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Draft Genome Sequences of a Unique t324-ST541-V Methicillin-Resistant *Staphylococcus aureus* Strain from a Pig

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Methicillin-resistant *Staphylococcus aureus* (MRSA), the major causative agent of nosocomial infection, has also been reported from non-human sources. A sequence type (ST) 541 MRSA isolate designated K12PJN53 was isolated from a healthy pig in 2012. The genome of K12PJN53 consists of 44 contiguous sequences (contigs), totalling 2,880,108 bases with 32.88% GC content. Among the annotated contigs, 14, 17, and 18 contained genes related to antimicrobial resistance, adherence, and toxin genes, respectively. The genomic distance of strain K12PJN53 was close to the ST398 strains. This is the first report of the draft genome sequence of a novel livestock-associated MRSA ST541 strain.

Keywords: MRSA, pig, genomics

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major causative agent of nosocomial infection, and it has also been reported from non-human sources [7, 15]. Although most MRSA infections occurred by skin infections, colonized MRSA in the gastrointestinal tract can also be transmitted through contaminated feces. Furthermore, food-related MRSA infection has been reported from the United States [8] and the Netherlands [17]. Livestock-associated MRSA (LA-MRSA) belonging to the ST398 lineage is common among pigs and other animals in several countries [2, 14]. Although LA-MRSA from animals has rarely colonized humans, several cases of human infection by MRSA have been reported [8, 17]. In Korea, two different lineages (*i.e.*, human-associated type (ST5, ST59, and ST72) and livestock-associated (LA) type (ST398, ST541, and ST692)) of MRSA have been identified in the livestock sector [12, 14]. However, recently LA-MRSA ST398 and ST541 have been more frequently detected than the human-associated type [12, 14]. In particular, *spa* type t324, ST541, and staphylococcal cassette chromosome *mec* element (SCC*mec*) type V (t324-ST541-V) is a unique and predominant clone in the pig rearing industry [12, 14]. To understand its occurrence, genetic repertoire, and relatedness with other MRSA types, whole-genome sequencing is essential.

A t324-ST541-V MRSA strain designated K12PJN53 was isolated from a nasal sample obtained from a live healthy pig in Gyeong-buk province, Korea in 2012. After enrichment in Mueller-Hinton broth (Becton Dickinson, Sparks, MD, USA) supplemented with 6.5% NaCl and cefoxitin (3.5 mg/l), the strain was identified as MRSA on the basis of the detection of 16S rRNA, *clfA*, and *mecA* genes [13]. This strain was resistant to multiple antimicrobial agents, including tetracycline, erythromycin, and ciprofloxacin. Molecular typing revealed that it belonged to ST541 (allelic profile 3-35-19-60-20-26-39), *spa* type t324, and SCC*mec* type V [14]. The pellet suspension of the overnight-cultured MRSA ST541 strain was pre-incubated with 10 mg/ml lytic enzyme (lysostaphin; Sigma-Aldrich, St. Louis, MO, USA), followed by treatment using the Wizard Genomic DNA Isolation kit (Promega, Madison, WI, USA) protocol. To quantify and check contamination of the extracted DNA, the PicoGreen dsDNA Assay kit (Invitrogen, Carlsbad, CA, USA) and 16S rRNA gene sequencing (ABI 3730 DNA

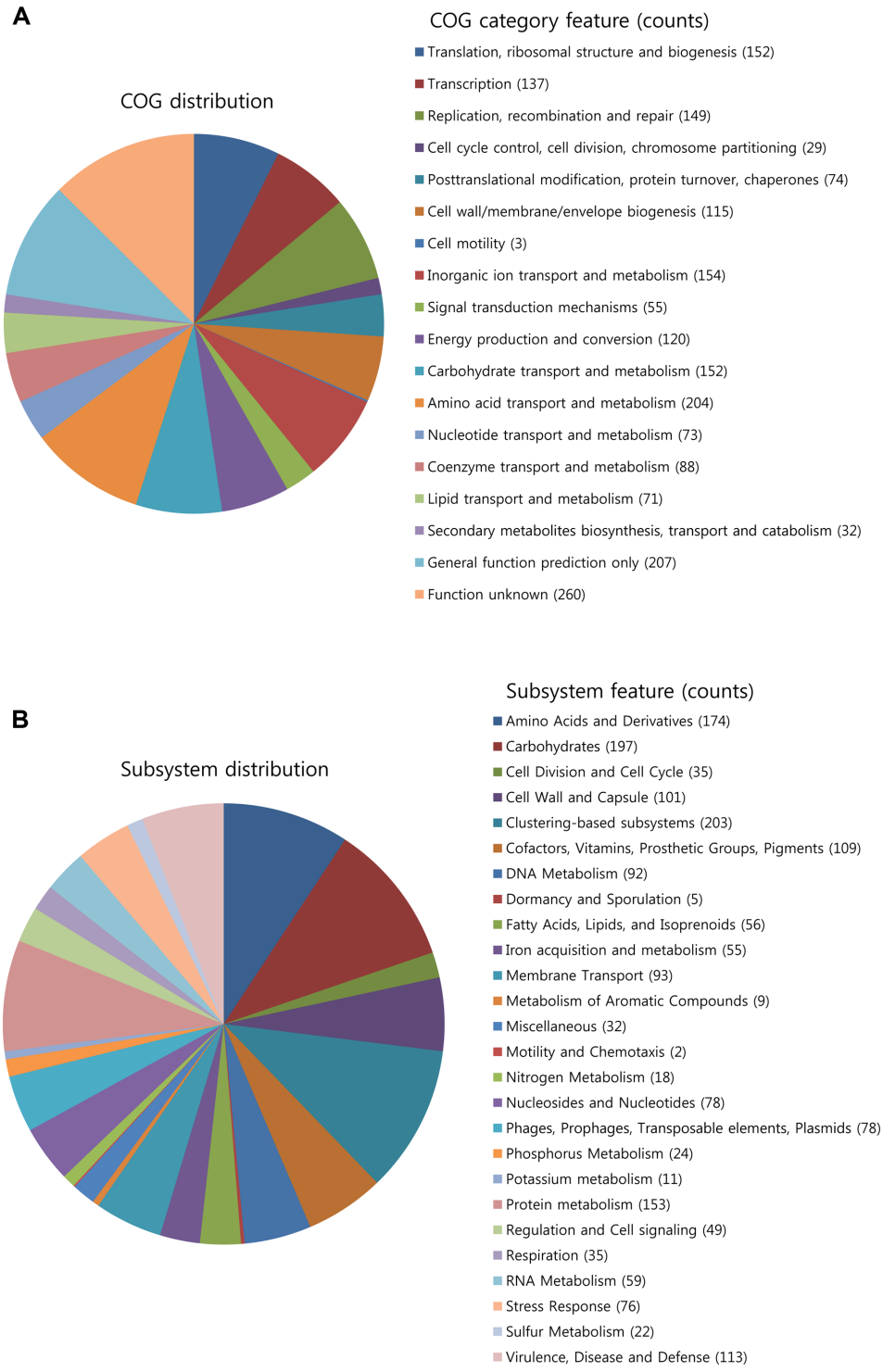

Fig. 1. Schematic representation of annotated genes of MRSA ST541 strain K12PJN53.
The annotated genes were based on COG (**A**) and SEED (**B**) databases.

sequencer; Applied Biosystems, Foster City, CA, USA) were used separately. The draft genome sequence of strain K12PJN53 was obtained by combined analysis of the results of Illumina MiSeq (300 bp paired-end sequencing)

Table 1. Antimicrobial resistance and virulence genes in the isolated MRSA ST541 strain K12PJN53.

Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
Antimicrobial resistance genes						
aac(6')-aph(2'')	100.00	1,440 / 1,440	JN53_26900	1..1440	Aminoglycoside resistance	M13771
aadD	99.73	744 / 771	JN53_26320	1..744	Aminoglycoside resistance Alternate name; *ant(4')-Ia* and *aadD2*	AF181950
spc	100.00	783 / 783	JN53_10330	1..783	Aminoglycoside resistance Alternate name; *aad9*, *ant(9)-Ia*	X02588
aadE	100.00	864 / 864	JN53_26160	1..864	Aminoglycoside resistance	KF421157
mecA	100.00	2,007 / 2,010	JN53_20930	1..2007	Beta-lactam resistance	AB512767
blaZ	95.98	846 / 846	JN53_13490	1..846	Beta-lactam resistance	AP003139
norA	91.95	1,167 / 1,167	JN53_01710	1..1167	Fluoroquinolone resistance	M97169
erm(A)	100.00	732 / 732	JN53_10320	1..732	Macrolide resistance	X03216
lnu(B)	99.88	804 / 804	JN53_26230	1..804	Lincosamide resistance	AJ238249
fexA	99.72	1,428 / 1,428	JN53_26500	1..1428	Phenicol resistance	AJ549214
tet(38)	99.33	1,353 / 1,353	JN53_21810	1..1353	Tetracycline resistance	FN433596
tet(L)	100.00	1,377 / 1,377	JN53_26110	1..1377	Tetracycline resistance	M29725
tet(K)	100.00	1,380 / 1,380	JN53_21060	1..1380	Tetracycline resistance	U38428
tet(M)	100.00	1,920 / 1,920	JN53_03470	1..1920	Tetracycline resistance	AM990992
Heavy metal resistance genes						
czrC	100.00	1,935/1,926	JN53_21120	1..1926	Cadmium/zinc resistance	KM369884.1
czcD	100.00	993/960	JN53_22130	1..960	Co/Zn/Cd efflux system component	CP003045.1
czcD	100.00	993/981	JN53_14940	1..981	Co/Zn/Cd efflux system component	CP003045.1
cadD	99.57	630/471	JN53_26340	1..471	Cadmium transporter	NG_041020.1
cadX	99.71	348/348	JN53_26350	1..348	Cadmium transporter	HF586889.1
Adherence-associated genes						
spa	97.89	1,332 / 1,044	JN53_21530	1..1044	Spa immunoglobulin G binding protein A	AJ938182.1
icaC	100.00	1,053 / 1,053	JN53_20150	1..1053	Intercellular adhesion protein C	CP003808.1
icaA	100.00	1,239 / 1,113	JN53_20130	1..1113	Intercellular adhesion protein A	CP003808.1
spa	96.08	1,308 / 1,200	JN53_23490	1..1200	Spa immunoglobulin G binding protein A	CP002110.1
spa	100.00	1,311 / 1,311	JN53_17650	1..1311	Spa immunoglobulin G binding protein A	AM990992.1
ebpS	100.00	1,455 / 1,455	JN53_08630	1..1455	Cell surface elastin-binding protein	AM990992.1
clfA	99.84	1,827 / 1,829	JN53_02570	1..1829	Fibrinogen-binding protein A, clumping factor	AM990992.1
vwb	98.94	1,887 / 1,887	JN53_07940	1..1887	von Willebrand factor-binding protein	CP002643.1
fib	100.00	330 / 318	JN53_05360	1..318	Fibrinogen-binding protein	CP003045.1
sdrE	95.13	3,408 / 3,408	JN53_00370	1..3402	Ser-Asp rich fibrinogen-binding protein E	FR821779.1
atl	99.97	3,747 / 3,747	JN53_04380	1..3747	Bifunctional autolysin Atl	AM990992.1
eap	98.83	426 / 426	JN53_15650	1..426	Extracellular adherence protein	CP002110.1
eap	98.85	435 / 435	JN53_03670	1..435	Extracellular adherence protein	CP002114.2
vwb	100.00	483 / 483	JN53_02610	1..483	von Willebrand factor-binding protein	AM990992.1
efb	100.00	498 / 498	JN53_05390	1..498	Extracellular fibrinogen-binding protein	CP003045.1
icaB	100.00	873 / 873	JN53_20140	1..873	Intercellular adhesion protein B	AM990992.1
sdrH	94.81	1,221 / 906	JN53_13800	355..1245	Ser-Asp rich fibrinogen-binding protein H	CP003194.1
S. aureus – toxin genes						
hlb	96.12	825 / 824	JN53_13700	170..993	Beta-hemolysin	CP001781.1
SExo	99.81	1,047 / 1,032	JN53_25050	1..1032	Exotoxin	CP003045.1
hld	100.00	138 / 135	JN53_13830	1..135	Delta-hemolysin	HE681097.1
set6	100.00	681 / 681	JN53_25030	1..681	Superantigen-like protein	AM990992.1

Table 1. Continued.

Resistance gene	%Identity	Query/HSP length	Contig	Position in contig	Predicted phenotype	Accession number
SExo	100.00	681 / 681	JN53_25130	1..681	Exotoxin	CP003045.1
set4	100.00	684 / 684	JN53_25100	1..684	Superantigen-like protein	AM990992.1
set1	100.00	696 / 696	JN53_25080	1..696	Superantigen-like protein	AM990992.1
set5	100.00	699 / 699	JN53_25090	1..699	Superantigen-like protein 5	CP003045.1
set3	99.86	705 / 705	JN53_25070	1..705	Superantigen-like protein	AM990992.1
SExo	100.00	762 / 717	JN53_05440	1..717	Exotoxin	CP003045.1
SExo	99.86	822 / 726	JN53_05450	1..726	Exotoxin	CP003045.1
SEntP	100.00	753 / 753	JN53_09810	1..753	Enterotoxin P	AM990992.1
eta	99.89	948 / 948	JN53_05500	1..948	Exfoliative toxin A	AM990992.1
hlgC	99.79	948 / 948	JN53_17680	1..948	Gamma-hemolysin component C	BX571856.1
SExo	100.00	969 / 954	JN53_25060	1..954	Exotoxin	CP003045.1
hla	100.00	960 / 960	JN53_05420	1..960	Alpha-hemolysin precursor	AM990992.1
hlgA	99.59	966 / 966	JN53_17660	1..966	Gamma-hemolysin chain II precursor	CP002110.1
hlgB	96.52	978 / 978	JN53_17690	1..978	Gamma-hemolysin component B precursor	HE681097.1
S. aureus–Exoenzyme						
sspA	93.88	1,011 / 1,029	JN53_04340	1..1029	Serine V8 protease	AP009351.1
sspB	99.14	1,167 / 1,167	JN53_13320	1..1167	Cysteine protease	CP002110.1
sspB	98.48	1,182 / 1,182	JN53_04330	1..1182	Cysteine protease	BX571856.1
geh	100.00	1,938 / 1,938	JN53_23630	1..1938	Glycerol ester hydrolase	AM990992.1
coa	98.34	2,016 / 1,983	JN53_22700	1..1983	Staphylocoagulase precursor	AM990992.1
geh	100.00	2,046 / 2,046	JN53_20160	1..2046	Glycerol ester hydrolase	AM990992.1
hysA	100.00	2,424 / 2,424	JN53_11780	1..2424	Hyaluronatelyase	AM990992.1
hysA	100.00	2,436 / 2,430	JN53_15610	1..2430	Hyaluronatelyase	CP003045.1
sspC	100.00	330 / 330	JN53_04320	1..330	Cysteine protease	CP003808.1
nuc	99.06	534 / 534	JN53_07060	1..534	Thermonuclease	BA000018.3
nuc	100.00	687 / 687	JN53_02620	1..687	Thermonuclease	CP003808.1

and Roche 454 FLX (8 kb insert paired-end sequencing). To prepare the sequencing library in the Illumina system and the Roche 454 system, the TruSeq DNA LT Sample Prep kit (Illumina, San Diego, CA, USA) and the GS FLX Titanium Rapid Library Preparation kit (Roche Diagnostics, Branford, CT, USA) were used, respectively. Sequencing reads obtained using the Illumina and Roche 454 sequencing systems were separately assembled by the CLC genomic workbench 5.5 (CLC Bio, Denmark) and the GS Assembler 2.6 (Roche Diagnostics). Assembled contigs were evaluated for accuracy and contiguity on the basis of the published *S. aureus* ATCC 51811 strain genomes. To get hybrid assembly results, contigs of both systems were processed with the CodonCode Aligner (CodonCode Co., MA, USA). Hybrid contigs and unassembled contigs were repeatedly reassembled until there was no change in the number of hybrid contigs. Short contigs (<500 bp) were excluded from the hybrid result file, and Glimmer 3 [3] was used to predict the genes. Annotation was done by homology search based on the Clusters of Orthologous Groups (COG) and SEED databases [4, 16]. To confirm assembled contigs, multilocus sequence typing was performed [5, 10]. The antimicrobial resistance genes and virulence genes were analyzed using a Web service of the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). A total of 458 genome sequences were obtained from *S. aureus* subsp. *aureus* from the EzGenome database (http://ezgenome.ezbiocloud.net) and compared with those of strain K12PJN53 by calculating average nucleotide identity (ANI) values [6]. The query genome was divided into 1,020 bp fragments, and high-scoring pairs were compared with two sequences by the BLAST algorithm [1]. After calculating ANI values with the unweighted pair group method, a dendrogram was constructed. Briefly, selected target homologous regions of query ORFs were identified with the BLASTN program, and genome comparison was performed using a pairwise

BLAST algorithm. From the subject contig, a resultant matched region was extracted and saved as a homolog. To prevent contamination, the 16S rRNA gene was amplified from the extracted DNA of strain K12PJN53 and compared

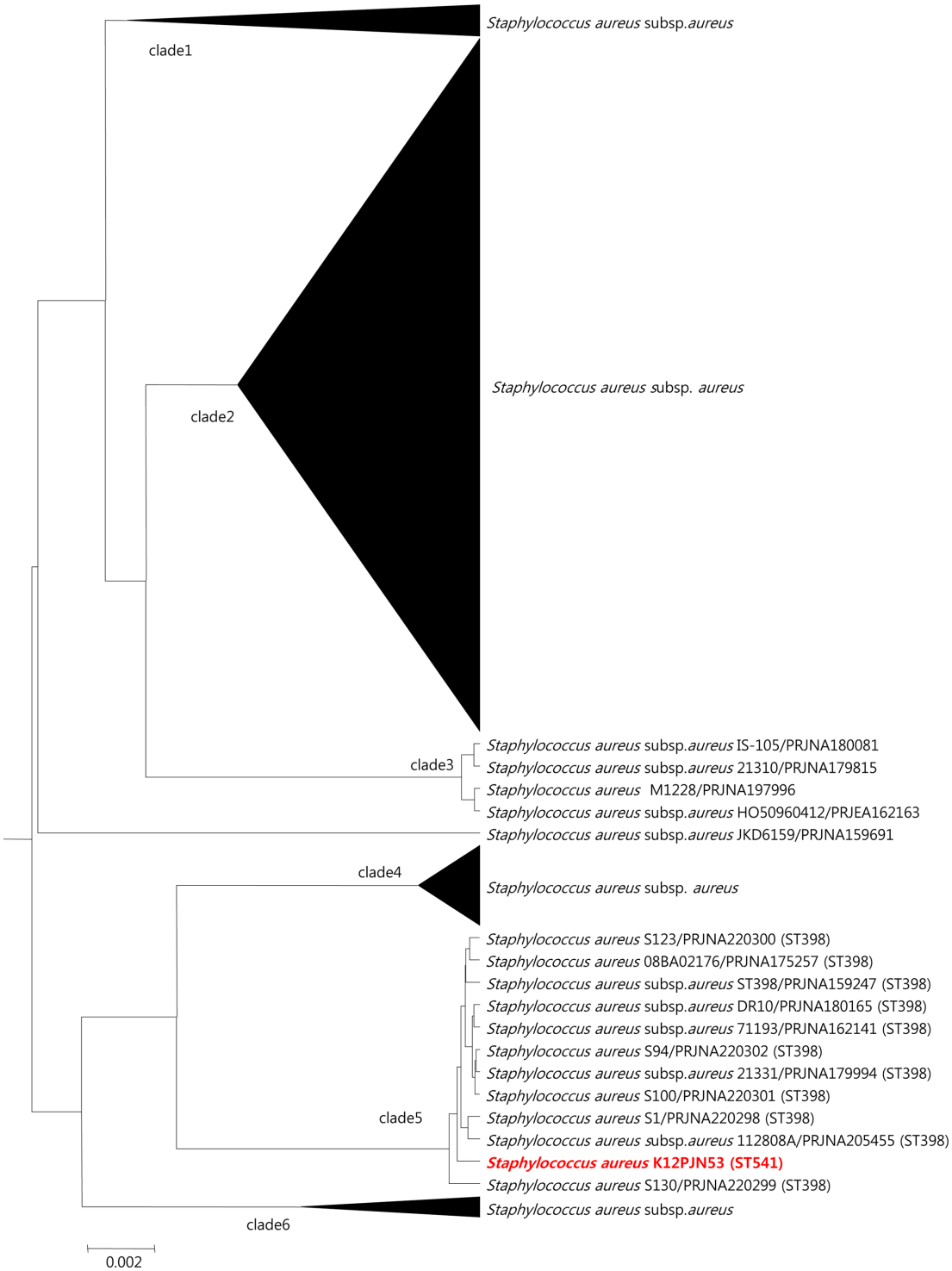

Fig. 2. Genomic relationship of MRSA strain K12PJN53 with other *S. aureus* genome sequences.
A total of 458 genome sequences were obtained from *S. aureus* subsp. *aureus* from the EzGenome database and compared with those of strain K12PJN53 by calculating average nucleotide identity values.

with the result of the draft genome sequence. Among the assembled contigs, 16S rRNA genes were found by rRNA Selector [11] and identified by the EzTaxon-e database [9]. Bioinformatics assembly was confirmed as compared with the published genome of species showing 99.6% similarity in ANI values [6].

In this study, 44 contigs (N50 = 164,393 bp) were generated using a hybrid assembly of reads from the Illumina (6,413,077 reads of 150 bp paired-end; >350-fold coverage) and Roche 454 (240,863 reads of 8-kb-insert paired-end; >14-fold coverage) systems. The size of the genome of the K12PJN53 strain was 2,880,108 bases, with a total coverage of 504.9× and a G+C content of 32.88%. The genome comprised 2,697 predicted protein-coding sequences, 57 tRNA genes, and 10 rRNA genes. Results of the genome annotation are shown in Fig. 1. For the COG distribution, function unknown (260 ORFs), general function prediction only (207 ORFs), and amino acid transport and metabolism (204 ORFs) were abundant categories (>9% of total COG-matched counts). Genes responsible for clustering-based subsystems (203 ORFs), carbohydrate metabolism (197 ORFs), amino acid metabolism (174 ORFs), and protein metabolism (153 ORFs) were abundant among the SEED subsystem categories.

MRSA strain K12PJN53 carried various antimicrobial resistance genes, heavy metal resistance genes, and virulence genes (Table 1). Sequence analysis revealed that strain K12PJN53 possessed SCC*mec* type V with a class C *mec* gene and *ccrC* gene complex. In addition, genes encoding aminoglycoside-modifying enzymes, tetracycline resistance, macrolide-lincosamide-streptogramin B resistance, and phenicol resistance were observed to have mobile genetic elements (insertion sequence (IS)*256*, IS*431*, IS*30*; transposon (Tn)*554*, Tn*552*, Tn*7*; tyrosine recombinases *XerD*). In addition, heavy metal resistance genes were also identified in the internal and external regions of SCC*mec* elements. Several virulence genes encoding adherence-associated proteins, toxins, and exoenzymes were present in K12PJN53; however, Panton-Valentine leukocidin was not detected.

A total of 458 *S. aureus* strains were chosen from the EzGenome database (http://ezgenome.ezbiocloud.net/). In the ANI tree analysis (Fig. 2), strain K12PJN53 showed a close relationship with the ST398 MRSA strains, which are prevalent in European countries [2, 15]. The close relationship with ST398 MRSA strains is supported by only a single locus variant of allelic profile (ST541: 3-35-19-60-20-26-39; ST398: 3-35-19-2-20-26-39), identical (t034) or a specific group of *spa* types with related repeat sequences (t011, t108, t899) and the same SCC*mec* type V that was most frequently observed in ST398 MRSA isolates [15].

This is the first report of the draft genome sequence of a novel LA-MRSA ST541 strain isolated from a pig in Korea. This sequence information will assist the understanding of features of the ST541 lineage, including antimicrobial resistance and virulence genes. Our findings provide the foundation for further research to decipher the pathogenesis of MRSA ST541 strains, which will also provide clues for devising approaches to control their spread.

The draft genome sequence of *Staphylococcus aureus* strain K12PJN53 is available under the accession number JSYA00000000 in GenBank. The version described in this paper is the first version JSYA00000000.

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