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Mini Review

Staphylococcus aureus genomics and the impact of horizontal gene transfer

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ABSTRACT

Whole genome sequencing and microarrays have revealed the population structure of *Staphylococcus aureus*, and identified epidemiological shifts, transmission routes, and adaptation of major clones. *S. aureus* genomes are highly diverse. This is partly due to a population structure of conserved lineages, each with unique combinations of genes encoding surface proteins, regulators, immune evasion and virulence pathways. Even more variable are the mobile genetic elements (MGE), which encode key proteins for antibiotic resistance, virulence and host-adaptation. MGEs can transfer at high frequency between isolates of the same lineage by horizontal gene transfer (HGT). There is increasing evidence that HGT is key to bacterial adaptation and success. Recent studies have shed light on new mechanisms of DNA transfer such as transformation, the identification of receptors for transduction, on integration of DNA pathways, mechanisms blocking transfer including CRISPR and new restriction systems, strategies for evasion of restriction barriers, as well as factors influencing MGE selection and stability. These studies have also lead to new tools enabling construction of genetically modified clinical *S. aureus* isolates. This review will focus on HGT mechanisms and their importance in shaping the evolution of new clones adapted to antibiotic resistance, healthcare, communities and livestock.

Introduction

S. aureus is a common cause of human and animal infection, causing a wide range of superficial through to invasive and toxin-mediated diseases. The normal habitat is the nares and mucous membranes, and 30% of humans are persistently colonized. Infection is generally with the host's own colonizing strain and is as a result of a breach of the protective skin layer, and a failure of the immune system – predominantly the innate system and neutrophils – to clear the organism (Lindsay, 2013).

The first *S. aureus* genomes were sequenced in 2001 and revealed the core metabolic, regulatory and virulent capacity of the organism, although the function of many genes is still unknown (Kuroda et al., 2001). Subsequent genomes differed substantially from the original sequences, and paved the way for population biology studies. Comparisons of whole genomes revealed *S. aureus* populations are clonal, with ten dominant lineages colonizing and infecting humans, and additional lineages found in animals (Lindsay et al., 2006; Sung et al., 2008). The lineages differ in hundreds of genes that are present/absent, or have variant regions within genes, and include surface proteins, gene regulators and immune evasion pathways. In addition, there are SNP differences in housekeeping and other stable genes. Isolates belonging to the same lineage are remarkably conserved, even when separated by time and space. For example, the original MW2 isolate of community-associated methicillin-resistant *S. aureus* (MRSA) of the lineage clonal complex (CC)1 from the USA and a methicillin-susceptible osteomyelitis CC1 isolate from the UK had only 285 differing SNPs within coding regions in the lineage specific genome (Holden et al., 2004). In contrast, the MGEs of these two strains differ markedly.

MGEs account for 15–20% of the genome of *S. aureus* isolates, and include bacteriophage, *S. aureus* pathogenicity islands, plasmids, transposons and staphylococcal cassette chromosomes (SCC) (Lindsay, 2010). MGEs vary substantially more than the core genomes of lineages. Snapshots of the genomes of *S. aureus* populations by whole genome sequencing reveals closely clustered organisms with remarkably different MGE profiles, indicating frequent transfer and loss of whole elements. This is important because the identification of genes encoded on MGEs is revealing important resistance, and host-adaptation mechanisms. They can also encode toxins such as Panton-Valentine leukocidin, the most important toxins for food poisoning such as entertoxins A, B and C, and toxic shock syndrome toxin. As we understand populations and their distribution better, it is becoming clearer that MGEs are key to the evolution of new clones that adapt to new niches and cause novel clinical and economic problems (Lindsay, 2010).

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Examples of these new clones include healthcare-associated MRSA (HA-MRSA) that are resistant to all types of β-lactam antibiotics as well as the fluoroquinolones. These isolates emerged in the 1980s, and are fitter than the early types of MRSA seen in hospitals (Knight et al., 2012). They did not replace sensitive *S. aureus* but caused an additional disease burden. High levels of MRSA in a hospital prevent the use of β-lactams as prophylactic or empirical therapy, meaning inferior antibiotics are relied upon for both preventing and treating many infections. Community-associated MRSA (CA-MRSA) cause severe skin and soft tissue infections in healthy patients in the community. These infections do not respond to β-lactams and often require hospitalization and systemic antibiotics, overloading hospital services. Livestock associated MRSA (LA-MRSA) heavily colonize pig and veal calves on farms, leading to colonization of farm workers and vets who are then susceptible to infection. In each of these cases, evolution and selection of these clones is strongly associated with resistance, immune evasion and virulence factors encoded on MGEs (Lindsay, 2010).

However, we do not see *S. aureus* isolates with all MGEs, and we do not even see *S. aureus* with all of the most important resistance or virulence or host adaption pathways. Therefore, there is much scope for new *S. aureus* emerging that could cause much greater problems for healthcare, agriculture and potentially other reservoirs by broadening host range, virulence factor and toxin carriage and resistance profile. Our greatest concerns might be fully drug resistant clones in healthcare, healthcare clones that cause severe infection in healthy patients, community clones that spread more easily, or livestock clones that frequently infect animals or carry toxins for food poisoning. The reasons why such clones have not emerged is not clear. However, we are discovering more about MGEs, the genes they carry, how they transfer, barriers to their transfer and factors that influence stability. This greater understanding helps us to predict which strains may evolve in the future and the environmental conditions that may enhance their emergence and selection.

Horizontal gene transfer mechanisms

The physical process of transferring DNA from one bacterial cell to another can be accomplished by generalized transduction, conjugation or transformation. Generalized transduction is likely to be the major method for transfer of DNA between *S. aureus* cells as phage required for transfer are prevalent in *S. aureus* populations and the process is very efficient in the laboratory.

Generalized transduction

Transduction simply describes the transfer of DNA from one cell to another via bacteriophage (phage), and typically refers to the transfer of the phage's own DNA from one cell to another and its integration into the bacterial chromosome at a specific site. An integrated bacteriophage (prophage) is generally stable in the chromosome and is passed to daughter cells during bacterial replication. Prophage can be induced under stress, leading to excision of the prophage DNA, replication of phage DNA, synthesis of new phage proteins including heads and tails, and packaging of the phage DNA into virulent phage particles. Lysis of the bacterial cell releases infectious phage particles which bind specifically to other *S. aureus* cells. The receptor for phage binding has recently been discovered to be glycosylated wall teichoic acid (Xia et al., 2011; Winstel et al., 2013). Phage particles inject their DNA into the recipient cell where it either integrates into the chromosome as a prophage (lysogenic pathway), or goes on to generate more infectious phage particles and kill the recipient host (lytic pathway).

These lysogenic, double-stranded DNA phage are common in *S. aureus*, with most naturally occurring isolates carrying between one and four different prophage types (Lindsay et al., 2006). At least eight families have been described, each with a unique integrase gene and corresponding insertion sites in the bacterial chromosome (McCarthy et al., 2012b). The phage genomes are typically 45 kb in size, and some are known to encode bacterial virulence factors such as enterotoxin A or Panton-Valentine leukocidin, while others encode immune evasion strategies such as chemotaxis inhibitory protein or complement inhibition proteins, and these presumably provide a benefit to the host bacterium.

Generalized transduction is a variation of the transduction process, where the newly forming phage particles package bacterial chromosomal or plasmid DNA instead of phage DNA. It is not known if this is accidental or programmed, and why only some bacteriophage do this but not others. The pseudo-phage particles are released during cell lysis, bind to *S. aureus* receptors on recipient cells, and inject the DNA into the new cell. This DNA is not phage, and therefore does not integrate as like the lysogenic phage, nor does it trigger the lytic lifecycle and kill the recipient cell.

Some host DNA appears to be preferentially packaged by generalized transducing phage leading to very high transfer frequencies. *S. aureus* pathogenicity islands are 15 kb mobile genetic elements related to phage, but that do not have the genes necessary for making phage particles (Lindsay et al., 1998). They can be packaged into miniature phage particles where the necessary proteins are donated by phage (Ruzin et al., 2001). Particular SaPI are preferentially packaged by particular phage (Maiques et al., 2007). SaPIs can encode toxins such as toxic shock syndrome toxin and enterotoxins B and C as well as resistances. Plasmids are also thought to be packaged into phage particles at higher frequency that other DNA, and package as concatemers of sequential phage DNA that is then resolved in the recipient cells (Novick et al., 1986). It is likely that all types of DNA can be packaged by generalized transduction, with a maximum load of around 45 kb.

Transduction does not require direct contact between the donor and recipient bacteria. Free phage particles can be released by growing cultures, and are relatively stable. Phage therapy studies have shown that they can also survive in mammalian hosts although some are sequestered by the reticuloendothelial system (Merril et al., 1996).

S. aureus bacteriophage are particularly host specific, and this ensures that *S. aureus* MGE are rarely found in other species or genera. Winstel et al. (2013) have shown that altered glycosylation of the *S. aureus* bacteriophage receptor is sufficient to block phage binding, but also to prevent HGT from other *S. aureus.* The divergent S. aureus lineage ST395 has altered glycosylation of wall teichoic acid and this could explain why it does not carry typical S. aureus MGE. Continued evolutionary divergence may lead to new sub-species or species.

Conjugation

Conjugation involves the transfer of DNA from one cell to another directly through a connecting tube (pilus) or pore (Grohmann et al., 2003). In *S. aureus* pili are not seen, and it is presumed that pores are formed between neighbouring cells in close proximity. The *tra* genes encode the necessary proteins and show similarity to type IV secretion systems (Guglielmini et al., 2013) and are carried exclusively on mobile genetic elements. Conjugative plasmids are large, generally over 45 kb and therefore too large to transfer by transduction, and can carry an extensive and variable range of antibiotic and heavy metal resistance genes (McCarthy and Lindsay, 2012; Liu et al., 2013). A recent survey of published and sequenced plasmids in *S. aureus* revealed *tra* genes in 4 of 39 plasmid groups and 13 of 243 plasmids (McCarthy and Lindsay, 2012).