AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of Two Methicillin-Susceptible *Staphylococcus aureus* Clinical Strains Closely Related to Community-Associated Methicillin-Resistant *S. aureus* USA300

Jo-Ann McClure,[a] Kunyan Zhang[a,b,c,d,e]

[a]Centre for Antimicrobial Resistance, Alberta Health Services/Alberta Public Laboratories/University of Calgary, Calgary, Alberta, Canada
[b]Department of Pathology & Laboratory Medicine, University of Calgary, Calgary, Alberta, Canada
[c]Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada
[d]Department of Medicine, University of Calgary, Calgary, Alberta, Canada
[e]The Calvin, Phoebe and Joan Snyder Institute for Chronic Diseases, University of Calgary, Calgary, Alberta, Canada

ABSTRACT Predominant community-associated methicillin-resistant *Staphylococcus aureus* strain USA300 is believed to have originated from an ancestral methicillin-susceptible strain, although the details of that evolution remain unknown. To help understand the emergence of this highly successful strain, we sequenced the genomes of two methicillin-susceptible *Staphylococcus aureus* clinical strains that are very closely related to USA300.

The evolutionary origins of the major methicillin-resistant *Staphylococcus aureus* (MRSA) clones are still poorly understood, although it is hypothesized that they repeatedly arose from epidemic methicillin-susceptible *S. aureus* (MSSA) strains through acquisition of the *mecA* gene via horizontal transfer (1–5). The highly successful community-associated MRSA (CA-MRSA) strain USA300 has become predominant in North America, causing significant morbidity and mortality (6–12). It is believed to have descended from an ancestral USA500-like MSSA strain through acquisition of multiple mobile genetic elements (MGEs) and clonal expansion (13, 14). As with the other major MRSA clones, more work is needed to fully understand its emergence and success. To that end, we selected two MSSA isolates that are closely related to USA300 for whole-genome sequencing, with the goal of elucidating the genetic and evolutionary relationships between these MSSA isolates and the highly successful USA300 MRSA group. Strain H489 was isolated by our clinical microbiological laboratory from the sputum of a patient from our local health care region in Calgary, Canada, in 1993, well before our USA300 outbreak began in 2004. Likewise, strain C3948 was isolated from a patient in 2002, just before the USA300 outbreak. Multilocus sequence analysis of the isolates indicated that, similarly to the USA300 outbreak strain, they belonged to sequence type 8 (ST8).

Genomic DNA was isolated by phenol-chloroform extraction of overnight cultures started from a single colony. Library preparation, DNA sequencing, contig assembly, and genome circularization were performed at the Génome Québec Innovation Centre in Montreal, Canada. Sheared large-insert libraries were prepared with Covaris g-TUBES and the SMRTbell template prep kit 1.0. Sequencing was done using the Pacific Biosciences (PacBio) RSII sequencing technology, with one single-molecule real-time (SMRT) cell. Contig assembly was done using the RS Hierarchical Genome Assembly Process (HGAP) protocol version 2.3.0.140936.p5 (15–17), with read quality controlled by aligning short reads on longer reads using BLASR (15). The genomes were circularized using Circlator version 1.4.1 and adjusted to the origin of replication (18). Gene annotation was done using the NCBI's Prokaryotic Genome Annotation Pipeline version 4.1, using the best-placed reference protein set (GeneMarkS+) (19).

Citation McClure J-A, Zhang K. 2019. Complete genome sequences of two methicillin-susceptible *Staphylococcus aureus* clinical strains closely related to community-associated methicillin-resistant *S. aureus* USA300. Microbiol Resour Announc 8:e00356-19. https://doi.org/10.1128/MRA.00356-19.

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

Address correspondence to Kunyan Zhang, kzhang@ucalgary.ca.

Received 26 March 2019
Accepted 30 March 2019
Published 25 April 2019

Two contigs were assembled for MSSA strain C3948 from 85,037 raw reads, covering 1,142,835,600 sequenced bases, with an N_{50} value of 2,823,074 bp. The estimated genome coverage was 368×, and the GC content was 32.82%. On the assembled chromosome of 2,795,888 bp, 2,924 genes were identified, of which 2,842 were coding sequences (CDS), 82 were RNA genes, and 76 were pseudogenes. Four contigs were assembled for MSSA strain H489 from 94,559 raw reads covering 1,215,818,661 sequenced bases, with an N_{50} value of 2,761,569 bp. The estimated genome coverage was 392×, and the GC content was 32.79%. On the chromosome of 2,757,748 bp, 2,874 genes were identified, of which 2,789 were CDS, 85 were RNA genes, and 94 were pseudogenes.

A complete analysis is under way to look at the major genetic components in these MSSA isolates and compare them with those found in MRSA USA300 to help shed light on the evolutionary path of the highly successful USA300 strain.

Data availability. The chromosomal genome sequences have been deposited at GenBank under the accession numbers CP020957 (C3948) and CP020959 (H489), with SRA accession numbers SRX5551895 (C3948) and SRX5552203 (H489).

ACKNOWLEDGMENTS

This work was supported in part by operation grants (FRN: ARE-147623 and ARF-151557) from the Canadian Institutes of Health Research, Canada, and in part by an operating fund from the Centre for Antimicrobial Resistance (CAR), Alberta Health Services, Alberta, Canada.

REFERENCES

1. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A 99:7687–7692. https://doi.org/10.1073/pnas.122108599.
2. Fitzgerald JR, Sturdevant DE, Mackie SM, Gill SR, Musser JM. 2001. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. Proc Natl Acad Sci U S A 98:8821–8826. https://doi.org/10.1073/pnas.161098098.
3. Kreiswirth B, Kornblum J, Arbeit RD, Eisner W, Maslow JN, McGeer A, Low DE, Novick RP. 1993. Evidence for a clonal origin of methicillin resistance in *Staphylococcus aureus*. Science 259:227–230. https://doi.org/10.1126/science.8093647.
4. Robinson DA, Enright MC. 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 47:3926–3934. https://doi.org/10.1128/AAC.47.12.3926-3934.2003.
5. Lakhundi S, Zhang K. 2018. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin Microbiol Rev 31:e00020-18. https://doi.org/10.1128/CMR.00020-18.
6. Carrel M, Perencevich EN, David MZ. 2015. USA300 methicillin-resistant *Staphylococcus aureus*, United States, 2000–2013. Emerg Infect Dis 21:1973–1980. https://doi.org/10.3201/eid2111.150452.
7. Diekema DJ, Richter SS, Heilmann KP, Dohrn CL, Riahi F, Tendolkar S, McDanel JS, Doern GV. 2014. Continued emergence of USA300 methicillin-resistant *Staphylococcus aureus* in the United States: results from a nationwide surveillance study. Infect Control Hosp Epidemiol 35:285–292. https://doi.org/10.1086/675283.
8. Jenkins TC, McCollister BD, Sharma R, McFann KK, Madinger NE, Barron M, Bessesen M, Price CS, Burman WJ. 2009. Epidemiology of healthcare-associated bloodstream infection caused by USA300 strains of methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals. Infect Control Hosp Epidemiol 30:233–241. https://doi.org/10.1086/595963.
9. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, Harrison LH, Lynfield R, Dumyati G, Townes JM, Craig AS, Zell ER, Fosheim GE, McDougal LK, Carey RB, Fridkin SK, Active Bacterial Core surveillance (ABCs) MRSA Investigators. 2007. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. JAMA 298:1763–1771. https://doi.org/10.1001/jama.298.15.1763.
10. Kreisel KM, Stine OC, Johnson JK, Perencevich EN, Shardell MD, Lesse AJ, Gordin FM, Climo MW, Roghmann MC. 2011. USA300 methicillin-resistant *Staphylococcus aureus* bacteremia and the risk of severe sepsis: is USA300 methicillin-resistant *Staphylococcus aureus* associated with more severe infections? Diagn Microbiol Infect Dis 70:285–290. https://doi.org/10.1016/j.diagmicrobio.2011.03.010.
11. McCaskill ML, Mason EO, Jr, Kaplan SL, Hammerman W, Lamberth LB, Hulten KG. 2007. Increase of the USA300 clone among community-acquired methicillin-susceptible *Staphylococcus aureus* causing invasive infections. Pediatr Infect Dis J 26:1122–1127. https://doi.org/10.1097/INF.0b013e31814536e0.
12. Simor AE, Gilbert NL, Gravel D, Mulvey MR, Bryce E, Loeb M, Matlow A, McGeer A, Louie L, Campbell J, Canadian Nosocomial Infection Surveillance Program. 2010. Methicillin-resistant *Staphylococcus aureus* colonization or infection in Canada: national surveillance and changing epidemiology, 1995–2007. Infect Control Hosp Epidemiol 31:348–356. https://doi.org/10.1086/651313.
13. Tenover FC, Goering RV. 2009. Methicillin-resistant *Staphylococcus aureus* strain USA300: origin and epidemiology. J Antimicrob Chemother 64:441–446. https://doi.org/10.1093/jac/dkp241.
14. Jamrozy DM, Harris SR, Mohamed N, Peacock SJ, Tan CY, Parkhill J, Anderson AS, Holden MT. 2016. Pan-genomic perspective on the evolution of the *Staphylococcus aureus* USA300 epidemic. Microb Genom 2:e000058. https://doi.org/10.1099/mgen.0.000058.
15. Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using Basic Local Alignment with Successive Refinement (BLASR): application and theory. BMC Bioinformatics 13:238. https://doi.org/10.1186/1471-2105-13-238.
16. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
17. Myers EW, Sutton GG, Delcher AL, Dew IM, Fasulo DP, Flanigan MJ, Kravitz SA, Mobarry CM, Reinert KH, Remington KA, Anson EL, Bolanos RA, Chou HH, Jordan CM, Halpern AL, Lonardi S, Beasley EM, Brandon RC, Chen L, Dunn PJ, Lai Z, Liang Y, Nusskern DR, Zhan M, Zhang Q, Zheng X, Rubin GM, Adams MD, Venter JC. 2000. A whole-genome assembly of *Drosophila*. Science 287:2196–2204. https://doi.org/10.1126/science.287.5461.2196.
18. Hunt M, De Silva N, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol 16:294. https://doi.org/10.1186/s13059-015-0849-0.
19. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.