Complete Genome Sequence of a Panton-Valentine Leukocidin-Negative Staphylococcus aureus Strain Isolated from a Patient with Pervasive Necrotizing Soft Tissue Infection

Yoshifumi Aiba, Shinya Watanabe, Rieko Tsukahara, Naoka Umemoto, Kanate Thitiananpakorn, Tanit Boonsiri, Feng-Yu Li, Kotaro Kiga, Yusuke Sato, Xin-Ee Tan, Yusuke Taki, Aa Haeruman Azam, Yuancheng Zhang, Tepeei Sasahara, Toshio Demitsu, Longzhu Cui

ABSTRACT The association of Panton-Valentine leukocidin (PVL) toxin with necrotizing soft tissue infection (NSTI) caused by Staphylococcus aureus remains controversial. Here, we report the complete genome sequence of the PVL-negative S. aureus strain JMUB1273, isolated from a patient with pervasive NSTI.

Necrotizing soft tissue infection (NSTI) is a life-threatening infection with a substantially high mortality rate (11% to 36%) and is occasionally caused by Staphylococcus aureus (1, 2). There are many staphylococcal toxins or extracellular products that are associated with the pathogenesis of NSTIs, including Panton-Valentine leukocidin (PVL) toxin, toxic shock syndrome toxin 1 (TSST-1), enterotoxin (SE) or enterotoxin-like (SEI) toxin, exfoliative toxin (ET), and phenol-soluble modulin (PSM). However, their individual involvement in the pathogenesis of NSTIs remains controversial and yet to be fully understood.

S. aureus strain JMUB1273 was isolated from a 61-year-old man with an NSTI on his upper back that occurred during dietary modification under glycemic control. Upon physical examination, a huge black lesion covering over half of the upper back beyond what appeared to be the confines of infection was observed, and pus was oozing from the area of the lesion. The patient’s laboratory risk indicator for necrotizing fasciitis (LRINEC) score was 8. Eventually, the patient was successfully treated with surgical drainage, negative pressure wound therapy, antibiotics, and glycemic control, followed by skin grafting.

Bacterial strain was isolated from surgical wound drainage by growing it on a sheep blood agar plate (Nissui, Tokyo, Japan) under 5% CO2 at 35°C for 48 h. The strain was identified as S. aureus and oxacillin susceptible by the RAISUS S4 (Nissui, Tokyo, Japan) system for rapid bacterial identification and antimicrobial susceptibility testing.

Genomic DNA extraction and whole-genome sequence analysis were performed as described previously (3). Briefly, JMUB1273 genomic DNA was extracted from an overnight culture in tryptic soy broth (BD, NJ, USA) at 37°C using the NucleoBond AXG kit (TaKaRa Bio, Inc., Japan). A genomic library was prepared using a rapid barcoding sequencing kit (SQK-RBK004) with an input DNA amount of 400 ng according to the manufacturer’s protocol. To determine the whole-genome sequence, a MinION Mk-1B device (Oxford Nanopore Technologies [ONT], Oxford, United Kingdom) integrated with a FLO-MIN106 (R9.4.1) flow cell (ONT) was used, and a total of 126,370 reads (average size, 5,004 bp) was obtained using MinKNOW software (version 1.14.1; ONT). The ONT sequences were then demultiplexed and the potential remaining adaptors were trimmed using Porechop tools (version 0.2.4; https://github.com/rrwick/porechop); they were de novo assembled into a single contig by using Canu (version 1.8; http://canu.readthedocs.org/). To correct errors in the assembled sequences, 986,906 short...
reads were generated using the Nextera mate pair preparation kit (Illumina, Inc.) for library construction and the MiSeq reagent kit version 3 (Illumina, Inc.) for sequencing (4, 5). The short reads generated with the MiSeq platform (2 × 301-bp, paired-end format) were trimmed with the FASTQ toolkit version 2.0.0 (Illumina, Inc.) with a quality level of 30. Using the MiSeq short reads, the assembled sequences generated from the ONT reads were polished with Pilon (version 1.22; https://github.com/broadinstitute/pilon/) (6) and the CLC Genomics Workbench version 9.5.3 (Qiagen, Hilden, Germany). Gene annotation was performed with the Microbial Genome Annotation Pipeline or Prokka (version 1.13.36) (7). Default parameters were used for all software unless otherwise noted.

The whole-genome sequence determination and annotation showed that JMUB1273 harbors a single circular chromosome that is 2,733,771 bp long (coverage depth, 61.7x) with a GC content of 32.8% and encodes 2,640 predicted proteins, 59 tRNAs, and 6 rRNAs. Following a detailed analysis using multilocus sequence typing (MLST 2.0), we found that JMUB1273 belonged to sequence type 188 (ST188) and clonal complex CC1 (8, 9). Many staphylococcal toxins known to be associated with NSTIs, including PVL, TSST-1, SE, and ET, were not found in this genome. However, other potentially associated genes (1, 10) encoding virulence factors, such as gamma-hemolysin, leukocidin, aureolysin, serine proteases, staphylokinase, putative SEI protein, PSMβ1, and staphylococcal complement inhibitor, were identified in this genome.

Data availability. The genome sequence was deposited in DDBJ/GenBank under the accession number AP018922. The associated BioProject and BioSample accession numbers are PRJDB7266 and SAMD00134450, respectively. The raw data reads have been deposited in the DDBJ/Sequence Read Archive under the accession numbers DRA009801 (ONT) and DRA009505 (Illumina).

ACKNOWLEDGMENTS

This study was partly funded by JSPS KAKENHI (grant numbers 15H05654 and 19K08960 to S.W., 18K15149 to K.K., 17K15691 to Y.S., and 17K19570 to L.C.), the Takeda Science Foundation (S.W., L.C.), the JSPS International Research Fellow (grant number 17F17713 to L.C.), and the Japan Agency for Medical Research and Development J-PRIIDE (grant number JP19fm0208028 to L.C.). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Shumba P, Mairpady Shambat S, Siemens N. 2019. The role of streptococcal and staphylococcal exotoxins and proteases in human necrotizing soft tissue infections. Toxins 11:332. https://doi.org/10.3390/toxins11060332.
2. Jabbour G, El-Menyar A, Peralta R, Shaikh N, Abdelrahman H, Mudali IN, Ellabib M, Al-Thani H. 2016. Pattern and predictors of mortality in necrotizing fasciitis patients in a single tertiary hospital. World J Emerg Surg 11:40. https://doi.org/10.1186/s13017-016-0097-y.
3. Watanabe S, Cui B, Kiga K, Alba Y, Tan X-E, Sato’o Y, Kawauchi M, Boonsiri T, Thitiananpakorn K, Taki Y, Li F-Y, Azam AH, Nakada Y, Sasahara T, Cui L. 2019. Composition and diversity of CRISPR-Cas13a systems in the genus Leptotrichia. Front Microbiol 10:2838. https://doi.org/10.3389/fmicb.2019.02838.
4. Watanabe S, Sasahara T, Arai N, Sasaki K, Alba Y, Sato’o Y, Cui L. 2016. Complete genome sequence of Streptococcus pyogenes strain JMUB1235 isolated from an acute phlegmonous gastritis patient. Genome Announc 4:e01133-16. https://doi.org/10.1128/genomeA.01133-16.
5. Watanabe S, Alba Y, Tan X-E, Li F-Y, Boonsiri T, Thitiananpakorn K, Cui B, Sato’o Y, Kiga K, Sasahara T, Cui L. 2018. Complete genome sequencing of three human clinical isolates of Staphylococcus caprae reveals virulence factors similar to those of S. epidermidis and S. capitis. BMC Genomics 19:810. https://doi.org/10.1186/s12866-018-5185-9.
6. Walker BJ, Abeel T, Shea T, Priest M, Abouelli A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
7. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
8. Enright MC, Day NPJ, Davies CE, Peacock SJ, Spratt BG. 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. J Clin Microbiol 38:1008–1015. https://doi.org/10.1128/JCM.38.3.1008-1015.2000.
9. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. 2012. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50:1355–1361. https://doi.org/10.1128/JCM.00904-11.
10. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B. 2005. Necrotizing fasciitis caused by community-associated methicillin-resistant Staphylococcus aureus in Los Angeles. N Engl J Med 352:1445–1453. https://doi.org/10.1056/NEJMo042683.