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An Unexpected Location of the Arginine Catabolic Mobile Element (ACME) in a USA300-Related MRSA Strain

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Abstract

In methicillin resistant Staphylococcus aureus (MRSA), the arginine catabolic mobile element (ACME) was initially described in USA300 (t008-ST8) where it is located downstream of the staphylococcal cassette chromosome mec (SCCmec). A common health-care associated MRSA in Copenhagen, Denmark (t024-ST8) is clonally related to USA300 and is frequently PCR positive for the ACME specific arcA-gene. This study is the first to describe an ACME element upstream of the SCCmec in MRSA. By traditional SCCmec typing schemes, the SCCmec of t024-ST8 strain M1 carries SCCmec IVa, but full sequencing of the cassette revealed that the entire J3 region had no homology to published SCCmec IVa. Within the J3 region of M1 was a 1705 bp sequence only similar to a sequence in S. haemolyticus strain JCS1435 and 2941 bps with no homology found in GenBank. In addition to the usual direct repeats (DR) at each extremity of SCCmec, M1 had two new DR between the arc gene and the J3 region of the SCCmec. The region between the arcX DR (DR1) and DR2 contained the ccrAB4 genes. An ACME II-like element was located between DR2 and DR3. The entire 26,468 bp sequence between DR1 and DR3 was highly similar to parts of the ACME composite island of S. epidermidis strain ATCC12228. Sequencing of an ACME negative t024-ST8 strain (M299) showed that DR1 and the sequence between DR1 and DR3 was missing. The finding of a mobile ACME II-like element inserted downstream of arcX and upstream of SCCmec indicates a novel recombination between staphylococcal species.

Introduction

The arginine catabolic mobile element (ACME) has been described in both coagulase negative staphylococci and MRSA. The prevalence is high in S. epidermidis and S. haemolyticus [1,2] where it is found in several different genetic backgrounds, whereas it has only been described in a few MRSA lineages [3]. ACME in MRSA has mostly been described in the USA300 clone, where it is located downstream of the staphylococcal cassette chromosome mec (SCCmec) [4]. The prototype USA300 (USA300-0114) belongs to sequence type (ST) 8, is spa type 008 and harbours SCCmec IVa [5]. The most common healthcare associated MRSA in Copenhagen, Denmark is t024-ST8-IVa [6] and by pulsed-field gel electrophoresis (PFGE) the t024 clone cannot be discriminated from USA300 [7]. Furthermore, most t024 isolates are PCR positive for the ACME-specific arcA gene allele. We have previously shown that the BD GeneOhm MRSA assay failed to detect most of our t024 isolates [8]. While all eleven t008-IVa isolates in the study were positive by the BD GeneOhm MRSA assay, this was the case for only five of thirty-three t024-IVa isolates and those five isolates were PCR positive in a late cycle indicating a false positive result. These results indicated that the J3 region of SCCmec IVa in t008 and t024 were not identical. In an attempt to study this phenomenon we discovered that PCR in the arcX region yielded no products by long range PCR and that the t024 isolates lacked the 2 kb downstream constant segment (dcs) [9] found in the J3 region of most SCCmec IV subtypes [10]. This suggested substantial changes in the J3 region and prompted us to sequence the entire genome of one ACME-positive and one ACME-negative t024 isolate. In this paper the SCCmec and the arcX near region of these two isolates are characterized.

Materials and Methods

Strains: From a collection of 311 t024 isolates (88 % PCR positive for the ACME specific arcA gene allele) we selected two MRSA strains (M1 and M299) that were t024-ST8-IVa and Panton-Valentine leukocidin (PVL) negative. M1 was PCR positive for the ACME-specific arcA gene allele [4] and M299 was PCR negative. The isolates were healthcare associated (HCA)-MRSA isolated in 2003 and 2005, respectively and had been stored at minus 80 degrees.

Typing and sequencing: spa typing [6], SCCmec typing [11,12], MLST [6], PCR for PVL [6] and ACME [4] were performed as previously described. DNA was extracted from colonies on 5 % blood agar plates (Statens Serum Institut, Copenhagen, Denmark) using the Ultraclean microbial DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA). DNA concentration was
measured on a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Whole genome sequencing was performed on a GS FLX (454 Life Sciences, a Roche company, CT, USA). For the M1 strain approximately two µg DNA was used to build a single stranded (ss) library according to GS FLX Library preparation manual (Roche). The ssDNA library was quantified by qPCR using primers targeting the A & B adaptors. Library preparation manual (Roche). The ssDNA library was used to build a single stranded (ss) library according to GS FLX Titanium chemistry on one-quarter Titanium PTP. The M1 and M299 DNA was deposited in the GenBank/EMBL/DDBJ databases under accession no. HM030720 and HM030721, respectively.

**Results**

Whole genome sequencing of M1 and M299 had a 40-fold and 20-fold coverage, respectively. In M1, the sequence from orfX to the right extremity junction of SCCmec was localized on two contigs and after gap closure the entire sequence was 53,863 bps of which the last 27,380 bps constituted the SCCmec. In M299, the SCCmec was adjacent to orfX. It was localized on seven contigs and after gap closure the entire sequence was 27,380 bps. M1 and M299 contained a type 2 ccr gene complex and a class B mec gene complex characteristic of SCCmec type IV [10].

In addition to the usual direct repeats (DR) at each extremity of SCCmec (DR1 and DR4 in Table S1 and Figure 1), two DR were identified in M1 (DR2 and DR3 in Table S1 and Figure 1). The entire sequence from DR1 to DR3 was highly similar to the ACME composite island of strain S. epidermidis ATCC12228 (AE015929) [4]. The sequence included the arginine deiminase pathway (arc) cluster and the crrAB4 genes. However, compared to the ACME composite island of ATCC12228 it lacked about 28.5 kbs of centrally placed genes including the genes coding for mercury and cadmium resistance. In addition, the ACME II-like element of M1 was shorter than the ACME II described in S. epidermidis ATCC12228 so we presume that it originated from a S. epidermidis background with another variant of the ACME composite island, alternatively these resistance genes could have been lost in M1 because of a high fitness cost. The arc gene cluster in M1 was surrounded by sequences highly similar to the ones in S. epidermidis ATCC12228 so we presume that it originated from a S. epidermidis although ACME has also been found to be prevalent in S. haemolyticus [2]. Surprisingly, in M1, ACME was localized upstream of SCCmec and not downstream of SCCmec as in MRSA USA300 [4]. Therefore it would be interesting to identify the location of ACME in other non-USA300 MRSA isolates. Large SCC elements are usually believed to create low fitness, but the ACME-positive t024-ST8-IV clone has spread in nursing homes in Copenhagen since 2003 and only

**Discussion**

The present study revealed recombination events in the J3 region of two MRSA SCCmec IVa and the presence of a sequence highly similar to the ACME composite island of S. epidermidis ATCC12228 in one isolate. The 53,863 bp sequence ranging from orfX to the right extremity junction of SCCmec in M1 contained two ccr gene complexes (ccrAB2 and ccrAB4), one mec gene complex and an ACME II-like element. Two new DR were identified in M1 between DR1 and the J3 region of SCCmec IVa. Assuming that these DR are recognized by ccr genes, the sequence between orfX and the right extremity junction of SCCmec in M1 can be considered a composite element consisting of three parts. In agreement with the recently published guidelines for naming the SCC elements [10], we name the 53,863 bp composite element as follows: SCCmec;ψSCCmec, SCCmec IVa (2B); J1, subtype 1-specific ORFs; J3, subtype 3-specific ORFs for M1. The SCCmec of MRSA isolate M299 was 27,380 bp long and contained only one ccr gene complex (ccrAB2). The SCCmec of M299 was identical to the SCCmec IVa of M1.

Most of the sequence between DR1 and DR3 in M1 was highly homologous to the S. epidermidis ACME composite island in strain ATCC12228 (AE015929) [4] but interestingly the genes coding for mercury and cadmium resistance were not present in M1 (Figure 2). This could indicate that the genetic material acquired by M1 came from a S. epidermidis background with another variant of the ACME composite island, alternatively these resistance genes could have been lost in M1 because of a high fitness cost. The arc gene cluster in M1 was surrounded by sequences highly similar to the ones in S. epidermidis ATCC12228 so we presume that it originated from a S. epidermidis although ACME has also been found to be prevalent in S. haemolyticus [2]. Surprisingly, in M1, ACME was localized upstream of SCCmec and not downstream of SCCmec as in MRSA USA300 [4]. Therefore it would be interesting to identify the location of ACME in other non-USA300 MRSA isolates. Large SCC elements are usually believed to create low fitness, but the ACME-positive t024-ST8-IV clone has spread in nursing homes in Copenhagen since 2003 and only
due to increased infection control and focus on this particular clone, the number of cases has decreased and the clone has been under control for the last couple of years. The ACME-negative M299 clone has been less successful. The first ACME-negative isolates were identified in 2005, two years after the ACME-positive clone appeared, and did not replace the ACME-positive clone (data not shown). Diep et al [4] have described ACME in USA300 and have shown in a rabbit model that it enhances fitness and pathogenicity of the clone [3], but this finding could not be confirmed in rat models [14]. Interestingly, a study by Miragaia et al [1] on ACME in S. epidermidis suggests that ACME leads to an enhanced colonization and transmission rather than increased pathogenicity. This is consistent with the fact that the ACME-positive t024-ST8-IVa clone spread easily in nursing homes and decolonization treatments often failed, and it might explain why the clone had success in spite of a large composite element. Since ACME-positive t024-MRSA appeared before ACME-negative t024-MRSA in Copenhagen, we presume that the ccr genes in M1 are responsible for the excision of 26,483 bps resulting in the M299 variant.

The 5,354 bps in J3 of M1 and M299 were an interesting mixture of 2,941 bps with no significant similarity to published sequences, 707 bps that were mostly similar to USA300 and 1,705 bps that were highly homologous to S. haemolyticus. The sequence without significant similarity had a GC content of 25 % and according to Takeuchi et al, low GC content is found in non-coding regions of Staphylococcal species [15]. Nevertheless, four possible ORFs in this sequence potentially coded for proteins similar to proteins found in S. epidermidis, indicating that this region might originate from a S. epidermidis not yet sequenced. Recently, Miragaia et al [1] described a Danish S. epidermidis strain (DEN077) containing both ccrA, ccrB and ACME, but the location of ACME was not determined. The presence, in M1, of a composite element including 31,837 bps with apparent diverse genetic background indicates a high rate of genetic exchange and recombination between staphylococcal species which has also been found by others [16–18]. Whether the recombination events occurred in S. aureus or in coagulase negative staphylococci remains to be determined.

The eleven SNPs between M1 and M299 found by GS FLX sequencing were not reproduced by standard PCR and sequencing. Ten of the SNPs were in regions with homopolymers. GS FLX errors in homopolymers is a well-known problem [19]. It is not surprising that all the errors were in M299 since sequencing of this strain had a coverage of 20 compared to 40 for the M1 strain.

The substantial changes found in J3 of our two isolates are missed by our routine SCCmec IV subtyping [12]. As a result, isolates that are considered to have identical SCCmec might have substantial differences that are unrecognized. In addition, the real
time PCR assay BD GeneOhm MRSA amplifies in the J3 region and failed to detect the two isolates [8]. This illustrates a continuous need to update PCR based MRSA detection in the orfX/J3 region, as would be necessary to detect our dominant t024-ST8-IVa clone. The microevolution of SCCmec cassettes seem to be rather fast, and new primers that target changing J3 regions can only be designed after variant clones are identified and analysed.

In conclusion, we have found ACME to be located upstream of SCCmec in a USA300-related MRSA strain. Furthermore, we have identified a SCCmec IVa with an entire novel J3 region including a sequence from S. haemolyticus. Our data suggest that considerable genetic exchange occurs between staphylococcal species and can lead to diversity in the SCCmec that is only recognized by complete nucleotide sequencing.

Supporting Information

Table S1  # Possible ORFs using ORF finder, Genbank. ^ The gene with the highest identity is listed. Only in cases where genes from several isolates have the same identity, all are listed. *The last 5 bps of the gene are missing in the M1 sequence. ^Only the last 118 of 330 bps. 2Only the last 612 of 867 bps. 3Possible direct repeats.

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Author Contributions

Conceived and designed the experiments: MDB LHH KB SJS HW. Performed the experiments: MDB LHH KB. Analyzed the data: MDB LHH KB HW. Contributed reagents/materials/analysis tools: MDB LHH KB SJS HW. Wrote the paper: MDB.

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