ST3268 – a geographically widespread primate MRSA clone

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Text

Sir,

We read with interest the work of Soge and co-workers describing two unusual methicillin-resistant *Staphylococcus aureus* (MRSA) clones found in macaques at a U.S. primate research centre in 2015.[1] The first, ST188-MRSA-IV, had previously been reported in humans and primates, whereas the second, ST3268-MRSA-V, had initially been discovered in quarantined primates originating outside Washington in the same year, but had subsequently also been identified in their primate colony.[1] We had also identified ST3268-MRSA-V and its single-locus variant (SLV) ST2817-MRSA-V among long-tailed macaques (*Macaca fascicularis*) used in experimental surgery in 2014, and describe our experience here, comparing our isolates to the two U.S. ST3268 isolates via genomic analysis.[1]

The SingHealth Experimental Medicine Centre (SEMC) has facilities at three separate locations in Singapore, with limited movement of animals between them. The macaques in these facilities originated primarily from Vietnam, and had been imported at various times starting from 2009. One macaque developed a MRSA wound infection in January 2014 following head implant surgery: *S. aureus* was identified via MALDI-TOF and the isolate was resistant to cefoxitin. This led to mass screening of all remaining 51 macaques (surgical site, nasal and perianal swabs) as well as all 28 of their human contacts (nasal swabs) across the three facilities, using MRSA plates (bioMérieux SA, Marcy l'Etoile, France).

All macaques and two humans were colonized. The macaques were treated with either sulfamethoxazole-trimethoprim or vancomycin, and the MRSA cluster was eventually cleared by isolating the colonised macaques, and decolonising both

humans and macaques by bathing with 0.05% chlorhexidine solution and applying mupirocin to the nares and any wound sites.

All MRSA isolates underwent antimicrobial susceptibility testing according to the CLSI standards,[2] and multi-locus VNTR analysis (MLVA).[3] The results are available in the Supplementary Table. Both human isolates and randomly selected representative macaque isolates from each of six MLVA profiles were selected for WGS, which was performed as previously described.[4] The MLSTs of these isolates were inferred from the WGS output.[5] WGS data for the TXA and TXB isolates described by Soge et al. were obtained from the SRA (accession number SRP067697).[1] Paired-end sequence reads were mapped to the chromosome of reference strain CA-357,[6] with SNPs identified as previously described.[4] Regions of homologous recombination were predicted by using Gubbins.[7] A phylogenetic tree was constructed from core genome SNPs, minus recombination regions, using RAxML.[8]

All but one of the macaque MRSA isolates belonged to MLVA clusters where representative sequenced isolates were assigned to either ST3268-MRSA-V or ST2817-MRSA-V. The final macaque MRSA was found to be ST22-MRSA-IV, the major human healthcare-associated MRSA clone in Singapore.[4] One human who performed animal husbandry, including blood collection from macaques, was colonized with ST3268-MRSA-V. The second human performed surgical procedures on macaques and carried ST2817-MRSA-V. WGS analysis demonstrated that it was phylogenetically related to Singaporean human isolates (data not shown), suggesting an anthroponotic source. [4]

Comparative genomic analysis revealed evidence of homologous recombination in the chromosomes of the ST2817 and ST3268 isolates. Phylogenetic

68 analysis, removing SNP variation associated with recombination, revealed the clonal
69 relationship of the ST2817 and ST3268 isolates (Figure 1), and the also evidence of
70 close genetic relationships between isolates belonging to each of the STs, supportive
71 of local transmission. Moreover, inclusion of the ST3268 isolates from the
72 Washington primate facility[1] revealed they belong to the same clone as the Singapore
73 isolates, with comparable genetic relatedness. Phylogenomic analysis of the ST2817
74 isolates revealed they emerged from ST3268 population, and that there were
75 numerous recombination events that accompanied the emergence of the ST; 21
76 recombination regions encompassing 7362 SNPs, and 40 SNPs core SNPs (Figure 1).
77 The phylogenetic relationships and genetic diversity indicates that this was
78 not a single point source outbreak originating at the SEMC primate facilities, although
79 some of the SNP distances between the isolates suggest that there could have been
80 localized transmission of some parts of the population. Interestingly, TXA and TXB
81 from the Washington facility are both placed within the larger cluster of ST3268
82 isolates from Singapore and are predicted to be within 36 SNPs of the nearest SEMC
83 isolate. Unfortunately, we were unable to ascertain the country of origin of the U.S.
84 primates, or whether these had shared a common transit facility with any of the
85 Singaporean macaques.
86 Collectively, our experience and results suggest that ST3268-MRSA-V and its
87 SLV ST2817-MRSA-V is probably a macaque-specific MRSA clone that is capable
88 of zoonotic transmission, in much the same way as the porcine- and bovine-specific
89 MRSA clones.[9,10] The genomic diversity of the macaque isolates coupled with the
90 paucity of ST3268-MRSA-V and its SLV in humans argue against a human-to-
91 macaque transmission with subsequent spread in the caged macaques. More studies

need to be done to determine if this clone is endemic in macaques or represent an exceptional expansion of uncommon *S. aureus* types in defined monkey populations.

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Transparency Declaration

All authors: none to declare.

Figure

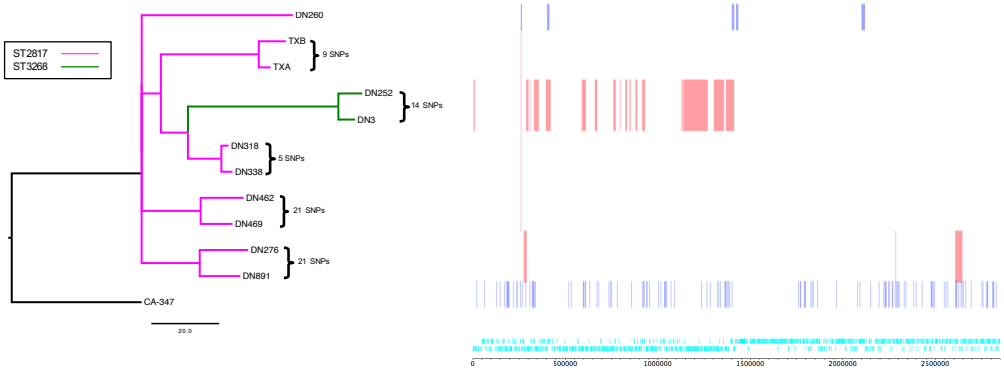

Figure 1

Prediction of recombination in the *S. aureus* isolate chromosomes. Regions of variation in the genomes of the 11 clinical *S. aureus* and the reference strain CA-347, which are predicted to have arisen by homologous recombination, are shown on the right. Red blocks indicate recombination predicted to have occurred on internal nodes, and blue indicates taxon-specific recombination. Isolates are ordered according to the phylogenetic tree displayed on the left. The tree is a maximum likelihood tree constructed with core chromosomes SNP, with SNPs in recombination regions removed, and rooted with the sequence type (ST) 45 CA-347 reference. The branches are colour coded according to the ST with the key indicated in the figure. The track along the bottom of the figure displays the CA-347 chromosome and annotation, in which protein-coding sequences (CDS) are indicated in light blue.

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