AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Genome Sequencing of Methicillin-Resistant and Methicillin-Susceptible *Mammaliicoccus sciuri* from Diseased Animals

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ABSTRACT *Mammaliicoccus sciuri* (previously *Staphylococcus sciuri*) is a frequent colonizer of mammals. We report the draft genomes of a methicillin-resistant strain (2254A) isolated from an armadillo and a methicillin-susceptible strain (6942A) from a cow. Genomes were sequenced using long-read Nanopore sequencing.

Five *Staphylococcus* species belonging to the *Staphylococcus sciuri* group (*S. sciuri*, *S. fleurettii*, *S. lentus*, *S. stepanovicii*, *S. vitulinus*) were recently reassigned to the novel genus *Mammaliicoccus* (1). The *M. sciuri* group has been hypothesized to carry the evolutionary ancestor of the *mecA* gene (2), which encodes an alternative penicillin-binding protein PBP2a and confers resistance to broad-spectrum beta-lactams (3). *M. sciuri* is a potential reservoir of resistance and virulence genes that can be acquired by other species (4). It has been reported in humans and animals (5, 6), and has been implicated in disease (7, 8).

Methicillin-resistant *M. sciuri* (MRMS) strain 2254A and methicillin-susceptible *M. sciuri* (MSMS) strain 6942A were sampled from animals with confirmed clinical infections (Table 1). Pure isolates were cultured in commercially prepared tryptic soy agar with 5% sheep red blood cells (Thermo Scientific Remel) at 37°C for 24–48 h. Initial species identification was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in Bruker Biotyper.

Total genomic DNA was isolated using Zymo Quick-DNA high molecular weight Magbead Kit. We used 400 μL of the bacterial cells grown in brain heart infusion broth (BD Difco) containing diluted bacteria and 550 μL of Bashing Bead Buffer in the initial step. We quantified DNA concentration using Qubit fluorometer (Invitrogen) and DNA quality using NanoDrop spectrophotometer (Thermo Scientific).

Sequencing libraries were prepared using the Genomic DNA by Ligation (SQK-LSK110) kit from Oxford Nanopore Technologies (ONT), including the DNA repair step prior to adapter ligation. Long-read sequencing was performed using the MinION platform with R9 flow cells. Sequencing quality was monitored using the MinKNOW v4.5.4 GUI interface. Sequences were base called and demultiplexed using Guppy v5.1.12 (9).

Genomes were assembled using open-source scripts from ONT, including the EPI2MELABS wf-bacterial-genomes pipeline, and ran on the nextflow platform (10). Raw fastq files that passed quality controls in MinKNOW were concatenated with fastcat v0.4.10 and assembled with Flye v2.9 (11). Variants, consensus sequences, and polished contigs were obtained with Medaka v1.6.0 (12). Assembly completeness was assessed using BUSCO v5.3.2 (13) and CheckM v1.1.3 (14). Genome contamination, GC content, N50, and number of contigs were estimated using QUAST v5.0.2 (15). Genomes were annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) v6.1 (16) in the National Center for Biotechnology Information (NCBI) (Table 1). Antimicrobial and heavy metal resistance genes were detected using Abricate

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

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The authors declare no conflict of interest.

Received 14 July 2022
Accepted 3 September 2022
Published 20 September 2022

TABLE 1 Genome characteristics of the two *M. sciuri* from diseased animals

Features	MRMS 2254A	MSMS 6942A
Animal source	Eye of a pet armadillo (order *Cingulata)*	Right rear mammary gland of a cow (*Bos taurus*)
Date of sampling	April 2018	September 2018
Location	West Nottingham, New Hampshire, USA	Durham, New Hampshire, USA
No. of reads	768,000 reads	3,384,000 reads
Bases called	9,345,898,253 bases	17,538,066,197 bases
Contigs	One	Two
Genome size	2,774,130 bp	2,772,237 bp, 32,841 bp
N50	2,774,130 bp	2,772,237 bp
GC content	32.68%	32.54%, 29.1%
Genome completeness	89.5%	96.8%
Genome contamination	4.1%	6.3%
BUSCO ("bacteria_odb10")	49.2%	85.5%
BUSCO ("bacillales_odb10")	47.6%	83.8%
CDS	3,044	2,949
Ribosomal RNAs	19	19
Transfer RNAs	58	57
Noncoding RNAs (ncRNAs)	4	4
Antimicrobial resistance genes	*mecA* (methicillin resistance), *salA* (pleuromutilin-lincosamide-streptogramin A resistance)	*salA*
Heavy metal resistance genes	*arsB* (arsenic resistance)	*asrC* (arsenic resistance), *cadD* (cadmium resistance)
mecA1 (*mecA* precursor)	Present	Present

(https://github.com/tseemann/abricate), AMRfinderPlus (17), and the Comprehensive Antibiotic Resistance Database (18). We used fastANI v1.33 (19) to compare the average nucleotide identity against 14 complete genomes named as either *M. sciuri* or *S. sciuri* that were available in NCBI as of June 2022 (Fig. 1).

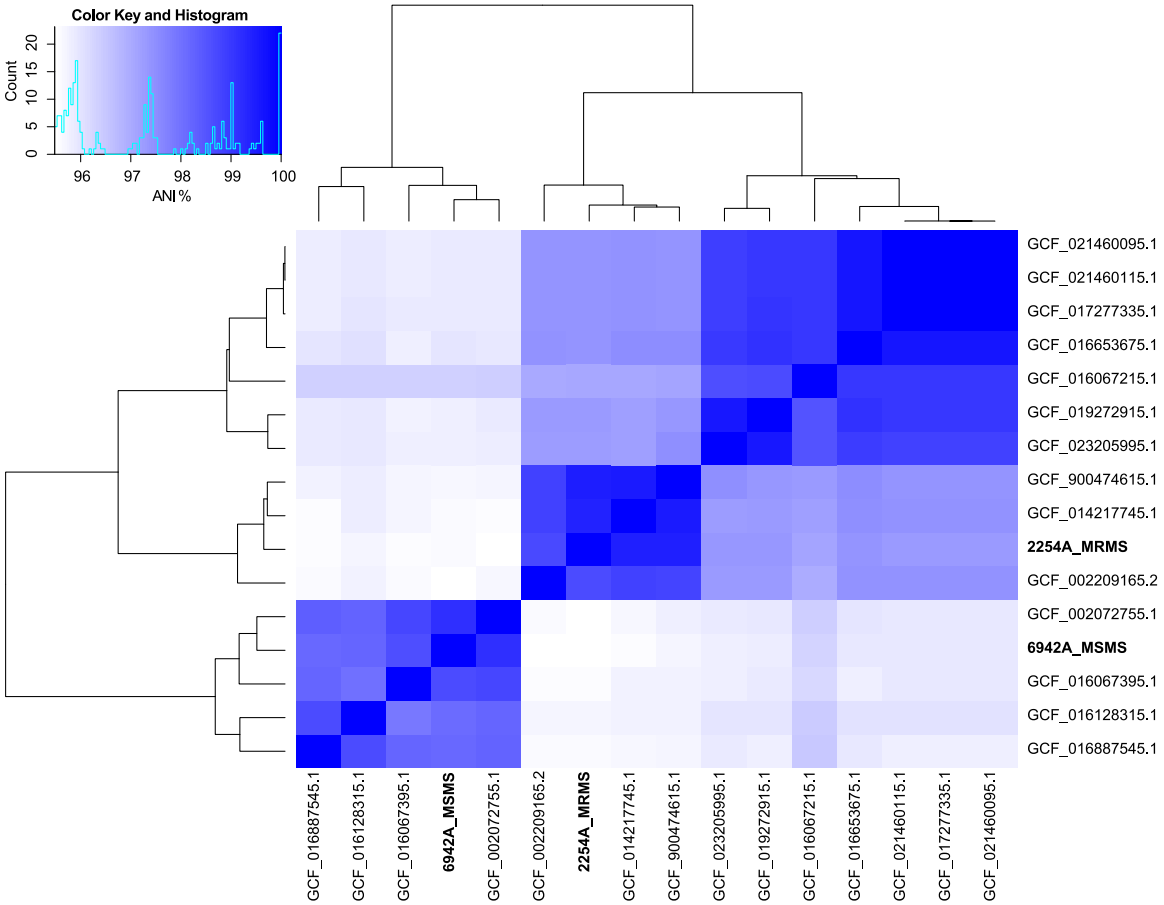

FIG 1 Pairwise comparison of the average nucleotide identity (ANI) between the newly sequenced MRMS 2254A and MSMS 6942A strains, and 14 complete *S. sciuri* or *M. sciuri* genomes available in NCBI. Genomes with at least 95% ANI threshold (inset) were considered the same species (19).

Default parameters were used for all software unless otherwise specified.

Data availability. Raw sequence reads have been deposited in the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA851703, with SRA accession numbers SRR19779535 (2254A) and SRR19790864 (6942A). Genome assemblies are available at NCBI under accession numbers CP100353 (2254A) and CP099816/CP099817 (6942A).

ACKNOWLEDGMENTS

We thank the Albany Research Technology Services where all bioinformatics analyses were carried out. We are also grateful to the staff of NHVDL for laboratory support.

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