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Research paper

First description of novel arginine catabolic mobile elements (ACMEs) types IV and V harboring a *kdp* operon in *Staphylococcus epidermidis* characterized by whole genome sequencing

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ABSTRACT

The arginine catabolic mobile element (ACME) was first described in the methicillin-resistant *Staphylococcus aureus* strain USA300 and is thought to facilitate survival on skin. To date three distinct ACME types have been characterized comprehensively in *S. aureus* and/or *Staphylococcus epidermidis*. Type I harbors the *arc* and *opp*3 operons encoding an arginine deaminase pathway and an oligopeptide permease ABC transporter, respectively, type II harbors the *arc* operon only, and type III harbors the *opp3* operon only.

To investigate the diversity and detailed genetic organization of ACME, whole genome sequencing (WGS) was performed on 32 ACME-harboring oro-nasal *S. epidermidis* isolates using MiSeq- and PacBio-based WGS platforms. In nine isolates the ACMEs lacked the *opp3* operon, but harbored a complete *kdp* operon (*kdp*E/D/A/B/C) located a maximum of 2.8 kb upstream of the *arc* operon. The *kdp* operon exhibited 63% DNA sequence identity to the native *S. aureus kdp* operon. These findings identified a novel, previously undescribed ACME type (designated ACME IV), which could be subtyped (IVa and IVb) based on distinct 5′ flanking direct repeat sequences (DRs).

Multilocus sequence typing (MLST) sequences extracted from the WGS data identified the sequence types (STs) of the isolates investigated. Four of the nine ACME IV isolates belonged to ST153, and one to ST17, a single locus variant of ST153.

A tenth isolate, identified as ST5, harbored another novel ACME type (designated ACME V) containing the *kdp*, *arc* and *opp3* operons and flanked by DR_F, and DR_B but lacked any internal DRs. ACME V was colocated with a staphylococcal chromosome cassette *mec* (SCC*mec*) IV element and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in a 116.9 kb composite island.

The extensive genetic diversity of ACME in *S. epidermidis* has been further elucidated by WGS, revealing two novel ACME types IV and V for the first time.

1. Introduction

The arginine catabolic mobile element (ACME) was first described in the methicillin-resistant *Staphylococcus aureus* (MRSA) strain USA300 and is thought to contribute to the transmission, colonization and persistence of this pathogen on human skin (Diep et al., 2008; Planet et al., 2013). Like the staphylococcal chromosomal cassette *mec* (SCC*mec*) element, ACME integrates into the staphylococcal chromosomal *orfX* locus using the *attB* attachment site and is flanked by direct repeat sequences (DRs) at integration sites. Like SCC*mec*, ACME is thought to have originated in coagulase negative staphylococci (CoNS), specifically *Staphylococcus epidermidis*, in which the prevalence and diversity of both SCC*mec* and ACME is significantly greater than in *S. aureus*. In many cases, these ACMEs also contain internal DRs, indicating that these elements are assembled in a stepwise, modular manner (Thurlow et al., 2013).

To date, three distinct ACME types have been characterized in detail; type I harbors both the *arc* and *opp*3 operons which encode an arginine deaminase pathway and an oligopeptide permease ABC transporter, respectively, type II harbors the *arc* operon only, and type

Abbreviations: ACME, arginine catabolic mobile element; WGS, whole genome sequencing; DRs, direct repeat sequences; MLST, multilocus sequence typing; STs, sequence types; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; MRSA, methicillin-resistant *Staphylococcus aureus*; SCC*mec*, staphylococcal chromosomal cassette *mec*; CoNS, coagulase negative staphylococci

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III harbors the *opp3* operon only. ACME types I and II and variants thereof have been described in *S. aureus* (Diep et al., 2006; Rolo et al., 2012; Shore et al., 2011) and all three ACME types and variants thereof have been described in *S. epidermidis* (Barbier et al., 2011; McManus et al., 2017; Miragaia et al., 2009; Onishi et al., 2013; Soroush et al., 2016). ACME variants have been described in *S. epidermidis* based on distinct PCR-based scanning patterns of 30 overlapping segments of DNA sequence, 1–2 kb in size (Miragaia et al., 2009). Additional studies have identified distinct ACME-*arc* and ACME-*opp3* allotypes by PCR-based amplification and DNA sequence analysis of the ACME-*arcA* and -*opp3AB* genes (Barbier et al., 2011), respectively. Other studies identified distinct, truncated variants of ACME type I (designated types Δ1.1–3) and ACME type II (designated type ΔII) in *S. epidermidis* and MRSA, using PCR-profiling and Sanger-based sequencing. These truncated variants of ACME were based on variations in the nucleotide sequence of the regions surrounding the *arc* and *opp3* operons or of the *opp3* operon itself (Onishi et al., 2013; Urushibara et al., 2016).

The importance of *S. epidermidis* as a causative agent of various community acquired diseases and infections associated with indwelling medical devices is being increasingly recognised and in this context, ACME likely plays a significant role in successful host colonization and the potential accumulation and spread of genes encoding antimicrobial resistance. Furthermore, the evolution of ACME in *S. epidermidis* could have important consequences for the epidemiology of *S. aureus* due to the capability of this species to serve as a genetic reservoir for *S. aureus*.

As part of a larger study investigating the prevalence and structural diversity of ACME, 32 oro-nasal *S. epidermidis* isolates recovered from orally healthy patients with or without dental implants, and from patients with periodontal disease or peri-implantitis, in which ACME was detected using ACME-*arc*- and ACME-*opp3*- specific primers, as previously described (McManus et al., 2017) were further characterized using whole genome sequencing (WGS). This was undertaken to elucidate the detailed genetic organization and diversity of these ACMEs, as such investigations may yield new insights into the evolutionary origins and spread of each ACME type. Analysis of the WGS data revealed a structurally unique group of ACMEs which consistently harbored a *kdp* operon encoding a ABC transporter upstream and adjacent to the *arc* operon in 10 *S. epidermidis* isolates, which indicated that these ACMEs represented highly distinct, previously undescribed ACME types. In addition, the presence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were identified downstream of the *kdp* and *arc* operons in one of these isolates.

2. Materials and methods

2.1. Isolates

The isolates investigated in this study were recovered from nasal swabs, subgingival sites or oral rinse samples taken by qualified Dentists from patients attending the Dublin Dental University Hospital, Ireland. Ethical approval was granted by the Faculty of Health Sciences Ethics Committee of Trinity College Dublin in February 2014.

Subgingival sites were sampled by inserting a PerioPaper™ gingival fluid collection strip (Oroflow Inc., NY, USA) into the subgingival crevice for 30 s. Following sampling the collection strips were placed in sterile 2 ml screw-capped tubes (Sarstedt AG & Co., Numbrecht, Germany) containing 1 ml of nutrient broth (Oxoid Ltd., Hampshire, UK). Oral rinse samples were collected by providing participants with sterile 100 ml polypropylene containers (Sarstedt AG & Co., Wexford, Ireland) containing 25 ml sterile phosphate buffered saline and instructing participants to rinse their mouths for 30 s before returning the fluid to the container. Following sampling, all samples were transported immediately to the microbiology laboratory and processed within 4 h.

Vials containing PerioPaper™ strips suspended in nutrient broth were vortexed at maximum speed for 1 min and 100 µl aliquots of the resulting cell suspension were plated onto mannitol salt agar and Sa*Select* (Bio-Rad, Hertfordshire, United Kingdom) agar. Oral rinse samples were processed by transferring a 1 ml aliquot to a sterile 1.5 ml Eppendorf Safe-lock™ microfuge tube (Eppendorf, Hamburg, Germany) and centrifuged at 20,000 × *g* for 1 min, after which the supernatant was discarded and the pellet resuspended in 200 µl nutrient broth. To isolate staphylococcal colonies, 100 µl aliquots of this cell suspension were plated on mannitol salt agar and Sa*Select*, both of which were incubated at 37 °C for 48 h in a static incubator (Gallenkamp, Leicester, United Kingdom).

Bacterial isolates were cultured on Columbia blood agar (Fannin Ltd., Dublin, Republic of Ireland) at 37 °C for 48 h prior to identification by Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) technology using the VITEK® MS system (bioMérieux, Marcy L'Etoile, France) according to the manufacturer's instructions. All isolates were stored on Microbank™ storage beads (Pro-lab diagnostics, Cheshire, UK) at −80 °C.

2.2. Whole genome sequence analysis

The genome sequence of 32 isolates, selected as representatives of different patients, patient groups, oro-nasal sample sites and each previously described ACME type was determined using a MiSeq sequencer (Illumina, Essex, United Kingdom). One additional isolate was sequenced using a Pacific Biosciences (PacBio) RS sequencing platform (CA, USA) at an average coverage of 302× with subsequent Hierarchal Genome Assembly Process (HGAP.3) analysis (The Genome Analysis Centre [TGAC], Norwich, United Kingdom). Genomic DNA extraction and library construction was performed as previously described (Earls et al., 2017).

For each isolate, reads were checked for quality and then aligned to a selection of ACMEs and SCC*mec* elements previously characterized in *S. aureus* and *S. epidermidis* (Diep et al., 2006; McManus et al., 2017; Zhang et al., 2003) in order to select the most appropriate reference ACME type to use as a scaffold. This was performed using the Burrows-Wheeler aligner (BWA) tool in SPAdes version 3.6 (http://cab.spbu.ru/software/spades/). Following these analyses, the ACME I sequence from MRSA USA300 strain FPR3757 (GenBank accession number CP000255.1) was selected as the most appropriate scaffold for isolates harboring both the ACME-*arc* and ACME-*opp3* genes and the ACME II sequence from *S. epidermidis* strain ATCC12228 (GenBank accession number AE015929) was selected as the most appropriate scaffold for ACMEs harboring only the ACME-*arc* genes.

For each isolate, contigs were generated by BWA assembly and aligned to the most appropriate reference scaffold. Contigs containing sequences previously associated with SCC*mec* or ACME were selected and annotated using the BioNumerics Genome Analysis Tool (GAT) plug-in version 7.6 (Applied Maths, Sint-Martens-Latem, Belgium). For each isolate investigated, ACME–associated genes were identified on between one and six separate contigs (Table 1). These contigs were organized and reorientated as appropriate using the relevant ACME scaffold and Artemis sequence viewer (Berriman and Rutherford, 2003) and Artemis Comparison Tool (Carver et al., 2005). Further annotation was carried out using BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

In order to confirm the genetic organization and orientation of contigs, primers were designed using the Artemis sequence viewer (Berriman and Rutherford, 2003) that targeted a minimum distance of 200 nucleotides from the contig boundaries. The target specificity of primers was confirmed using BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Supplementary Table S1). All primers were supplied by Sigma-Aldrich Ltd. (Wicklow, Republic of Ireland). Amplification products were subjected to Sanger-based sequencing carried out commercially by Source BioScience (Waterford, Republic of Ireland).