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Shuffling of mobile genetic elements (MGEs) in successful healthcare-associated MRSA (HA-MRSA)

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Abbreviations: CC, Clonal complex; HA, Hospital-associated; HGT, Horizontal gene transfer; MGE, Mobile genetic element; MLST, Multi-locus sequence typing; MRSA, Methicillin-resistant *Staphylococcus aureus*; PFGE, Pulse-field gel electrophoresis; RM, Restriction-modification; SAM-62, 62-strain *S. aureus* microarray; SaPI, *S. aureus* pathogenicity island; SCC, staphylococcal cassette chromosome; SSTI, Skin and soft tissue infections

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Methicillin-resistant *Staphylococcus aureus* (MRSA) CC22 SCC*mec*IV is a successful hospital-associated (HA-) MRSA, widespread throughout the world, and now the dominant clone in UK hospitals. We have recently shown that MRSA CC22 is a particularly fit clone, and it rose to dominance in a UK hospital at the same time as it began acquiring an increased range of antibiotic resistances. These resistances were not accumulated by individual CC22 isolates, but appear to shuffle frequently between isolates of the MRSA CC22 population. Resistances are often encoded on mobile genetic elements (MGEs) that include plasmids, transposons, bacteriophage and *S. aureus* pathogenicity islands (SaPIs). Using multi-strain whole genome microarrays, we show that there is enormous diversity of MGE carried within a MRSA CC22 SCC*mec*IV population, even among isolates from the same hospital and time period. MGE profiles were so variable that they could be used to track the spread of variant isolates within the hospital. We exploited this to show that the majority of patients colonised with MRSA at hospital admission that subsequently became infected were infected with their own colonising isolate. Our studies reveal MGE spread, stability, selection and clonal adaptation to the healthcare setting may be key to the success of HA-MRSA clones, presumably by allowing rapid adaptation to antibiotic exposure and new hosts.

Staphylococcus aureus are commensals of humans and animals, and 25% of healthy humans are colonised in the nose. *S. aureus* are a common cause of skin and soft tissue infections (SSTIs), and one of the major causes of infection in hospitalised patients. Methicillin-resistant *S. aureus* (MRSA) have acquired the methicillin resistance (*mecA*) gene, which confers resistance to all β-lactamase resistant β-lactam antibiotics including methicillin, flucloxacillin, carbapenems and cephalosporins. This class of antibiotics is highly effective and commonly used to prevent and treat susceptible staphylococcal infections. The proportion of *S. aureus* infections caused by MRSA in many developed countries is 10–50%, leading to enormous financial burdens, and substantial morbidity and mortality.

HA-MRSA Clones

S. aureus isolates can be divided into independently evolving clonal complex (CC) lineages. Each CC lineage has a unique and stable combination of surface protein variants and a unique restriction-modification (RM) system combination that controls horizontal gene transfer (HGT) between lineages.[1-3] While there are ten major human *S. aureus* lineages, only some lineages have acquired *mecA* which is carried on variants of the relatively stable staphylococcal cassette chromosome (SCC). The most successful hospital-associated (HA)-MRSA clones include CC22 SCC*mec*IV, CC30 SCC*mec*II, ST239 SCC*mec*III, CC5 SCC*mec*II, and CC45 SCC*mec*IV. These MRSA clones have evolved during the last two to three decades and in some cases have spread

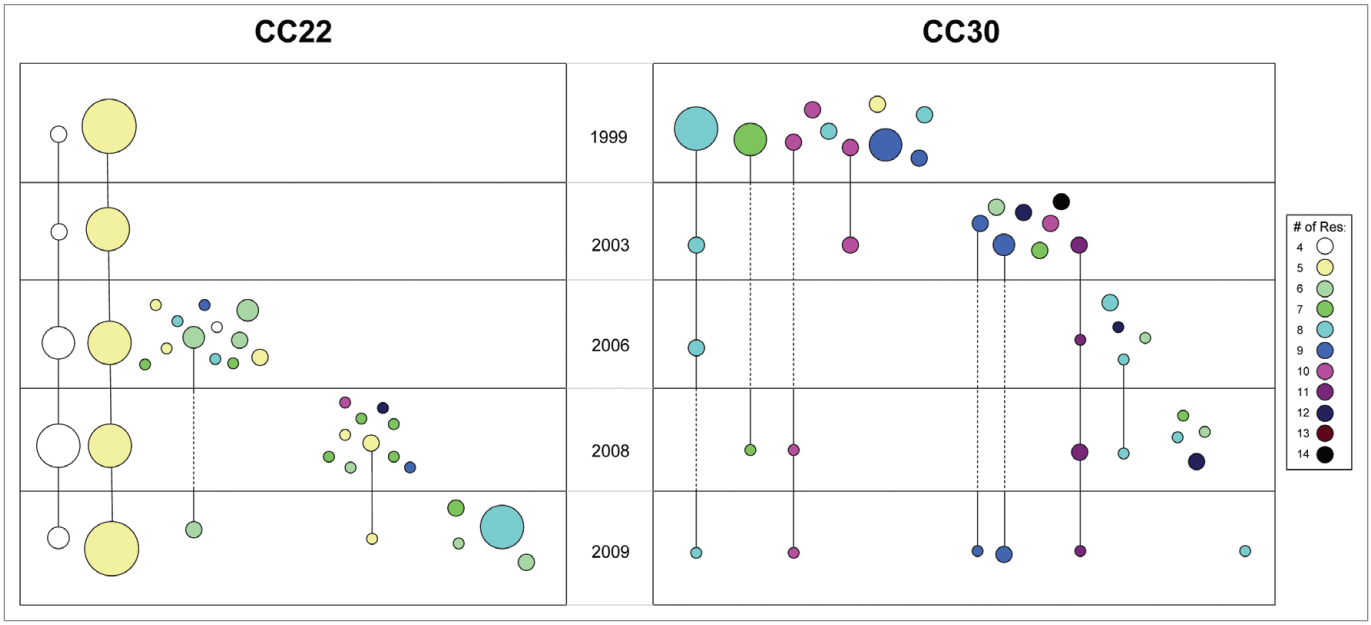

Figure 1. Changes in antibiograms within MRSA clones CC22 and CC30 over time. Each circle represents an individual antibiogram. Circle size corresponds to the proportion of total MRSA in each year and color represents the number of phenotypic resistances contained within the antibiogram.[11] Vertical lines indicate an antibiogram present in multiple years. CC22 became the dominant lineage by 2006 corresponding with an expanded resistance profile. Over time the most resistant antibiograms are not the most successful. Instead the frequent shuffling of resistance determinants is seen; new antibiograms appear each year and resistances do not accumulate.

worldwide. In more recent years, independently evolving clones have caused infections predominately in the community [CC8 SCC*mec*IV (USA300), CC1 SCC*mec*IV and CC59 SCC*mec*IV] and in pigs and persons with livestock contact (CC398 SCC*mec*IV).[4,5]

MGEs

Mobile genetic elements (MGEs) account for approximately 15–20% of the *S. aureus* genome.[5] They include SCC elements, bacteriophage, *S. aureus* pathogenicity islands (SaPIs), plasmids and transposons. These elements can move from one bacterium to another via HGT mechanisms. A range of resistance, virulence, toxin, immune evasion and host specificity factors can be encoded on these MGEs.[5] This suggests MGEs have pivotal roles in *S. aureus* environmental adaptation. Indeed, the emerging MRSA clones in the community and livestock have acquired stable and distinct MGEs that are responsible for their ability to adapt to new niches and hosts.[6] In addition, over 80 whole genome sequencing projects are currently in the public domain, and these already show an enormous variety of MGEs in different isolates.[7-9] However, relatively little is known about MGE stability, movement and selection during evolution of *S. aureus* populations.

Evolution of HA-MRSA in Hospital

We investigated the evolution of MRSA in a single acute-care teaching hospital in London, UK over a ten-year period.[10] We saw MRSA CC22 SCC*mec*IV become the dominant clone concurrent with acquisition of resistance to a range of antibiotics including aminoglycosides, chloramphenicol, clindamycin, fusidic acid, mupirocin, tetracyclines and trimethoprim (**Fig. 1**). Most of these resistance phenotypes are due to genes encoded on plasmids and transposons. Interestingly, no isolate of CC22 became resistant to all antibiotics. Instead, the antibiograms of isolates from both CC22 SCC*mec*IV and CC30 SCC*mec*II MRSA over time were highly variable, and could only have arisen due to frequent exchange of resistances, as well as frequent loss.

As MRSA CC22 SCC*mec*IV became dominant in our hospital, it displaced MRSA CC30 SCC*mec*II and MRSA ST239 SCC*mec*III.[10] MRSA CC22 was the significantly fitter clone in growth, competition and desiccation assays. MRSA CC30 was also able to shuffle resistance genes, but MRSA ST239 was not (**Fig. 1**). This suggests that both fitness and shuffling may provide an advantage for successful HA-MRSA clones.

MGE Variation

In order to investigate MGE variation, we characterized MRSA CC22 SCC*mec*IV isolates (n = 40) found in our hospital during summer 2009 in more detail.[11] We compared their genomes by using a recently constructed 62-strain *S. aureus* microarray (SAM-62) that contains 60-mer probes to all the predicted genes in the first 62 whole genome sequencing projects and 153 plasmids.[9]

The range of MGE variation among the 40 isolates was enormous, despite all isolates being found in the same hospital at the same time, and all belonging to the same MRSA clonal type. **Figure 2** illustrates variation in known antibiotic, heavy metal and biocide resistance genes. McCarthy et al.[11] shows additional substantial variation in other MGEs, including 4/8 known bacteriophage families,

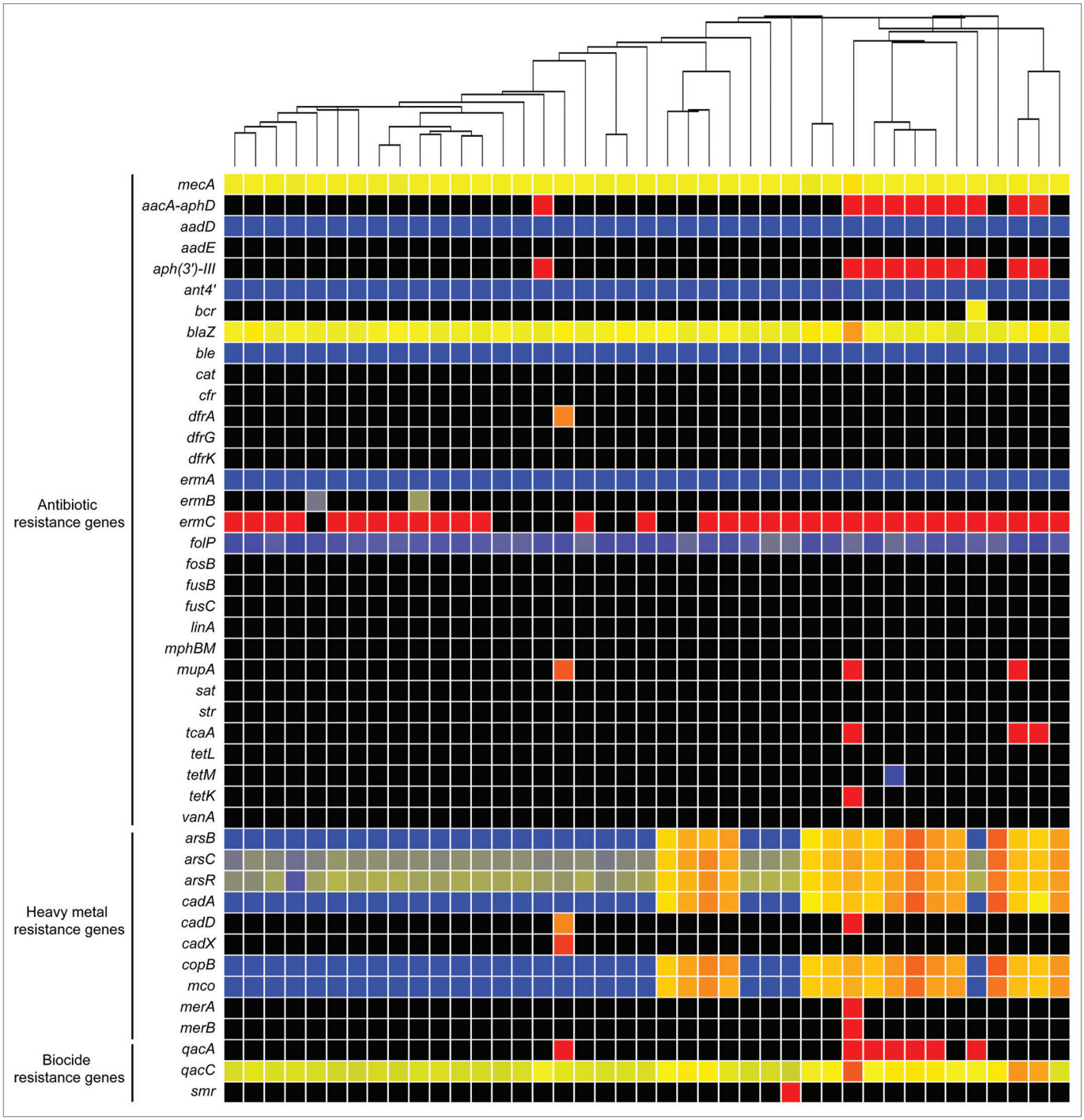

Figure 2. Clustering of HA-MRSA CC22-*SCCmec*IV isolates and distribution of MGE-carried antibiotic, heavy metal and resistance genes by microarray. Each vertical line represents an isolate. Isolates have been clustered using data from 60-mers that represent all genes on mobile genetic elements (MGEs). The relationship between isolates is shown at the top of the figure. Horizontal lines represent different 60-mer oligo probes specific to 43 different antibiotic, heavy metal and biocide resistance genes. The color in the main figure is an indicator of test over reference signal ratio. Thus; (1) yellow indicates presence in both test and reference (MRSA252) isolates, (2) red indicated presence in the test but not the reference isolate, (3) blue indicates absence in the test but not the reference isolate, and (4) black indicates absence in both test and reference isolates. Color intensity is an indicator of signal intensity and may vary due to copy number or due to differences in probe homology.

4/5 known SaPI families, 9/18 known plasmid *rep* families, and 1/4 known transposon families detected in at least one of the isolates.

This amount of MGE variation has potential to be used as a typing tool to discriminate between isolates of clonal MRSA. To test this, we followed up patients from whom we had previously isolated nasal MRSA at hospital admission and subsequently developed MRSA infection.[12] In 6/8 cases, the admission

isolate was identical to the infecting isolate, and in one case the two isolates differed by one MGE.[11] This shows that the majority of patients colonised with MRSA at admission, that subsequently develop an infection, became infected with their own endogenous strain rather than circulating hospital strains. This raises important issues for our understanding of how MRSA and *S. aureus* spread between patients and how we can best combat infection.

We identified two small clusters of related isolates in our hospital that had very similar MGE profiles. Upon returning to the patient's notes, we were able to uncover a link between the patients suggesting previously unrecognized transmission of MRSA in the hospital.[11]

Our results reveal interesting dynamics of MRSA spread in hospitalised patients. At least 1.8% of patients admitted to hospital are positive on nasal screen for MRSA.[12] Colonisation is the greatest risk factor for subsequent infection,[14] and this is because the colonising isolate is usually the same as the infecting isolate.[11] If MRSA can spread from infected patient to infected patient,[11] then patients must also be at risk of becoming colonised with MRSA in hospital. The proportion of colonised patients in hospital or at discharge are unknown, but more than half of admitted patients have previous hospital exposure,[13] generating a cycle of MRSA isolates transmitted, discharged and readmitted to hospital. The diversity of MGE seen in the CC22 population could indicate this substantial reintroduction of CC22 variants to the hospital by newly admitted patients.

Investigation of MRSA spread is supported by adequate typing methods. Our study showed that MGE profiles have the potential to develop into rapid and inexpensive typing methods for tracking hospital spread and outbreaks in the healthcare setting. Current typing methods for MRSA concentrate on the CC lineage (such as multi-locus sequencing typing (MLST) or restriction-modification (RM) typing) and SCC*mec* type. However, these patterns are relatively stable and do not include the fine detail necessary for a typing method to discriminate between isolates to measure transmission and spread and to identify outbreaks. *Spa*-typing allows some variants of clonal types to be distinguished, but is ineffective for many clones such as MRSA CC22 SCC*mec*IV.[15] Variation in MGE carriage could be developed into a rapid and inexpensive method using PCR or microarray/hybridization platforms. Alternatively, once bench-top genomic sequencing is widespread, data interpretation based on MGE content may provide the clearest and most reliable interpretive data.

MGE Stability

These studies also raise very interesting questions about the stability of MGEs in *S. aureus* and MRSA populations. While we saw substantial diversity in MGE profiles among MRSA of the same clonal group, the MGEs were stable enough to use as a typing method to identify isolates from the same patient or reservoir. This suggests the MGEs are relatively stable, but our data also showed evidence of low level transfer and loss of a wide range of MGE. During the time scale of a hospital stay, there can be some variation in MGE carriage in clonal isolates from the same patient, but within a hospital the variation is large, possibly due to the accumulation of differences over longer time scales.

If we look back through the literature, we can now put this into context. Based on epidemiology, early typing methods such as pulse field gel electrophoresis (PFGE) grouped isolates as the "same" if they had four or less band pattern differences.[16] As a band shift is usually due to acquisition or loss of an MGE, this argues that MGE movement in isolates spreading in the hospital setting is frequent in epidemiologically linked isolates. More recently, the acquisition and loss of bacteriophage from *S. aureus* during cystic fibrosis infection in individual patients has been well described.[17]

The mechanism of HGT in MRSA is likely to be transduction, as transformation has not been demonstrated in *S. aureus*, and conjugative plasmids and transposons are found in only a small proportion of isolates.[5,8] Transduction is dependent on bacteriophage; it occurs when DNA is packaged into a bacteriophage and then transferred from a donor cell to a recipient cell. All clinical MRSA have prophage integrated into their chromosome and these bacteriophage can be induced to replicate and lyse the host cell by stresses such as UV light, mitogens and antibiotics.[5] Some bacteriophage heads are able to package bacterial or plasmid DNA, efficiently delivering their payload to *S. aureus* of the same lineage.[2,7]

MGEs and resistances did not systematically accumulate suggesting the loss of MGE is very important. They may have a fitness cost on the host, or there may be mechanisms to limit the total size of the genome or number of variants of particular MGE types.[10] In the laboratory, most MGEs are stable, and the mechanisms for MGE loss are unknown.

At this stage it is difficult to definitively prove that shuffling of MGEs is responsible for the success of HA-MRSA clones. We are continuing to investigate a genetic explanation for how MRSA CC22 SCC*mec*IV became more amenable to MGE acquisition over time. Perhaps MRSA clones that can easily acquire or lose MGEs may adapt more quickly to new environmental conditions such as antibiotic prescribing or transfer between patients. This may give them an advantage over other commensals that do not adapt so readily, leading to successful colonisation of patients, and ultimately to infection.

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