Genome Sequence of a Recently Emerged, Highly Transmissible, Multi-Antibiotic- and Antiseptic-Resistant Variant of Methicillin-Resistant *Staphylococcus aureus*, Sequence Type 239 (TW)

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A 2-year outbreak of a highly transmissible methicillin-resistant \textit{Staphylococcus aureus} (MRSA) strain (designated TW) in an intensive care unit (ICU) in London was recently reported (12). Acquisition of TW MRSA was four times more likely to be associated with bacteremia than was acquisition of other commonly found MRSA strains \(\geq 95\%\) epidemic (E)MRSA-15 or EMRSA-16. TW MRSA was also significantly more frequently isolated from vascular access device cultures but less frequently from carriage sites (anterior nares, axilla, and perineum), suggesting that TW differs in its colonization capacity from other MRSA strains. TW was initially defined by its extended antibiotic resistance pattern, being resistant to penicillin, methicillin, erythromycin, ciprofloxacin, gentamicin, neomycin, trimethoprim, and tetracycline (12). TW also had elevated minimum bactericidal concentrations (MBCs) for chlorhexidine and was resistant to a chlorhexidine-based antiseptic protocol effective against other MRSA strains (MBCs) for chlorhexidine and trimethoprim (12).

The 3.1-Mb genome of an outbreak methicillin-resistant \textit{Staphylococcus aureus} (MRSA) strain (TW20) contains evidence of recently acquired DNA, including two large regions (635 kb and 127 kb). The strain is resistant to a wide range of antibiotics, antiseptics, and heavy metals due to resistance genes encoded on mobile genetic elements and also mutations in housekeeping genes.

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Received 18 September 2009/Accepted 17 November 2009

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a large block of DNA (26). The approximate boundaries of the recombination were identified from pairwise comparisons of the TW20 chromosome with MRSA 252 (CC30) and USA300 TCH1516 (CC8). A marked shift in DNA percent identity of approximately 1 percentage point was observed across the approximate recombination breakpoints (data not shown), demonstrating that 635 kb (7.4% of the TW20 chromosome; SATW20_26800 to SATW20_03960) may have been transferred from a CC30 donor. This transfer event also contributes to the high level of reciprocal Fasta matches between TW20 and MRSA252 (ST36).

The origins of SCCmecIII in the TW20 genome are unclear, since SCCmecIII has not been found in the CC30 lineage. Each of the SCC elements contains further MGEs: SCCmercury contains Tn554, encoding a streptomycin 3′-adenylyltransferase and an erythromycin resistance protein, ErmA1, and SCCmec contains an integrated plasmid, pT181, and Tn554, containing cadmium resistance CDSs. In addition to Tn554 and Tn554 in the SCCmec region, the TW20 chromosome contains an additional Tn554 and a Tn532 transposon, encoding the β-lactamase BlaZ, within an integrative conjugative element (ICE) (31).

Further resistance determinants are found on plasmid pTW20_1. Importantly, it carries a gene encoding an antiseptic
resistance protein, QacA, that confers resistance to antiseptics such as cationic biocides, quaternary ammonium salts, and diaminodienes via an export-mediated mechanism (29). In addition, part of the plasmid is highly similar (98 to 100% DNA identity) to the mer operon of the SCCmercury region found on the chromosome (Fig. 2). pTW20_1 also contains a homolog of the gene encoding the cadmium-transporting ATPase CadA, found in ΨTn554 of SCCmec. This region in pTW20_1 is bordered by IS431 elements, as it is in the chromosomal copy of SCCmercury. Notably, upstream of the SCCmercury mer operon, there is a CDS that encodes a putative NADH-binding protein. A fragment homologous to the 3′ end of this CDS is also present on pTW20_1 upstream of the mer operon and is truncated by an IS431 element. The absence of the 5′ region of this CDS on pTW20_1 suggests that this region, including the mer operon, may have arisen on the plasmid by recombination between chromosomal and plasmid IS431 elements. It is therefore possible that IS431-mediated recombination plays a role in the evolution of the SCC region.

Two other drug resistance genes, encoding a tetracycline resistance protein, TetM, and a trimethoprim-resistant dihydrofolate reductase, DfrG, are found in a 31.3-kb region (Tn5801-like), similar to transposons/ICE found in the genomes of S. aureus strains Mu50 (20) and Mu3 (23) and Staphylococcus agalactiae strain COH1 (35). In comparison to the Tn5801 elements in Mu50 and Mu3, the TW20 element contains an additional four CDSs, including dfrG, in the central region of the element.

There are three prophage within the TW20 genome, two of which are similar to those previously found in sequenced S. aureus genomes: δSa1(TW20) is 43.3 kb in size, is integrated within the 5′ region of a lipase gene, and does not carry CDSs with homology to known virulence factors; δSa3(TW20) is 44.7 kb in size, is integrated in the phospholipase C gene, and carries the staphylococcal complement inhibitor SCIN (28), staphylokinase (30), and enterotoxin A (7) genes associated with virulence. Genes for two other enterotoxins, enterotoxins K and Q, are carried on a Staphylococcus aureus pathogenicity island (SaPI), SaPI1.

At 127.2 kb, the third prophage, δSPβ-like(TW20), is markedly larger than the other two and does not display similarity with other S. aureus prophage. δSPβ-like(TW20) exhibits extended similarity with the δSPβ-like region in the Staphylococcus epidermidis RP62a genome (13) (Fig. 3). Comparison of the two sequences reveals a region of sequence divergence and rearrangement in the center of the prophage. In δSPβ-like(TW20), this region contains CDSs associated with aminoglycoside resistance and streptothricin resistance (Fig. 3). In addition, δSPβ-like(TW20) contains a CDS that may have a role in promoting persistence of TW20 in the hospital setting. S. aureus possesses many surface-anchored proteins with the LPxTG motif, which bind host molecules (27). SATW20_21850 encodes an LPxTG motif surface-anchored protein that does not have orthologs in any of the genomes of the other sequenced S. aureus strains currently available. A highly similar CDS (95.1% amino acid identity), sesI (8), is present in the S. epidermidis δSPβ-like region (Fig. 3). A recent study by Söderquist et al. found that sesI was absent from normal S. epidermidis flora of healthy individuals without any health care association but was found in approximately 50% of clinical isolates causing invasive infections, leading them to suggest that this gene was a potential marker of invasive capacity (32). The presence of an LPxTG motif surface-anchored protein on an MGE in TW20 suggests that this strain has augmented its array of this family of functionally important proteins through a recent acquisition event and therefore this LPxTG motif surface-anchored protein may not be widely distributed in related strains. Genome sequencing of a global collection of ST239 strains revealed only 7% (3/42) of isolates were positive for orthologs of this CDS (14a). Work is under way to survey...
the wider distribution of this gene in the *S. aureus* population and investigate the function of the encoded protein.

Evidence of adaptation to survive in a health care environment is also found in the core genome. Several housekeeping genes have alleles associated with antibiotic resistance. The TW20 DNA gyrase subunit A (GyrA) contains a leucine residue at position 84. The more-widespread residue in *S. aureus* GyrA proteins is serine, suggesting this is the plesiomorphic amino acid at this position. *In vitro* studies have demonstrated that substitution of Ser84Leu generates resistance to quinolones in *S. aureus* (34). TW20 exhibits low-level resistance to mupirocin. The TW20 isoleucyl-tRNA synthetase contains a phenylalanine residue at position 588. The substitution of Val588Phe has been shown to confer chromosomal low-level mupirocin resistance in *S. aureus* without significantly affecting fitness (17).

In conclusion, genomic analysis of TW20 provides evidence of its adaptation to survive in a health care setting through acquisition of drug and antiseptic resistance genes carried on MGEs, large chromosomal insertions, and point mutations in housekeeping genes. The large size of the TW20 genome reflects the ability of the ST239 lineage to undergo prolonged and continuing evolution to adapt to the hospital environment. Further studies are under way to elucidate the components of the genome that promote transmission and interaction with the host.

**Nucleotide sequence accession numbers.** The sequence and annotation of the TW20 genome have been deposited in the EMBL database under the accession numbers FN433596, FN433597, and FN433598.

The Sanger Institute is core funded by the Wellcome Trust. Funding for the sequencing of the TW20 genome was provided by Guy’s and St. Thomas’ Charity. J.D.E. receives funding from the Department of Health via the NIHR Comprehensive Biomedical Research Centre award to Guy’s and St Thomas’ NHS Foundation Trust in partnership with King’s College London.

We thank the Sanger Institute’s Pathogen Production Group for shotgun and finishing sequencing and the core Informatics Group for support.

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