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GENOME SEQUENCES

Draft Genome Sequences of 12 Panton-Valentine Leucocidin-Positive and Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* Strains Isolated from an Intensive Care Unit in Pakistan

Kidon Sung,[a] Dereje D. Gudeta,[a] Miseon Park,[a] Jungwhan Chon,[b] Sungguk Kim,[c] Mohamed S. Nawaz,[a] Steven L. Foley,[a] Saeed A. Khan[a]

[a]Division of Microbiology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas, USA
[b]Department of Pet Total Care, Division of Nursing and Welfare, Kyung-in Women's University, Incheon, South Korea
[c]Office of Scientific Coordination, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, Arkansas, USA

ABSTRACT Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogenic bacterium responsible for difficult-to-treat staphylococcal infections due to multidrug resistance. Twelve Panton-Valentine leucocidin (PVL)-positive and multidrug-resistant clinical MRSA isolates from hospitals in Pakistan were sequenced and annotated to investigate genetic markers associated with antimicrobial resistance, virulence, and biofilm formation.

Staphylococcus aureus is a commensal bacterium that is found in the skin and nasal cavity (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of morbidity and death and can cause soft tissue infections, endocarditis, pneumonia, sepsis, bloodstream infections, and toxic shock syndrome (2). MRSA is known to harbor a wide range of extracellular enterotoxin genes, virulence factors, and Panton-Valentine leucocidin (PVL) genes that help it to infect its host and evade host defenses (3). Therefore, it is important to have the genomic sequences of MRSA strains to analyze the factors responsible for their pathogenicity, virulence, diagnosis, and evolution.

MRSA strains used in this study were isolated (6 from nares and 6 from rectum) from 12 patients admitted to an intensive care unit in Rawalpindi, Pakistan, and were sequenced. Four of the patients died due to complications resulting from infections. MRSA isolates were characterized by *nucA* and coagulase profiles and identified using Vitek 2 identification cards. Details of their growth conditions and isolation have been described previously (4, 5). The isolates were grown in tryptic soy broth (TSB) (Thermo Fisher Scientific, Waltham, MA) at 35°C for 18 h, and the pellet was obtained after centrifugation at 10,000 × *g* for 5 min. Pellets of MRSA strains were incubated at 37°C for 1 h in TE buffer (10 mM Tris-HCl plus 1 mM EDTA [pH 8.0]) containing lysostaphin (MilliporeSigma, St. Louis, MO). This was followed by the addition of proteinase K (MilliporeSigma) and AL buffer (Qiagen, Germantown, MD) and incubation at 56°C for 30 min and then at 95°C for 15 min. A Nextera XT DNA library preparation kit (Illumina, San Diego, CA) was employed to make DNA sequencing libraries. Samples were multiplexed using a combination of two indexes with a Nextera XT index kit (Illumina) according to the manufacturer's instructions. Whole-genome sequencing (WGS) reactions were carried out on a MiSeq sequencer (Illumina) in 2 × 300-bp paired-end format (4).

Removal of the adapter sequences, filtering of low-quality sequence reads, quality control, and *de novo* assembly of high-quality sequences were performed using the Trim Reads, QC for Sequencing Reads, and De Novo Assembly tools of CLC Genomics Workbench v21.0.4 (Qiagen) (5, 6). QUAST v5.1 (http://quast.sourceforge.net) was employed to check the assembly quality and the completeness of the draft genomes and to compare contig and scaffold statistics (7). The draft genome assembly was first annotated using the Rapid

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Address correspondence to Saeed A. Khan, saeed.khan@fda.hhs.gov.

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TABLE 1 WGS analyses of 12 MRSA isolates

Isolate	Year of isolation	Source	MLST sequence type	*spa* type	SCC*mec* type	Total genome size (bp)	Total no. of reads	Genome coverage (×)	N_{50} (bp)	No. of contigs[a]	No. of CDSs[b]	G+C content (%)[c]	No. of tRNAs[b]	No. of rRNAs[b]	No. of antibiotic resistance genes[d]	No. of virulence genes[e]	GenBank accession no.
16S	2010	Nare	239	t632	III(3A)	2,886,329	2,088,656	259	59,651	176	2,916	32.70	54	5	46	77	JAHNFC000000000
25S	2010	Nare	8	t064	IV(2B)	2,860,610	1,737,038	257	36,678	240	2,909	32.66	52		47	69	JAHNFD000000000
31S	2011	Nare	239	t030	III(3A)	2,882,928	2,117,788	258	17,384	118	2,889	32.68	51	5	46	72	JAHNFE000000000
33S	2011	Rectum	239	t030	III(3A)	2,935,581	2,363,144	263	79,937	185	2,988	32.75	55	5	45	74	JAHNFF000000000
34S	2011	Rectum	239	t030	III(3A)	2,965,579	2,513,282	262	45,957	178	3029	32.73	51	5	46	73	JAHNFG000000000
37S	2011	Nare	8	t064	IVa(2B)	2,853,783	77,766	256	64,946	167	2,871	32.70	55	4	47	69	JAHNFH000000000
39S	2011	Rectum	8	t064	IVa(2B)	2,831,079	60,384	254	25,082	271	2,873	32.71	37	4	47	76	JAHNFI000000000
43S	2011	Nare	30	t148	III(3A)	2,831,307	67,224	254	29,415	292	2,889	32.74	51	5	48	71	JAHNFJ000000000
41S	2011	Rectum	239	t030	III(3A)	2,940,057	91,218	264	73,184	121	2,961	32.74	56	5	45	71	JAHNFK000000000
48S	2011	Rectum	239	t030	III(3A)	2,849,750	127,630	255	6,195	934	3,125	32.89	49	4	45	89	JAHNFL000000000
50S	2011	Rectum	503	t138	IVg(2B)	2,830,515	63,526	254	59,806	103	2,810	32.71	50	3	43	73	JAHNFM000000000
52S	2010	Rectum	15	t1509	IVa(2B)	2,649,066	31,976	237	9,228	565	2,718	32.77	33	3	39	70	JAHNFN000000000

[a] The numbers of contigs were determined following *de novo* assembly with CLC Genomics Workbench v21.0.4.

[b] The numbers of CDSs, tRNAs, and rRNAs were determined by NCBI PGAP.

[c] The G+C content was determined by PATRIC.

[d] Searched by PATRIC.

[e] Searched in the Virulence Factor Database (VFDB).

Annotations using Subsystem Technology (RAST) tool kit (RASTtk) within Pathosystems Resource Integration Centre (PATRIC) v3.6.9 (https://www.patricbrc.org) (8). The NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.2 was then employed to annotate the publicly available genome sequences (9). Multilocus sequence typing (MLST), *spa* typing, and staphylococcal cassette chromosome *mec* element (SCC*mec*) typing were conducted with MLST v2.0, spaTyper v1.0, and SCC*mec*Finder v1.2, respectively (10, 11). Default parameters were used for all software unless otherwise specified. The isolation year, source, MLST, *spa*, and SCC*mec* types, total genome sizes, numbers of contigs, coding sequences (CDSs), tRNA genes, rRNA genes, antibiotic resistance genes, virulence genes, genome coverage, N_{50} values, G+C contents, and GenBank accession numbers are listed in Table 1.

Data availability. The draft genome sequences reported in this study were deposited in DDBJ/ENA/GenBank with the accession numbers listed in Table 1. The raw sequence reads were deposited in the SRA with accession numbers SRX11183745 (isolate 16S), SRX11183746 (isolate 25S), SRX11183749 (isolate 31S), SRX11183751 (isolate 33S), SRX11183751 (isolate 34S), SRX11183752 (isolate 37S), SRX11183753 (39S), SRX11183755 (isolate 41S), SRX11183754 (isolate 43S), SRX11183756 (isolate 48S), SRX11183747 (isolate 50S), and SRX11183748 (isolate 52S) under BioProject accession number PRJNA737117.

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