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

Jodi A. Lindsay,* Gwenan M. Knight,† Emma L. Budd and Alex J. McCarthy
Centre for Infection and Immunity; Division of Clinical Sciences; St George’s; University of London; London UK
†Current Affiliation: London School of Hygiene and Tropical Medicine; Infectious Disease Epidemiology; London UK

Methicillin-resistant *Staphylococcus aureus* (MRSA) CC22 SCCmecIV 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 SCCmecIV 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.

*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. 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 SCCmecIV, CC30 SCCmecII, ST239 SCCmecIII, CC5 SCCmecII, and CC45 SCCmecIV. These MRSA clones have evolved during the last two to three decades and in some cases have spread...
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 SCCmeCIV isolates (n = 40) found in our hospital during summer 2009 in more detail. 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. 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. shows additional substantial variation in other MGEs, including 4/8 known bacteriophage families.
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. In 6/8 cases, the admission
isolate was identical to the infecting isolate, and in one case the two isolates differed by one MGE.\textsuperscript{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 \textit{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.\textsuperscript{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.\textsuperscript{12} Colonisation is the greatest risk factor for subsequent infection,\textsuperscript{14} and this is because the colonising isolate is usually the same as the infecting isolate.\textsuperscript{11} If MRSA can spread from infected patient to infected patient,\textsuperscript{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,\textsuperscript{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\textit{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. \textsl{Spa}-typing allows some variants of clonal types to be distinguished, but is ineffective for many clones such as MRSA CC22 SCC\textit{mec}IV.\textsuperscript{11} 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 \textit{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.\textsuperscript{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 \textit{S. aureus} during cystic fibrosis infection in individual patients has been well described.\textsuperscript{17}

The mechanism of HGT in MRSA is likely to be transduction, as transformation has not been demonstrated in \textit{S. aureus}, and conjugative plasmids and transposons are found in only a small proportion of isolates.\textsuperscript{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.\textsuperscript{2} Some bacteriophage heads are able to package bacterial or plasmid DNA, efficiently delivering their payload to \textit{S. aureus} of the same lineage.\textsuperscript{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.\textsuperscript{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\textit{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.

**References**

1. Lindsay JA, Moore CE, Day NP, Peacock SJ, Witney AA, Stabler RA, et al. Microarrays reveal that each of the ten dominant lineages of \textit{Staphylococcus aureus} has a unique combination of surface-associated and regulatory genes. \textit{J Bacteriol} 2006; 188:669-76; PMID:16385056; http://dx.doi.org/10.1128/JB.188.2.669-676.2006.

2. Waldron DE, Lindsay JA. Saul: a novel lineage-specific type I restriction-modification system that blocks horizontal gene transfer into \textit{Staphylococcus aureus} and between \textit{S. aureus} isolates of different lineages. \textit{J Bacteriol} 2006; 188:5578-85; PMID:16855248; http://dx.doi.org/10.1128/JB.00418-06.

3. McCarthy AJ, Lindsay JA. Genetic variation in \textit{Staphylococcus aureus} surface and immune evasion genes is lineage associated: implications for vaccine design and host-pathogen interactions. \textit{BMC Microbiol} 2010; 10:173; PMID:20950675; http://dx.doi.org/10.1186/1471-2180-10-173.

4. Robinson DA, Enright MC. Evolutionary models of the emergence of meticillin-resistant \textit{Staphylococcus aureus}. \textit{Antimicrob Agents Chemother} 2003; 47:3926-34; PMID:14638503; http://dx.doi.org/10.1128/AAC.47.12.3926-3934.2003.

5. Lindsay JA, Holden MTG. Understanding the rise of the superbug: investigation of the evolution and genomic variation of \textit{Staphylococcus aureus}. Funct Integr Genomics 2006; 6:186-201; PMID:16453141; http://dx.doi.org/10.1007/s10142-005-0019-7.
6. Lindsay JA. Genomic variation and evolution of *Staphylococcus aureus*. Int J Med Microbiol 2010; 300:98-103; PMID:19811948; http://dx.doi.org/10.1016/j.ijmm.2009.08.013.

7. McCarthy AJ, Witney AA, Lindsay JA. *Staphylococcus aureus* temporate bacteriophage: carriage and horizontal gene transfer (HGT) is lineage associated. Front Cell Infect Microbiol 2012; 2:6; PMID:22919598; http://dx.doi.org/10.3389/fcimb.2012.00006.

8. McCarthy AJ, Lindsay JA. The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. BMC Microbiol 2012; 12:104; PMID:22691167; http://dx.doi.org/10.1186/1471-2180-12-104.

9. McCarthy AJ, Witney AA, Gould KA, Moodley A, Guardabassi L, Voss A, et al. The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country. Genome Biol Evol 2011; 3:1164-74; PMID:21920902; http://dx.doi.org/10.1093/gbe/evr092.

10. Knight GM, Budd EL, Whitney L, Thornley A, Al-Ghusein H, Planche T, et al. Shift in dominant hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) clones over time. J Antimicrob Chemother 2012; 67:2514-22; PMID:22761351; http://dx.doi.org/10.1093/jac/dks245.

11. McCarthy AJ, Breathnach A, Lindsay JA. Detection of mobile genetic element (MGE) variation between colonising and infecting hospital-associated (HA)-MRSA isolates. J Clin Microbiol 2012; 50:1073-5; PMID:22170916; http://dx.doi.org/10.1128/JCM.09938-11.

12. Krebes J, Al Ghusein H, Feasey N, Breathnach A, Lindsay JA. Are nasal carriers of *Staphylococcus aureus* more likely to become colonized or infected with methicillin-resistant *Staphylococcus aureus* on admission to a hospital? J Clin Microbiol 2011; 49:430-2; PMID:20980558; http://dx.doi.org/10.1128/JCM.02039-10.

13. Wyllie DH, Walker AS, Peto TE, Crook DW. Hospital exposure in a UK population, and its association with bacteraemia. J Hosp Infect 2007; 67:301-7; PMID:18022283; http://dx.doi.org/10.1016/j.jhin.2007.08.018.

14. Safdar N, Bradley EA. The risk of infection after nasal colonization with *Staphylococcus aureus*. Am J Med 2008; 121:310-5; PMID:18374690; http://dx.doi.org/10.1016/j.amjmed.2007.07.034.

15. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 2012; 39:273-82; PMID:22230333; http://dx.doi.org/10.1016/j.ijantimicag.2011.09.030.

16. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J Clin Microbiol 1995; 33:551-5; PMID:7751356.

17. Goerke C, Wirtz C, Flückiger U, Wolz C. Extensive phage dynamics in *Staphylococcus aureus* contributes to adaptation to the human host during infection. Mol Microbiol 2006; 61:1673-85; PMID:16968231; http://dx.doi.org/10.1111/j.1365-2958.2006.05394.x.