

Molecular Characterization of Methicillin- and Multidrug-Resistant *Staphylococcus pseudintermedius* Strain Isolated from a Case of Feline Otitis Externa

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ABSTRACT

Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is an emerging zoonotic pathogen in veterinary medicine. Whole-genome sequencing (WGS) is highly preferred today as a valid method for molecular typing of bacterial pathogens due to its high discriminatory power and characterization efficiency. This study describes the WGS-based characterization of an MRSP strain named HMKU-VET-MRSP-2020 from a case of cat otitis externa in Türkiye. The strain was classified as MLST sequence type 71 and SCC_{mec} type IIIA. WGS analysis indicated the presence of several antimicrobial resistance genes and mutations consistent with the resistance phenotype. Phylogenetic analysis showed that the strain was clustered with the isolates from dog clinical cases previously reported from different countries of Europe and one human isolate from the USA. The study is the first report on the isolation and molecular characterization of MRSP from a cat in Türkiye and provides insights into the zoonotic potential of this microorganism.

Keywords: Cat; Methicillin Resistance; *Staphylococcus pseudintermedius*; Whole-Genome Sequencing.

INTRODUCTION

Staphylococcus pseudintermedius is an opportunistic pathogen that colonizes companion animals' skin and mucous membranes asymptotically (1). *S. pseudintermedius* is the most frequent cause of skin and soft tissue infections in dogs than in cats (2). The lower colonization rates observed in cats are attributed to less adhesion of *S. pseudintermedius* to feline corneocytes than to canine corneocytes (3). However, in cats, *S. pseudintermedius* can be isolated from various infections related mostly to the skin, including dermatitis, otitis, and wound infections (1).

In 2006, methicillin-resistant *S. pseudintermedius* (MRSP) emerged and reached worldwide high prevalence rates (4).

The frequent reports of multi-drug resistance (MDR) in MRSP isolates greatly complicate veterinary clinicians' therapeutic interventions (5, 6). MRSP has an epidemic clonal population structure (4), and certain dominant MDR MRSP lineages were frequently reported in particular continents, namely ST71 and ST258 in Europe, ST68 in North America, and ST45 and ST112 in Asia (4). *S. pseudintermedius*-related human infections have also been reported due to transmission from pet animals to humans (2, 7, 8).

This study was, therefore, conducted to perform a Whole-genome sequencing (WGS)-based characterization of an MDR *S. pseudintermedius* strain isolated from a case of feline otitis externa in Türkiye.

MATERIALS AND METHODS

Isolation and identification

The MRSP strain was isolated from a 3.5-year-old female Tekir cat's left ear with otitis externa in the Microbiology Laboratory, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University. The strain was first identified using phenotypic tests (colony morphology, Gram staining, and catalase test) for genus level. The species identification was performed by the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer using the Biotyper 3.1 software (Bruker Daltonics, Germany) and the Vitek2® automated system (bioMérieux, Marcy l'Etoile, France) in parallel.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the strain was determined using the standard disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (9). The following commercial disks were used: penicillin (1 U, P), oxacillin (1 µg, OXA), erythromycin (15 µg, E), gentamicin (10 µg, CN), clindamycin (2 µg, DA), trimethoprim-sulphamethoxazole (1.25/23.75 µg, SXT), ciprofloxacin (5 µg, CIP), tetracycline (30 µg, TE), and fusidic acid (10 µg, FA). *S. aureus* ATCC 25213 was used as a control strain.

DNA extraction, library preparation, and sequencing

The genomic DNA was extracted from the strain using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quantity and quality of the extracted DNA were measured using a Qubit 3.0 (Thermo Fisher Scientific, Waltham, MA, USA). The sequencing libraries of genomic DNA were prepared with the Illumina TruSeq DNA Nano Library Prep Kit (Illumina, San Diego, CA, USA) and paired-end (2×150 bp) sequencing was performed on the NovaSeq platform (Illumina, San Diego, CA, USA). After trimming low-quality reads and removing adapter sequences using Trimmomatic v 0.36 (10), the quality of both raw reads and trimmed reads was assessed using FastQC v 0.11.9 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>; accessed on 26 March 2022). The *de novo* genome assembly was conducted using the SPAdes algorithm (v 3.14.1) by applying the default parameters (11). The quality of assembly

was evaluated using QUAST 4.5 (12), and contigs longer than >200 bp were included in further analysis. The genome assembly data were deposited at NCBI under accession no. JAJICA000000000.

WGS-based characterization of MRSP strain

The MLST type, SCC_{mec} type, and the presence of acquired antimicrobial resistance genes of the strain were searched using the bioinformatic tools available at the Center for Genomic Epidemiology (CGE) platform (<http://www.genomicepidemiology.org/>). Mutations in the topoisomerase II (*gyrA*) and topoisomerase IV (*grlA*) genes that mediate fluoroquinolone resistance in the strain were analyzed using BLASTn. To this end, reference *gyrA* and *grlA* sequences were downloaded from strain CCUG 49543^T (NCBI accession numbers AM262968 and AM262971, respectively) and queried against a custom BLAST database. Gene predictions and annotations of the *de novo* assembled genomes were annotated using NCBI Prokaryotic Genome Annotation Pipeline for annotation (13). For the phylogenetic comparison of the strain, WGS of 46 *S. pseudintermedius* isolates of animal and human origin from different countries were retrieved from PATRIC and NCBI databases. The tree constructed using the presence and absence of accessory genes were provided in Roary outputs (accessory_binary_genes.fa.newick). The phylogenetic tree was visualized using an interactive web tool iTOL (14).

RESULTS

The strain was found to be resistant to penicillin, oxacillin, tetracycline, ciprofloxacin, erythromycin, clindamycin, gentamicin and trimethoprim-sulphamethoxazole, and was named HMKU-VET-MRSP-2020.

The sequenced reads were assembled into a draft genome consisting of 141 contigs, with a total length of 2 726 834 bp and N₅₀ of 14816 bp. The average G+C content was 37.34%. *In silico* analysis of the assembled genome revealed eight antimicrobial resistance (AMR) genes conferring resistance to: (i) beta-lactams (*blaZ* and *mecA*); (ii) aminoglycosides (*aac(6')-aph(2'')*, *aph(3')-III* and *ant(6)-Ia*); (iii) macrolide, lincosamide and streptogramin (MLS) group (*ermB*); (iv) tetracyclines (*tetK*); and (v) trimethoprim (*dfpG*). Based on the MLST, the strain belonged to sequence type (ST) 71 and harbored SCC_{mec} type III (3A).

Tree scale: 0.1

- COUNTRY**
- ARGENTINA
 - AUSTRALIA
 - BELGIUM
 - BOTSWANA
 - CANADA
 - CZECHIA
 - DENMARK
 - GERMANY
 - INDIA
 - IRELAND
 - ISRAEL
 - ITALY
 - JAPAN
 - NETHERLAND
 - NEW ZEALAND
 - SOUTH KOREA
 - SPAIN
 - SRI LANKA
 - SWEDEN
 - SWITZERLAND
 - THAILAND
 - TURKEY
 - UK
 - USA
- HOST**
- CAT
 - DOG
 - HORSE
 - HUMAN

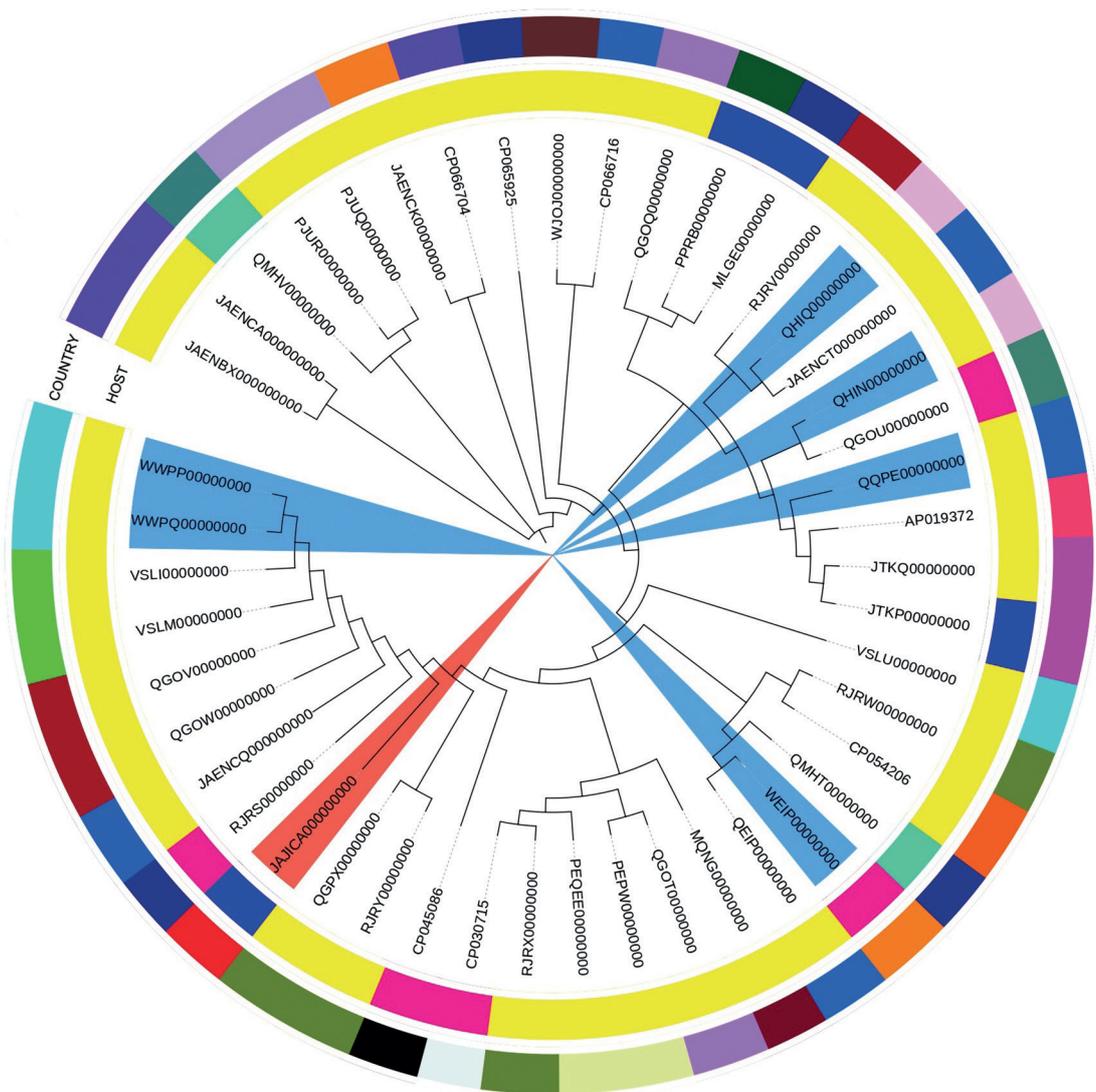


Figure 1. The phylogenetic tree is based on the core genome of *S. pseudintermedius* from different regions of the world. The strain from this study is highlighted with a red color shade. MRSP isolates were highlighted with a blue color shade.

The phylogenetic analysis based on genomes of 46 *S. pseudintermedius* of human and animal origin revealed three main clades. The strain is closely related to dog clinical isolates from Italy, Switzerland, the UK, and Germany and one human isolate from the USA (Figure).

DISCUSSION

This study shows the WGS-based molecular characterization of the MRSP strain isolated from otitis externa of a cat in Türkiye. The WGS revealed that the strain belonged to ST71, which is also the most frequently reported MDR clone in

Europe for dogs, cats, and humans including both clinical patients and healthy carriers (1, 4, 15) and is increasingly reported worldwide (16-18). It has been reported that the presence of ST71 in humans is often connected with previous contact with dogs (19, 20). Therefore, it could be speculated that owners of cats are also at risk of being infected with MRSP. More importantly, Latronico *et al.* (21) showed that MRSP ST71 strains of human origin adhered equally well to canine and human corneocytes. Thus, it could be said that MRSP ST71 strains have a high adhesion ability to human skin.

Thus far, both methicillin-susceptible and resistant *S.*

pseudintermedius have not been reported to be isolated from cats in Türkiye. The cat was admitted to our faculty clinics with the complaint of otitis externa that did not respond to treatment. Although it is not possible to determine the exact source of the infection, previously reported risk factors by Lehner *et al.* (22), such as previous hospitalization, frequent visits to veterinary settings, close contact with dogs, along with the administration of glucocorticosteroids and antibacterial chemotherapeutics might have played a role as the source of infection.

As previously reported in Europe, the MRSP strains belonging to ST71 have been significantly associated with resistance to beta-lactams, gentamicin, erythromycin, clindamycin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole (15, 17). In concordance with these studies, similar genetic determinants responsible for resistance were also present in the strain. This makes increasingly selecting of antimicrobial therapy very limited. Due to the zoonotic potential of MRSP and the horizontal transfer of resistance genes, continuous monitoring of phenotypic and genotypic antimicrobial profiles is important.

In conclusion, this study provides important data on phenotypic and genotypic features of MDR MRSP from a clinical case of a cat. According to our knowledge, this study is the first report on WGS analysis of *S. pseudintermedius* from the cat in Türkiye. The findings of the study also indicate an urgent need for national surveillance of MRSP in companion animals, veterinary clinics, and veterinary personnel to reduce the burden of this important veterinary pathogen, with particular emphasis on the detection of emerging MDR MRSP clones such as ST71.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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