Journal of Bacteriology

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

Matthew T. G. Holden, Jodi A. Lindsay, Craig Corton, Michael A. Quail, Joshua D. Cockfield, Smriti Pathak, Rahul Batra, Julian Parkhill, Stephen D. Bentley and Jonathan D. Edgeworth

J. Bacteriol. **2010, 192(3):888. DOI: 10.1128/JB.01255-09. Published Ahead of Print 30 November 2009.**

JOURNAL OF BACTERIOLOGY, Feb. 2010, p. 888–892 Vol. 192, No. 3
0021-9193/10/$12.00 doi:10.1128/JB.01255-09

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

Matthew T. G. Holden,[1]* Jodi A. Lindsay,[2] Craig Corton,[1] Michael A. Quail,[1] Joshua D. Cockfield,[2] Smriti Pathak,[3] Rahul Batra,[4] Julian Parkhill,[1] Stephen D. Bentley,[1] and Jonathan D. Edgeworth[3,4]

The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom[1]; Centre for Infection, Department of Cellular & Molecular Medicine, St George's, University of London, Cranmer Terrace, London, United Kingdom[2]; Department of Infectious Diseases, King's College London, Guy's, King's and St Thomas' Medical School, Guy's Hospital, London, United Kingdom[3]; and Directorate of Infection, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom[4]

Received 18 September 2009/Accepted 17 November 2009

The 3.1-Mb genome of an outbreak methicillin-resistant *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.

A 2-year outbreak of a highly transmissible methicillin-resistant *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 [>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 in the ICU (6). TW20 (strain 0582) was a representative bacteremic isolate cultured on 21 October 2003 (12).

Multilocus sequence typing (MLST) identified TW20 as sequence type 239 (ST239), an international health care-associated (HA) MRSA lineage prevalent in Asia (19, 38), South America (2, 37), and Eastern Europe (5, 33), which includes EMRSA-1, -4, -7, and -11 and the Brazilian, Portuguese, Hungarian, and Viennese clones (24). To investigate the genetic basis for increased resistance and transmissibility, the TW20 genome was completely sequenced, assembled, and finished and annotated as described previously (16, 25). The final finished genome (10) assembly contained 64,087 capillary reads, giving an average coverage of 13.3. At 3,075,806 bp, the TW20 genome is the largest *S. aureus* genome sequenced thus far. It consists of a single chromosome of 3,043,210 bp in size (Fig. 1) and 2 plasmids (pTW20_1 and pTW20_2), of 29,585 bp and 3,011 bp.

TW20 belongs to clonal complex 8 (CC8), which contains strains NCTC8325 (ST8) (14), Newman (ST8) (3), USA300 (ST8) (11), and COL (ST250) (13). Comparative genomic analysis with these strains by reciprocal Fasta analysis (36) revealed that between 83.7 and 82.7% of protein coding sequences (CDSs) in the TW20 chromosome have reciprocal matches with CC8 members. The highest numbers of matches in any sequenced *S. aureus* strain, however, was with MRSA 252 (85.9% of CDSs). MRSA 252 (ST36) belongs to CC30 and is a representative of EMRSA-16 that has been a dominant MRSA clone in United Kingdom hospitals for more than 10 years (16). In comparison to CC8, most of the additional matches to MRSA 252 are to CDSs in horizontally acquired mobile genetic elements (MGEs) rather than to orthologous CDSs. A significant component of the *S. aureus* genome is derived from MGEs that contribute to the accessory genome (21). In the TW20 genome, 16.2% of the CDSs (12.6% of the total genomic DNA) are found in MGEs (Fig. 1). Both TW20 and MRSA 252 are representatives of successful hospital-associated MRSA lineages and have large accessory genomes that contain many of the CDSs associated with drug resistance.

Methicillin resistance is conferred by a *mecA* gene on a type III staphylococcal cassette chromosome (SCC) *mec* element (SCC*mec*III). TW20 has a composite SCC region of two SCC elements, SCC*mercury* and SCC*mec*III, identical in structure to the type III SCC*mec* region found by Ito et al. (18) in an isolate from New Zealand in 1985. The SCC*mec*III region is present in a part of the chromosome hypothesized to have been transferred from CC30 into a CC8 background as part of

* Corresponding author. Mailing address: The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge CB10 1SA, United Kingdom. Phone: 44 (0)1223 494761. Fax: 44 (0)1223 494919. E-mail: mh3@sanger.ac.uk.

▽ Published ahead of print on 30 November 2009.

† The authors have paid a fee to allow immediate free access to this article.

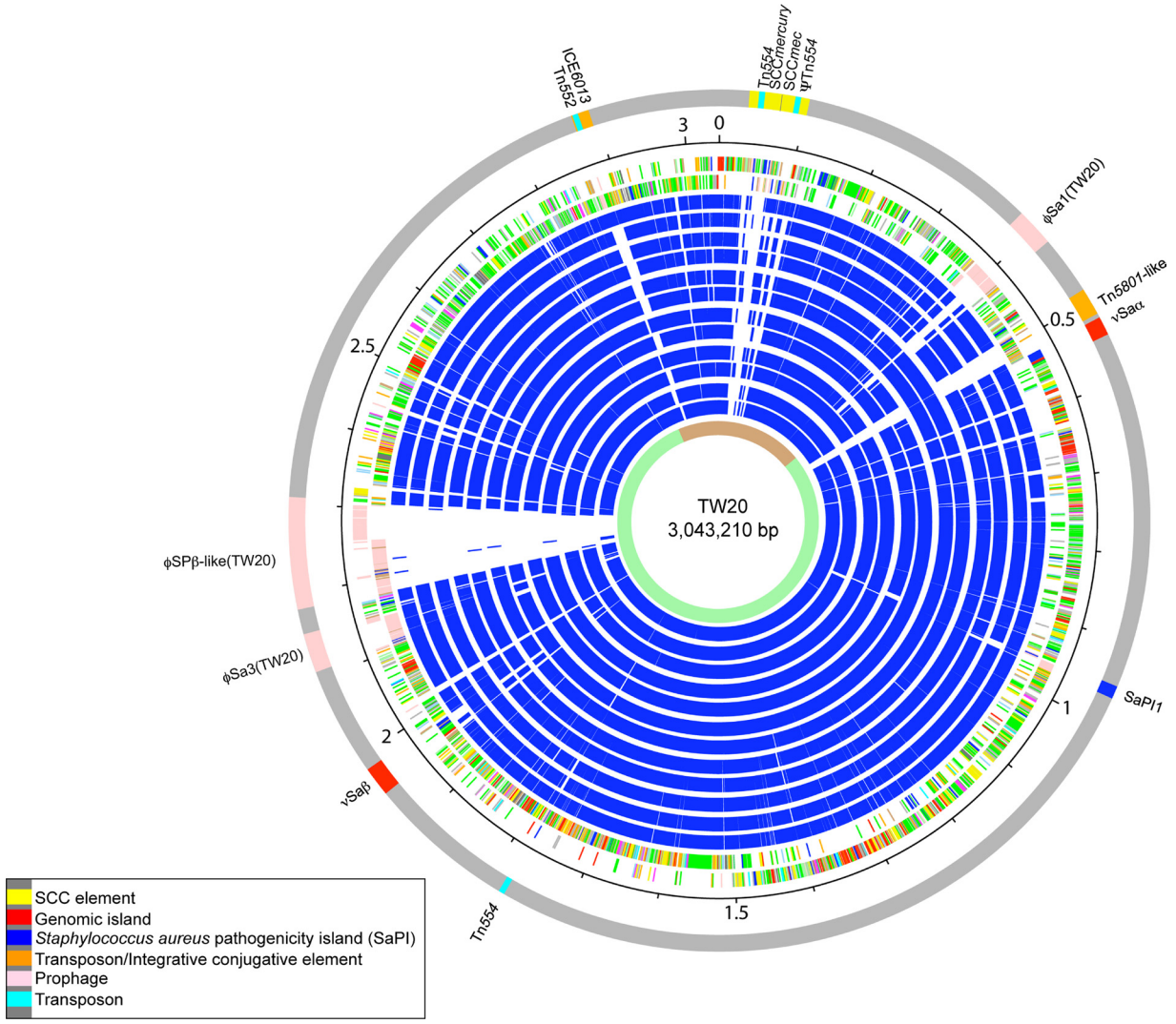

FIG. 1. Schematic circular diagram of the *S. aureus* TW20 chromosome. Key for the circular diagram (outer to inner): outer colored segments on the gray outer ring represent genomic islands and horizontally acquired DNA (see the key in the figure); scale (in Mb); annotated CDSs colored according to predicted function are shown on a pair of concentric circles, representing both coding strands; *S. aureus* reciprocal Fasta matches shared with the *S. aureus* strains: MRSA252, (accession number BX571856) (16), MSSA476 (accession number BX571857) (16), MW2 (accession number BA000033) (4), N315 (accession number BA000018) (20), Mu50 (accession number BA000017) (20), Mu3 (accession number AP009324) (23), COL (accession number CP000046) (13), NCTC8325 (accession number CP000253) (14), USA3000 FPR3757 (accession number CP000255) (11), JH9 (accession number CP000703) (22), Newman (accession number AP009351) (3), and RF122 (accession number AJ938182) (15); regions of the chromosome derived from a CC8 ancestor (light green) or the CC30 ancestor (brown). Color coding for TW20 CDS functions: dark blue, pathogenicity/adaptation; black, energy metabolism; red, information transfer; dark green, surface associated; cyan, degradation of large molecules; magenta, degradation of small molecules; yellow, central/intermediary metabolism; pale green, unknown; pale blue, regulators; orange, conserved hypothetical; brown, pseudogenes; pink, phage and IS elements; gray, miscellaneous.

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 (~20.6% 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 SCC*mec*III in the TW20 genome are unclear, since SCC*mec*III has not been found in the CC30 lineage. Each of the SCC elements contains further MGEs: SCC*mercury* contains Tn*554*, encoding a streptomycin 3′-adenylyltransferase and an erythromycin resistance protein, ErmA1, and SCC*mec* contains an integrated plasmid, pT181, and ΨTn*554*, containing cadmium resistance CDSs. In addition to Tn*554* and ΨTn*554* in the SCC*mec* region, the TW20 chromosome contains an additional Tn*554* and a Tn*552* 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

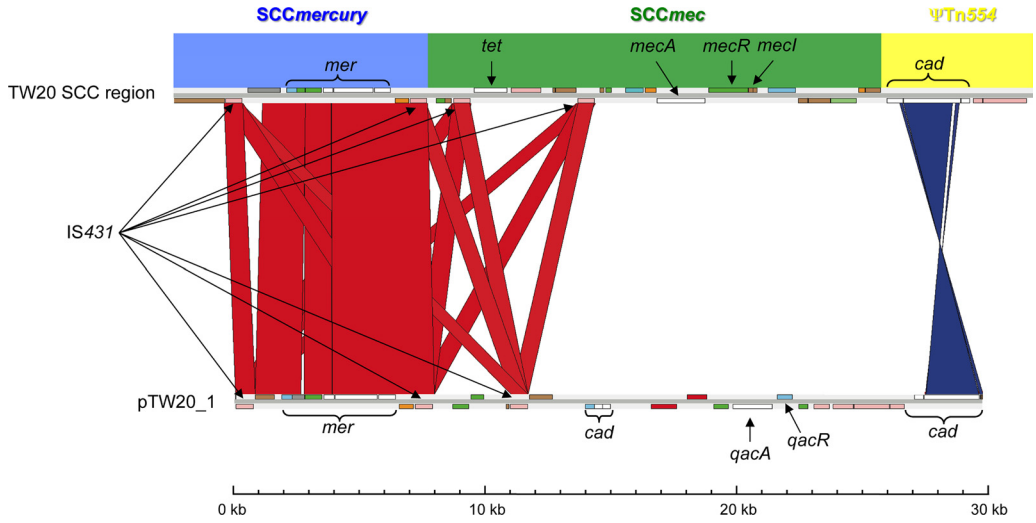

FIG. 2. Comparative analysis of the TW20 plasmid pTW20_1 with the *mer* operon of the TW20 SCC region. Pairwise comparisons of the TW20 SCC region containing the *mer* operon from the TW20 chromosome (top) with the TW20 plasmid pTW20_1 (bottom) using the Artemis Comparison Tool (ACT) (9) are shown. The colored bars separating each sequence (red and blue) represent matches identified by BlastN (1); red lines link matches in the same orientation, and blue lines link matches in the reverse orientation. CDSs associated with metal and drug resistance are marked, as are IS*431* elements. Colored bars at the top of the figure indicate parts of the sequence found in the SCC*mercury* (blue) and SCC*mec* (green) elements, including Ψ*Tn554* (yellow), that make up this region.

resistance protein, QacA, that confers resistance to antiseptics such as cationic biocides, quaternary ammonium salts, and diamidines 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 SCC*mercury* region found on the chromosome (Fig. 2). pTW20_1 also contains a homologue of the gene encoding the cadmium-transporting ATPase CadA, found in Ψ*Tn554* of SCC*mec*. This region in pTW20_1 is bordered by IS*431* elements, as it is in the chromosomal copy of SCC*mercury*. Notably, upstream of the SCC*mercury 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 IS*431* 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 IS*431* elements. It is therefore possible that IS*431*-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 (Tn*5801*-like), similar to transposons/ICE found in the genomes of *S. aureus* strains Mu50 (20) and Mu3 (23) and *Streptococcus agalactiae* strain COH1 (35). In comparison to the Tn*5801* 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: ϕSa1(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; ϕSa3(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, ϕSPβ-like(TW20), is markedly larger than the other two and does not display similarity with other *S. aureus* prophage. ϕSPβ-like(TW20) exhibits extended similarity with the ϕSPß-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 ϕSPβ-like(TW20), this region contains CDSs associated with aminoglycoside resistance and streptothricin resistance (Fig. 3). In addition, ϕSPβ-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* ϕSPβ-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

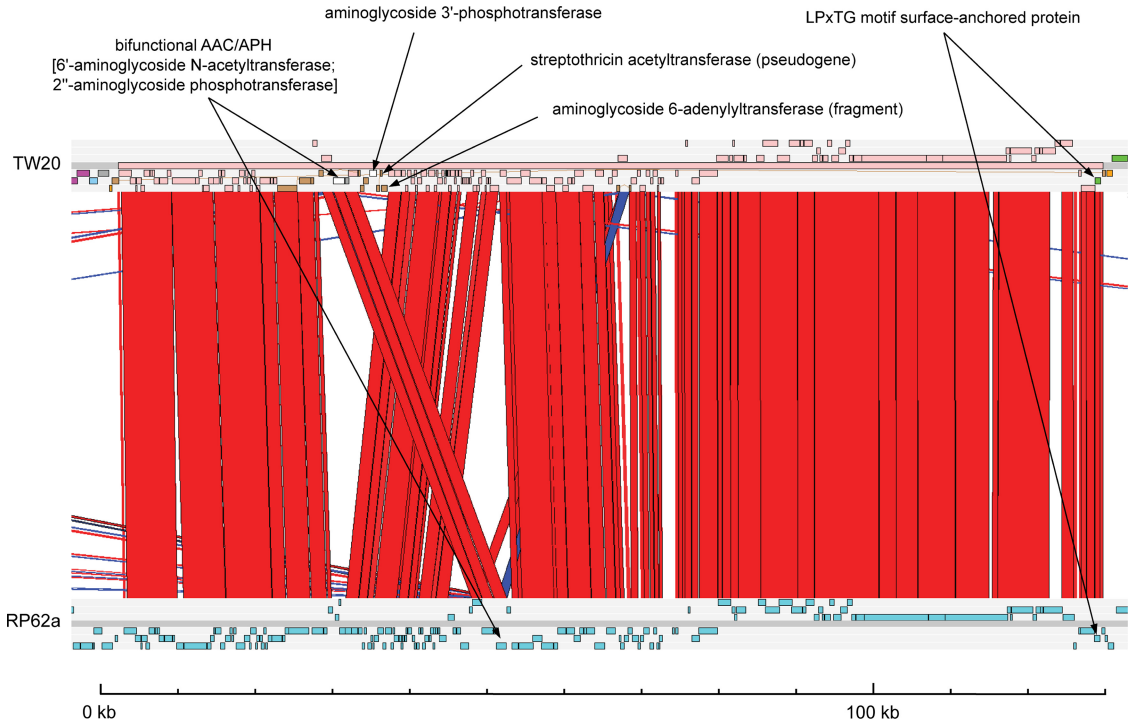

FIG. 3. Comparative analysis of ϕSPβ-like(TW20) prophage with the *S. epidermidis* RP62a ϕSPß-like prophage. Pairwise BlastN comparison of the *S. aureus* TW20 prophage ϕSPβ-like(TW20) region from the TW20 chromosome (top) with the *S. epidermidis* RP62a ϕSPß-like prophage region from the RP62a chromosome (bottom) (13) displayed in ACT is shown. The extent of the ϕSPβ-like(TW20) prophage in the TW20 sequence, which extends from SATW20_20290 to SATW20_21850, is marked by the pink horizontal bar.

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.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic Local Alignment Search Tool. J. Mol. Biol. **215:**403–410.
2. **Amaral, M. M., L. R. Coelho, R. P. Flores, R. R. Souza, M. C. Silva-Carvalho, L. A. Teixeira, B. T. Ferreira-Carvalho, and A. M. Figueiredo.** 2005. The predominant variant of the Brazilian epidemic clonal complex of methicillin-resistant *Staphylococcus aureus* has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. J. Infect. Dis. **192:**801–810.
3. **Baba, T., T. Bae, O. Schneewind, F. Takeuchi, and K. Hiramatsu.** 2008. Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J. Bacteriol. **190:**300–310.
4. **Baba, T., F. Takeuchi, M. Kuroda, H. Yuzawa, K. Aoki, A. Oguchi, Y. Nagai, N. Iwama, K. Asano, T. Naimi, H. Kuroda, L. Cui, K. Yamamoto, and K. Hiramatsu.** 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet **359:**1819–1827.
5. **Bartels, M. D., A. Nanuashvili, K. Boye, S. M. Rohde, N. Jashiashvili, N. A. Faria, M. Kereselidze, S. Kharebava, and H. Westh.** 2008. Methicillin-resistant *Staphylococcus aureus* in hospitals in Tbilisi, the Republic of Georgia, are variants of the Brazilian clone. Eur. J. Clin. Microbiol. Infect. Dis. **27:**757–760.
6. **Batra, R., B. S. Cooper, C. Whiteley, A. Patel, D. Wyncoll, and J. D. Edge-**

worth. The TW variant of MRSA sequence type 239 is not controlled by a surface antiseptic protocol effective at preventing transmission of endemic MRSA on an intensive care unit. Clin. Infect. Dis., in press.

7. **Betley, M. J., and J. J. Mekalanos.** 1988. Nucleotide sequence of the type A staphylococcal enterotoxin gene. J. Bacteriol. **170:**34–41.
8. **Bowden, M. G., W. Chen, J. Singvall, Y. Xu, S. J. Peacock, V. Valtulina, P. Speziale, and M. Hook.** 2005. Identification and preliminary characterization of cell-wall-anchored proteins of *Staphylococcus epidermidis*. Microbiology **151:**1453–1464.
9. **Carver, T. J., K. Rutherford, M. Berriman, M. A. Rajandream, B. Barrell, and J. Parkhill.** 2005. ACT: the Artemis comparison tool. Bioinformatics **21:**3422–3423.
10. **Chain, P. S., D. V. Grafham, R. S. Fulton, M. G. Fitzgerald, J. Hostetler, D. Muzny, J. Ali, B. Birren, D. C. Bruce, C. Buhay, J. R. Cole, Y. Ding, S. Dugan, D. Field, G. M. Garrity, R. Gibbs, T. Graves, C. S. Han, S. H. Harrison, S. Highlander, P. Hugenholtz, H. M. Khouri, C. D. Kodira, E. Kolker, N. C. Kyrpides, D. Lang, A. Lapidus, S. A. Malfatti, V. Markowitz, T. Metha, K. E. Nelson, J. Parkhill, S. Pitluck, X. Qin, T. D. Read, J. Schmutz, S. Sozhamannan, P. Sterk, R. L. Strausberg, G. Sutton, N. R. Thomson, J. M. Tiedje, G. Weinstock, A. Wollam, and J. C. Detter.** 2009. Genomics. Genome project standards in a new era of sequencing. Science **326:**236–237.
11. **Diep, B. A., S. R. Gill, R. F. Chang, T. H. Phan, J. H. Chen, M. G. Davidson, F. Lin, J. Lin, H. A. Carleton, E. F. Mongodin, G. F. Sensabaugh, and F. Perdreau-Remington.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet **367:**731–739.
12. **Edgeworth, J. D., G. Yadegarfar, S. Pathak, R. Batra, J. D. Cockfield, D. Wyncoll, R. Beale, and J. A. Lindsay.** 2007. An outbreak in an intensive care unit of a strain of methicillin-resistant *Staphylococcus aureus* sequence type 239 associated with an increased rate of vascular access device-related bacteremia. Clin. Infect. Dis. **44:**493–501.
13. **Gill, S. R., D. E. Fouts, G. L. Archer, E. F. Mongodin, R. T. DeBoy, J. Ravel, I. T. Paulsen, J. F. Kolonay, L. Brinkac, M. Beanan, R. J. Dodson, S. C. Daugherty, R. Madupu, S. V. Angiuoli, A. S. Durkin, D. H. Haft, J. Vamathevan, H. Khouri, T. Utterback, C. Lee, G. Dimitrov, L. X. Jiang, H. Y. Qin, J. Weidman, K. Tran, K. Kang, I. R. Hance, K. E. Nelson, and C. M. Fraser.** 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacteriol. **187:**2426–2438.
14. **Gillaspy, A. F., V. Worrell, J. Orvis, B. A. Roe, D. W. Dyer, and J. J. Iandolo.** 2006. The *Staphylococcus aureus* NCTC 8325 genome, p. 381–412. *In* V. Fischetti, R. Novick, J. Ferretti, D. Portnoy, and J. Rood (ed.), Gram positive pathogens. ASM Press, Washington, DC.

14a. **Harris, S., E. J. Feil, M. T. G. Holden, M. A. Quail, E. K. Nickerson, N. Chantratita, S. Gardete, A. Tavares, N. Day, J. A. Lindsay, J. D. Edgeworth, H. de Lencastre, J. Parkhill, S. J. Peacock, and S. D. Bentley.** Evolution of MRSA during hospital transmission and intercontinental spread. Science, in press.

15. **Herron-Olson, L., J. R. Fitzgerald, J. M. Musser, and V. Kapur.** 2007. Molecular correlates of host specialization in *Staphylococcus aureus*. PLoS One **2:**e1120.
16. **Holden, M. T. G., E. J. Feil, J. A. Lindsay, S. J. Peacock, N. P. J. Day, M. C. Enright, T. J. Foster, C. E. Moore, L. Hurst, R. Atkin, A. Barron, N. Bason, S. D. Bentley, C. Chillingworth, T. Chillingworth, C. Churcher, L. Clark, C. Corton, A. Cronin, J. Doggett, L. Dowd, T. Feltwell, Z. Hance, B. Harris, H. Hauser, S. Holroyd, K. Jagels, K. D. James, N. Lennard, A. Line, R. Mayes, S. Moule, K. Mungall, D. Ormond, M. A. Quail, E. Rabbinowitsch, K. Rutherford, M. Sanders, S. Sharp, M. Simmonds, K. Stevens, S. Whitehead, B. G. Barrell, B. G. Spratt, and J. Parkhill.** 2004. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. Proc. Natl. Acad. Sci. U. S. A. **101:**9786–9791.
17. **Hurdle, J. G., A. J. O'Neill, and I. Chopra.** 2004. The isoleucyl-tRNA synthetase mutation V588F conferring mupirocin resistance in glycopeptide-intermediate *Staphylococcus aureus* is not associated with a significant fitness burden. J. Antimicrob. Chemother. **53:**102–104.
18. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, C. Tiensasitorn, and K. Hiramatsu.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **45:**1323–1336.
19. **Ko, K. S., J. Y. Lee, J. Y. Suh, W. S. Oh, K. R. Peck, N. Y. Lee, and J. H. Song.** 2005. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. J. Clin. Microbiol. **43:**421–426.
20. **Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Z. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Q. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu.** 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet **357:**1225–1240.
21. **Lindsay, J. A., and M. T. G. Holden.** 2004. *Staphylococcus aureus*: superbug, super genome? Trends Microbiol. **12:**378–385.
22. **Mwangi, M. M., S. W. Wu, Y. Zhou, K. Sieradzki, H. de Lencastre, P. Richardson, D. Bruce, E. Rubin, E. Myers, E. D. Siggia, and A. Tomasz.** 2007. Tracking the *in vivo* evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. Proc. Natl. Acad. Sci. U. S. A. **104:**9451–9456.
23. **Neoh, H. M., L. Cui, H. Yuzawa, F. Takeuchi, M. Matsuo, and K. Hiramatsu.** 2008. Mutated response regulator *graR* is responsible for phenotypic conversion of *Staphylococcus aureus* from heterogeneous vancomycin-intermediate resistance to vancomycin-intermediate resistance. Antimicrob. Agents Chemother. **52:**45–53.
24. **Oliveira, D. C., A. Tomasz, and H. de Lencastre.** 2001. The evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*: identification of two ancestral genetic backgrounds and the associated *mec* elements. Microb. Drug Resist. **7:**349–361.
25. **Parkhill, J., M. Achtman, K. D. James, S. D. Bentley, C. Churcher, S. R. Klee, G. Morelli, D. Basham, D. Brown, T. Chillingworth, R. M. Davies, P. Davis, K. Devlin, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, S. Leather, S. Moule, K. Mungall, M. A. Quail, M. A. Rajandream, K. M. Rutherford, M. Simmonds, J. Skelton, S. Whitehead, B. G. Spratt, and B. G. Barrell.** 2000. Complete DNA sequence of a serogroup A strain of *Neisseria meningitidis* Z2491. Nature **404:**502–506.
26. **Robinson, D. A., and M. C. Enright.** 2004. Evolution of *Staphylococcus aureus* by large chromosomal replacements. J. Bacteriol. **186:**1060–1064.
27. **Roche, F. M., R. Massey, S. J. Peacock, N. P. J. Day, L. Visai, P. Speziale, A. Lam, M. Pallen, and T. J. Foster.** 2003. Characterization of novel LPXTG-containing proteins of *Staphylococcus aureus* identified from genome sequences. Microbiology **149:**643–654.
28. **Rooijakkers, S. H., M. Ruyken, A. Roos, M. R. Daha, J. S. Presanis, R. B. Sim, W. J. van Wamel, K. P. van Kessel, and J. A. van Strijp.** 2005. Immune evasion by a staphylococcal complement inhibitor that acts on C3 convertases. Nat. Immunol. **6:**920–927.
29. **Rouch, D. A., D. S. Cram, D. DiBerardino, T. G. Littlejohn, and R. A. Skurray.** 1990. Efflux-mediated antiseptic resistance gene *qacA* from *Staphylococcus aureus*: common ancestry with tetracycline- and sugar-transport proteins. Mol. Microbiol. **4:**2051–2062.
30. **Sako, T., and N. Tsuchida.** 1983. Nucleotide sequence of the staphylokinase gene from *Staphylococcus aureus*. Nucleic Acids Res. **11:**7679–7693.
31. **Smyth, D. S., and D. A. Robinson.** 2009. Integrative and sequence characteristics of a novel genetic element, ICE*6013*, in *Staphylococcus aureus*. J. Bacteriol. **191:**5964–5975.
32. **Soderquist, B., M. Andersson, M. Nilsson, A. Nilsdotter-Augustinsson, L. Persson, O. Friberg, and S. Jacobsson.** 2009. Staphylococcus epidermidis surface protein I (SesI): a marker of the invasive capacity of *S. epidermidis*? J. Med. Microbiol. **58:**1395–1397.
33. **Szczepanik, A., M. Koziol-Montewka, Z. Al-Doori, D. Morrison, and D. Kaczor.** 2007. Spread of a single multiresistant methicillin-resistant *Staphylococcus aureus* clone carrying a variant of staphylococcal cassette chromosome mec type III isolated in a university hospital. Eur. J. Clin. Microbiol. Infect. Dis. **26:**29–35.
34. **Tanaka, M., T. Wang, Y. Onodera, Y. Uchida, and K. Sato.** 2000. Mechanism of quinolone resistance in *Staphylococcus aureus*. J. Infect. Chemother. **6:**131–139.
35. **Tettelin, H., V. Masignani, M. J. Cieslewicz, C. Donati, D. Medini, N. L. Ward, S. V. Angiuoli, J. Crabtree, A. L. Jones, A. S. Durkin, R. T. Deboy, T. M. Davidsen, M. Mora, M. Scarselli, I. Margarit y Ros, J. D. Peterson, C. R. Hauser, J. P. Sundaram, W. C. Nelson, R. Madupu, L. M. Brinkac, R. J. Dodson, M. J. Rosovitz, S. A. Sullivan, S. C. Daugherty, D. H. Haft, J. Selengut, M. L. Gwinn, L. Zhou, N. Zafar, H. Khouri, D. Radune, G. Dimitrov, K. Watkins, K. J. O'Connor, S. Smith, T. R. Utterback, O. White, C. E. Rubens, G. Grandi, L. C. Madoff, D. L. Kasper, J. L. Telford, M. R. Wessels, R. Rappuoli, and C. M. Fraser.** 2005. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: implications for the microbial "pan-genome". Proc. Natl. Acad. Sci. U. S. A. **102:**13950–13955.
36. **Thomson, N. R., S. Howard, B. W. Wren, M. T. Holden, L. Crossman, G. L. Challis, C. Churcher, K. Mungall, K. Brooks, T. Chillingworth, T. Feltwell, Z. Abdellah, H. Hauser, K. Jagels, M. Maddison, S. Moule, M. Sanders, S. Whitehead, M. A. Quail, G. Dougan, J. Parkhill, and M. B. Prentice.** 2006. The complete genome sequence and comparative genome analysis of the high pathogenicity *Yersinia enterocolitica* strain 8081. PLoS Genet. **2:**e206.
37. **Vivoni, A. M., B. A. Diep, A. C. de Gouveia Magalhaes, K. R. Santos, L. W. Riley, G. F. Sensabaugh, and B. M. Moreira.** 2006. Clonal composition of *Staphylococcus aureus* isolates at a Brazilian university hospital: identification of international circulating lineages. J. Clin. Microbiol. **44:**1686–1691.
38. **Xu, B. L., G. Zhang, H. F. Ye, E. J. Feil, G. R. Chen, X. M. Zhou, X. M. Zhan, S. M. Chen, and W. B. Pan.** 2009. Predominance of the Hungarian clone (ST 239-III) among hospital-acquired meticillin-resistant *Staphylococcus aureus* isolates recovered throughout mainland China. J. Hosp. Infect. **71:**245–255.