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Global acquisition of genetic material from different bacteria into the staphylococcal cassette chromosome elements of a *Staphylococcus epidermidis* isolate

Huping Xue [a,1], Zhaowei Wu [a,1], Dandan Qiao [a], Chao Tong [a], Xin Zhao [a,b,*]

[a] College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China
[b] Department of Animal Science, McGill University, Quebec, Canada

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ABSTRACT

Staphylococcus epidermidis has been suggested as a main reservoir of methicillin resistance and virulence genes facilitating the evolution of *Staphylococcus aureus* as a successful pathogen. However, it remains a mystery where and how *S. epidermidis* obtains these numerous genes to serve as the reservoir. In this study, methicillin-resistant *S. epidermidis* isolate NW32 from a mastitic milk sample was sequenced and its staphylococcal cassette chromosome (SCC) elements were characterised. The SCC composite island covered 3.5% of the genome and consisted of three intact SCC elements carrying resistance genes against β-lactam antibiotics, several heavy metals and polyamines as well as genes for utilisation of sorbitol as a carbon source. Analysis of the postulated evolutionary route suggested that the three SCC elements were assembled from genetic material from various bacterial species (staphylococci, streptococci, salinicocci and *Lysinibacillus*) from three habitats (human, soil and cow) in different countries (Asia, North America, South America and Europe). We propose that the *hsdS* restriction-modification profile and the lack of CRISPR (clustered regularly interspaced short palindromic repeat) sequences in this bacterium may facilitate the genetic exchange of SCC elements among different staphylococcal species.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a notorious pathogen and is one of the most frequent causes of infection and death in hospitalised patients. *Staphylococcus epidermidis* has been suggested as a main reservoir of genes facilitating the evolution of methicillin-susceptible *S. aureus* into MRSA by genetic exchange [1]. Genetic exchange is a frequent event in the evolution of most bacterial species and this process often plays a greater role in genomic diversification than de novo mutation. Genetic material exchange between *S. aureus* and *S. epidermidis* appears unidirectional, since *S. epidermidis* does not acquire genes from *S. aureus* [1]. Yet it remains a mystery where and how *S. epidermidis* obtains so many genes to serve as the reservoir.

Two systems are mainly responsible for protection of bacteria against foreign DNA, namely the CRISPR (clustered regularly interspaced short palindromic repeat) sequences and restriction-modification (R-M) systems [2,3]. The existence of CRISPR systems is rarely reported in *S. epidermidis* [4,5]. R-M systems are much more intensively studied in *S. aureus* compared with *S. epidermidis.* It appears that *S. aureus* populations exchange mobile genetic elements (MGEs) at a high frequency, but this is restricted to isolates from related clones and lineages [6]. On the other hand, unique R-M systems in different common clonal complexes of *S. aureus* contribute to inhibition of genetic exchange between *S. aureus* lineages [7,8]. This finding is supported by bioinformatics studies showing that the distribution of MGEs is lineage-dependent and each lineage has evolved more or less independently [9–11]. However, the role of R-M systems in genetic exchange among different staphylococcal species is still largely unknown.

Staphylococcal cassette chromosome (SCC) elements are genomic islands ubiquitously disseminated among staphylococci that often carry antimicrobial resistance and, in some cases, virulence-associated genes [12,13]. Staphylococcal cassette chromosome *mec* (SCC*mec*) elements are the most representative SCCs and harbour the methicillin resistance determinant *mecA*. Studies undertaken over the last decade have revealed an ever-increasing complexity among SCC elements [12]. The region flanking SCC elements is one of the strongest exchange hotspots in staphylococci, with genes that are laterally transferred between *S. aureus* and *S. epidermidis* commonly being associated with SCC elements. Nevertheless, how and why genetic exchange of SCC elements occurs among different

* Corresponding author. Department of Animal Science, McGill University, Quebec, Canada.
E-mail address: xin.zhao@mcgill.ca (X. Zhao).
[1] These two authors contributed equally to this work.

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staphylococcal species represent two major unsolved questions. With the increasing availability of whole-genome data sets for bacterial populations, it is now possible to explore the role of specific staphylococci and other bacterial genera in the evolution of SCC elements [14,15].

The greatly increased use of antimicrobials in animal agriculture represents an increasing selective pressure on staphylococci that is met with a rapid change in their genomes. In this study, a methicillin-resistant *S. epidermidis* (MRSE) isolate NW32 from a mastitic milk sample was sequenced and its SCC elements were characterised. Based on a comparative genomic analysis, probable pathways for global genetic acquisition among different bacterial species in the formation of the three SCC elements were constructed and it was proposed that the *hsdS* R-M profiles and the lack of CRISPR sequences in this bacterium were responsible for such genetic exchanges.

2. Materials and methods

2.1. Bacterial strain and genome sequencing

Isolate NW32 was identified as a *S. epidermidis* based on 16S rRNA sequencing. Genomic DNA was extracted and whole-genome sequencing (WGS) was performed both using Illumina MiSeq PE300 (MajorBio, Shanghai, China) and HiSeq 2000 PE100 (BGI, Shenzhen, China) sequencing technology. The MiSeq reads were assembled de novo into contigs using SOAPdenovo v.2.21 (http://soap.genomics.org.cn/soapdenovo.html) and sequences were corrected by read mapping with HiSeq 2000 reads. The contigs were joined into scaffolds using paired-end information. The order of the scaffolds was determined by alignment with a reference genome of S. *epidermidis* strain RP62A (GenBank accession no. **NC002976**) using the SOAPaligner v.2.21 (http://soap.genomics.org.cn/soapaligner.html). The genome sequence of NW32 was deposited in GenBank under accession no. **LJIF00000000**.

2.2. Assembly and identification of SCC elements in NW32

To map SCC elements, every scaffold of NW32 was searched against marker genes including *orfX*, *mec* gene complex and *ccr* gene complex using BLASTN. Positive scaffolds were aligned against SCC elements of *S. aureus* strains TSGH17 (GenBank accession no. **AB512767**) to reveal their genetic organisation. Gaps were closed by PCR followed by sequencing. Gene prediction and annotation was performed using the Rapid Annotations Subsystems Technology (RAST) server (http://rast.nmpdr.org/). Nucleotide sequences of predicted genes and their deduced amino acid sequences were subsequently compared against the nucleotide collection (nr/nt) database and the non-redundant protein database provided by the National Center for Biotechnology Information (NCBI).

2.3. PCR for detecting excision of SCC elements

PCR reactions were performed to investigate the excision of SCC elements as previously described [16]. Extracted genomic DNA of NW32 from log-phase cultures was used as a template. The products were then cloned into a pEASY®-Blunt Zero vector (TransGen, Beijing, China) for Sanger sequencing. The primer sequences are listed in Supplementary Table S1.

2.4. Determination of minimum inhibitory concentrations (MICs)

MICs for various antimicrobial agents (gentamicin, penicillin, oxacillin, cefoxitin, tetracycline, lincomycin, ciprofloxacin, teicoplanin, trimethoprim and sulfamethoxazole) were determined for NW32 by the agar dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [17] (Table 1). MICs of heavy metal ions were measured as previously described [18]. Bacterial suspensions of 0.5 McFarland standard were prepared for the test. Mueller–Hinton agar plates with different final concentrations of heavy metal ions were prepared ($CuSO_4 \cdot 5H_2O$ for copper, $ZnSO_4 \cdot 7H_2O$ for zinc and $HgCl_2$ for mercury. All reagents were purchased from Sigma-Aldrich (St Louis, MO). *Staphylococcus aureus* strain RN4220 served as a control for MIC determination both for antibiotics and heavy metal ions.

2.5. Bioinformatics analyses

To determine phylogenetic relationships among the composite SCC in NW32 and other reported composite SCCs, the NCBI nucleotide blast software was used for genome alignments in the nr/nt and WGS databases with default parameters (https://blast.ncbi.nlm.nih.gov/Blast.cgi; accessed 3 May 2016). The query sequence was the identified nucleotide sequence of CI_{32} or three SCC elements. Comparison of different composite SCCs was conducted using BLASTN. To explore the origination of the mercury tolerance region (orf43–orf45) in NW32, which has not been reported in any other staphylococcal isolates, a phylogenetic tree of the region from different isolates was constructed employing the MEGA6 program (http://www.megasoftware.net/) on a neighbour-joining algorithm and a maximum likelihood method.

3. Results

3.1. General resistance features and the SCC composite island in *Staphylococcus epidermidis* NW32

Isolate NW32 was identified as an oxacillin-susceptible *S. epidermidis* despite the presence of the *mecA* gene (Fig. 1A; Table 1). However, NW32 was converted to an oxacillin-resistant phenotype (MIC > 256 mg/L) under treatment with oxacillin for only 1 h. Moreover, a set of antibiotic resistance genes (*blaZ*, *msrA*, *lnuA*, *vgaA* and *dfrA*O) was identified, conferring resistance to a range of antimicrobial agents including penicillin, cefoxitin, lincomycin, ciprofloxacin, teicoplanin, trimethoprim and sulfamethoxazole. Moreover, NW32 was also tolerant to certain heavy metals, with the MICs for copper, zinc and mercury of 4, 4 and 0.1 mM, respectively (Table 1).

The genome of NW32 was sequenced and analysed, especially for the SCC element structure. Among the assembled scaffolds, seven

Table 1
Results of susceptibility testing against heavy metal compounds and antibiotics of methicillin-resistant *Staphylococcus epidermidis* isolate NW32.

Strain	MIC (mM) of metal[a]			MIC (μg/mL) of antimicrobial agent[a]									
	$CuSO_4$	$ZnSO_4$	$HgCl_2$	GEN	PEN	OXA	FOX	TET	LIN	CIP	TEC	TMP	SMX
S. epidermidis NW32	4	4	0.1	1	32	1	8	0.5	>256	32	32	>256	128
Staphylococcus aureus RN4220	4	4	0.05	1	1	0.5	2	1	1	2	2	1	>256

MIC, minimum inhibitory concentration; GEN, gentamicin; PEN, penicillin G; OXA, oxacillin; FOX, cefoxitin; TET, tetracycline; LIN, lincomycin; CIP, ciprofloxacin; TEC, teicoplanin; TMP, trimethoprim; SMX, sulfamethoxazole.
[a] All MIC determinations were performed by agar dilution assay.