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Staphylococcus aureus Strains That are Hypersusceptible to Resistance Gene Transfer from Enterococci▽

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We identified naturally occurring *Staphylococcus aureus* mutants of the restriction modification pathway SauI, including bovine lineage ST151. In a model of vancomycin resistance transfer from *Enterococcus faecalis*, ST151 isolates are 500 times more susceptible than human *S. aureus* isolates. The eradication of "hyper-recipient" strains may reduce the evolution of vancomycin-resistant *S. aureus*.

Six cases of fully vancomycin-resistant *Staphylococcus aureus* (VRSA) in U.S. hospitals have been described since 2002 (1, 16). VRSA strains have acquired vancomycin resistance genes, such as *vanA*, from vancomycin-resistant enterococci (VRE) (6, 16). Both VRE and methicillin-resistant *S. aureus* (MRSA) are widespread in hospitals (3, 7), and it is not uncommon for a patient to be colonized or infected with both and treated with vancomycin. In addition, VRE are found in the agricultural setting despite the banning of glycopeptides and *S. aureus* strains are widespread in animals and are a major cause of dairy cow mastitis. The emergence and spread of VRSA in hospitals is an enormous threat, with resistance to all new antibiotics already reported for *S. aureus* and no vaccine on the horizon.

The mechanism of the spread of an antibiotic resistance gene from enterococci to *S. aureus* was first described by Clewell et al. in 1985 (2). Some *Enterococcus faecalis* strains carry large pheromone-responsive plasmids, which in turn can carry other mobile pieces of DNA, such as transposons encoding resistance genes. These plasmids respond to a lipoprotein signal produced by *S. aureus*, triggering conjugation. The transposon jumps to the *S. aureus* chromosome, while the plasmid is unable to replicate in *S. aureus* and is lost. In this model, the *S. aureus* recipient strain was 879R4RF, a putative "restriction-deficient" isolate.

A laboratory transfer of vancomycin resistance from enterococci to *S. aureus* recipient B111 was reported in 1992 (12). While the process was not genetically characterized, it showed that *vanA* could be transferred to and expressed in *S. aureus*. When the first naturally occurring VRSA strain was isolated in Michigan (6, 16), the mechanism of transfer appeared to be similar to that described by Clewell et al. (2). The donor plasmid from *E. faecalis*, pAM830, facilitated the transfer of a resident *vanA* Tn*1546*-like element to *S. aureus* and then was lost. However, pAM830 was not pheromone responsive and was more closely related to the enterococcal broad-host-range plasmid pIP501 (6).

We have recently described the major mechanism that blocks the horizontal transfer of DNA into *S. aureus* (15). It is the Sau1 (or Sau1I) restriction modification (RM) system. A restriction enzyme composed of subunits encoded by *sau1hsdR* (restriction) and one of two *sau1hsdS* (specificity) genes identifies and binds to a specific DNA sequence and digests the DNA. This protects the bacterial cell from deleterious foreign DNA, such as that from a bacteriophage. To protect its own DNA, *S. aureus* also produces a modification enzyme composed of subunits encoded by one of two *sau1hsdM* genes and the same *sau1hsdS* gene. This modification enzyme recognizes the same specific DNA sequence and methylates it, protecting it from restriction. The Sau1 system blocks uptake of DNA from *Escherichia coli* and reduces uptake from enterococci. In addition, it prevents the transfer of DNA between the dominant lineages of *S. aureus* (9), which each have unique *sau1hsdS* gene variants (15). The standard *S. aureus* laboratory strain that accepts foreign DNA, RN4220, is deficient in *sau1hsdR* (15).

The aim of this study was to investigate whether all of the strains of *S. aureus* are capable of accepting resistance genes from enterococci or whether only a select few "restriction-deficient" strains could do this.

***S. aureus* isolates.** The *S. aureus* isolates included the standard laboratory strain 8325-4; its *sau1hsdR*-deficient mutant RN4220 (15); 879R4RF, which has a "restriction-deficient" phenotype (3); and B111, kindly donated by Sue Howell (12).

The human *S. aureus* isolates represented the major dominant lineages from hospitals and the community, including hospital MRSA. They included 13 epidemic hospital MRSA isolates, representing the lineages CC30, CC22, CC5, CC8, and CC45 (4, 11, 13); 11 hospital methicillin-susceptible *S. aureus* (MSSA) isolates, representing lineages CC8, CC30, CC45, and CC15 (10); and 15 community-acquired MSSA isolates, representing all 10 dominant human lineages, CC1, CC5, CC8, CC12, CC15, CC22, CC25, CC30, CC45, and CC51 (9).

Isolates from animals were kindly collected by David Lloyd and colleagues at the Royal Veterinary College, United Kingdom. These isolates were from cows ($n = 19$; predominantly mastitis), horses ($n = 13$), sheep ($n = 2$), goats ($n = 2$), and a camel ($n = 1$). A further 18 United Kingdom bovine *S. aureus* strains were kindly donated by Chris Teale, Veterinary Laboratories Agency. The lineage of animal strains was determined by microarray (9, 17), and representative isolates of each lin-

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TABLE 1. Conjugation frequency

Strain(s)[a]	No. of transconjugants per 10^8 donors		
	Mean	SD	SE
ST151 from cows ($n = 14$)[b]	12,385	10,507	
Other animal isolates ($n = 39$)	596	2,096	
Human isolates, including MRSA ($n = 39$)	32	57	
Laboratory isolates			
8325-4	14		6.8
RN4220	426		199
879R4RF	1,770		320
B111	12,750		250

[a] All animal and laboratory isolates were tested in duplicate.
[b] Significantly different from other animal isolates ($P < 0.001$) and from human isolates ($P < 0.001$).

eage were confirmed using multilocus sequence typing (5); a more complete description of this population will be published separately. RF122 was kindly donated by Ross Fitzgerald. Three animal isolates were naturally tetracycline resistant and not studied further. Antibiotic resistance was tested on Mueller-Hinton agar with discs according to CLSI (formerly NCCLS) guidelines.

Conjugation assays. We used the conjugation assay previously described by Clewell (2, 15). One group of isolates accepted enterococcal DNA from JH2-2 at an extremely high transfer frequency (Table 1; Fig. 1). These isolates were all of the same lineage, ST151, and all came from dairy cows in the United Kingdom. The sequencing of the two *sau1hsdS* genes required for Sau1 activity in five ST151 strains revealed that they each had exactly the same two stop mutations, one in each of the two *sau1hsdS* gene copies. This genotype is predicted to prevent the restriction (and modification) of foreign DNA and would explain the enhanced ability to accept resistance genes from enterococci. The RF122 sequence shows that it is ST151 and carries two identical mutations. Thus, all strains from this lineage are probably "hyperrecipient." In our collection, one-third of 39 bovine isolates were from the ST151 lineage. ST151 isolates have been reported in cows in Norway (www.mlst.net), but in a study of U.S. and South American bovine isolates, they were not found (14). There are no reports of ST151 in humans.

The *S. aureus* B111 recipient strain used by Noble et al. (12) was hyperrecipient (Table 1). However, after sequencing the five *hsd* genes in this strain, we had not identified any obvious mutations. B111 belongs to CC1, and another hyperrecipient CC1 isolate was identified from a horse. Other CC1 isolates were not hyperrecipient. The 879R4RF isolate used by Clewell et al. (2) was also found to be hyperrecipient. We have not identified an *hsd* mutation in this strain. It belongs to CC51, but a second CC51 isolate was not hyperrecipient. Thus, some hyperrecipient strains have developed independently of their lineages. Furthermore, there is likely to be a second pathway in *S. aureus* that blocks the horizontal transfer of foreign DNA or an unknown but necessary step that is essential for Sau1 activity. Other RM pathways have been described for some isolates of *S. aureus*, including on mobile genetic elements, and they may be implicated (8, 15).

Conclusions. The discovery that certain *S. aureus* lineages and strains have deficiencies in the dominant RM pathway and a hyperrecipient phenotype is key for predicting how VRSA may arise in the future. The high incidence of antibiotic resis-

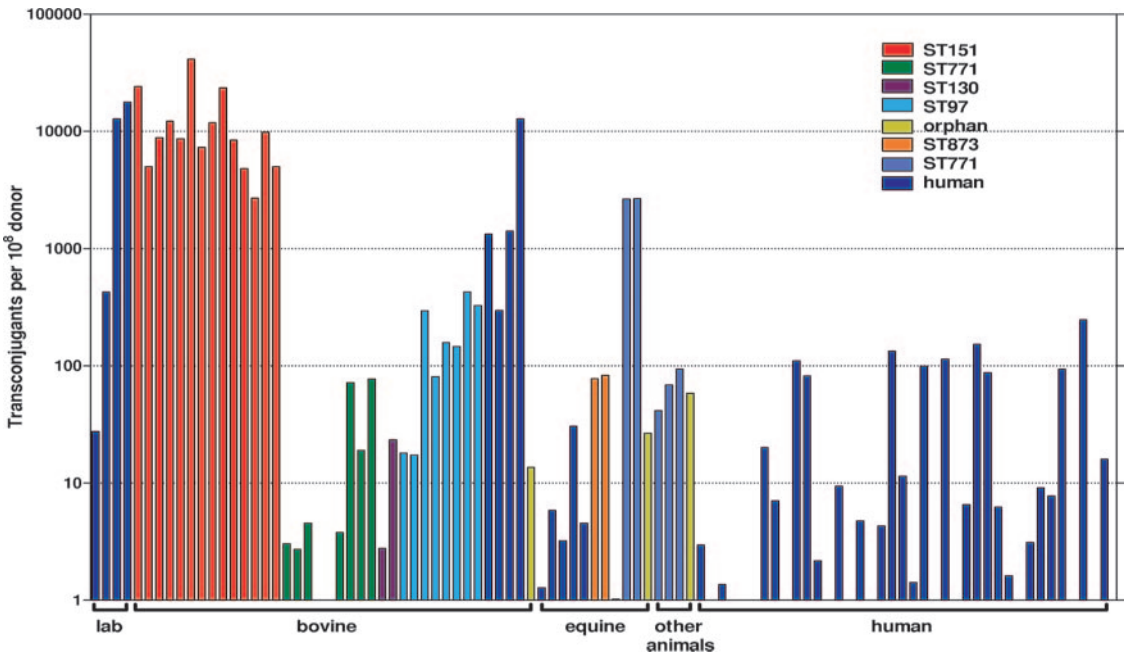

FIG. 1. Susceptibility of strains to conjugational transfer. Data are presented as a log scale of numbers of transconjugants per 10^8 donors. Strains are grouped according to source, with laboratory strains in the order of 8325-4, RN4220, 879R4RF, and then B111, followed by bovine, equine, and other animal and human sources as indicated below the figure. The lineage of each isolate is indicated in color (see key), with ST151 isolates in red. All isolates from human lineages are navy blue and include one ST1 and three ST188 from bovine sources and four ST1 and one ST22 from equine sources.

tance gene transfer into animal strains strongly supports the decision to ban glycopeptide antibiotics, such as avoparcin, for agricultural use (18). The incidence also supports the surveillance of *S. aureus* populations for hyperrecipient strains, particularly when they are in close contact with VRE, so that high-risk situations can be identified and contained.

Nucleotide sequence accession number. During the course of this project, *S. aureus* RF122, an isolate from bovine mastitis in Ireland, was sequenced and deposited in GenBank under accession number AJ938182.

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