J Vet Sci. 2020 Jan;21(1):e11
https://doi.org/10.4142/jvs.2020.21.e11
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Rapid Communication
Microbiology

Check for updates

Complete genome sequence of a methicillin-resistant *Staphylococcus schleiferi* strain from canine otitis externa in Korea

Gi Yong Lee, Soo-Jin Yang*

School of Bioresources and Bioscience, Chung-Ang University, Anseong 17546, Korea

OPEN ACCESS

Received: Aug 21, 2019
Revised: Oct 16, 2019
Accepted: Oct 23, 2019

***Corresponding author:**
Soo-Jin Yang
School of Bioresources and Bioscience, Chung-Ang University, 4726 Seodong-daero, Daedeok-myeon, Anseong 17546, Korea.
E-mail: soojin@cau.ac.kr

ORCID iDs
Gi Yong Lee
https://orcid.org/0000-0001-5308-0065
Soo-Jin Yang
https://orcid.org/0000-0003-3253-8190

Funding
This work was supported by the Cooperative Research Program for Agriculture Science & Technology Development (Grant No. PJ012811) funded by Rural Development Administration, Republic of Korea.

Conflict of Interest
The authors declare no conflicts of interest.

ABSTRACT

The increase in canine skin and soft tissue infections, such as pyoderma and otitis, caused by *Staphylococcus schleiferi* strains, is of significant zoonotic concern. In this study, we report the first complete genome sequence for a methicillin-resistant clinical isolate of *S. schleiferi* (MRSS) designated as SS4, obtained from a dog with otitis externa, in Korea. The genome of SS4 strain was of 2,539,409 bp and presented high G+C content ratio (35.90%) with no plasmid. Comparative analysis of SS4 genome revealed that it is closely related to 2142-05 and 5909-02 strains isolated from the canine skin infections in the USA.

Keywords: *Staphylococcus schleiferi*; dogs; genomics; otitis

INTRODUCTION

Staphylococcus schleiferi subsp. *schleiferi* (*S. schleiferi*) was initially described in 1988 and has been reported to be associated with a number of infections in companion animals and humans [1]. Although coagulase-positive *Staphylococcus pseudintermedius* has most frequently caused canine otitis externa and skin and soft tissue infections [2], recent studies reported that coagulase-negative *S. schleiferi* is becoming more prevalent in canine otitis externa and pyoderma cases [2-4]. Furthermore, the increased frequency of methicillin-resistance in *S. schleiferi* (MRSS) is of significant public health concern worldwide [4-6]. A recent study in our laboratory has indicated that ~26% of *S. schleiferi* strains of the canine origin were MRSS, often presenting increased levels of multiple drug resistance phenotype [6]. Genotypic characterization of these canine-associated MRSS strains revealed that the methicillin resistance was frequently conferred by staphylococcal cassette chromosome *mec* type V (SCC*mec* V) [6]. The widespread occurrence of SCC*mec* V has been reported in other methicillin-resistance staphylococci, in particular, in livestock-associated methicillin-resistant *S. aureus* (MRSA) [7], indicating dissemination of the SCC*mec* V among various staphylococcal species. Although MRSS strains have been increasingly isolated from the infected dogs over the past decades worldwide [3,4], complete whole-genome sequences are available for only several *S. schleiferi* strains isolated in the USA and Japan. Thus, a representative canine-associated MRSS strain with SCC*mec* V isolated from Korea was

Author Contributions

Conceptualization: Yang SJ; Data curation: Yang SJ, Lee GY; Formal analysis: Yang SJ, Lee GY; Funding acquisition: Yang SJ; Investigation: Lee GY; Methodology: Yang SJ, Lee GY; Project administration: Yang SJ, Lee GY; Writing - original draft: Yang SJ, Lee GY; Writing - review & editing: Yang SJ.

selected for complete genome sequence analysis in order to understand its prevalence, genetic repertoire, and relationship to the previously sequenced MRSS strains.

MATERIALS AND METHODS

A SCC*mec* V-MRSS strain designated as SS4 was isolated from an ear swab sample of a dog with otitis externa in Seongnam, Korea, in 2017 [6]. The strain was identified as *S. schleiferi* using the Vitek 2 system (BioMérieux, France), sequencing of 16S rRNA and *tuf* gene (Cosmogenetech, Korea), and coagulase production test. This strain was resistant to several antimicrobial agents, including ampicillin, enrofloxacin, kanamycin, and oxacillin. Genotypic analyses revealed that this strain possessed SCC*mec* V and five of the staphylococcal enterotoxin genes (*seg*, *sei*, *sell*, *selm*, and *selq*). This strain had oxacillin MIC of 8 μg/mL. The *S. schleiferi* strain was grown in tryptic soy broth (TSB, Difco Laboratories).

Genomic DNA (gDNA) of *S. schleiferi* SS4 strain was extracted using the Wizard genomic DNA Isolation Kit (Promega, USA), according to the manufacturer's protocols. Quantity and quality of the extracted gDNA was assessed via fluorescence-based quantification and NanoDrop 2000c spectrophotometer (Thermo Scientific, USA), respectively.

Whole genome sequence data of *S. schleiferi* SS4 strain were generated by a combination of PacBio RS II (Pacific Biosciences, USA) and Illumina HiSeq platform (Illumina, USA). Library preparation of *de novo* genome assembly was carried out by PacBio reads under the Hierarchical Genome Assembly protocol (version 3.0) in SMRT Portal version (2.3.0). PacBio sequencing yielded 136,862 reads covering 1,305,140,901 bp with genome coverage of 514X. After the *de novo* assembly, Illumina HiSeq generating 150 bp paired-end reads was applied for rectifying the error using the Pilon program (version 1.21). Following the hybrid assembly, 5,277,928 reads covering 796,967,128 bp with 313X coverage were generated from Illumina sequencing. Genome annotation was performed using Prokka 1.12b.

For comparative genomic analyses, the previously published genome sequences of five *S. schleiferi* strains were obtained from NCBI. The five *S. schleiferi* strains were all isolated from canine skin infections (accession numbers of the five *S. schleiferi* strains were 1360-13; CP009470, 2142-05; CP009762, 5909-02; CP009676, 2317-03; CP010309 and TSCC54; AP014944, respectively) [8,9].

Functional genome analysis of *S. schleiferi* strains was performed via genome-wide approach, such as proteomics and metabolomics. Gene annotation based on orthology and functionality was done by Cluster of Orthologous Groups and RAST server of SEED databases (http://rast.theseed.org/FIG/rast.cgi) [10]. The presence of virulence genes and antimicrobial resistance genes was confirmed by using BLAST algorithm and Center for Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/). For identification of antimicrobial resistance genes, ResFinder of CGE and the Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/) were integrated. Mobile genetic elements (MGEs), such as insertion sequences (ISs) and prophages, were detected by using the IS Finder database (https://isfinder.biotoul.fr/) and PHAST (http://phast.wishartlab.com/), respectively.

Phylogenetic analyses of *S. schleiferi* strains relied on two comparative parameters: average nucleotide identity (ANI) values and 16S rRNA gene sequencing. The ANI values were

calculated based on BLAST algorithm (ANIb) using JSpecies [11], and modified ANI was calculated using OrthoANI (version 0.93) [12]. A phylogenic tree of 16S rRNA sequences was generated using the MEGA-X software, and these sequences were aligned with ClustalW. The phylogenic tree was constructed using maximum likelihood analysis implemented with general time reversible model. Pan-genomic analysis of *S. schleiferi* strains was also performed using the Roary [13].

RESULTS AND DISCUSSION

The complete genome of MRSS SS4 strain comprised a single circular chromosome of 2,539,409 bp with a guanine-cytosine (GC) content of 35.90% and no plasmid. We identified 2310 open reading frames (ORFs), 59 tRNAs, and 16 rRNAs in this genome. The genome size and GC content of the SS4 were similar to those of the five *S. schleiferi* strains sequenced previously (**Supplementary Table 1**).

As presented in **Fig. 1A and B**, the functional categorization of the SS4 genome revealed that categories E (amino acid transport and metabolism), P (inorganic ion transport and metabolism), and J (translation, ribosomal structure and biogenesis) were most abundant (**Fig. 1A**). SEED data also revealed that 1708 ORFs (70%) encode proteins with known functions, whereas 741 ORFs (30%) produce proteins with unknown functions (**Fig. 1B**). Among the predicted ORFs, genes involved in the metabolism of amino acids and their

Table 1. Antimicrobial resistance genes and virulence genes of *Staphylococcus schleiferi* SS4 strain

Genes	% Identity	Query/template length	Position in contig	Protein function
Antimicrobial resistance genes				
aac(6′)-aph(2″)	100	1,440/1,440	462605..264044	Aminoglycoside resistance
ant(4′)-Ib	100	771/771	449980..450750	Aminoglycoside resistance
mecA	100	2,007/2,007	1067400..1069406	Beta-lactams resistance
Heavy metal resistance genes				
cadA	100	1,471/1,471	67268..69430	Cd-transporting ATPase
cadC	100	262/262	69414..69797	Cd resistant regulatory protein
czcD	100	629/629	1460820..1461761	Cd, Co and Zn antiporter
Adhesion-associated genes				
spa	100	1,039/1,039	1169851..1171404	Immunoglobulin G-binding protein A
ebps	100	876/876	2368531..2369838	Elastin-binding protein
fndA	100	2,034/2,034	221624..224641	Fibronectin-binding protein A
pfbA1	100	1,328/1,328	505559..507514	Fibronectin-binding protein A
pfbA2	100	759/759	1926166..1927272	Fibronectin-binding protein A
fib	100	226/226	220932..221276	Fibrinogen-binding protein
sdrD	100	1,060/1,060	1300673..1302217	Ser-Asp repeat-containing protein D
sdrD	100	1,131/1,131	1303189..1304856	Ser-Asp repeat-containing protein D
sdrD	100	3,916/3,916	1306579..1312791	Ser-Asp repeat-containing protein D
sdrE	100	2,029/2,029	362084..365110	Ser-Asp repeat-containing protein E
Toxin genes				
hlgB	100	667/667	2053736..2054713	Gamma-hemolysin component B
hlgB	100	665/665	2056281..2057258	Gamma-hemolysin component B
etb	94	282/282	1537673..1538125	Exfoliative toxin B
lukS	100	645/645	2052792..2053733	Leukocidin-S subunit
lukS	100	639/639	2055334..2056278	Leukocidin-S subunit
Exoenzymes				
lip	87	1,055/1,055	943518..945620	Lipase
lip	100	1,295/1,295	1314264..1316132	Lipase
lip	100	1,316/1,316	1458361..1460268	Lipase
nucH	100	347/347	25367..25876	Thermonuclease

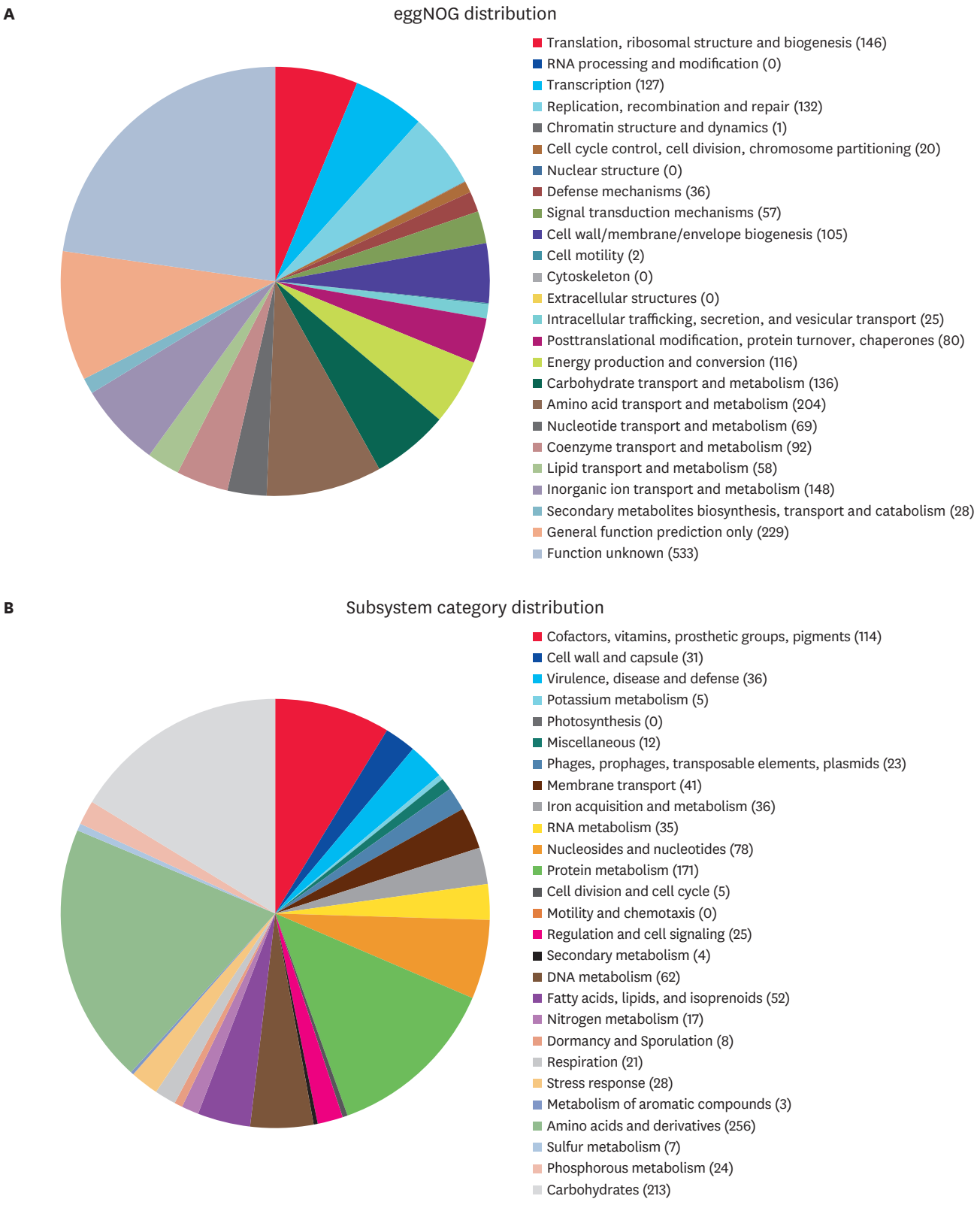

Fig. 1. Functional categorization of annotated genes in the *Staphylococcus schleiferi* SS4 strain using (A) Cluster of Orthologous Group and (B) SEED databases.

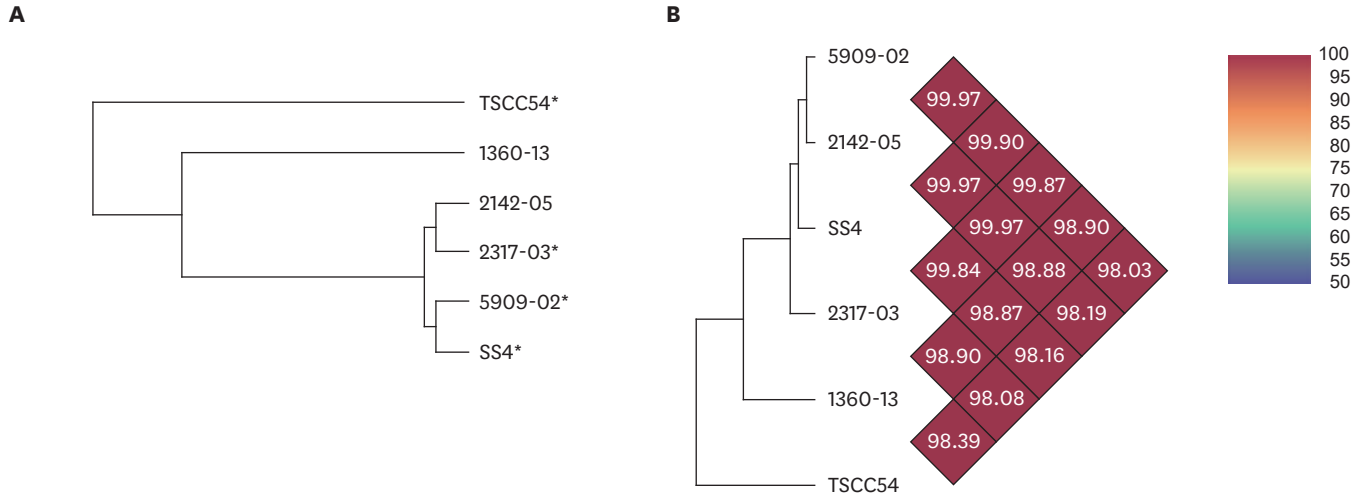

Fig. 2. Phylogenetic analysis of six genomes of *Staphylococcus schleiferi* strains based on the pangenomes with Roary (A) and OrthoANI (B).
*Methicillin-resistant *S. schleiferi* strains: TSCC54, SS4, and 2317-03 had SCC*mec* V and 5909-02 strain had SCC*mec* IVa.

derivatives (256 ORFs) were most abundant, followed by those involved in carbohydrate (213 ORFs) and protein (171 ORFs) metabolism. Interestingly, the number of phages, prophages, and transposable elements was higher in the SS4 strain than the other *S. schleiferi* strains (data not shown). Correlating with the higher number of MGEs, five different enterotoxin genes (*seg*, *sei*, *sell*, *selm*, and *selq*), which is usually carried by phages, were identified in the SS4 strains [6]. Different host factors and environmental conditions affecting staphylococcal/microbial communities on canine hosts may have caused the differences in prevalence of phages among the six *S. schleiferi* strains.

In accordance with the previous publication [6], the SS4 strain harbored *mecA* gene within SCC*mec* type V. Although the phenotypic antimicrobial susceptibility profiles of the five *S. schleiferi* strains were unavailable, three of the five *S. schleiferi* strains (2317-03, 5909-02, and TSCC54) also had *mecA* in their genome. Notably, the 2317-03 and TSCC54 strains isolated from dogs in the USA and Japan, respectively, carried SCC*mec* V, and 5909-2 strain from the USA had SCC*mec* IVa for methicillin resistance. Analyses of antimicrobial resistant genes in the SS4 and five *S. schleiferi* genomes revealed that SS4 and 2713-03 strains carried two aminoglycoside resistant gene cassettes, AAC(6′)-Ie-APH(2″)-Ia and ANT(4′)-Ib. Furthermore, another beta-lactam resistant gene, *blaZ*, was identified only in the 2317-03 strain (**Supplementary Table 2**). The three antimicrobial resistance genes identified in the SS4 strain (**Table 1**) were all located within MGEs.

CGE and genome BLAST search revealed that the MRSS strain SS4 comprises various virulence factors associated with its potential pathogenicity. As presented in **Table 1**, three heavy metal resistance genes, eight adhesion-associated genes, four toxin genes, and two exoenzyme genes were identified in the SS4 strain. Of these virulence-related genes, multiple copies of *sdrD, hldB, lukS,* and *lip* genes were identified in the SS4 strain. Sequence analysis around the *sdrD*, *hldB*, *lukS*, and *lip* genes revealed that there are no MGEs or characteristics of translocatable sequences, suggesting that homologous recombination may have caused the amplification of the genes.

Phylogenomic analysis of *S. schleiferi* strains based on 16S rRNA sequences revealed that all the six *S. schleiferi* strains were genetically identical (data not shown). In the Roary analysis, *S. schleiferi* strain SS4 exhibited a close relationship to the 5909-02 strain isolated from infected

dogs in the USA (**Fig. 2A**). Correlating with the Roaryanalysis result, OrthoANI values also indicated that SS4, 2142-05, and 5909-02 are closely related. These data suggested that the MRSS SS4 strain is more closely related to the *S. schleiferi* strains isolated from the USA than to the TSCC54 strain isolated from Japan. Multiple genome alignment of the six *S. schleiferi* strains using Mauve algorithm [14] also identified two distinct blocks of genome only in SS4 and 2317-03 strains comprising aminoglycoside resistant gene cassette and cell division-associated proteins, respectively (data not shown).

To our knowledge, this is the first report of complete genome sequence of an MRSS-SCC*mec* V strain isolated from a dog with otitis externa in Korea. This whole genome sequence information will contribute to the understanding of genetic features of canine-associated MRSS strains, such as antimicrobial resistance and virulence-related genes. More detailed comparative analysis of the *S. schleiferi* genomes to those of other staphylococcal species, such as MRSA and methicillin-resistant *S. pseudintermedius* (MRSP) is necessary for future investigation.

The complete genome sequence of methicillin-resistant *S. schleiferi* strain SS4 has been deposited in GenBank under the accession number CP035007.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Genomic features of *Staphylococcus schleiferi* isolates

Click here to view

Supplementary Table 2

Antimicrobial resistance genes of *Staphylococcus schleiferi* strains isolated from dogs with otitis externa

Click here to view

REFERENCES

1. Freney J, Brun Y, Bes M, Meugnier H, Grimont F, Grimont PA, Nervi C, Fleurette J. *Staphylococcus lugdunensis* sp. nov. and *Staphylococcus schleiferi* sp. nov., two species from human clinical specimens. Int J Syst Bacteriol 1988;38:168-172.
CROSSREF
2. Penna B, Varges R, Medeiros L, Martins GM, Martins RR, Lilenbaum W. Species distribution and antimicrobial susceptibility of staphylococci isolated from canine otitis externa. Vet Dermatol 2010;21:292-296.
PUBMED | CROSSREF
3. May ER, Kinyon JM, Noxon JO. Nasal carriage of *Staphylococcus schleiferi* from healthy dogs and dogs with otitis, pyoderma or both. Vet Microbiol 2012;160:443-448.
PUBMED | CROSSREF
4. Penna B, Mendes W, Rabello R, Lilenbaum W. Carriage of methicillin susceptible and resistant *Staphylococcus schleiferi* among dog with or without topic infections. Vet Microbiol 2013;162:298-299.
PUBMED | CROSSREF
5. Cain CL, Morris DO, Rankin SC. Clinical characterization of *Staphylococcus schleiferi* infections and identification of risk factors for acquisition of oxacillin-resistant strains in dogs: 225 cases (2003–2009). J Am Vet Med Assoc 2011;239:1566-1573.
PUBMED | CROSSREF

6. Lee GY, Lee HH, Hwang SY, Hong J, Lyoo KS, Yang SJ. Carriage of *Staphylococcus schleiferi* from canine otitis externa: antimicrobial resistance profiles and virulence factors associated with skin infection. J Vet Sci 2019;20:e6.
PUBMED | CROSSREF
7. Hau SJ, Frana T, Sun J, Davies PR, Nicholson TL. Zinc resistance within swine-associated methicillin-resistant *Staphylococcus aureus* isolates in the United States is associated with multilocus sequence type lineage. Appl Environ Microbiol 2017;83:83.
PUBMED | CROSSREF
8. Misic AM, Cain CL, Morris DO, Rankin SC, Beiting DP. Complete genome sequence and methylome of *Staphylococcus schleiferi*, an important cause of skin and ear infections in veterinary medicine. Genome Announc 2015;3:3.
PUBMED | CROSSREF
9. Sasaki T, Tsubakishita S, Kuwahara-Arai K, Matsuo M, Lu YJ, Tanaka Y, Hiramatsu K. Complete genome sequence of methicillin-resistant *Staphylococcus schleiferi* strain TSCC54 of canine origin. Genome Announc 2015;3:3.
PUBMED | CROSSREF
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. The RAST server: rapid annotations using subsystems technology. BMC Genomics 2008;9:75.
PUBMED | CROSSREF
11. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 2007;57:81-91.
PUBMED | CROSSREF
12. Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016;66:1100-1103.
PUBMED | CROSSREF
13. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, Fookes M, Falush D, Keane JA, Parkhill J. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 2015;31:3691-3693.
PUBMED | CROSSREF
14. Darling AC, Mau B, Blattner FR, Perna NT. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 2004;14:1394-1403.
PUBMED | CROSSREF