12-1-2014

Whole Genome Sequencing of a Methicillin-Resistant Staphylococcus aureus Pseudo-Outbreak in a Professional Football Team.

Deverick J. Anderson

Simon R. Harris

Eliot Godofsky

Todd Toriscelli

Thomas H. Rude

See next page for additional authors

Authors
Deverick J. Anderson, Simon R. Harris, Eliot Godofsky, Todd Toriscelli, Thomas H. Rude, Kevin Elder, Daniel J. Sexton, Elliot J. Pellman, Thom Mayer, Vance G. Fowler, and Sharon J. Peacock

Whole Genome Sequencing of a Methicillin-Resistant *Staphylococcus aureus* Pseudo-Outbreak in a Professional Football Team

Deverick J. Anderson,[1] Simon R. Harris,[2] Eliot Godofsky,[3] Todd Toriscelli,[4] Thomas H. Rude,[1] Kevin Elder,[5,6] Daniel J. Sexton,[1] Elliot J. Pellman,[7,8] Thom Mayer,[9,10] Vance G. Fowler Jr.,[1] and Sharon J. Peacock[11]

[1]Duke University Medical Center, Durham, North Carolina; [2]Wellcome Trust Sanger Institute, Hinxton, United Kingdom; [3]Bach & Godofsky, Bradenton, Florida; [4]St. Thomas Sports Park, Nashville, Tennessee; [5]BayCare Medical Group; [6]University of South Florida, Tampa; [7]Icahn School of Medicine at Mount Sinai; [8]ProHEALTH Care Associates LLP, New York, New York; [9]George Washington University School of Medicine, District of Columbia; [10]University of Virginia School of Medicine, Charlottesville; and [11]University of Cambridge, United Kingdom

Two American football players on the same team were diagnosed with methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft tissue infections on the same day. Our investigation, including whole genome sequencing, confirmed that players did not transmit MRSA to one another nor did they acquire the MRSA from a single source within the training facility.

Keywords. American football; MRSA; outbreak investigation; whole genome sequencing.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the leading cause of skin and soft tissue infections (SSTIs) in the United States [1], and it is particularly common among American football players. Outbreaks in these athletes have been linked to contaminated whirlpools, water bottles, and shared equipment [2, 3]. Suspected MRSA outbreaks are traditionally investigated using bacterial genotyping approaches such as pulsed-field gel electrophoresis (PFGE), but recent studies have demonstrated superior discrimination of whole genome sequencing (WGS) [4, 5]. In this study, we describe a suspected MRSA outbreak in a professional football team in which WGS was required to accurately resolve the relationship between isolates and definitively exclude transmission.

The study was approved by the Institutional Review Boards of both Duke University Health System and Mt. Sinai Medical Centers. Two professional American football players on the same team developed symptoms consistent with SSTI on the same day during the 2013 football season (day 1). Three cultures were obtained from Player 1 (P1a, P1b, P1c), and 2 cultures were obtained from Player 2 (P2a, P2b). These cultures were sent to a local microbiology laboratory for culture, antimicrobial susceptibility testing, and polymerase chain reaction (PCR) for MRSA using standard techniques. Both players were evaluated by team physicians and started on antimicrobial therapy. On day 2, specimens from both players were PCR-positive for MRSA. Both players were withheld from team activities, and investigators from the Duke Infection Control Outreach Network were contacted to investigate the suspected outbreak and potential for further spread among team members. Local and national media reported that an outbreak of MRSA infection was occurring within the team facility. On day 3, all 5 samples were culture-positive for MRSA. Duke Infection Control Outreach Network investigators noted that the antimicrobial susceptibility results were not identical for isolates from the 2 players, with variation in susceptibility to trimethoprim-sulfamethoxazole (Supplementary Table 1). The MRSA isolates from Player 1 had a minimum inhibitory concentration (MIC) ≥ 320, and the MRSA isolates from Player 2 had an MIC ≤ 10. Duke Infection Control Outreach Network investigators concluded that these MRSA infections were unlikely to be related based on this phenotypic difference.

The MRSA isolates were sent for molecular typing to provide supportive evidence for this conclusion. PFGE was performed for the 5 isolates from the football players together with control isolates for USA100 through to USA800, using *Sma*I restriction as previously described [6]. PFGE banding patterns were analyzed using BioNumerics software (Applied Maths, Kortrijk, Belgium). A similarity coefficient of 80% was used to define pulsed-field type cluster, and PFGE profiles were interpreted according to a published typing schema. PFGE of the 5 isolates demonstrated 3 distinct banding patterns (Figure 1*A*). The 3 isolates from Player 1 exhibited 2 distinct PFGE patterns, suggesting mixed MRSA infection. Two isolates (P1a, P1b) had an identical PFGE profile that was most closely related to the

Received 15 July 2014; accepted 2 October 2014.

Correspondence: Deverick J. Anderson, MD, MPH, Associate Professor of Medicine, Duke University Medical Center, DUMC Box 102359, Durham, NC 27710 (deverick.anderson@duke.edu).

Open Forum Infectious Diseases

DOI: 10.1093/ofid/ofu096

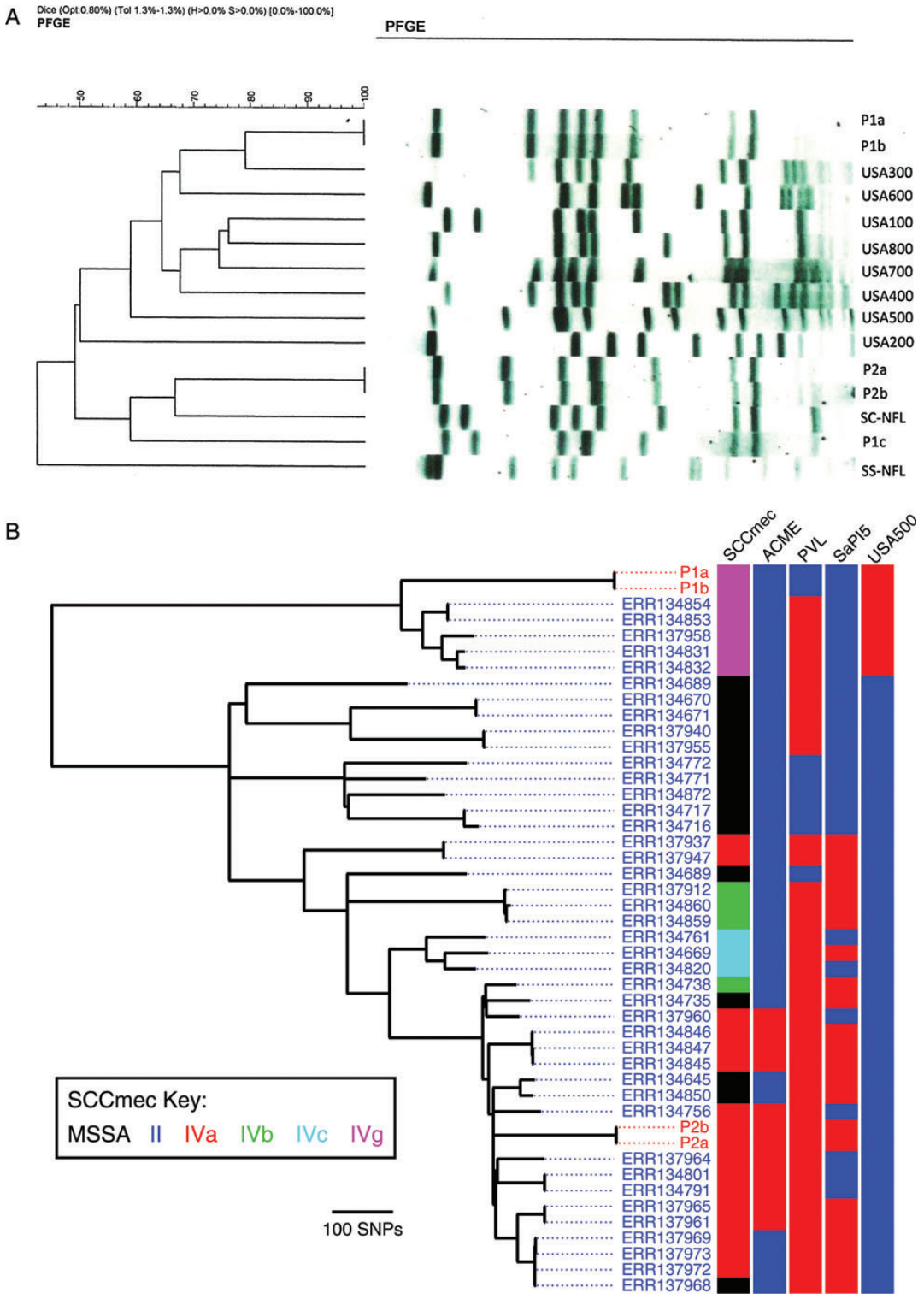

Figure 1. Phylogenetic analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from 2 professional football players. (*A*) Pulsed-field gel electrophoresis (PFGE) analysis of 5 isolates obtained from 2 football players with MRSA infections. (*B*) Core genome maximum likelihood phylogenetic tree of the 4 ST8 isolates obtained from 2 football players and a sample of 42 isolates from a single collection of 387 isolates, chosen to represent the diversity of ST8. These samples provide context to the diversity in genome sequence between the isolates from the 2 players, highlighted in red. The scale bar represents 100 single-nucleotide polymorphisms. The first column of colored blocks to the right of the tree indicates the SCC*mec* type of the samples, with a key inset. Red indicates the presence of specific genetic elements, whereas blue indicates the absence of the genetic element. The final column indicates isolates that are classified as USA500 (red) rather than USA300 (blue). Abbreviations: ACME, arginine catabolic mobile element; MSSA, methicillin-susceptible *Staphylococcus aureus*; PVL, Panton-Valentine leukocidin; SCC, staphylcococcal chromosomal cassette; SNPs, single-nucleotide polymorphisms; ST, sequence type.

USA300 control, whereas P1c had a different pattern that was nontypeable. Both isolates from Player 2 had an identical PFGE profile that was nontypeable. The isolates from Player 1 and Player 2 seemed to be unrelated by PFGE, but the generation of 3 of 5 test results that were effectively "null" is reflective of an imprecise method. Therefore, we performed WGS on the clinical isolates to provide definitive genotypic data.

To facilitate multilocus sequence typing (MLST) and WGS, sequencing libraries were prepared from DNA extracted from each of the 5 isolates, as previously described [7]. WGS was performed using an Illumina HiSeq to generate 150 base pair-paired end reads, and results were interpreted by an investigator who was blinded to all clinical, epidemiological, or microbiological information. Sequence reads were aligned to the chromosome of a USA300 reference isolate (FPR3757, defined by MLST as sequence type [ST] 8) using SMALT (http://www.sanger.ac.uk/resources/software/smalt/). First, we derived the in silico ST by assembling de novo sequence reads from each isolate using velvet [8], and we identified their MLST alleles by using Basic Local Alignment Search Tool (BLAST) [9] to search the *S aureus* MLST database (http://saureus.mlst.net/). Isolates from Player 1 were identified as ST8 (P1a, P1b) and ST5 (P1c), and both isolates from Player 2 (P2a, P2b) were ST8. This demonstrated that isolates from both players were indistinguishable by MLST and that this method failed to resolve uncertainty regarding a transmission event.

Additional informatics analysis of WGS data was undertaken on the ST8 isolates (excluding the unrelated ST5 isolate). The presence of genome-wide single-nucleotide polymorphisms (SNPs) and insertions or deletions (indels) were identified for each isolate using a combination of samtools, mpileup, and bcftools [10], and filtered as previously described [11]. Data for 42 published ST8 isolates chosen to represent the diversity of the ST were analyzed in the same way to allow the isolates from football players to be placed into a wider context [12]. Comparison of the 2 ST8 isolates from Player 1 demonstrated only 3 SNPs in the core genome, which is consistent with the 2 isolates being the same strain. Likewise, the 2 ST8 isolates from Player 2 exhibited no SNP differences. By contrast, the ST8 isolates from the 2 players differed by a mean of 1488 SNPs (1487–1489), which, based on the known rate of evolution for *S aureus*, definitively excluded a direct transmission event. We then placed the 4 ST8 isolates into a broader genetic analysis by comparing their genomes with 42 published ST8 isolates that had been chosen to represent the diversity of this ST and had been mapped to the same reference genome. A maximal likelihood phylogenetic reconstruction of the core variable sites for all isolates was performed with RAxML using a generalized time-reversible model of evolution with a gamma parameter to correct for among-site rate heterogeneity. The resulting phylogenetic tree confirmed that the isolates from the 2 players were minimally related (Figure 1*B*).

We explored the added value that could be derived from the genome sequence by predicting the antimicrobial resistance profile and curating a list of toxin genes (Supplementary Table 1). Each of the isolates was searched for known antibiotic resistance genotypes. Gene presence- and absence-based resistance determinants and staphylcococcal chromosomal cassette *mec*, arginine catabolic mobile element, Panton-Valentine leukocidin, and SaPI5 elements were identified using BLAST to search the de novo assemblies of each isolate, whereas known SNP-based resistances were identified from the mapped variant calls. Deletions upstream of *erm*C and *erm*A, which are known to be involved in inducible clindamycin resistance and clindamycin sensitivity, respectively, were also identified from the mapped data (Supplementary Table 1).

WGS data identified important limitations in the ability to infer epidemiologic associations using antibiogram patterns of clinical MRSA isolates. Player 1 was infected with 2 different MRSA lineages that shared the same antibiogram, whereas MRSA isolates with the same lineage (ST8) but different antibiograms were isolated from the 2 cases. Our results demonstrate (1) the inherent weaknesses of PFGE and MLST and (2) the potential of WGS to accurately resolve the genetic relationship between MRSA associated with high value putative outbreaks. Our findings refute incorrect speculation by local and national media reports that a point-source outbreak of MRSA had occurred within the facility of the team. In summary, our investigation and WGS provided the necessary information to conclude that ongoing transmission of MRSA was not occurring in the facility, thereby ensuring that infection control resources could be allocated appropriately.

Supplementary Material

Supplementary material is available online at *Open Forum Infectious Diseases* (http://OpenForumInfectiousDiseases.oxfordjournals.org/).

Acknowledgments

We thank Beth Blane and her technician team at the University of Cambridge for DNA extraction.

Financial support. This work was supported by grants from the National Institutes of Health (Grants K23-AI 095357 [to D. J. A.] and 2R01-AI0168804 and K24-AI093969 [to V. G. F.]); UK Clinical Research Collaboration Translational Infection Research Initiative; the Medical Research Council (Grant G1000803), with contributions from the Biotechnology and Biological Sciences Research Council, the National Institute for Health Research on behalf of the UK Department of Health, and the Chief Scientist Office of the Scottish Government Health Directorate (to S. J. P.); and the Wellcome Trust Sanger Institute (Grant WT098051 [to S. R. H.]).

Potential conflicts of interest. S. J. P. is a consultant for Pfizer Inc. V. G. F. served as Chair of V710 Scientific Advisory Committee (Merck), has received grant support from Cerexa, Pfizer, Advanced Liquid Logic, MedImmune, has been a paid consultant for Merck, Astellas, Affinium, Theravance, Cubist, Cerexa, Durata, Pfizer, NovaDigm, Novartis,

Medicines Company, Biosynexus, MedImmune, and Inimex, and has received honoraria from Merck, Astellas, Cubist, Pfizer, Theravance, and Novartis. T. M. is the Medical Director of the NFL Players Association.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med **2006**; 355:666–74.
2. Centers for Disease Control and Prevention (CDC). Methicillin-resistant *Staphylococcus aureus* among players on a high school football team–New York City, 2007. MMWR Morb Mortal Wkly Rep **2009**; 58:52–5.
3. Kazakova SV, Hageman JC, Matava M, et al. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N Engl J Med **2005**; 352:468–75.
4. Harris SR, Cartwright EJ, Torok ME, et al. Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. Lancet Infect Dis **2013**; 13:130–6.
5. Koser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. N Engl J Med **2012**; 366:2267–75.
6. McDougal LK, Steward CD, Killgore GE, et al. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol **2003**; 41:5113–20.
7. Quail MA, Otto TD, Gu Y, et al. Optimal enzymes for amplifying sequencing libraries. Nat Methods **2012**; 9:10–1.
8. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res **2008**; 18:821–9.
9. Altschul SF, Gish W, Miller W, et al. Basic local alignment search tool. J Mol Biol **1990**; 215:403–10.
10. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics **2009**; 25:2078–9.
11. Harris SR, Feil EJ, Holden MT, et al. Evolution of MRSA during hospital transmission and intercontinental spread. Science **2010**; 327:469–74.
12. Uhlemann AC, Dordel J, Knox JR, et al. Molecular tracing of the emergence, diversification and transmission of *S. aureus* sequence type 8/USA300 in a New York community. **2014**; Proc Natl Acad Sci U S A 111:6738–43.