The role of horizontal gene transfer in Staphylococcus aureus host adaptation

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Staphylococcus aureus is an important human pathogen that also causes economically important infections of livestock. In a recent paper, we employed a population genomic approach to investigate the molecular basis of ruminant host adaptation by S. aureus. The data suggest that the common pathogenic clone associated with small ruminants originated in humans but has since adapted to its adopted host through a combination of allelic diversification, gene loss and acquisition of mobile genetic elements. In particular, a new subfamily of staphylococcal pathogenicity islands (SaPI) was identified encoding a novel von Willebrand factor-binding protein (vWBP) with ruminant-specific coagulase activity. The wide distribution of vWBP-encoding SaPIs among ruminant strains implies an important role in host-adaptation. In the current article we summarize the findings of the paper and comment on the implications of the study for our understanding of the molecular basis of bacterial host adaptation.

Staphylococcus aureus is a notorious human pathogen that can also cause important diseases of animals including mastitis in ruminants, dermatitis in rabbits and skeletal infections in poultry. Strains of S. aureus with combinations of phenotypes which were unique to different host species were first identified in the 1930s, leading to the description of host-specific ecological variants (ecovars). Subsequently, numerous population genetic studies have demonstrated that the majority of S. aureus animal infections are caused by pathogenic clones which are not commonly found in association with humans, implying that they are host-specialized and largely host-restricted. While the molecular basis of the adaptation of S. aureus to animal hosts has not been well examined, recent studies have identified genetic determinants that correlate with infection of a particular host. For example, Lowder et al. provided the first clear evidence of a human-to-poultry host jump by a bacterial pathogen, which led to the emergence of an avian-adapted pandemic clone. The origin of the clone was traced to a subtype of the common human clonal complex CC5 about 40 years ago but it has since undergone inter-continental dissemination due to the globalized nature of the poultry industry. Of note, a large-scale acquisition of mobile genetic elements (MGE) from other S. aureus strains resident on birds was discovered, suggesting a key role for horizontal gene acquisition in avian host adaptation. Furthermore, analysis of the genome sequence of a bovine mastitis strain RF122 of the ST151 clone of revealed novel MGE present in other bovine strains but absent in human isolates. Recently, we employed a combination of population genetics, comparative genomics and functional analysis to investigate the evolutionary origin of the major clonal complex affecting small ruminants (CC133). The data suggest that the CC133 clone originated as a result of a host jump from humans to ruminants followed by genetic adaptation through...
a combination of allelic diversification, genome decay and acquisition of mobile elements containing virulence genes with enhanced activity in ruminants. In comparison to *S. aureus* strains of human origin, allelic variation was identified in genes involved in toxin production, metabolism, adherence, replication and repair, and regulatory pathways, including many which were under diversifying selective pressure. Gene decay is a common feature of bacteria undergoing niche adaptation, and this has previously been observed among *S. aureus* clones adapted to cows and poultry. Similarly, ED133 contained pseudogenes resulting in loss of function of putative virulence factors such as toxins and lipoproteins. Of note, several pseudogenes were shared with the bovine clone ST151 but contained unique underlying mutations suggestive of a strong selective pressure for loss of function in ruminants. The most striking feature of the genome of ED133 was a unique complement of MGE that had not been previously identified among strains from humans or other animals. In total, two new members of the *S. aureus* pathogenicity island (SaPI) family and three novel prophages were identified. Of these, two of the prophages and both SaPIs were widely distributed among other isolates of the CC133 lineage but not among other ruminant, avian or human lineages examined, consistent with a function which is specific for the small ruminant niche occupied by CC133 strains.

The capacity of ruminant strains of *S. aureus* to coagulate bovine or ovine plasma has been employed as a component of the traditional biotyping scheme used to differentiate *S. aureus* host-specific ecovars. However, the molecular basis for this phenotype was previously unknown. A novel SaPI (SaPlov2), widespread among CC133 strains encoded a unique variant of the core genome-encoded von-willebrand factor binding protein (vWBp) gene.

Similar to the chromosomally encoded vWBp, vWBpSov2 contained a predicted coagulase domain which has previously been implicated in *S. aureus* pathogenesis. We speculated that SaPlov2 may confer the capacity to coagulate ruminant plasma and constructed isogenic CC133 ruminant strains, differing only in the presence of SaPlov2. In each case, the presence of SaPlov2 conferred the ability to coagulate ruminant plasma, revealing the basis for a defining phenotype of the *S. aureus* biotyping scheme. Independently, Viana et al. identified 3 additional SaPIs encoding variant vWBPs among strains of bovine and equine origin, and the authors demonstrated that the plasma coagulation phenotype was due to the activity of the encoded vWBPs. It appears that diversification of the N-terminal coagulase domain of vWBp has resulted in the capacity to activate equine or ruminant prothrombin leading to the conversion of soluble fibrinogen to insoluble fibrin. Note of note, an equine strain of the same clonal complex as ovine strain ED133 (CC133) contained a SaPI encoding a vWBp with the capacity to coagulate equine plasma, consistent with a central role for this family of SaPIs in the host-tropism of *S. aureus*. Recently, Cheng et al. demonstrated the importance of the archetypal *S. aureus* coagulase and the chromosomal copy of vWBp in the formation of abscesses in a mouse model. The role of plasma coagulase activity in ruminant mastitis is unclear but we speculate that it may contribute to the formation of micro-abscesses during intramammary infection, which may facilitate persistence during chronic infection.

An understanding of the timescale for the evolution of livestock-associated *S. aureus* is important to appreciate the ecological context of their emergence and the capacity for new pathogenic clones affecting animals to evolve. To predict the date of the putative human-to-ruminant host jump that led to the CC133 clonal complex, a relaxed molecular clock based method was employed with the program BEAST. The estimated host switch was calculated to have occurred about 115 to 1204 years ago, based on a mutation rate of 3.3 x 10^-6 per site per year calculated for contemporary isolates of the important human clone ST239. However, the relevance of this mutation rate for predicting long term evolutionary events is unclear as the effects of purifying selection may limit the applicability of mutation rates calculated over shorter timescales. Furthermore, our current appreciation of the variation in mutation rates for different *S. aureus* clones occupying diverse niches is very limited. It is reasonable to assume that our prediction is the minimum date a host switch could have happened but it may have been much earlier. We predict that it is likely to have happened since the domestication of small ruminants (approximately ~11,000 years ago), which would have resulted in ample opportunities for the transfer of bacteria between human and livestock. However, these important questions remain to be answered.

Overall, this population genomics study and other related works have improved our understanding of the evolutionary origin of livestock-associated *S. aureus* and identified some of the genetic determinants that differentiate animal strains from human pathogenic clones. In particular, although possibly not surprising, MGE appear to play a fundamental role in facilitating the adaptation of *S. aureus* to different host species. The origin of the MGE is unclear but it is likely that other staphyloccoccal species that are normally resident on animal hosts may represent the source. It is feasible that the functional characterization of the role of the identified MGE in bacterial adaptation to animal hosts may lead to the design of novel approaches for controlling livestock infections.

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