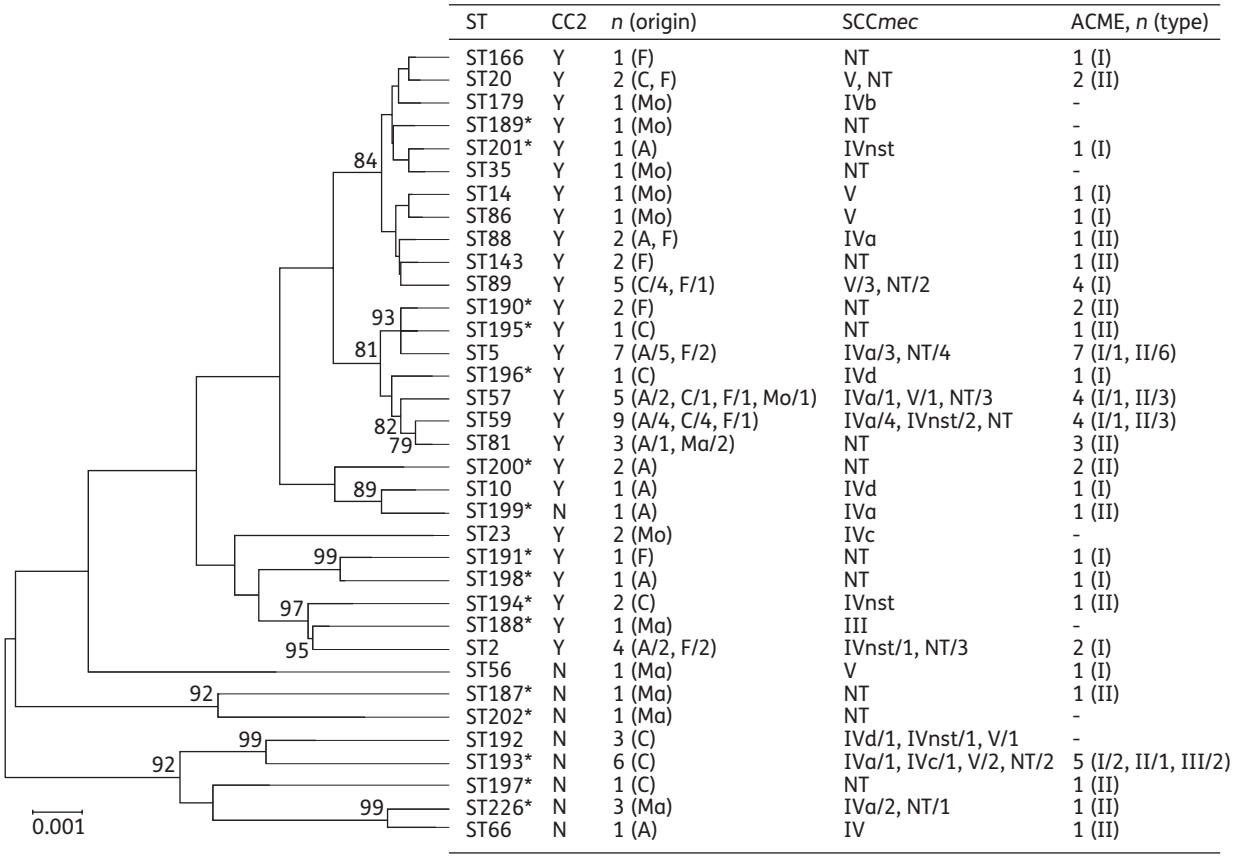
Of all MRSE strains studied, 51 (65.4%) and 16 (20.5%) carried ACME-*arcA* and ACME-*opp3AB*, respectively. The prevalence of ACME-*arcA* among MRSE strains differed significantly from one country to another (Algeria, 90.9%; Cambodia, 50.0%; France, 73.3%; Mali, 55.5%; Moldova, 37.5%; $P=0.01$). ACME-*opp3AB* carriage ranged from 11.1% in strains from Mali to 33.3% in strains from France and Cambodia ($P=$NS). Nineteen (24.4%), 32 (41.0%) and 2 (2.6%) MRSE strains carried an ACME type I, II and III, respectively. The distribution of ACME types according to MLST data is shown in Figure 1. ACME-*arcA* and ACME-*opp3AB* were identified in strains from numerous distinct STs, including new singletons described in this study. Neither these two loci nor ACME types were significantly associated with CC2. Most notably, there was no significant difference in the carriage of ACME type I between strains from CC2 and strains from other lineages (16/60 and 3/18, respectively, $P=0.58$).

Distribution of ACME by SCCmec types

SCC*mec* were characterized as type III ($n=1$ strain), type IV ($n=29$), type V ($n=11$) and NT ($n=37$) (Table 2). Among the 37 strains with NT SCC*mec*, 26 carried *ccrAB2* (including 24 with multiple *ccr*) and 22 carried *ccrC* (including 21 with multiple *ccr*). Overall, an ACME was carried by 14 (48.3%) strains with an SCC*mec* IV, 9 (81.8%) strains with an SCC*mec* V and 30 (81.1%) strains with an NT SCC*mec* ($P=0.01$). ACME type I was more frequent in strains carrying an SCC*mec* V than in those with other SCC*mec* types (54.5% versus 19.4%, $P=0.03$). There was no significant association between ACME types II/III and SCC*mec* types. When considered by *ccr* recombinase allotypes, the prevalence of ACME was higher in strains carrying *ccrC* than in those with *ccrAB2* (82.4% versus 60.0%, $P=0.048$).

ACME-arcA sequencing

ACME-*arcA* sequences (671 bp) from the 51 positive MRSE strains were classified in four allotypes designated allotypes a to d. These ACME-*arcA* sequences constituted one phylogenetic group clearly separated from those of *S. epidermidis* and *S. aureus* native *arcA* (Figure 2). All but one (C229-2, allotype d, 22 point substitutions) of the 51 positive MRSE strains harboured an ACME-*arcA* (allotypes a, b and c) displaying >99.7% nt identity with its counterpart in USA300-FPR3757. Seven strains (from France, Cambodia and Moldova, $n=2$ for each origin, and from Algeria, $n=1$) carried the same ACME-*arcA* (allotype a) as



ST	CC2	*n* (origin)	SCC*mec*	ACME, *n* (type)
ST166	Y	1 (F)	NT	1 (I)
ST20	Y	2 (C, F)	V, NT	2 (II)
ST179	Y	1 (Mo)	IVb	-
ST189*	Y	1 (Mo)	NT	-
ST201*	Y	1 (A)	IVnst	1 (I)
ST35	Y	1 (Mo)	NT	-
ST14	Y	1 (Mo)	V	1 (I)
ST86	Y	1 (Mo)	V	1 (I)
ST88	Y	2 (A, F)	IVa	1 (II)
ST143	Y	2 (F)	NT	1 (II)
ST89	Y	5 (C/4, F/1)	V/3, NT/2	4 (I)
ST190*	Y	2 (F)	NT	2 (II)
ST195*	Y	1 (C)	NT	1 (II)
ST5	Y	7 (A/5, F/2)	IVa/3, NT/4	7 (I/1, II/6)
ST196*	Y	1 (C)	IVd	1 (I)
ST57	Y	5 (A/2, C/1, F/1, Mo/1)	IVa/1, V/1, NT/3	4 (I/1, II/3)
ST59	Y	9 (A/4, C/4, F/1)	IVa/4, IVnst/2, NT	4 (I/1, II/3)
ST81	Y	3 (A/1, Ma/2)	NT	3 (II)
ST200*	Y	2 (A)	NT	2 (II)
ST10	Y	1 (A)	IVd	1 (I)
ST199*	N	1 (A)	IVa	1 (II)
ST23	Y	2 (Mo)	IVc	-
ST191*	Y	1 (F)	NT	1 (I)
ST198*	Y	1 (A)	NT	1 (I)
ST194*	Y	2 (C)	IVnst	1 (II)
ST188*	Y	1 (Ma)	III	-
ST2	Y	4 (A/2, F/2)	IVnst/1, NT/3	2 (I)
ST56	N	1 (Ma)	V	1 (I)
ST187*	N	1 (Ma)	NT	1 (II)
ST202*	N	1 (Ma)	NT	-
ST192	N	3 (C)	IVd/1, IVnst/1, V/1	-
ST193*	N	6 (C)	IVa/1, IVc/1, V/2, NT/2	5 (I/2, II/1, III/2)
ST197*	N	1 (C)	NT	1 (II)
ST226*	N	3 (Ma)	IVa/2, NT/1	1 (II)
ST66	N	1 (A)	IV	1 (II)

Figure 1. Neighbour-joining tree constructed on concatenated sequences of the seven housekeeping gene fragments included in the *S. epidermidis* MLST scheme (total length, 2522 bp) and the corresponding distribution of SCC*mec* elements and ACMEs in 78 carriage isolates of MRSE. Asterisks indicate new STs described in this study. All STs not belonging to CC2 were singletons or part of duplets. Origin: A, Algeria; C, Cambodia; F, France; Ma, Mali; Mo, Moldova. ACME types were defined as type I (*arcA*+/*opp3AB*+), type II (*arcA*+/*opp3AB*−) and type III (*arcA*−/*opp3AB*+). Note that ST199 differs from CC2 STs (including ST10) for at least three of the seven alleles, explaining why ST199 does not belong to this CC according to the eBURST algorithm.

USA300-FPR3757. The remaining 43 strains showed a G73941A substitution, with (allotype b, $n=4$) or without (allotype c, $n=39$) an additional A73548G substitution. However, the 223 amino acid sequences deduced from allotypes a, b and c were identical. The distribution of ACME-*arcA* allotypes according to MLST data suggested frequent intraspecies transfers. Indeed, strains sharing a common ST were found to carry distinct ACME-*arcA* allotypes, e.g. allotypes a, b and d in strains belonging to singleton ST193 (Figure 2). Likewise, identical allotypes were found in strains from distinct STs within CC2, as in singletons not belonging to CC2.

ACME-opp3AB sequencing

The 1183 bp long sequences of ACME-*opp3AB* from the 21 positive MRSE strains split into 11 distinct allotypes, with 57 sites of point mutations (Figure 3). Four strains harboured an ACME-*opp3AB* gene displaying 100% nt identity with the one sequenced in USA300-FPR3757. These four strains also carried the same ACME-*arcA* (allotype a) as USA300-FPR3757. The 17 remaining strains carried ACME-*opp3AB* allotypes with 95%–98% identity when compared with their counterpart in USA300-FPR3757, with a number of nt substitutions ranging from 1 to 31.

LR-PCR of ACME type I

LR-PCR 1 yielded an ∼3.5 kb amplicon in all MRSE strains with ACME type I ($n=19$), indicating that they carried an ACME-*arc* locus similar in length and orientation to those harboured by MRSA strain USA300-FPR3757 and MSSE strain ATCC 12228 (reference length, 3533 bp). LR-PCR 2 did not provide any amplicon in four strains, including three strains with NT SCC*mec* and one with SCC*mec* V. An ∼9 kb amplicon was obtained in the remaining 15 strains with ACME type I as in USA300-FPR3757 (reference length, 9095 bp), suggesting a similar distance between ACME-*arcA* and ACME-*opp3AB*. The region between *opp3AB* and the chromosome junction was amplified by LR-PCR 3 in only 11 strains (2/2, 2/2, 4/6 and 3/9 strains with SCC*mec* IVa, IVd, V and NT, respectively), with amplicon sizes ranging from 6 to 9 kb. These results are compatible with a structural polymorphism on the 3′-end of ACME type I, or the insertion of another mobile genetic element downstream of ACME.

Table 2. Association of ACME and SCC*mec* in 78 carriage isolates of MRSE

SCC*mec*	MRSE strains, *n*	ACME, *n* (%)			
		total	type I	type II	type III
Types					
SCC*mec* III	1	—	—	—	—
SCC*mec* IV	29	14 (48.3)[a]	4 (13.8)	9 (31.0)	1 (3.4)
SCC*mec* V	11	9 (81.8)[a]	6 (54.5)[b]	3 (27.3)	—
NT SCC*mec*	37	30 (81.1)[a]	9 (24.3)	20 (54.0)	1 (2.7)
total	78	53 (67.9)	19 (24.3)	32 (41.0)	2 (2.6)
SCC*mec* IV subtypes					
SCC*mec* IVa	15	9 (60.0)	2 (13.3)	6 (40.0)	1 (6.7)
SCC*mec* IVb	1	0	—	—	—
SCC*mec* IVc	3	0	—	—	—
SCC*mec* IVd	3	2 (66.6)	2 (66.6)	—	—
SCC*mec* IVnst	7	3 (43.3)	—	3 (43.0)	—

NT SCC*mec*, non-typeable SCC*mec*; SCC*mec* IVnst: non-subtypeable SCC*mec* IV.
[a]$P=0.01$ for the global comparison between SCC*mec* types.
[b]$P=0.035$ for the comparison between SCC*mec* V and other SCC*mec* types.

GenBank accession numbers

The two new ACME-*arcA* allotypes described here are available at www.ncbi.nlm.nih.gov/GenBank under accession numbers HQ315759 to HQ315758 and HQ315759 for allotypes c and d, respectively. The 10 new ACME-*opp3AB* allotypes were deposited under accession numbers HQ315760 to HQ315776.

Discussion

We describe here the prevalence of ACME allotypes and their association with SCC*mec* elements and genomic background in 78 carriage strains of MRSE colonizing outpatients from five distinct geographical origins. Data on the diffusion of ACME within the species *S. epidermidis* are currently scarce.[1,7] Miragaia *et al.*[7] recently reported a 51% overall prevalence of ACME in a collection of 127 *S. epidermidis* isolates (including 79 carriage isolates). Whether these strains were isolated from hospitalized subjects or outpatients is not known. In our work, ACME was found to be widely disseminated in MRSE strains spreading out of the hospital setting, with 68% of them carrying one of the three allotypes described to date.

The archetypal ACME I-0.1 carried by the USA300 lineage of CA-MRSA does not act as a virulence factor,[25] but is associated with enhanced fitness and ability to colonize the skin and the mucosal surfaces.[3,11] Most notably, the additional arginine deiminase system encoded by the ACME-*arc* cluster may improve bacterial survival and growth by facilitating both pH regulation and ATP production in acidic environments such as the human skin.[12] In our work, ACME types I and II, i.e. containing an ACME-*arc* cluster, accounted for 96.2% of ACME-positive MRSE strains. ACME-*arcA* exhibited highly conserved sequences, and MLST analysis argued for frequent transfers and acquisitions involving strains from CC2 as well as strains from other phylogenic lineages (Figure 2). Moreover, we report here for the first time the carriage of ACME type I in strains not belonging to CC2. These observations, together with those currently available,[7] further suggest that the horizontal acquisition of ACME-*arc* may provide a selective advantage in terms of colonization ability for *S. epidermidis*, a major component of the normal human skin and nasal microbiota.

The potential benefit conferred by ACME-*opp3* remains less clear. ACME-*opp3* is a putative oligopeptide permease operon belonging to the same family as *opp1* and *opp2*, two natural, chromosomal operons that encode ABC transporters involved in nutrient uptake from the bacterial environment.[26] In our study, the prevalence of ACME-*opp3* in MRSE strains was relatively low when compared with ACME-*arc* (26.9% versus 65.4%, respectively). Moreover, ACME-*opp3AB* displayed a wide diversity of sequences, with 57 sites of point mutations yielding 11 distinct allotypes in the 21 positive strains, contrasting with the highly conserved feature of ACME-*arcA*. The fact that ACME-*opp3* is less prevalent and less conserved might indicate that this cluster is less crucial than ACME-*arc* in terms of fitness benefit for carriage strains of MRSE. This hypothesis is consistent with the findings obtained in ACME-positive MRSA lineages other than USA300 (e.g. ST5-USA100 and ST59-USA1000), which carry a highly conserved ACME-*arc* cluster without ACME-*opp3*.[3] *In vitro* studies are needed to clarify the precise functions of ACME-*arc* and ACME-*opp3* and to assess their respective impacts on the fitness of ACME-positive *S. aureus* and *S. epidermidis* strains.

To the best of our knowledge, this is the first study focused on the linkage between ACME and SCC*mec*, the mobile element carrying *mecA*, in MRSE isolates colonizing outpatients. Recent studies have emphasized the high prevalence of MRSE nasal carriage in non-hospitalized subjects,[16–19] including those with no previous exposure to the healthcare system.[19] This situation is worrisome given the growing number of reports on community-acquired diseases involving *S. epidermidis*, e.g. native valve endocarditis and late-onset infections of prosthetic heart valves, pacemakers and orthopaedic prostheses.[13–15] Horizontal transfer of ACME and SCC*mec* among staphylococci may depend on linked mechanisms. Indeed, these two classes of mobile genetic islands are integrated in the same chromosomal site, designated *orfX*, are flanked by homologous IR/DR sequences and are mobilized by the SCC-encoded *ccr* recombinases.[1,3,7] Thus, the acquisition of an SCC element, most notably SCC*mec*, could constitute an auspicious background for the chromosomal integration of ACME. The selective advantage conferred by ACME in terms of fitness and ability to colonize the host may subsequently enhance the spread of MRSE in an out-of-hospital environment largely devoid of antibiotic selection pressure (including β-lactams) when compared with the hospital setting, as suggested for the USA300 clone of CA-MRSA.[12] Whether ACME confers a similar benefit for MRSE in healthcare settings with large antibiotic use is plausible, but remains to be investigated.

ACME was found in MRSE strains displaying highly heterogeneous SCC*mec* patterns, including 37 strains carrying an NT SCC*mec* element (Table 2). Thus, ACME acquisition in *S. epidermidis* does not depend on the carriage of a given SCC*mec* type,

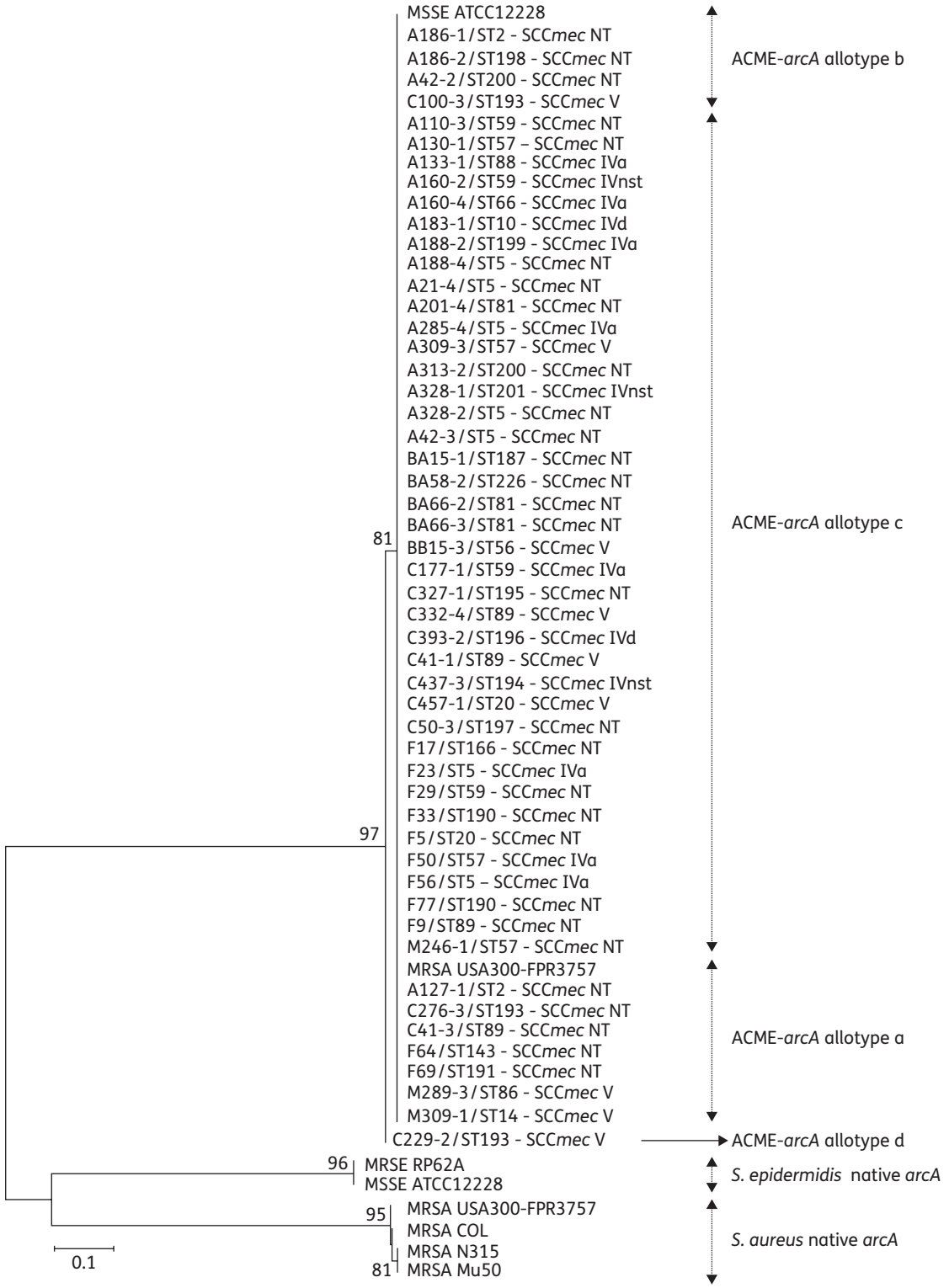


Figure 2. Neighbour-joining tree based on the comparison of 671 bp internal regions of ACME-associated *arcA* (ACME-*arcA*) genes from 51 MRSE strains and reference sequences of ACME-*arcA* and native *arcA* from *S. aureus* and *S. epidermidis*. ACME-*arcA* allotype a ($n=7$) was 100% homologous to its counterpart in MRSA strain USA300-FPR3757. ACME-*arcA* allotypes b and c displayed >99.7% nt identity with the one sequenced in USA300-FPR3757, with 1 and 2 nt substitutions, respectively. ACME-*arcA* allotype d displayed 22 point substitutions compared with allotype a and was only found in one strain.

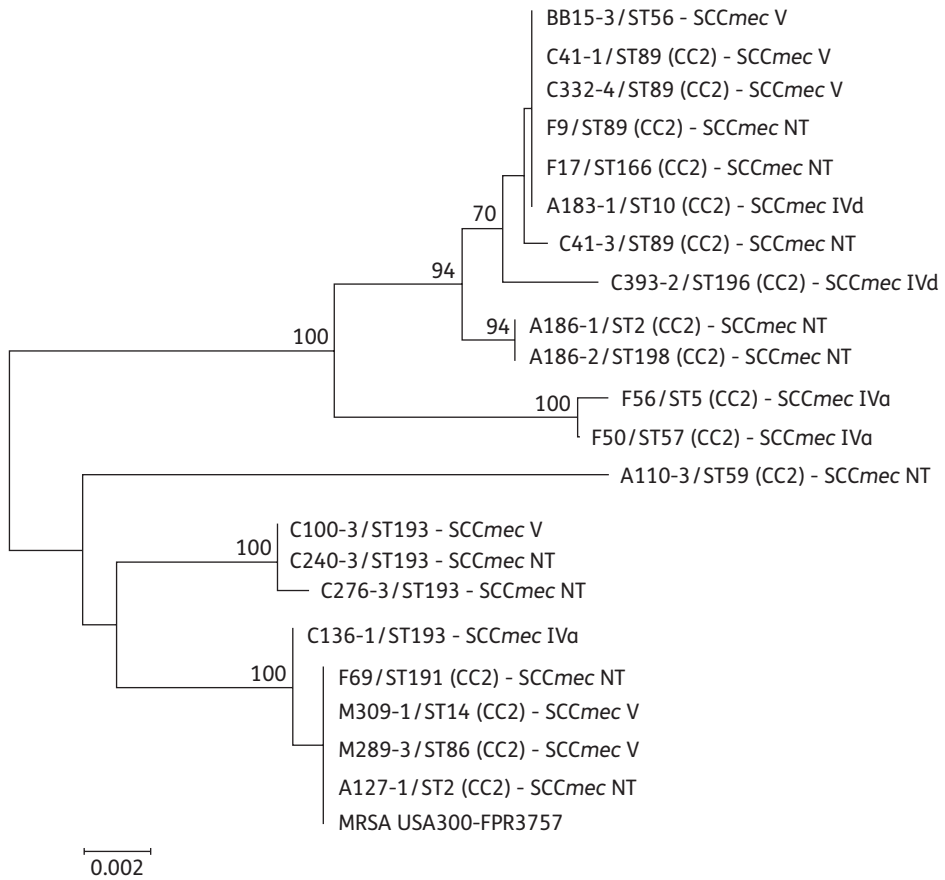


Figure 3. Neighbour-joining tree based on the comparison of 1183 bp internal regions of ACME-associated *opp3AB* (ACME-*opp3AB*) genes from 21 MRSE strains and MRSA strain USA300-FPR3757.

which is similar to the situation in MRSA, which may carry ACME in association with SCC*mec* type II, IVa, or V.[3–5] However, we found that ACME was more prevalent in strains carrying *ccrC*, with either a type V or an NT SCC*mec*, than in those carrying *ccrAB2*, the most common *ccr* recombinase allotype in *S. epidermidis* (82.4% versus 60.0%, respectively, $P=0.048$).[17] Even though we cannot exclude a fortuitous association, this result may suggest that the carriage of *ccrC* represents a more favourable background for ACME acquisition when compared with *ccrAB2*. Studies focusing on the mobilization of ACME in *S. epidermidis* are needed to further assess this hypothesis.

That USA300 had acquired ACME type I by horizontal transfer from *S. epidermidis* is strongly suspected.[1,7] Indeed, an ACME type I-0.2 variant displaying >99% nt identity with its counterpart in USA300 was recently reported in *S. epidermidis* isolates.[7] In our study, 19/78 MRSE strains (24.4%) harboured an ACME type I, and LR-PCR indicated structural homologies with the one carried by USA300 for 15 of them. These results may indicate that MRSE constitutes an important reservoir of ACME for *S. aureus* in the community. That we did not investigate the diffusion of ACME among carriage isolates of MSSE may represent a limitation of our study. Indeed, Miragaia *et al.*[7] recently reported a 64.7% prevalence of ACME in a collection of 34 MSSE isolates, with about one-third of them carrying one of the type I variants. These data suggest that MSSE could also act as a reservoir of ACME for *S. aureus*. Further studies are thus needed to assess the prevalence of ACME in MSSE isolates colonizing outpatients.

In conclusion, we found that the carriage of ACME is highly prevalent in MRSE strains colonizing outpatients, regardless of their SCC*mec* types, with further evidence of frequent intraspecies exchanges. These results bring new insights into the current context of MRSE spread out of the hospital setting. Indeed, by providing a selective advantage in terms of fitness and ability to colonize the host, ACME may enhance the dissemination of these MRSE strains in the community.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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