CASE REPORT

Implanted Port Catheter System Infection Caused by Methicillin-resistant *Staphylococcus pseudintermedius* ST71-SCCmec type III

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Abstract:

*Staphylococcus pseudintermedius* is commonly associated with skin and soft tissue infections in dogs. However, infections caused by *S. pseudintermedius* are only rarely reported in humans, and this pathogen is frequently misidentified as *S. aureus*. We herein report a case of an implanted port catheter system infection caused by methicillin-resistant *S. pseudintermedius* (MRSP) in a patient with hepatocellular carcinoma. The patient was also a dog owner. *S. pseudintermedius* was first identified using the Vitek2 system (BioMérieux). Whole-genome sequencing revealed that this MRSP was a sequence type 71-carrying Staphylococcal cassette chromosome mec type III (ST71-SCCmec III) isolate.

Key words: *staphylococcus pseudintermedius*, *staphylococcus intermedius* group, bloodstream infection, bacteremia, human

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Introduction

*Staphylococcus pseudintermedius* is a novel, coagulase-positive *Staphylococcus* species that was identified in the last decade (1). It belongs to the *S. intermedius* group (SIG), which also comprises *S. cornubiensis*, *S. intermedius*, and *S. delphini* (1-3). *S. pseudintermedius* commonly colonizes the skin and mucosal surfaces of animals and particularly dogs, and it is associated with skin and soft tissue infections (SSTIs) (4). The emergence and rapid spread of the veterinary pathogen methicillin-resistant *S. pseudintermedius* (MRSP) has become a problem worldwide (5). Epidemic clones of sequence type 71-carrying Staphylococcal cassette chromosome mec type III (previously described as II-III (6); ST71-SCCmec III) MRSP were previously identified in Europe (7, 8), and have now spread worldwide (5). Although human infections with *S. pseudintermedius* remain rare, such isolates are increasingly recognized as a cause of human infection. *S. pseudintermedius* is associated with SSTIs and dog-bite wounds (9, 10), although this pathogen may also cause other human infections such as implanted device infection, infective endocarditis, and bone infection (10-16). However, as *S. pseudintermedius* is frequently misidentified as *S. aureus* in clinical laboratories (9), the true incidence of *S. pseudintermedius* as a cause of human infection may be underestimated. In addition, there is only one report about *S. pseudintermedius* infection from Japan (13). We herein report a case of implanted port catheter system infection caused by ST71-SCCmec III MRSP.

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Case Report

A 69-year-old man visited our hospital with fever and pain in the right groin. The patient had a medical history of liver cirrhosis caused by hepatitis C infection and multiple hepatocellular carcinoma (HCC). A port catheter system had been implanted two years previously in the right femoral artery (right groin) for hepatic arterial infusion chemotherapy (HAIC) and transcatheter arterial chemoembolization therapy, which the patient underwent for HCC. In addition, he was a dog owner.

On arrival at the hospital, the patient’s body temperature was 37.7°C. A physical examination showed pain and redness at the site of the implanted port system without purulent discharge. Laboratory testing revealed a white blood cell count of 4,670 cells/μL and a C-reactive protein level of 2.03 mg/dL. A plain computed tomography (CT) scan revealed fluid collection at the site of the implanted port system and an infection was suspected. The patient was admitted, the implanted port system was removed from the right groin, and pus was found at the surgical site. Gram staining of the pus revealed gram-positive, cluster-forming cocci and the pus was sent for a culture. After obtaining two sets of blood cultures, we empirically administered vancomycin (1 g every 12 hours, intravenously).

On day 2, both sets of blood cultures and surgical specimen cultures became positive and gram-positive staphylococci were detected. On day 3, the isolates were identified as *S. pseudintermedius* (identification accuracy, 92%) using the Vitek2 system with gram-positive ID cards (BioMérieux Japan Ltd., Tokyo, Japan). The strains showed white-gray color colonies on sheep-blood agar plates with β-hemolysis, and a Staphylococcus latex agglutination test performed using PS latex (Eiken Chemical Co., Ltd, Tokyo, Japan) was negative. A single isolate obtained from the blood culture and a Staphylococcus latex agglutination test performed using Shovill v1.0.9 (https://github.com/tseemann/shovill) and were annotated using Prokka version 1.13 (18). The assembled contigs were submitted to ResFinder 3.2 to identify drug-resistance genes, MLST 1.8 for multilocus sequence typing (MLST), and SCC mec Finder 1.2 for SCC mec typing (all available on the Center for Genomic Epidemiology (CGE) website: http://www.genomicepidemiology.org/) (19-21). For drug-resistance and toxin genes, >99.5% identity and 100% reference sequence length were accepted as positive.

We also performed whole-genome sequencing (WGS) of JH6152 for further genetic characterization. Genomic DNA was extracted from the JH6152 isolate using a QIAamp DNA Minikit (Qiagen, Hilden, Germany). The DNA library was prepared using the QIAseq FX DNA Library Kit (Qiagen) and sequenced as paired-end reads using an Illumina HiSeq X FIVE platform (300 cycles; Illumina, Inc., San Diego, CA, USA). Illumina reads were assembled de novo using Shovill v1.0.9 (https://github.com/tseemann/shovill) and were annotated using Prokka version 1.13 (18). The assembled contigs were submitted to ResFinder 3.2 to identify drug-resistance genes, MLST 1.8 for multilocus sequence typing (MLST), and SCC mec Finder 1.2 for SCC mec typing (all available on the Center for Genomic Epidemiology (CGE) website: http://www.genomicepidemiology.org/) (19-21). For drug-resistance and toxin genes, >99.5% identity and 100% reference sequence length were accepted as positive.

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Based on the 16S rRNA gene sequencing, MLST, and WGS SCC mec typing results, we finally identified the JH 6152 strain as ST71-SCC mec III MRSP. This strain harbored the antimicrobial resistance genes *aac(6’)-aph(2”)*, *ant(6’)-Ia, aph(3’)-III*, and *sat4A* (aminoglycoside resistance), *mecA* and *blaZ* (*β*-lactam resistance), *erm(B)* (macrolides resistance), and *dfrG* (trimethoprim resistance). JH6152 was negative for *tetM* and *tetK* (tetracycline resistance). JH6152 carried the fluorquinolone resistance-conferring *GyrA* Ser84Leu and *GrlA* Ser80Ile mutations. In addition, the identification of the JH6159 strain was confirmed via matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS; MALDI Biotyper, Bruker Daltonics, Inc., Billerica, MA, USA) using the using the MBT Compass library research-use-only (RUO) Version 8, 7855. JH6159 was identified as *S. pseudintermedius* (score value 2.109), match pattern *Staphylococcus pseudintermedius* 472 RLT (National Center for

### Table. Antimicrobial Susceptibility of the *Staphylococcus Pseudintermedius* Isolate Detected.

| Antimicrobial agent | MIC (µg/mL) | Interpretation |
|---------------------|-------------|----------------|
| Oxacillin           | >4          | R              |
| Ampicillin          | >8          | N/A            |
| Gentamicin          | 16          | R              |
| Ciprofloxacin       | >2          | R              |
| Levofloxacin        | >4          | R              |
| Minocycline         | ≤1          | S              |
| Clindamycin         | >2          | R              |
| Trimethoprim-sulfamethoxazole | >2/38 | R |
| Vancomycin          | 1           | S              |
| Teicoplanin         | ≤0.5        | S              |
| Linezolid           | 1           | S              |
| Daptomycin          | 0.5         | S              |

MIC: minimum inhibitory concentration, S: susceptible, N/A: categorical interpretation not available, R: resistant.
Biotechnology Information (NCBI) No. 283734).

**Discussion**

Although MRSP has emerged worldwide over the past decade (5), human infections with MRSP remain rare. The *S. pseudintermedius* isolate was classified as ST71-SCCmec III after WGS. The clonal complex (CC) 71 clone-carrying SCCmec III (also known as II-III) was previously regarded as the European epidemic clone of MRSP ((7, 8) but it has now spread worldwide (5). This clone also appears to be a major clone in MRSP isolated from dogs in Japan ((22, 23). *S. pseudintermedius* ST71-SCCmec III is the predominant multidrug-resistant (MDR) MRSP clone in European countries ((7, 8). The characteristic antimicrobial resistance genes and mutations in JH6152 were similar to those of ST71-SCCmec III isolated from dogs in Japan (23), and the worldwide-disseminated ST71-SCCmec III clone (24).

Although the carriage of *S. pseudintermedius* in healthy humans seems to be uncommon, the colonization of *S. pseudintermedius* in dog owners and veterinary staff has been reported ((25, 26). As the patient in this case was a dog owner, the transmission of *S. pseudintermedius* from dog to owner was therefore suspected. However, we did not investigate ST71-SCCmec III MRSP colonization of the patient’s dog or the patient’s skin and nasal cavity. Clinicians should be aware of companion animal contact when *S. pseudintermedius* is detected in patients. However, human infection caused by *S. pseudintermedius* has previously been described without any known dog-human contact, as has a cluster of MRSP infections among patients in a tertiary hospital (27).

The clinical characteristics of human infections caused by *S. pseudintermedius* remain unclear. Reported *S. pseudintermedius* infections occur mainly as SSTIs, and particularly dog-bite wounds (9, 10). Implanted device infections such as the one described here have also been reported (11-13). In this case, the patient had no history of dog bite before admission. It is suspected that MRSP had previously colonized the patient’s skin and entered the device site when the device was used, thus causing a bloodstream infection.

The accurate identification of *S. pseudintermedius* has important implications for the interpretation of antimicrobial susceptibility testing data to detect the presence of the *mecA* gene, as cefoxitin-based methods perform poorly in detecting the presence of *mecA* in *S. pseudintermedius* ((17, 28). In addition, the MIC breakpoint of oxacillin for *S. pseudintermedius* is different from that for *S. aureus* (17).

In this case, isolates were identified as *S. pseudintermedius* using the Vitek2 system and this was further confirmed via 16s rRNA gene sequencing and PCR targeting a *nuc* gene. SIG members are frequently misidentified as *S. aureus* via phenotypic and biochemical methods, as both are coagulase- and catalase-positive staphylococci that show β-hemolysis on blood agar plate ((9, 29). The Vitek2 system database, which is used in our clinical laboratory, includes *S. pseudintermedius*. In contrast, other commercially available automated biochemical identification platforms such as the MicroScan WalkAway (Beckman Coulter, Inc., Brea, CA, USA) and BD Phoenix (BD Biosciences, Franklin Lakes, NJ, USA) database, do not include *S. pseudintermedius* (30). The true incidence of *S. pseudintermedius* as a cause of human infection may therefore be underestimated.

In Japan, the prevalence of methicillin-resistant *S. aureus* (MRSA) with SCCmec III isolated from human clinical specimens is low ((31, 32). Therefore, MRSP with SCCmec III may be misidentified as MRSA with SCCmec III, especially in strains identified using automated biochemical identification platforms.

Recent studies have showed MALDI-TOF MS to be a rapid and accurate method for the species-level identification of SIG group members (30, 33, 34). However, the accurate species level identification of *S. pseudintermedius* depends on the database. In this study, JH6152 was identified as *S. pseudintermedius* with a score of 2.109 via MALDI Biotyper using the MBT Compass library RUO Version 8, 7855. However, this library is not usually used in clinical laboratories.

In conclusion, we herein described a rare case of implanted port catheter system infection caused by ST71-SCCmec III MRSP possibly transmitted by the patient’s dog. Human infections caused by *S. pseudintermedius* may be underestimated because of misidentification as *S. aureus*. Further studies using high-resolution methods such as nucleic acid-based methods and MALDI-TOF MS are needed to clarify the real prevalence of *S. pseudintermedius* in human infections.

The authors state that they have no Conflict of Interest (COI).

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**Accession Numbers**

The raw sequence data of JH6152 have been deposited in DDBJ/ENA/GenBank under the BioProject number PRJDB9857.

**Ethical Approval**

Written informed consent was obtained from the patient for publication of this case report.

**Authorship Statement**

All authors met the ICMJE authorship criteria: (1) conception
and design of the study, or acquisition of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

References

1. Devriese LA, Vancanneyt M, Baelle M, et al. Staphylococcus pseudintermedius sp. nov., a coagulase-positive species from animals. Int J Syst Evol Microbiol 55: 1569-73, 2005.

2. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata S, Hiramatsu K. Reclassification of phenotypically identified staphylococcus intermedius strains. J Clin Microbiol 45: 2770-8, 2007.

3. Murray AK, Lee J, Bendall R, et al. Staphylococcus cornubiensis sp. nov., a member of the Staphylococcus intermedius Group (SIG). Int J Syst Evol Microbiol 68: 3404-8, 2018.

4. Bannehr J, Guardabassi L. Staphylococcus pseudintermedius in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. Vet Dermatol 23: 253-66, 2012.

5. Pires Dos, Santos T, Damborg P, Moodley A, Guardabassi L. Systematic Review on Global Epidemiology of Methicillin-Resistant Staphylococcus pseudintermedius: Inference of Population Structure from Multilocus Sequence Typing Data. Front Microbiol 7: 1599, 2016.

6. Descloux S, Rossano A, Perreten V. Characterization of new staphylococcal cassette chromosome mec (SCCmec) and topoisomerase genes in fluoroquinolone- and methicillin-resistant Staphylococcus pseudintermedius. J Clin Microbiol 46: 1818-23, 2008.

7. Perreten V, Kadlec K, Schwarz S, et al. Clonal spread of methicillin-resistant Staphylococcus pseudintermedius in Europe and North America: an international multicentre study. J Antimicrob Chemother 65: 1145-54, 2010.

8. Ventrella G, Moodley A, Grandolfo E, et al. Frequency, antimicrobial susceptibility and clonal distribution of methicillin-resistant Staphylococcus pseudintermedius in canine clinical samples submitted to a veterinary diagnostic laboratory in Italy: A 3-year retrospective investigation. Vet Microbiol 211: 103-106, 2017.

9. Borjesson S, Gomez-Sanz E, Ekstrom K, Torres C, Gronlund U. Staphylococcus pseudintermedius can be misdiagnosed as Staphylococcus aureus in humans with dog bite wounds. Eur J Clin Microbiol Infect Dis 34: 839-844, 2015.

10. Somayaji R, Priyantna MA, Rubin JE, Church D. Human infections due to Staphylococcus pseudintermedius, an emerging zoonosis of canine origin: report of 24 cases. Diagn Microbiol Infect Dis 85: 471-476, 2016.

11. VanHoovels L, Vankeerberghen A, Boel A, Van Vaerenbergh K, De Beernhouver H. First case of Staphylococcus pseudintermedius infection in a human. J Clin Microbiol 44: 4609-4612, 2006.

12. Chuang CY, Yang YL, Hsieh PR, Lee PI. Catheter-related bactemia caused by Staphylococcus pseudintermedius refractory to antibiotic-lock therapy in a hemophiliac child with dog exposure. J Clin Microbiol 48: 1497-1498, 2010.

13. Nomoto H, Kutsuna S, Nakamura K, et al. Totally implantable venous access port infection caused by Staphylococcus pseudintermedius: Possible transmission from a companion dog to a human. J Infect Chemother 26: 1305-1308, 2020.

14. Riegel P, Jesel-Morel L, Laventie B, Boisset S, Vandenesch F, Prevost G. Coagulase-positive Staphylococcus pseudintermedius from animals causing human endocarditis. Int J Med Microbiol 301: 237-239, 2011.

15. Darlow CA, Paidakakos N, Sikander M, Atkins B. A spinal infection with Staphylococcus pseudintermedius. BMJ Case Rep 2017, 2017.

16. Sasaki T, Tsubakishita S, Tanaka Y, et al. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J Clin Microbiol 48: 765-769, 2010.

17. CLSI. Performance standards for antimicrobial susceptibility testing; 28th informational supplement. CLSI document M100-S28. Clinical and Laboratory Standards Institute, Wayne, PA. 2018.

18. Seemann T, Prokka: rapid prokaryotic genome annotation. Bioinformatics 30: 2068-2069, 2014.

19. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67: 2640-2644, 2010.

20. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol 50: 1355-1361, 2012.

21. Kaya H, Hasman H, Larsen J, et al. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data. mSphere 3: e00612-e00617, 2018.

22. Ishihara K, Koizumi A, Saito M, Muramatsu Y, Tamura Y. Detection of methicillin-resistant Staphylococcus pseudintermedius ST 169 and novel ST354 SCCmec II-III isolates related to the worldwide ST71 clone. Epidemiol Infect 144: 434-442, 2016.

23. Kasai T, Saegusa S, Shirai M, Murakami M, Kato Y. New categories designated as healthcare-associated and community-associated methicillin-resistant Staphylococcus pseudintermedius in dogs. Microbiol Immunol 60: 540-551, 2016.

24. Brooks MR, Padilla-Vélez L, Khan TA, et al. Prophage-Mediated Disruption of Genetic Competence in Staphylococcus pseudintermedius. mSystems 5: e00684-19, 2020.

25. Wawer B, Hermes J, Cuny C, et al. Sharing more than friendship–nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS One 7: e35197, 2012.

26. Fessler AT, Schuenemann R, Kadlec K, et al. Methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-resistant Staphylococcus pseudintermedius (MRSP) among employees and in the environment of a small animal hospital. Vet Microbiol 221: 153-158, 2018.

27. Starlander G, Borjesson S, Gronlund-Andersson U, Tellgren-Roth C, Melhus A. Cluster of infections caused by methicillin-resistant Staphylococcus pseudintermedius in humans in a tertiary hospital. J Clin Microbiol 52: 3118-3120, 2014.

28. Wu MT, Burnham CA, Westblade LF, et al. Evaluation of Oxacillin and Cefoxitin Disk and MIC Breakpoints for Prediction of Methicillin Resistance in Human and Veterinary Isolates of Staphylococcus intermedius Group. J Clin Microbiol 54: 535-542, 2016.

29. Pottumarthi S, Schapiro JM, Prentice JL, et al. Clinical isolates of Staphylococcus pseudintermedius masquerading as methicillin-resistant Staphylococcus aureus. J Clin Microbiol 42: 5881-5884, 2004.

30. Canver MC, Tekle T, Compton ST, et al. Performance of Five Commercial Identification Platforms for Identification of Staphylococcus delphini. J Clin Microbiol 57: e00721-19, 2019.

31. Harada D, Nakaminami H, Miyajima E, et al. Change in genotype of methicillin-resistant Staphylococcus aureus (MRSA) affects the antibiogram of hospital-acquired MRSA. J Infect Chemother 24: 563-569, 2018.

32. Aung MS, Urushibara N, Kawaguchiya M, et al. Clonal Diversity and Genetic Characteristics of Methicillin-Resistant Staphylococcus aureus Isolates from a Tertiary Care Hospital in Japan. Microb Drug Resist 25: 1164-1175, 2019.

33. Decrystofhriss P, Fasola A, Benagli C, Tonolla M, Petruzzi O. Identification of Staphylococcus intermedius Group by MALDI-MS. Syst Appl Microbiol 34: 45-51, 2011.

34. Murugaiyan J, Wawer B, Stamm I, et al. Species differentiation within the Staphylococcus intermedius group using a refined MALDI-TOF MS database. Clin Microbiol Infect 20: 1007-1015, 2014.
