AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# Complete Genome Sequences of Eight Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from Patients in Japan

**Miyako Hikichi,[a] Miki Nagao,[b] Kazunori Murase,[a] Chihiro Aikawa,[a] Takashi Nozawa,[a] Akemi Yoshida,[c] Taisei Kikuchi,[d] Ichiro Nakagawa[a]**

[a]Department of Microbiology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[b]Clinical Laboratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[c]Laboratory of Genomics, Frontier Science Research Center, University of Miyazaki, Miyazaki, Japan
[d]Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

**ABSTRACT** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen causing nosocomial infections, and the clinical manifestations of MRSA range from asymptomatic colonization of the nasal mucosa to soft tissue infection to fulminant invasive disease. Here, we report the complete genome sequences of eight MRSA strains isolated from patients in Japan.

*Staphylococcus aureus* is a causative agent that triggers a wide range of clinical infections, such as bacteremia, catheter-associated infections, and surgical site infections (1, 2). Among *S. aureus* strains, methicillin-resistant *Staphylococcus aureus* (MRSA) is a representative drug-resistant bacterium and one of the most successful modern pathogens, accounting for 60% of clinical *S. aureus* infections in Japan (3). Vancomycin and daptomycin are the agents of choice for acceptable treatment of invasive MRSA infections. Alternative agents that may be used for second-line or salvage therapy include telavancin, ceftaroline, and linezolid (4). However, it has recently been reported that acquisition of the *vanA* gene cluster during antibiotic therapy leads to resistance to vancomycin (5). Here, we report the complete genome sequence of eight MRSA strains isolated in Japan, which showed multidrug resistance and possessed related genes in their plasmids. This genome information could help understanding of the multidrug resistance machinery of *S. aureus*.

Eight MRSA strains were isolated from the specimens of patients during the period of 2004 through 2018 and identified according to CLSI approved standard M100. All clinical samples were cultured on sheep blood agar, chocolate agar, and Drigalski agar at 35°C for 24 h, and the colonies were selected and then identified using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). The Kyoto University institutional review board approved the protocol for use of the clinical data and strains.

Eight MRSA strains were incubated for 15 h at 37°C in Trypticase soy medium. The bacterial cells were lysed with lysostaphin and lysozyme, and then the genomic DNA was extracted by the phenol-chloroform extraction method. Extracted DNA was sequenced by a combination of short-read and long-read sequencing platforms. An Illumina short-read library was prepared using a Nextera DNA library prep kit, and paired-end reads were generated using a MiSeq reagent kit (v3-600) on the MiSeq platform (Illumina). A MinION long-read library was prepared from unsheared genomic DNA using the Rapid Barcoding kit (catalog number SQK-RBK004) and sequenced with an R9 flow cell (FLO-MIN106) on a MinION device (Oxford Nanopore Technologies). The Illumina data were preprocessed using Trimmomatic (v0.36) to remove adapter and low-quality sequences (6), and hybrid assembly with the MinION long reads was

**Citation** Hikichi M, Nagao M, Murase K, Aikawa C, Nozawa T, Yoshida A, Kikuchi T, Nakagawa I. 2019. Complete genome sequences of eight methicillin-resistant *Staphylococcus aureus* strains isolated from patients in Japan. Microbiol Resour Announc 8:e01212-19. https://doi.org/10.1128/MRA.01212-19.

**Editor** Irene L. G. Newton, Indiana University, Bloomington



Address correspondence to Ichiro Nakagawa, nakagawa.ichiro.7w@kyoto-u.ac.jp.

**Received** 26 September 2019
**Accepted** 28 October 2019
**Published** 21 November 2019

**TABLE 1** Assembly statistics, general genome information, and relevant characteristics of eight MRSA strains

| Statistic or characteristic | Data for MRSA strain[b]: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | KUN1163 | KUH140013 | KUH140046 | KUH140087 | KUH140331 | KUH180038 | KUH180062 | KUH180129 |
| Strain information | | | | | | | | |
| Origin | Sputum | Sputum | Nasal cavity | Blood | Blood | Pus | Sputum | Blood |
| Clinical symptom | Colonization | Pneumonia | Colonization | Sepsis | Sepsis | Skin and soft tissue infection | Colonization | Sepsis |
| Yr of isolation | 2014 | 2014 | 2014 | 2014 | 2014 | 2018 | 2018 | 2018 |
| Antibiotic resistance (MIC [μg/ml])[a] | CEZ (≥64), IPM (≥32), LVFX (≥16), GM (≥32), EM (≥16), CLDM (≥16), MPIPC (≥16) | CEZ (≤8), IPM (2), LVFX (>4), GM (>8), EM (>4), CLDM (>2) | CEZ (>16), IPM (8), LVFX (>4), GM (>8), EM (>4), CLDM (>2), MPIPC (>2) | | | CEZ (16), IPM (8), LVFX (>4), GM (>8), EM (>4), CLDM (>2), MPIPC (>2) | CEZ (>16), IPM (>8), LVFX (>4), GM (>8), EM (>4), MINO (>8), CLDM (>2), MPIPC (>2) | |
| Assembly and genome statistics | | | | | | | | |
| No. of reads | | | | | | | | |
| MinION | 91,288 | 79,719 | 88,426 | 120,397 | 56,527 | 100,511 | 108,241 | 70,353 |
| MiSeq | 451,782 | 1,059,412 | 904,752 | 810,867 | 1,021,192 | 876,198 | 891,514 | 749,820 |
| Total no. of bases | | | | | | | | |
| MinION | 621,945,741 | 582,298,636 | 654,264,184 | 578,007,623 | 379,853,580 | 811,349,149 | 815,396,551 | 490,139,316 |
| MiSeq | 186,281,854 | 415,398,345 | 375,301,178 | 334,508,054 | 399,768,647 | 349,878,661 | 354,906,810 | 323,657,748 |
| Coverage (×) | 274.46 | 351.62 | 360.06 | 330.43 | 283.05 | 406.50 | 394.08 | 280.88 |
| Chromosome description | | | | | | | | |
| Genome size (bp) | 2,914,567 | 2,804,820 | 2,825,163 | 2,761,631 | 2,751,401 | 2,822,409 | 2,939,465 | 2,876,735 |
| G+C content (%) | 32.91 | 32.78 | 32.78 | 32.42 | 32.85 | 32.78 | 32.89 | 32.95 |
| No. of CDSs[c] | 2704 | 2560 | 2588 | 2511 | 2513 | 2584 | 2724 | 2682 |
| No. of rRNAs | 5 | 6 | 6 | 6 | 6 | 6 | 5 | 5 |
| No. of tRNAs | 61 | 62 | 60 | 61 | 61 | 62 | 59 | 61 |
| No. of tmRNAs[d] | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| GenBank accession no. | AP020324 | AP020311 | AP020313 | AP020315 | AP020316 | AP020318 | AP020320 | AP020322 |
| Plasmid description | | | | | | | | |
| Genome size (bp) | 30,220 | 32,574 | 34,268 | | 2,987 | 34,268 | 30,217 | 20,547 |
| G+C content (%) | 29.15 | 28.72 | 29.36 | | 29.53 | 29.36 | 29.16 | 29.04 |
| No. of CDSs | 35 | 45 | 40 | | 3 | 41 | 35 | 24 |
| GenBank accession no. | AP020325 | AP020312 | AP020314 | | AP020317 | AP020319 | AP020321 | AP020323 |

[a] MIC values (μg/ml) were determined by the broth microdilution method approved by CLSI and are shown in parentheses next to antibiotics.
[b] CEZ, cefazolin; IPM, imipenem/cilastatin; LVFX, levofloxacin; GM, gentamicin; EM, erythromycin; MINO. minocycline; CLDM, clindamycin; MPIPC, oxacillin.
[c] CDSs, coding sequences.
[d] tmRNAs, transfer-messenger RNAs.

performed using the Unicycler pipeline (v0.4.7b) with default parameters (7). Next, the assembled genome sequence was annotated using Prokka (v1.11) with default settings (8). Assembly statistics, general genome information, and relevant characteristics are summarized in Table 1.

Among the eight MRSA genomes, seven strains (excluding strain KUH140087) have a 3- to 30-kb plasmid carrying similar antimicrobial or antiseptic resistance genes [*aac(6′)-Ie*, *aph(2″)-Ia*, *qacA*, *qacB*, *aph(2″)-If*, *fosD*, and *blaZ*] in different combinations. It has been reported that genes for drug resistance are often derived from plasmids (9, 10), suggesting the importance of the investigation of antibiotic resistance plasmids for understanding *S. aureus* infection and its future treatments.

**Data availability.** The complete genome sequences of eight MRSA strains in this study have been deposited in DDBJ/EMBL/GenBank under accession numbers AP020311 to AP020325. The raw Illumina and MinION read data can be found in the DDBJ Sequence Read Archive/NCBI SRA under accession number DRA008776.

## ACKNOWLEDGMENTS

We thank Keisuke Katsura (University of Miyazaki, Japan) for sequence data analysis.

This work was supported in part by Grants-in-Aid for Scientific Research (grants 16H05188, 16K08775, 17K19552, 18KK0193, and 19H03471), the Yakult Bio-Science Foundation, the Joint Research Project of the Institute of Medical Science, the University of Tokyo, the Research Program on Emerging and Re-emerging Infectious Diseases (grants 19fk0108073h0002 and 19fk0108044h0203), and J-PRIDE (grant 19fm0208030h0003) from the Japan Agency for Medical Research and Development (AMED).

## REFERENCES

1. Lowy FD. 1998. *Staphylococcus aureus* infections. N Engl J Med 339:520–532. https://doi.org/10.1056/NEJM199808203390806.
2. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. Jr. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28:603–661. https://doi.org/10.1128/CMR.00134-14.
3. Kohno S, Yamaguchi K, Aikawa N, Sumiyama Y, Odagiri S, Aoki N, Niki Y, Watanabe S, Furue M, Ito T, Croos-Dabrera R, Tack KJ. 2007. Linezolid versus vancomycin for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* in Japan. J Antimicrob Chemother 60:1361–1369. https://doi.org/10.1093/jac/dkm369.
4. Choo EJ, Chambers HF. 2016. Treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Infect Chemother 48:267–273. https://doi.org/10.3947/ic.2016.48.4.267.
5. Rossi F, Diaz L, Wollam A, Panesso D, Zhou Y, Rincon S, Narechania A, Xing G, Di Gioia TSR, Doi A, Tran TT, Reyes J, Munita JM, Carvajal LP, Hernandez-Roldan A, Brandão D, van der Heijden IM, Murray BE, Planet PJ, Weinstock GM, Arias CA. 2014. Transferable vancomycin resistance in a community-associated MRSA lineage. N Engl J Med 370:1524–1531. https://doi.org/10.1056/NEJMoa1303359.
6. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
7. 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.
8. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
9. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, Holland TL, Fowler VG. Jr. 2019. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nat Rev Microbiol 17:203–218. https://doi.org/10.1038/s41579-018-0147-4.
10. Foster TJ. 2017. Antibiotic resistance in *Staphylococcus aureus*: current status and future prospects. FEMS Microbiol Rev 41:430–449. https://doi.org/10.1093/femsre/fux007.