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Characterization of CRISPR-Cas system in clinical *Staphylococcus epidermidis* strains revealed its potential association with bacterial infection sites

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ABSTRACT

Staphylococcus epidermidis is considered as a major cause of nosocomial infections, bringing an immense burden to healthcare systems. Virulent phages have been confirmed to be efficient in combating the pathogen, but the prensence of CRISPR-Cas system, which is a bacterial immune system eliminating phages was reported in few *S. epidermidis* strains. In this study, the CRISPR-Cas system was detected in 12 from almost 300 published genomes in GenBank and by PCR of *cas6* gene in 18 strains out of 130 clinical isolates obtained in Copenhagen. Four strains isolated in 1965–1966 harboured CRISPR elements confirming that this immunity system was not recently acquired by *S. epidermidis.* In these CRISPR-positive strains, 44 and 12 spacers were found to belong to CRISPR1 and CRISPR2 elements, respectively. However, only 15 spacers displayed homology to reported phages and plasmids DNA. Interestingly, 5 different spacers located in the CRISPR1 locus with homolgy to virulent phage 6ec DNA sequences, and 19 strains each carrying 2 or 3 different spacers recognizing this phage, implied that the CRISPR-Cas immunity could be abrogated by nucleotide mismatch between the spacer and its target phage sequence, while new spacers obtained from the evolved phage could recover the CRISPR interference. In addition, phylogenetic analysis of the 29 CRISPR-positive isolates divided them into four lineages, with 81% human blood isolates as a distinct sub-lineage, suggesting that the CRISPR difference is closely related to diverse habitats. Knowledge of CRISPR and its prevalence may ultimately be applied in the understanding of origin and evolution of CRISPR-positive *S. epidermidis* strains.

1. Introduction

Staphylococcus aureus and *Staphylococcus epidermidis* are among the most common pathogens leading to nosocomial infections (Lim and Webb, 2005; Lowy, 1998; von Eiff et al., 2002). While most studies focus on *S. aureus,* less is known of *S. epidermidis*, the most common of the coagulase-negative staphylococci (CoNS) from human epithelia (Otto, 2009, 2012) and of other CoNS *Staphylococci* such as, *S. capitis* found in the human microbiota (Rasigade et al., 2012).

With the completion of genome sequences of *Staphylococci* strains, the presence of the CRISPR-Cas (clustered regularly interspaced short palindromic repeats–CRISPR-associated proteins) system has been reported in some strains (Holt et al., 2011; Lemriss et al., 2014; Marraffini and Sontheimer, 2008). As an adaptive immune system against phage or plasmids, the CRISPR-Cas system is prevalent in many bacterial species and nearly all archaea (Sorek et al., 2008). The CRISPR-Cas system is composed of *cas* genes and CRISPR loci, which is repeat elements intercalated with short "spacer" sequences matching phage or plasmid genomes (Goldberg et al., 2014). The identity of spacers to phage or plasmid sequences dictates the bacteria for immunity to these foreign DNA elements. According to the difference of *cas* genes content and organization, the CRISPR-Cas systems could be divided into three types (I–III) with 12 subtypes (A-F) (Goldberg et al., 2014; Makarova et al., 2011). Although only a few *Staphylococci* strains have been reported to harbour this system, the spacers in these strains have

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Table 1
S. epiderimidis isolates included in this study.

Isolate (number)	Source	Year of isolation	Reference
S. epidermidis (38)	Blood stream infections/contaminants	1965–1966	Skovgaard et al.,2013
S. epidermidis (64)	Blood stream infections/contaminants	2009–2011	Skovgaard et al.,2013
S. epidermidis (28)	Scrub samples from patients and nurses	2010–2011	Skovgaard et al.,2013
S. epidermidis R62a (ATCC 35984)	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures	1962	ATCC

Table 2
Primers of sequences used in this study.

Primer name	Sequences (5′–3′)	Annealing temperature (AT)	Amplification target	Size of PCR product (bp)
Cas6-F	AGGAAGTATTTTACATGGTGT	55 °C	*cas6* gene in *S. aureus* and *S. epidermidis*	568
Cas6-R	AACCTGAAAATTCGCCAAAC			
SECR1-F	CTATTTTCCTTCGCAGTAAC	58 °C	CRISPR1 in *S. epidermidis*	Different size
SECR1-R	TCTTGTAGTGAGGGAACGTC			
SECR2-F	TGTCTTGAGAACTAGGAATACC	58 °C	CRISPR2 in *S. epidermidis*	Different size
SECR2-R	CGTTTACAGGAGAAATGGTG			

been confirmed to be functional against plasmids or phages carrying homologus sequences indicating that the system is active (Marraffini and Sontheimer, 2008). Recently it was demonstrated that *S. aureus* MSHR1132, 08BA02716, *S. epidermidis* R62a, and *S. capitis* CR01 have the type III-A CRISPR-Cas system comprising the Cas proteins, Cas1, Cas2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, and Cas6 (Golding et al., 2010, 2012; Holt et al., 2011; Lemriss et al., 2014; Makarova et al., 2011; Marraffini and Sontheimer, 2008).

As the CRISPR-Cas system is not prevalent in *Staphylococci* species, there are conflicting thoughts as to whether the presence of this system in these CRISPR-positive *Staphylococcus* strains is ancestral or recently acquired. Except for *S. epidermidis* R62a isolated in 1962, most of the other reported CRISPR-positive strains were isolated in 21 st century (Golding et al., 2010, 2012; Holt et al., 2011; Lemriss et al., 2014). In addition, CRISPR regions are closely associated with SCC*mec* cassette encoding methicillin resistance in *S. aureus*, it is speculated that the CRISPR itself may be a mobile genetic element, which could be transferred into *Staphylococcus* strains (Holt et al., 2011). In order to address the source of CRISPR-Cas system in *Staphylococci*, we identified its distribution among *Staphylococci*, especially *S. epidermidis*.

2. Materials and methods

2.1. Bacterial strains

The reference strain of *S. epidermidis* R62a with positive CRISPR-Cas element was obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures. And the CRISPR-positive *S. aureus* strain 08BA2176 was offered by Dr. Michael R. Mulvey (Makarova et al., 2011). 130 *S. epidermidis* strains were collected from nurses and patients recruited at the Copenhagen University Hospital, Hvidovre, Denmark (Skovgaard et al., 2013). Among the 130 strains, 64 and 38 blood isolates were detected during 2009–2011 and 1965–1966, respectively (Table 1) The other 28 isolates were collected from nurses and patients by swapping the dominant hand and nares (Skovgaard et al., 2013).

2.2. Analysis of CRISPR elements in published genomes of Staphylococcus

In order to identify CRISPR elements in *Staphylococcus*, the CRISPR-Cas loci in *S. epidermidis* R62a and *S. aureus* 08BA2176 were determined in the CRISPR database (http://crispr.u-psud.fr/Server/). As it is demonstrated that *cas* genes were stable and conserved, *cas6* gene (SERP_RS12030, NC_002976.3) was selected to search CRISPR-Cas array in other published *Staphylococcus* strains using BLASTX (Default Parameters, nr database). The CRISPR elements from *cas6* positive genomes were submitted to the CRISPR database to display the components of spacers. The phylogenic tree based on the arrangement of spacers was formed by Bionumericus 7.0 software (Applied Maths, Belgium).

2.3. PCR amplification

Primers listed in Table 2 was designed to amplify, *cas6*, CRISPR1 and CRISPR2 from *S. epidermidis* according to the conservative sequences in CRISPR elements. PCR reactions were performed in a total volume of 20 μl: 3 μl template, 10 μl PCR Master Mix (2×) (Fermentas), 1 μl of each 10 mM primer and 5 μl water. PCR reaction conditions were as follows and the annealing temperature (AT) are listed in Table 2: 10 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 1 min at AT and 3 min at 72 °C; a final extension step was done at 72 °C for 10 min.

2.4. DNA sequencing and phylogenetic analysis

PCR products were sequenced at the Macrogen (http://www.macrogen.com/); All the PCR fragments were sequenced in both the forward and reverse directions to obtain double-stranded sequence. Sequences were assembled using SeqMan software, and then submitted to the CRISPR-finder database (http://crispr.u-psud.fr/Server/). Repeats were removed to determine the homology of spacers among CRISPR-positive strains, the CRISPR spacers were aligned in the CRISPR-finder database and named manually. Each strain has a CRISPR array composed of the arrangement of CRISPR spacers. To analyse the phylogenetic relationship among these strains based on CRISPR diversity, the CRISPR array was transformed into binary file and analyzed using Bionumeriucs 7.0 software.

2.5. Information of CRISPR spacers

Based on the sequences of CRISPR spacers, BLASTN (Default Parameters, nr database, short queries) was used to identify plasmid, phage genes or DNA fragments harbouring sequences with homology to these spacers. And combined with the arrangement of spacers, we could speculate the phage-host interactions and the evolutionary changes between phages and the bacteria.