First Detailed Genetic Characterization of the Structural Organization of Type III Arginine Catabolic Mobile Elements (ACMEs) Harbored by *Staphylococcus epidermidis* using Whole Genome Sequencing

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ABSTRACT

The type III arginine catabolic mobile element (ACME) was detected in three *Staphylococcus epidermidis* oral isolates recovered from separate patients (one healthy, one healthy with dental implants and one with periodontal disease) based on ACME-*arc* operon and -*opp3* operon directed PCR. These isolates were subjected to whole genome sequencing to characterize the precise structural organization of ACME III for the first time and also revealed that all three isolates were the same sequence type, ST329.

The arginine catabolic mobile element (ACME) was first described in the *Staphylococcus aureus* strain USA300 (1) and is thought to aid colonization and persistence on skin. Since first described, ACME has been identified in other staphylococcal species including *Staphylococcus epidermidis* (2-5), ranging from 30-34 kb in size (1, 4). The element is primarily characterized by the presence of two distinct operons; the *arc* operon (*arcR/A/D/B/C)* encoding an arginine deaminase pathway and the *opp3* operon (*opp3A/B/C/D/E)* encoding an oligopeptide permease ABC transporter. To date, three distinct types of ACME have been described based on the presence of both the *arc* and *opp3* operons (type I), the *arc* operon only (type II) and the *opp3* operon only (type III). The genetic structure and organization of ACME types I & II in staphylococci have been illucidated in detail previously including by the use of whole genome sequencing (WGS) (1, 4). In contrast, the corresponding genetic structural organization of ACME type III have not been comprehensively characterized to date. What is known about the ACME III elements in staphylococci is based on PCR-based scanning/tiling methods using primer pairs designed against the reference ACME type I in USA300 (6, 7), or based on PCR amplification and subsequent sequence analysis of ACME-*arc* and *opp3*- genes (2, 3, 5, 8). Comprehensive characterization of ACME III could yield useful information regarding important features of ACME and its conservation, evolution and spread, such as into the epidemic methicillin-resistant *S. aureus* strain USA300.

We detected ACME III in 9/142 (6.3%) oral methicillin-susceptible *S. epidermidis* isolates from separate patient groups who i) were orally healthy, ii) had dental implants or iii) had periodontal disease**,** using PCR primers directed towards ACME-*arcA* (6) and ACME-*opp3* (ACME-opp3B_F 5′-

GGATTCGCCCAAGTGATGACC-3′ and ACME-opp3B_R 5′-GACTGCTGGGTATGACGT-3′), using the USA300 strain M05/0060 (9) which harbors both the ACME-*arc* and *opp3* operons, as a positive PCR control. We did not detect ACME III in any of the 54 *S. aureus* isolates investigated from the same three patient groups. The genetic structure of three of these ACME IIIs harbored by *S. epidermidis* isolates recovered by oral rinse sampling of three separate patients (one with periodontal disease [P16OR1], one healthy patient [204OR1] and one healthy patient with a dental implant [I11OR1]) were characterized in detail using WGS. To our knowledge, this is the first comprehensive description of the structural organization of ACME III. Isolates were first sequenced using a MiSeq sequencer (Illumina, Essex, United Kingdom) performing genomic DNA extraction and library construction as previously described (10). Reads were checked for quality, trimmed and contigs were generated by *de novo* assembly using SPAdes version 3.6 (http://bioinf.spbau.ru/en/spades). For each isolate subjected to MiSeq-based WGS, ACME–associated genes were identified on four different contigs. As the genes in these contigs differed considerably in composition and orientation to those previously described in ACME types I and II and an appropriate reference ACME to use as a sequence scaffold was lacking, these isolates were also sequenced using a Pacific Biosciences (PacBio) RS sequencing system (CA, USA) with subsequent Hierarchal Genome Assembly Process (HGAP.3) analysis (The Genome Analysis Centre [TGAC], Norwich, United Kingdom) at an average coverage of 265x. For each isolate, all ACME-associated genes were identified on the same contig, thus confirming the orientation and synteny of all ACME III-associated genes.

The bioinformatic tools used for annotation and analysis were the BioNumerics Genome Analysis Tool (GAT) plug-in version 7.6 (Applied Maths, Sint-Martens-Latem, Belgium), Artemis sequence viewer (11), Artemis Comparison Tool (12) and BLAST software (http://blast.ncbi.nlm.nih.gov.elib.tcd.ie/Blast.cgi). Final elucidated genomic structures were confirmed using specific PCR primers (Table S1). The multilocus sequence type (MLST) of all three isolates investigated were determined by submitting the relevant genomic regions to the *S. epidermidis* MLST online database (https://pubmlst.org/sepidermidis/).

Each ACME III harbored the *opp3* genes but lacked the *arc* operon and ranged from 21.2-21.5 kb in size. Adjacent staphylococcal cassette chromosome (SCC) elements were identified upstream of ACME III in two isolates (Fig. 1). Five distinct direct repeat sequences (DRs) (1-A, 1-B & 2-4) were identified amongst the ACMEs characterized. Four (DR1-A and 2-4; Fig. 1) were identified in the ACMEs harbored by isolates 204OR1 and I11OR1, whereas three (DRs1-B, 3 & 4) were detected in the isolate P16OR1 ACME (Fig. 1). There were four nucleotide differences identified between DR1-A and DR1-B (Fig. 1).

A comparative BLAST analysis of the DNA sequence for ACME III (the region between DRs 3 and 4 of isolate I11OR1) with ACME types I & II revealed that although the DNA sequence identity with ACME I (GenBank FPR3757) & ACME II (GenBank AE015929) was 99% and 96% respectively, the query cover was only 54% and 60% respectively, indicating high genetic similarity in distinct genomic regions only. These findings were confirmed using the Artemis Comparison Tool.

The *copA* gene and the *ars* operon were located directly upstream of ACME III for the first time. Previous studies described their location near the 3′ end and immediately downstream of ACME types I and II (1). In two of the elements sequenced (204OR1 and I11OR1), the *copA* and *ars* genes were located between DRs 2 and 3, whereas in the third ACME these genes were in the same location but DR2 was absent (Fig. 1). The genomic regions from the *copA* gene to DR4 exhibited >99% DNA sequence identity in all three ACMEs characterized. The relocation of these antimicrobial resistance genes has not been reported previously, although other genes encoding tetracycline, cadmium, mercury and beta-lactam resistance have been detected previously within ACME-SCC composite elements (1).

Genes previously associated with the SCC*pbp4*-ACME II composite element in *S. epidermidis* (1) were identified in two isolates investigated (Fig. 1), including the cassette chromosome recombinase (*ccr*) and *pbp4* genes. Together, these findings highlight the ability of ACMEs to accumulate antimicrobial resistance genes, particularly within composite elements and their potential to facilitate the spread of these genes to different strains and species.

The *speG* gene conferring polyamine resistance was identified in only one ACME III sequenced and previous research has suggested an association of this gene with *arcA,* which is absent in ACME III (13).

The main feature of ACME III is considered to be the presence of the *opp3* operon in the absence of the *arc* operon. The function of ACME-*opp3* has not been fully elucidated to date, but multiple different *opp*-operons have been identified in bacterial species and are reportedly involved in nutrient uptake, host cell attachment, cell wall metabolism,

121 resistance to antimicrobial peptides and chemotaxis (11, 12). This operon was detected
122 510 bp upstream of DR4 in all three ACMEs characterized, however a nucleotide
123 deletion identified at the +384 position of the *opp3A* gene in isolate 204OR1 resulted in a
124 frameshift mutation and the premature truncation of the encoded protein. These ACME-
125 *opp3* genes likely contribute little advantage, perhaps due to the presence of two native
126 *opp* operons in staphylococci and perhaps represent remnants from previous ACME
127 rearrangements.

128 The elements characterized were divided into modular segments by DRs (Fig. 1)
129 of which, the genomic regions between the *copA* gene and DR4 were highly conserved.
130 Only eight of the 20 open reading frames observed in ACME III shared >97% sequence
131 homology with the *opp3* operon and surrounding genomic regions of previously
132 described ACME I (1), however the *copA* and SE_0128 genes (corresponding to *copA*
133 and SAUSA300_0079 in FPR3757) at the 3′ end of ACME I have been internalized in
134 these ACME III-SCC composite elements (Fig. 1). Previous research has suggested a
135 stepwise assembly of modular ACME segments in *S. epidermidis* prior to transfer to
136 USA300 (14). The results of the present study support this hypothesis, demonstrate how
137 mobile genetic elements can be constructed in a stepwise manner at this genomic region,
138 and suggest ACME III is most likely a genetic remnant of these processes. Surprisingly
139 all three isolates were identified as belonging to multilocus sequence type ST329.
140 Previous MLST-based studies from this laboratory (unpublished) that investigated 36
141 independent oral *S. epidermidis* isolates identified 18 distinct STs, not including ST329.
142 ST329 has been identifed in only 3/1068 (0.3%) allelic profiles currently listed in the *S.*
143 *epidermidis* MLST database (accessed 8th June 2017) suggesting that this ST is rare and

144 is possibly the ST in which ACME rearrangements resulting in ACME type III originally
145 occurred.

146 **Nucleotide sequence accession numbers.** The nucleotide sequences of the three
147 ACME-SCC composite elements 204OR1, P16OR1 and I11OR1 have been submitted to
148 GenBank under accession numbers MF346683- MF346685, respectively.

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159 **FIGURE LEGEND**

160 FIG 1 Schematic diagram showing the genetic organization of previously described
161 ACME type I (a) and II (b) elements and the comparative organization of the three
162 ACME III elements (c-e) determined by whole genome sequencing in the present
163 investigation. Arrows indicate the position and orientation of open reading frames.
164 Genes commonly associated with antimicrobial resistance, SCC or ACME are shaded
165 in color; ACME-*arc* (red), *opp3* (blue), *speG* (dark grey), *copA* (lime green), *ars*
166 operon (yellow), *pbp* (dark green), *ccr* (navy) and *tetR* (mustard). The resistance gene
167 clusters encoding mercury and cadmium resistance in ACME type II_AE015929 are
168 indicated in pale green. For each ACME, *orfX* is indicated in black and specific direct
169 repeat sequences (DRs) identified are indicated [DR1-A,
170 GAAGCGTATCACAAATAA; DR1-B, GAAGCATATCATAAGTGA; DR2,
171 GAAGGGTATCATAAATAA; DR3, GAAGCGTATAATAAGTAA; DR4
172 GAAGCGTATCGTAAGTGA]. Genomic regions from *copA* to DR4 in each ACME
173 III exhibited >99% DNA sequence homology to each other and are enclosed in red
174 rectangles.

REFERENCE:

1. **Diep BA, Gill SR, Chang RF, Phan TH, Chen JH, Davidson MG, Lin F, Lin J, Carleton HA, Mongodin EF, Sensabaugh GF, Perdreau-Remington F.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet 367:731-739. DOI: 10.1016/S0140-6736(06)68231-7
2. **Barbier F, Lebeaux D, Hernandez D, Delannoy AS, Caro V, Francois P, Schrenzel J, Ruppe E, Gaillard K, Wolff M, Brisse S, Andremont A, Ruimy R.** 2011. High prevalence of the arginine catabolic mobile element in carriage isolates of methicillin-resistant *Staphylococcus epidermidis*. J Antimicrob Chemother 66:29-36. DOI: 10.1093/jac/dkq410
3. **Pi B, Yu M, Chen Y, Yu Y, Li L.** 2009. Distribution of the ACME-*arcA* gene among meticillin-resistant *Staphylococcus haemolyticus* and identification of a novel *ccr* allotype in ACME-*arcA*-positive isolates. J Med Microbiol 58:731-736. DOI: 10.1099/jmm.0.007351-0
4. **Shore AC, Rossney AS, Brennan OM, Kinnevey PM, Humphreys H, Sullivan DJ, Goering RV, Ehricht R, Monecke S, Coleman DC.** 2011. Characterization of a novel arginine catabolic mobile element (ACME) and staphylococcal chromosomal cassette *mec* composite island with significant homology to *Staphylococcus epidermidis* ACME type II in methicillin-resistant *Staphylococcus aureus* genotype ST22-MRSA-IV. Antimicrob Agents Chemother 55:1896-1905. DOI: 10.1128/AAC.01756-10
5. **Onishi M, Urushibara N, Kawaguchiya M, Ghosh S, Shinagawa M, Watanabe N, Kobayashi N.** 2013. Prevalence and genetic diversity of arginine catabolic mobile element (ACME) in clinical isolates of coagulase-

negative staphylococci: identification of ACME type I variants in *Staphylococcus epidermidis*. Infect Genet Evol 20:381-388. DOI: 10.1016/j.meegid.2013.09.018

6. **Diep BA, Stone GG, Basuino L, Graber CJ, Miller A, des Etages SA, Jones A, Palazzolo-Ballance AM, Perdreau-Remington F, Sensabaugh GF, DeLeo FR, Chambers HF.** 2008. The arginine catabolic mobile element and staphylococcal chromosomal cassette *mec* linkage: convergence of virulence and resistance in the USA300 clone of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 197:1523-1530. DOI: 10.1086/587907
7. **Miragaia M, de Lencastre H, Perdreau-Remington F, Chambers HF, Higashi J, Sullam PM, Lin J, Wong KI, King KA, Otto M, Sensabaugh GF, Diep BA.** 2009. Genetic diversity of arginine catabolic mobile element in *Staphylococcus epidermidis*. PLoS One 4:e7722. DOI: 10.1371/journal.pone.0007722
8. **Goering RV, McDougal LK, Fosheim GE, Bonnstetter KK, Wolter DJ, Tenover FC.** 2007. Epidemiologic distribution of the arginine catabolic mobile element among selected methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates. J Clin Microbiol 45:1981-1984. DOI: 10.1128/JCM.00273-07
9. **Shore AC, Rossney AS, Kinnevey PM, Brennan OM, Creamer E, Sherlock O, Dolan A, Cunney R, Sullivan DJ, Goering RV, Humphreys H, Coleman DC.** 2010. Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining *spa*, *dru*, and pulsed-field gel electrophoresis typing data. J Clin Microbiol 48:1839-1852. DOI: 10.1128/JCM.02155-09

10. **Earls MR, Kinnevey PM, Brennan GI, Lazaris A, Skally M, O'Connell B, Humphreys H, Shore AC, Coleman DC.** 2017. The recent emergence in hospitals of multidrug-resistant community-associated sequence type 1 and *spa* type t127 methicillin-resistant *Staphylococcus aureus* investigated by whole-genome sequencing: Implications for screening. PLoS One 12:e0175542. DOI: 10.1371/journal.pone.0175542
11. **Berriman M, Rutherford K.** 2003. Viewing and annotating sequence data with Artemis. Brief Bioinform 4:124-132.
12. **Carver TJ, Rutherford KM, Berriman M, Rajandream MA, Barrell BG, Parkhill J.** 2005. ACT: the Artemis Comparison Tool. Bioinformatics 21:3422-3423. DOI: 10.1093/bioinformatics/btn529
13. **Planet PJ, LaRussa SJ, Dana A, Smith H, Xu A, Ryan C, Uhlemann AC, Boundy S, Goldberg J, Narechania A, Kulkarni R, Ratner AJ, Geoghegan JA, Kolokotronis SO, Prince A.** 2013. Emergence of the epidemic methicillin-resistant *Staphylococcus aureus* strain USA300 coincides with horizontal transfer of the arginine catabolic mobile element and *speG*-mediated adaptations for survival on skin. MBio 4:e00889-00813. DOI: 10.1128/mBio.00889-13
14. **Thurlow LR, Joshi GS, Clark JR, Spontak JS, Neely CJ, Maile R, Richardson AR.** 2013. Functional modularity of the arginine catabolic mobile element contributes to the success of USA300 methicillin-resistant *Staphylococcus aureus.* Cell Host Microbe 13:100-107. DOI: 10.1016/j.chom.2012.11.012

Fig. 1

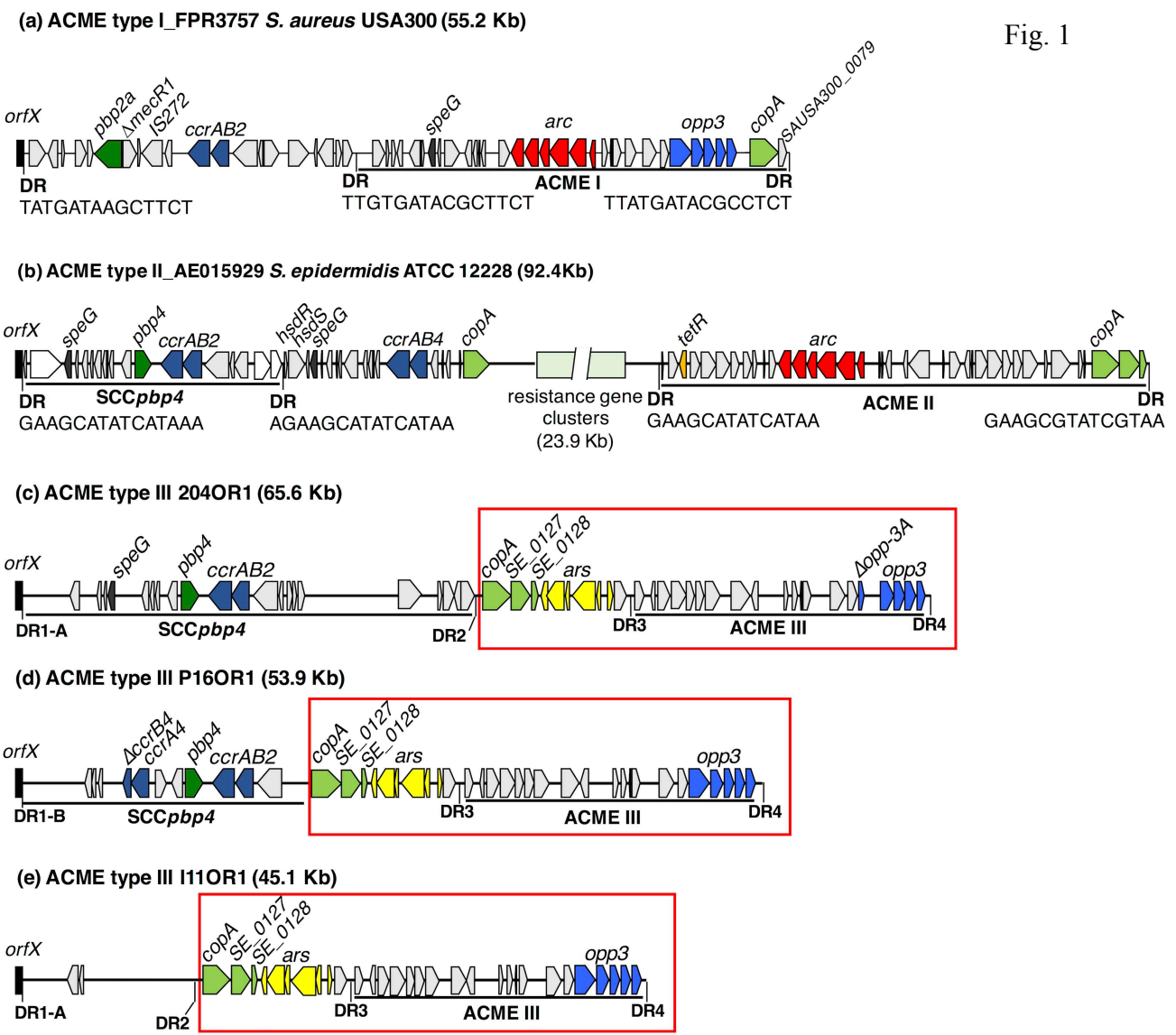
(a) ACME type I_FPR3757 S. aureus USA300 (55.2 Kb)
orfX
pbp2a
ΔmecR1
IS272
ccrAB2
speG
arc
opp3
copA
SAUSA300_0079
DR
TATGATAAGCTTCT
DR
TTGTGATACGCTTCT
ACME I
DR
TTATGATACGCCTCT
(b) ACME type II_AE015929 S. epidermidis ATCC 12228 (92.4Kb)
orfX
speG
pbp4
ccrAB2
hsdR
hsdS
speG
ccrAB4
copA
tetR
arc
copA
DR
SCCpbp4
GAAGCATATCATAAA
DR
AGAAGCATATCATAA
resistance gene clusters (23.9 Kb)
DR
GAAGCATATCATAA
ACME II
DR
GAAGCGTATCGTAA
(c) ACME type III 204OR1 (65.6 Kb)
orfX
speG
pbp4
ccrAB2
copA
SE_0127
SE_0128
ars
Δopp-3A
opp3
DR1-A
SCCpbp4
DR2
DR3
ACME III
DR4
(d) ACME type III P16OR1 (53.9 Kb)
orfX
ΔccrB4
ccrA4
pbp4
ccrAB2
copA
SE_0127
SE_0128
ars
opp3
DR1-B
SCCpbp4
DR3
ACME III
DR4
(e) ACME type III I11OR1 (45.1 Kb)
orfX
copA
SE_0127
SE_0128
ars
opp3
DR1-A
DR2
DR3
ACME III
DR4

Table S1. Primers used to confirm the structure of ACME III

Primer Name	Sequence (5′-3′)	Amplimer Size (bp)	Isolates from which amplimer was obtained
204-1F	CCGTTAAGGATTCATAAGGC	1481	204OR1
204-2R	GCAGTCCTGTTGTTACAGTTG		
204-3F	ATGCAGAAACGTTCAGAGA	3892	204OR1
204-4R	CTTCTGACAGCTCTTCTATTCC		
204-5F	ATCTTTGGAACCTGGACA	4999	204OR1, P16OR1
204-6R	CTGTTCTACTGGAGTATGTGGTC		
204-7F	TAGGTTCTCGTGCCATTG	2929	204OR1, P16OR1, I11OR1
204-8R	CTCATTACGGTCGCTTAGT		
204-9F	AGATGATGAGATGGCACG	2444	204OR1, P16OR1, I11OR1
204-10R	CTAAAGCCGTATCCTAAGTTG		
I11-1 F	GGTAAATACGTAATATCGGTTG	2493	I11OR1
I11-2 R	GGGTGCGAGATGAATTAC		
I11-3 F	CCACACACTTTAGCAGAATC	2943	I11OR1
I11-4 R	CTCTTATCGCCACTGATG		
I11-5F	GCTTGCTTAAAAATTGAGG	2027	204OR1, I11OR1
I11-6R	CCTGAGTGAAATTATTGACG		
P16-1F	GTCCACCTTTTTATTAATAGGG	2362	P16OR1
P16-2R	GGTCTTTTAGTTGATTCAATTC		
P16-3F	GATGGAAGTCACAGTATTCTTTG	5998	P16OR1
P16-4R	CTTTTATCGCCACTGATGG		