AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequence of Methicillin-Resistant *Staphylococcus aureus* Harboring Staphylococcal Cassette Chromosome *mec* Type IX, Isolated from a Fatal Bacteremic Pneumonia Case

Peechanika Chopjitt,[a] Thidathip Wongsurawat,[b] Piroon Jenjaroenpun,[b] Parichart Boueroy,[a] Watcharaporn Kamjumphol,[c] Rujirat Hatrongjit,[d] Anusak Kerdsin[a]

[a]Faculty of Public Health, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand
[b]Division of Bioinformatics and Data Management for Research, Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand
[c]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand
[d]Faculty of Science and Engineering, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon, Thailand

ABSTRACT Here, we report the whole-genome sequence of a methicillin-resistant *Staphylococcus aureus* strain harboring staphylococcal cassette chromosome *mec* (SCC*mec*) type IX, isolated from a fatal bacteremic pneumonia case. Genomic analysis revealed that the isolate was sequence type 9 and *spa* type t3446, carrying multiple antimicrobial resistance genes comprising *mecA*, *blaZ*, *aac(6′)-aph (2″)*, *aadD*, *ant(6)-Ia*, *lsa*(E), *dfrG*, *tet*(M), *fexA*, and *lnu*(B).

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are classified as hospital-acquired (HA), community-acquired (CA), and livestock-associated (LA) infections (1). MRSA strains carry different types of the staphylococcal cassette chromosome *mec* (SCC*mec* I to XIII). While SCC*mec* I to III are commonly found in HA-MRSA, SCC*mec* IV to XIII are usually detected in CA-MRSA and LA-MRSA (2).

Interestingly, MRSA sequence type 9 (ST9) harboring SCC*mec* IX was reported in humans as a newly identified CA-MRSA clone disseminating in Thailand (3). Herein, we determined the genome sequence of the MRSA strain carrying SCC*mec* IX (isolate M16), isolated in February 2019 from sputum from a 49-year-old man in northern Thailand with a fatal case of bacteremic pneumonia. The isolate was cultured on sheep blood agar at 37° C for 18 h and identified using conventional biochemical tests (4). Its resistance to methicillin was investigated using cefoxitin disk diffusion according to the 2020 Clinical and Laboratory Standard Institute (CLSI) guidelines (5). A pentaplex PCR assay was used to simultaneously identify the genus (*Staphylococcus*; 16S rRNA), the species (*S. aureus*; *femA*), and the methicillin resistance (*mecA*) and PVL toxin (*lukS*) genes (6). These assays demonstrated that the isolate was a MRSA strain with no *lukS* gene.

The bacterium was grown on tryptic soy agar at 37°C for 18 h. Genomic DNA was extracted from the colony using a ZymoBIOMICS DNA kit (Zymo Research, USA) and quantified using the Invitrogen Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (Thermo Fisher Scientific, MA, USA). Genomic libraries were generated using the NEBNext Ultra II DNA library prep kit for Illumina (New England BioLabs, USA) following the manufacturer's instructions. Whole-genome sequencing was performed using the MiSeq platform (Illumina, CA, USA) according to the manufacturer's instructions to obtain 250-bp paired-end reads (7). We applied Skewer v0.2.2 (8) for quality filtering and adapter trimming of the Illumina reads. Quality checking of the Illumina reads was performed using FastQC v0.11.8 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and the genome was *de novo* assembled using Unicycler v0.4.8 (9). The genome sequences were checked for quality using QUAST v5.0.2 (10). The

Citation Chopjitt P, Wongsurawat T, Jenjaroenpun P, Boueroy P, Kamjumphol W, Hatrongjit R, Kerdsin A. 2021. Draft genome sequence of methicillin-resistant *Staphylococcus aureus* harboring staphylococcal cassette chromosome *mec* type IX, isolated from a fatal bacteremic pneumonia case. Microbiol Resour Announc 10:e00616-21. https://doi.org/10.1128/MRA.00616-21.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Address correspondence to Anusak Kerdsin, anusak.ke@ku.th.

Received 15 June 2021
Accepted 12 July 2021
Published 29 July 2021

genomic sequences were submitted to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.12 for annotation. Default parameters were used for all software unless otherwise specified.

In total, 4,242,022 raw reads were obtained for isolate M16. Sixty-nine contigs were assembled, with an N_{50} value of 126,866 bp. On average, the assembled draft genome sequence was covered 230.42 times. The draft genome size was determined to be 2,761,167 bp and the GC content to be 32.72%. This isolate was identified as ST9, carried SCC*mec* IX, and had the *spa* type t3446 according to MLST 2.0, SCC*mec*Finder, and spaTyper 1.0, respectively (11–13). The arginine catabolic mobile element (ACME) was not detected using MyDbFinder (https://cge.cbs.dtu.dk/services/MyDbFinder/). The isolate genome included acquired antimicrobial resistance genes, namely, *mecA*, *blaZ*, *aac(6′)-aph (2″)*, *aadD*, *ant(6)-Ia*, *lsa*(E), *dfrG*, *tet*(M), *fexA*, and *lnu*(B), according to ResFinder v4.1 (14). We detected *tet*(M) located on the *mecA* contig, which suggested that it was carried on the *SCCmec* IX element. The VirulenceFinder tool revealed aureolysin (*aur*), enterotoxin types G, I, M, N, O, and U (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*), and γ-hemolysin (*hlgA*, *hlgB*, and *hlgC*) (15).

This study was reviewed and approved by the Ethics Review Board (ERB) of the Ministry of Public Health, Thailand. The ERB waived the requirement for informed consent because the study satisfied the conditions of the policy statement on ethical conduct for research involving humans. This study was conducted according to the principles of the Declaration of Helsinki.

Data availability. The results of this whole-genome shotgun project were deposited in DDBJ/ENA/GenBank under the BioProject accession no. PRJNA735605, BioSample accession no. SAMN19589956, and accession no. JAHKSK000000000.1. The Sequence Read Archive (SRA) number is SRR14802804.

ACKNOWLEDGMENTS

The Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand, provided a grant and English-editing assistance.

REFERENCES

1. 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.
2. Rongsanam P, Yano T, Yokart W, Yamsakul P, Sutammeng S, Udpaun R, Pichpol D, Tamdee D, Anukool U. 2020. Acquisition risk factors of the SCC*mec* IX-methicillin-resistant *Staphylococcus aureus* in swine production personnel in Chiang Mai and Lamphun provinces, Thailand. Antibiotics (Basel) 9:651. https://doi.org/10.3390/antibiotics9100651.
3. Lulitanond A, Ito T, Li S, Han X, Ma XX, Engchanil C, Chanawong A, Wilailuckana C, Jiwakanon N, Hiramatsu K. 2013. ST9 MRSA strains carrying a variant of type IX SCC*mec* identified in the Thai community. BMC Infect Dis 13:214. https://doi.org/10.1186/1471-2334-13-214.
4. Becker K, Skov RL, von Eiff C. 2011. Staphylococcus, Micrococcus and other catalase-positive cocci, p 639–657. *In* Jorgensen JH, Carroll KC, Funke G, Pfaller MA, Landry ML, Richter SS, Warnock DW (ed), Manual of clinical microbiology, 10th ed, vol 2. ASM Press, Washington, DC.
5. Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial susceptibility testing: CLSI document M100, 30th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
6. Al-Talib H, Yean CY, Al-Khateeb A, Hassan H, Singh K-KB, Al-Jashamy K, Ravichandran M. 2009. A pentaplex PCR assay for the rapid detection of methicillin-resistant Staphylococcus aureus and Panton-Valentine leucocidin. BMC Microbiol 9:113. https://doi.org/10.1186/1471-2180-9-113.
7. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560.
8. Jiang H, Lei R, Ding S-W, Zhu S. 2014. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinformatics 15:182. https://doi.org/10.1186/1471-2105-15-182.
9. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
10. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
11. 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.06094-11.
12. Kaya H, Hasman H, Larsen J, Stegger M, Johannesen TB, Allesøe RL, Lemvigh CK, Aarestrup FM, Lund O, Larsen AR. 2018. SCC*mec*Finder, a Web-based tool for typing of staphylococcal cassette chromosome *mec* in *Staphylococcus aureus* using whole-genome sequence data. mSphere 3:e00612-17. https://doi.org/10.1128/mSphere.00612-17.
13. Bartels MD, Petersen A, Worning P, Nielsen JB, Larner-Svensson H, Johansen HK, Andersen LP, Jarløv JO, Boye K, Larsen AR, Westh H. 2014. Comparing whole-genome sequencing with Sanger sequencing for *spa* typing of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 52:4305–4308. https://doi.org/10.1128/JCM.01979-14.
14. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wieczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. J Antimicrob Chemother 75:3491–3500. https://doi.org/10.1093/jac/dkaa345.
15. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. J Clin Microbiol 52:1501–1510. https://doi.org/10.1128/JCM.03617-13.