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Impact of recombination on genetic variability within *Staphylococcus aureus* clonal complexes

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ABSTRACT

The population structure of *Staphylococcus aureus* is generally described as highly clonal and is consequently subdivided into several clonal complexes (CCs). Recent data suggested that recombination might occur more frequently within than among CCs. To test this hypothesis as well as to understand how genetic diversity is created in *S. aureus*, we analyzed a collection of 182 isolates with MLST and five highly variable core adhesion (ADH) genes. As expected the polymorphism of ADH genes was higher than MLST genes. However both categories of genes showed low within CCs diversity with a dominant haplotype and its single nucleotide variants. Several recombination events were detected but none involved intra-CC recombination. This did not confirm the hypothesis of higher recombination within CCs. Nevertheless, molecular analyses of variance indicated that these few recombination events have a significant impact on the genetic diversity within CCs. In addition, although most ADH genes were under purifying selection, signs of positive selection associated with a recombinant group were detected. These data highlight the importance of recombination on the evolution of the highly clonal *S. aureus* and suggest that recombination when combined with demographic mechanisms as well as selection might favor the rapid creation of new clonal complexes.

1. Introduction

The ability of bacteria to generate genetic variation is crucial for their survival. Among the mechanisms that generate variation, horizontal DNA transfer allows bacteria to rapidly adapt to new ecological niches (Narra and Ochman, 2006). This mechanism includes lateral gene transfer resulting in the introduction of novel sequences in the chromosome (e.g. SCC*mec* in *Staphylococcus aureus*) as well as homologous recombination resulting in new haplotypes (Narra and Ochman, 2006). The introduction of new combinations of alleles in a bacterial population can play a role in its diversification process and thus in its potential for adaptation. For example, recombination can increase the pathogenicity or the ability of a bacteria to spread by diffusing genetic material throughout the rest of the population (Bruen et al., 2006).

S. aureus is a major human pathogen and is one of the most common nosocomial organisms. This bacterium can cause a variety of infections ranging from asymptomatic carriage to severe life-threatening diseases like pneumonia or septicemia. Methicillin-resistant *S. aureus* (MRSA) represents one of the greatest challenges for modern antimicrobial chemotherapy, especially with the emergence of *S. aureus* of intermediate susceptibility to glycopeptides. Recently, community-acquired MRSA have been increasingly reported worldwide, and represent a new challenge in terms of pathogenicity and antibiotic therapy. In addition, the capacity of some strains to grow in frequency and create epidemics highlights the importance of understanding the mechanisms of emergence of new strains with specific biological characteristics.

Using multilocus sequence typing (MLST), the population of *S. aureus* was classified into isolated sequence types (ST) and clusters of related strains defined as clonal complexes. Although several chromosomal replacement events were described for *S. aureus* (Narra and Ochman, 2006; Robinson and Enright, 2004), this species has been shown to be highly clonal using a variety of genes: MLST (Feil et al., 2003), cell surface *sas* genes (Robinson and Enright, 2003), cell surface core and accessory (i.e. not present in all the strains) adhesion genes (Kuhn et al., 2006), accessory exotoxin-like genes (Fitzgerald et al., 2003). Despite this clonal mode of reproduction *S. aureus* has a relatively high level of genetic diversity compared to monomorphic species like *Yersina pestis* (Feil, 2004; Achtman, 2008).

To understand how genetic diversity is created in a species, it is necessary to determine the relative contribution of the evolutionary processes that generate this variation: i.e. mainly point mutation and recombination. In addition, it is also necessary to

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understand the impact of the forces acting on that genetic diversity: i.e. selection and genetic drift. Most of the population genetic studies on *S. aureus* aimed at uncovering structure within the species but analyses of the genetic diversity considering this sub-structure are crucial for obtaining accurate inferences and avoid misleading results (Halkett et al., 2005; Tibayrenc, 1995). This is especially important since a recent study suggested that recombination in *S. aureus* might occur more frequently between closely related strains than between the distant phylogenetic lineages (Kuhn et al., 2006). This study indicated that for a small number of allelic differences between strains (up to two in the case of MLST genes and up to three in the case of core adhesion genes) there was no positive correlation with nucleotide diversity. This pattern is understood as the result of recombination overcoming the effects of divergence caused by de novo point mutations. This hypothesis was supported by Waldron and Lindsay (2006) who highlighted a restriction modification system that blocks horizontal transfers between *S. aureus* strains of different CCs. This system provides a mechanism by which gene flow and recombination may be higher within than between CCs (Waldron and Lindsay, 2006). Since barriers to gene flow are the basis of the biological species concept (de Queiroz, 2005), these data suggest that CCs could correspond to biological 'species' (Turner and Feil, 2007). This mode of population structure corresponds to the cryptic speciation model described by Smith et al. (1993), in which the species is actually subdivided into two or more genetic lineages, genetically isolated from one another, with each being panmictic. Finally, intra-lineage recombination might be one of the processes responsible for the relatively high genetic diversity observed in *S. aureus* and provide a possible mechanism of emergence of particularly epidemic, virulent or resistant strains.

A species like *S. aureus* with moderate to high levels of diversity can be initially subdivided into CCs on the basis of the housekeeping (MLST) gene variation. However, the variation within a single CC must be examined using more variable markers (Cooper and Feil, 2004). It is expected that genes encoding proteins which interact with the host or the external environment will be highly variable owing to strong diversifying selection (Cooper and Feil, 2006). Examples of these genes are adhesion cell surface genes implicated in host colonization and/or virulence, and which provide interesting nucleotide diversity (Hughes and Friedman, 2005; Kuhn et al., 2006). Our aims in the present study was to measure the relative levels of recombination within and between CCs in order to test the hypothesis that recombination levels are higher within CCs than between CCs and to understand how genetic diversity is created and maintained in the highly clonal species *S. aureus*. For this reason, we analyzed the genetic variability of a collection of 182 strains belonging to the four most common CCs with MLST and five highly variable core adhesion (ADH) genes.

Table 1
Geographical origins and clonal complexes (CC) of the *S. aureus* isolates analyzed in this study.

Origin	CC5	CC8	CC30	CC45
Belgium	3	7	–	2
Canada	–	–	–	1
England	–	–	1	–
Finland	1	–	–	1
France	11	6	–	5
Germany	–	1	–	2
Hungary	–	1	–	–
Norway	–	–	–	3
Poland	1	1	–	–
Portugal	–	8	–	–
Switzerland	24	19	33	36
The Netherlands	3	3	–	9
Total	43	46	34	59

2. Materials and methods

2.1. S. aureus strains

A total of 182 strains were analyzed in this study. These strains were selected from four common CCs (i.e. CC5, CC8, CC30 and CC45) to represent *S. aureus* isolates from different geographical origins (Table 1). In CC5, 24 strains were methicillin sensitive *S. aureus* (MSSA) and 15 were MRSA; in CC8, 16 strains were MSSA whereas 24 were MRSA; CC30 included 32 MSSA and two MRSA and finally CC45 was composed of 42 MSSA and 14 MRSA.

2.2. Culture, lysis and DNA extraction of bacterial strains

S. aureus strains were grown overnight at 37 °C in 1 ml brain heat liquid medium with agitation. Pelleted cells obtained from 200 μl of culture were lysed in 400 μl lysis buffer (1× Tris–EDTA buffer, 0.35 M NaCl and 0.05 mg/ml lysostaphin). DNA was extracted from 100 μl lysate as described previously (Kuhn et al., 2006).

2.3. Sequencing of MLST and core adhesion genes

The sequence of MLST genes was obtained as described earlier (Enright et al., 2000) whereas the sequences of ADH genes (*clfA, clfB, fnbA, map, sdrC*) were amplified using primers described by Kuhn et al. (2006). Four primers (SA_clfA_786 for, SA_clfB_562 for, SA_fnbA_534 for, SA_fnbA_1598 rev) were redesigned to match more conserved regions and improve the amplifications (Table 2). The annealing temperature for the newly designed *fnbA* primers was 54 °C whereas the other annealing temperatures remained unchanged (Kuhn et al., 2006).

Table 2
Locus and primer details of the regions analyzed in this study.

Locus name	Accession # of the reference sequence	Position on the reference sequence	Primer name	Primer sequence (5′–3′)
clfA	Z18852	786	SA_*clfA*_786 for[a]	CACCTCAAAATTCTACAAATGCGG
		1943	SA_*clfA*_1943 rev	GTTCAATTTCACCAGGCTCATCAG
clfB	AJ224764	561	SA_*clfB*_562 for[a]	ATTTCCAATGCGCAAGGAACTAGT
		1638	SA_*clfB*_1638 rev	GTCTTTCGGATTTACTGCTGAATC
fnbA	J04151	534	SA_*fnbA*_534 for[a]	AAGACAAGTAGATTTAACACCTAA
		1598	SA_*fnbA*_1598 rev[a]	CTGTATAAAACTAAACCATTATCC
map	AJ245439	164	SA_*map*_164 for	AATCATCAAGTWCRTTACAYCATGG
		1173	SA_*map*_1173 rev	CATTTACTCGAATTGTGTATGGTAC
sdrC	AJ005645	689	SA_*sdrC*_689 for	ATTCTGTTAAAGAGGGCGATAC
		1604	SA_*sdrC*_1604 rev	TTCATCTGTTGTCGTACGATCT

[a] Primers redesigned from Kuhn et al. (2006).