GENOME WATCH

# The grapes of wrath

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Viewed under the microscope, staphylococci can appear in 'grape-like' clusters. It is this morphological characteristic of these Gram-positive cocci that has given rise to their name; staphyle being Greek for 'a bunch of grapes'. The genus contains several species that cause infections in humans and animals, the most notorious of which is *Staphylococcus aureus*, a healthcare-associated pathogen with a talent for developing antibiotic resistance. Although *S. aureus* has attracted the lion's share of sequencing efforts within this genus, several other species have been sequenced, providing a refined view of the diversity of this genus and highlighting the common mechanisms of genome evolution that transcend taxonomic boundaries.

This month we highlight the most recent *Staphylococcus aureus* sequence: the community-acquired meticillin (formerly methicillin)-resistant *S. aureus* (MRSA) strain FPR3757 (REF. 1) and look at the contents of its genome to decipher the secret of its success. We also look at the genomes of three other pathogenic staphylococci: *Staphylococcus epidermidis* strain RP62A (REF. 2), *Staphylococcus haemolyticus* JCSC1435 (REF. 3) and *Staphylococcus saprophyticus* ATCC 15305 (REF. 4).

The fearsome reputation of *S. aureus* as a pathogen belies its close, and often harmless, relationship with humans. *S. aureus* is a frequently found resident of the nose and throat and, for most healthy individuals, *S. aureus* colonization poses no problems. However, if *S. aureus* leaves its natural niche and contaminates deeper tissues, it has the potential to go on to cause serious infections and life-threatening diseases. Patients in hospitals are often particularly prone to *S. aureus* infection owing to compromised immune systems and invasive procedures. The spread of drug resistance has also contributed to the success of *S. aureus* in the healthcare environment. Additionally, the past decade has seen an alarming increase in MRSA infections in healthy individuals who have acquired the infection in the community setting rather than in hospital.

*S. aureus* FPR3757 (REF. 1) is a recent addition to the swelling ranks of *S. aureus* genomes (currently eight other complete genomes are available), and it is a representative of the USA300 clone, a recently emerged community-acquired MRSA lineage that has been associated with outbreaks of severe skin and soft-tissue infection. Originally identified in the USA, the USA300 clone has been detected in Canada and Europe, and is also spreading to hospitals

The genome of FPR3757 consists of a circular chromosome of 2.87 Mb and three plasmids. Multilocus sequence typing places FPR3757 in the same clonal complex as the MRSA strain COL[2] and the same sequence type as *S. aureus* strain NCTC 8325 (REF. 5). In their manuscript, Diep *et al.* used the comparison to COL to identify the genomic differences that differentiate these closely related strains. As with other *S. aureus* genomes, much of the variable component of the genome is associated with mobile genetic elements (MGEs). These often carry strain-specific virulence and drug-resistance determinants, which supplement the considerable arsenal of core functions that *S. aureus* possesses.

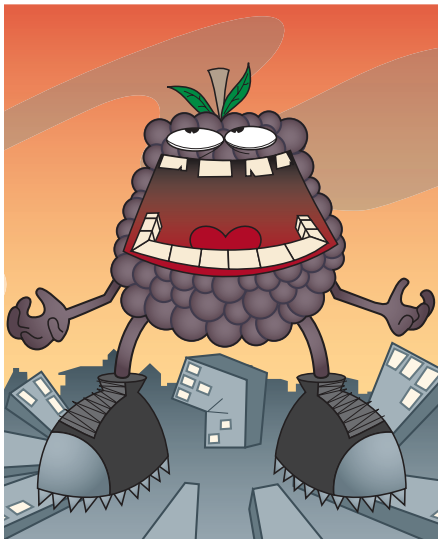
Although closely related to the MRSA strain COL, FPR3757 carries a different type of meticillin-resistance-encoding *Staphylococcus* cassette chromosome (SCC*mec*) element. SCC*mec* elements carry the *mecA* meticillin-resistance gene and site-specific recombinase genes, and integrate at a conserved location on the chromosome. These elements can be divided into different classes based on the combinations of genes in the element, and have been found in several species of staphylococci. The SCC*mec* in FPR3757 is a type IV element that is virtually the same as the SCC*mec* element in the community MRSA strain MW2 (REF. 6), whereas the SCC*mec* in COL is a type I element that was associated with the early emergence of MRSA. Two of the three plasmids in the genome carry antibiotic resistance genes — pUSA02 encodes tetracycline resistance and is highly similar to a plasmid in the genome of *Staphylococcus epidermidis* ATCC 12228 (REF. 7). The largest of the plasmids, pUSA03, is a conjugative plasmid that carries genes encoding constitutive resistance to macrolides, lincosamides and streptogramin B, and high-level resistance to mupirocin. This plasmid shares much of its backbone with pLW1043, a plasmid carrying a vancomycin-resistance transposon of *Enterococcus faecalis* origin, which was recently found in vancomycin-resistant *S. aureus*.

The chromosome of FPR3757 contains a novel staphylococcal pathogenicity island (SaPI5) that carries two enterotoxins, SEQ2 and SEK2, and two prophages, both of which carry virulence factors: ϕSA3usa encodes staphylokinase (Sak) and chemotaxis-inhibiting protein (Chp) and ϕSA2usa encodes the Panton-Valentine leucocidin (PVL). The prophage ϕSA2usa is similar to a PVL-encoding prophage in the community MRSA strain MW2 (REF. 6). PVL is a pore-forming cytotoxin that targets neutrophils and causes the necrosis of host tissues. It is rarely found in hospital-acquired *S. aureus* strains and, in recent years, has been associated with an increase in invasive skin infections in healthy individuals. More worryingly, PVL toxin strains have been associated with a severe necrotizing community-acquired pneumonia in children.

Downstream of SCC*mec* is an island region (the arginine catabolism mobile element, ACME I) encoding an arginine deiminase pathway that has a potential role in virulence. This region is flanked by insertion sequence

elements and possibly constitutes a composite transposon. In *Streptococcus pyogenes*, arginine deiminase activity has been shown to promote intracellular survival and survival at low pH. Diep *et al.* speculate that this region might enhance the capacity of *S. aureus* to survive in the acidic environment of the human skin, and also under the anaerobic conditions found in deeper tissues. None of the other sequenced *S. aureus* strains contain ACME elements, however a related element (ACME II) was found in the genome of *S. epidermidis* ATCC 12228, leading to suggestions that the ACME element in FPR3757 might have originated in other members of the genus. The close proximity of the ACME region to SCC*mec* adds circumstantial evidence to the idea that this element might have been transferred between species, possibly in conjunction with the adjacent SCC*mec*. In the past, much of the attention on SCC*mec* was focused on *S. aureus* because of the prominence of this pathogen, and the spread of meticillin resistance. As more genomes are sequenced from this genus it is becoming apparent that SCC elements are not confined to *S. aureus* and that they also carry other cargos, including alternative drug-resistance genes and virulence determinants.

*S. epidermidis* is the most common *Staphylococcus* species found on human skin, and is an opportunistic pathogen that is especially problematic for patients with indwelling medical devices owing to its ability to form biofilms. The genome of strain RP62A (REF. 2) is the second *S. epidermidis* genome to be sequenced. Unlike the previously sequenced strain, *S. epidermidis* ATCC 12228 (REF. 7), RP62A is a pathogenic isolate that is positive for intracellular cell adhesion (*ica*) and is also resistant to meticillin. *S. epidermidis* does not contain many of the virulence factors found in *S. aureus*, such as protein A, coagulase, haemolysins, surface proteins, enterotoxins, exotoxins or the capsule. Equally, this species contains fewer MGEs; RP62A carries a novel prophage (ϕSPβ) carrying a LPXTG surface protein, an integrated type I plasmid (νSe1) and a free plasmid (pSERP) carrying drug-resistance genes. This strain also has a type II SCC*mec* element that is closely related to SCC*mec* elements in some *S. aureus* strains, supporting the hypothesis that the origins of SCC*mec* might be outside *S. aureus*.



*S. haemolyticus* is another member of the bacterial flora of the human skin and mucous membranes. *S. haemolyticus* is often resistant to multiple antibiotics and, like *S. epidermidis*, is an opportunistic pathogen that causes infections in patients with indwelling devices. The publication by Takeuchi *et al.* describes the genome of a glycopeptide-resistant strain of *S. haemolyticus*, strain JCSC1435 (REF. 3). Although the *S. haemolyticus* genome is missing most of the *S. aureus* virulence factors, and some genes found in both *S. aureus* and *S. epidermidis*, it is replete with drug-resistance determinants carried on a large array of MGEs. The JCSC1435 genome contains three free plasmids (pSHaeA, pSHaeB and pSHaeC), two integrated plasmids (πSh1 and πSh2), two prophages (ϕSh1 and ϕSh2), five transposons (one copy of Tn*552* and a Tn*554*-like element, and three copies of Tn*554*) and an SCC (SCC*h1435*). Surrounding SCC*h1435* are the remnants of several other SCC elements, including an SCC*mec* element. These fragments are the scars of previous insertion and deletion events, and highlight the previous traffic of the SCC elements in the genome. Analysis of the JCSC1435 SCC region suggested that at least six SCC elements have been integrated into the chromosome at this site. The genome also contains 82 insertion sequences, which were shown to promote genome rearrangements and lead to phenotypic changes in this strain.

*S. saprophyticus* is the second most common cause of urinary-tract infection in females of child-bearing age. Like the other member of the genus, the genome of *S. saprophyticus* strain ATCC 15305 (REF. 4) contains a diverse collection of MGEs. These include: two SCC elements, a novel SCC with genes encoding a new capsule type ($SCC_{15305cap}$) and an SCC carrying a restriction–modification system ($SCC_{15305RM}$); a prophage remnant; two free plasmids (pSSP1 and pSSP2); and a genomic island ($\nu Ss_{15305}$) that carries resistance to streptomycin and fosfomycin and has an integrase that is related to the phage integrases.

Like other members of this genus, *S. saprophyticus* lacks the extensive array of virulence factors possessed by *S. aureus*. The inventory of the ATCC 15305 genome indicates that this species has tailored its virulence arsenal to its specific pathological niche. For example, there are extra ion-transport genes and high levels of urease activity, which might promote survival in the urinary tract. *S. saprophyticus* also encodes a single cell-wall-anchored protein, compared with the numerous cell-wall-anchored proteins found in *S. aureus* and other member of the genus. This large protein, uroadherence factor A, has haemagglutination properties and is responsible for the adherence of *S. saprophyticus* to human bladder cells.

*Staphylococcus aureus* was an early favourite of the bacterial genome sequencers and is now blessed with nine complete genomes. The recent appearance of genomes from other pathogenic members of the genus, and the promise of more to come, will provide invaluable data for studying this important group of bacteria. Comparative analyses will allow further investigation into the trafficking of MGEs in this genus, and might give insights into the evolution of the pathogenic and commensal manifestations of the different species.

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