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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*

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ABSTRACT

Arginine catabolic mobile element (ACME), a genomic island consisting of the *arc* and/or *opp3* gene clusters found in staphylococcal species, is related to increased bacterial adaptability to hosts. *Staphylococcus epidermidis* is considered a major ACME reservoir; however, prevalence and genetic diversity of ACME in coagulase-negative staphylococci (CNS) have not yet been well characterized for clinical isolates in Japan. A total of 271 clinical isolates of CNS in a Japanese hospital were investigated for the presence and genotype of ACME and SCC*mec*. The prevalence of ACME-*arcA* was significantly higher ($p < 0.001$) in *S. epidermidis* (45.8%) than in other CNS species (3.7%). ACME in *S. epidermidis* isolates ($n = 87$) were differentiated into type I ($n = 33$), variant forms of type I (ΔI, $n = 26$) newly identified in this study, type II ($n = 6$), and type ΔII ($n = 19$). ACME-type ΔI, which were further classified into three subtypes, lacked some genetic components between the *arc* and *opp3* clusters in archetypal type I, whereas the *arc* and *opp3* clusters were intact. The *arc* cluster exhibited high sequence identity (95.8–100%) to that of type I ACME; in contrast, the *opp3* cluster was highly diverse, and showed relatively lower identities (94.8–98.7%) to the identical regions in type I ACME. Twenty-one isolates of ΔI ACME-carrying *S. epidermidis* possessed SCC*mec* IVa and belonged to ST5 (clonal complex 2). Phylogenetic analysis revealed that isolates harboring ACME ΔI in this study clustered with previously reported *S. epidermidis* strains with other lineges, suggesting that *S. epidermidis* originally had some genetic variations in the *opp3* cluster. In summary, ACME type ΔI, a truncated variant of ACME-I, was first identified in *S. epidermidis*, and revealed to be prevalent in ST5 MRSE clinical isolates with SCC*mec* IVa.

1. Introduction

The arginine catabolic mobile element (ACME) is a novel genetic composite that was first identified in a community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) USA 300 clone (Diep et al., 2006). The function of ACME is believed to enhance bacterial adaptability and colonization ability to hosts, which was shown with a rabbit bacteraemia model by Diep et al. (2008). ACME consists of two major gene clusters: the *arc* cluster (*arcC-argR*) and *opp3* cluster (*opp3A-opp3E*). According to the presence/absence of those two clusters, three ACME types have been reported to date: ACME type I (possessing both the *arc* and *opp3* clusters), type II (possessing only the *arc* cluster), and type III (possessing only the *opp3* cluster) (Barbier et al., 2011; Diep et al., 2006). In addition to these three ACME types, a type II variant, type ΔII, was discovered in MRSA (Shore et al., 2011). It is believed that the *arc* cluster is involved in the arginine-deiminase pathway, and the *opp3* cluster encodes an olygopeptide permease system (Diep et al., 2006, 2008). An outstanding characteristic of the arginine-deiminase pathway is to produce ammonia, which makes USA300 clones to be tolerant of the acidic environment such as human skin (pH ~5.0); exogenously expressed ACME-*arc* gene cluster conferred full acid tolerance to the *S. aureus* strain Newman (Diep et al., 2008; Thurlow et al., 2013). Furthermore, in contrast to the chromosomal *arc* cluster, the arginine-deiminase pathway encoded by the ACME-*arc* cluster is constitutively expressed regardless of the presence of glucose and/or oxygen (Thurlow et al., 2013). These data imply that ACME may provide the similar benefits for other staphylococcus species including CNS.

ACME is located adjacent to staphylococcal cassette chromosome *mec* (SCC*mec*) in chromosome (Diep et al., 2006). SCC*mec*

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confers *mecA* gene, which is responsible for methicillin resistance, and has been classified into 11 types in *S. aureus* to date (www.Sccmec.org/Pages/SCC_TypesEN.html) (IWG-SCC, 2009). SCC*mec* typing has been used for the global epidemiological study of hospital-associated (HA) and CA-MRSA (David and Daum, 2010; Deurenberg and Stobberingh, 2008), and methicillin-resistant *S. epidermidis* (MRSE) and other MR-coagulase negative staphylococci (MR-CNS), too (Garza-Gonzalez et al., 2010; Miragaia et al., 2009; Ruppe et al., 2009). Among all the CNS species, *S. epidermidis* is believed to be clinically the most important not only because it is a common pathogen of infections caused by CNS species (Gordon et al., 2012; Piette and Verschraegen, 2009; Raad et al., 2009), but also it is considered a reservoir of mobile genetic elements such as SCC*mec* and ACME (Barbier et al., 2011; Hanssen and Ericson Sollid, 2006; Miragaia et al., 2009; Otto, 2013). ACME is believed to be excised and inserted by *ccr* gene complex in SCC*mec* and transferred with SCC*mec* among staphylococci, especially from *S. epidermidis* to *S. aureus*, which results in an increasing adaptability of MRSA to hosts and spread, as typically seen in CA-MRSA clone USA300 (Diep et al., 2006, 2008).

To date, a number of studies on ACME in *S. aureus* have been conducted, and ACME type I or ΔII adjacent to SCC*mec*-II to -V was reported in different geographic areas (Bartels et al., 2011; Espedido et al., 2012; Kawaguchiya et al., 2011, 2013; Shore et al., 2011; Takano et al., 2013; Urushibara et al., 2012). In contrast to studies performed on *S. aureus*, ACME in CNS has not yet been clarified enough (Barbier et al., 2011; Miragaia et al., 2009; Pi et al., 2009). In the present study, we analyzed the distribution and genetic diversity of ACME with SCC*mec* in recent clinical isolates of CNS from a single hospital. We report herein the divergent nature of ACME, particularly in *S. epidermidis*, and the presence of novel variants of type I ACME.

2. Materials and methods

2.1. Bacterial isolates

From January to October in 2012, a total of 271 clinical isolates of CNS were collected from various clinical specimens (blood, catheters, pus, or other) at Sapporo Medical University Hospital in Sapporo, Japan. The CNS isolates (one per patient) were preserved in Microbank (Pro-Lab Diagnostics) at −80 °C, and recovered and cultivated on trypticase soy agar with 5% sheep blood (Becton and Dickinson) at 37 °C for 24 h at which point they were analyzed. All the CNS isolates obtained in the study period were analyzed. In addition to the conventional biochemical methods for species identification, multiplex PCR (M-PCR) targeting 16S rRNA and thermonuclease genes was employed for the confirmation of staphylococcal species (Hirotaki et al., 2011; Zhang et al., 2008). When staphylococcal species were not identified by the M-PCR, a partial sequence of the 16S rRNA gene was determined with primers Epsilon F and 1510R (Heikens et al., 2005).

2.2. SCCmec and ACME typing

M-PCR targeting *mecA* and ACME-*arcA* genes was carried out on all CNS isolates as described previously (Zhang et al., 2008), using the TaKaRa EX Taq DNA polymerase (Takara Bio Inc., Shiga, Japan). Further PCRs for *mecA* and/or ACME-*arcA*-positive strains were performed with use of the PrimeSTAR GXL DNA polymerase (Takara Bio Inc., Shiga, Japan) according to the manufacture's instruction.

mec classes (A, B, and C) and *ccr* allotypes (*ccr*A1B1 to *ccr*A4B4, *ccr*A5B3 and *ccr*C) were determined by M-PCR as described previously (Higashide et al., 2008; Kobayashi et al., 1996; Pi et al., 2009; Urushibara et al., 2011). SCC*mec* types were classified into types I, II, III, IV, and V based on the combination of *mec* class and *ccr* allotypes (IWG-SCC, 2009). SCC*mec* was defined as non-typeable (NT) when *mec* class and/or *ccr* allotypes could not be determined, or a *mec-ccr* combination had not been described to date. SCC*mec* IV subtypes (IVa, IVb/IVF, and IVc/IVE) were determined by M-PCR with primers described previously (Milheirico et al., 2007). SCC*mec* IV subtype was described as "ND" when the PCR amplicon was not detected.

ACME typing for the differentiation of ACME type I, II, and ΔII was performed on all ACME-*arcA*-positive strains. Primers (arcA-R and AIPS46) previously described by Barbier et al. (2011) were used to identify ACME type I of which the amplicon size was 9095 bp. For the detection of ACME type II and ΔII, we used newly designed primers in this study: argR-F1 (5′-GAAATAGTTGTCAAGCTATAGTAAACTCC-3′) and SE0112-R1 (5′-AGCGCATTAAATACGGCGAAGGT-3′) to amplify 3900 bp-amplicon from ACME II, and argR-F1 and IS431-R1 (5′-GATATATCACGATAACTCAATGC-3′) to amplify 3400 bp-amplicon from ACME ΔII. A chi-squared test was performed to analyze the difference in the prevalence of ACME-*arcA* gene depending on the presence of *mecA* and CNS species. *P* value <0.05 was considered statistically significant.

2.3. Sequencing analysis and PCR profile of ACME

The sequence of the *arc* cluster, the *opp3* cluster, and a region between those clusters was determined for the representative strains with ACME type I including its variant by PCR and direct sequencing. PCR product was purified by using Agencourt® AMPure® XP (Beckman Coulter, Inc., Beverly, MA, USA) and its nucleotide sequence was determined on an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with use of a BigDye® Terminator Sequencing Kit (Applied Biosystems). The results were analyzed by pairwise alignment, and sequence identities were investigated with NCBI and DDBJ BLAST sequence searches (www.blast.ncbi.nlm.nhi.gov, www.blast.ddbj.nig.ac.jp, respectively). Their genetic compositions were compared to each other and those of reference strains, which were *S. epidermidis* strain ATCC12228, ACME type II, and an MRSA strain USA300-FPR3757, ACME type I (GenBank accession numbers were AE015929 and CP000255, respectively). Phylogenic analysis of a sequence between *opp3A* and *opp3B* (1107 bp) was performed on the strains analyzed in the present study, together with those representing ACME-*opp3AB* allotypes of *S. epidermidis* (17 strains) described by Barbier et al. (2011) and USA300-FPR3757, by the neighbor-joining algorithm method (Kimura-2 Parameter model) with use of MEGA v5.05 software.

The genetic structure of the ACME variant was analyzed through PCR profile which represents presence/absence of four portions of ACME type I, i.e., *copA* and the intergenic sequences of *arcC-arcA*, *opp3A-opp3E*, and *copA-SAUSA300_0079* or *copA-SE_0128* by using the primers shown in Table 1.

2.4. GenBank accession numbers

Sequences of ACME (*arc-opp3* clusters) from an ACME type I strain (CNS5), and three ACME I variant strains (CNS266, CNS115, CNS149) were deposited in the GenBank database under accession numbers AB817064 to AB817067.

2.5. Multilocus sequence typing (MLST)

For ACME-I variant *S. epidermidis* strains, MLST was carried out by sequence determination of the internal regions in seven housekeeping genes (*arcC, aroE, gtr, mutS, pry, tpi* and *yqiL*) (Thomas et al., 2007). Sequence types (STs) were identified with use of the MLST database (www.mlst.net/).