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【 CASE REPORT 】

Implanted Port Catheter System Infection Caused by Methicillin-resistant *Staphylococcus pseudintermedius* ST71-SCC*mec* 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-SCC*mec* 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-SCC*mec* 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-SCC*mec* 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℃. 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, 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, Tokyo, Japan) was negative. A single isolate obtained from the blood culture was designated as JH6152. We performed 16S ribosomal RNA (16S rRNA) gene sequencing on JH6152 using the universal primers 27F and 1492R. The sequences obtained (1,516 bp) were compared with those published in the GenBank database using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/blast). The closest match obtained was *S. pseudintermedius* KCTC 43135 (accession number CP045085.1; identity 100%). In addition, the identity of the isolates was further confirmed via a thermonuclease (nuc) gene polymerase chain reaction (PCR) test (16).

The minimum inhibitory concentrations (MICs) of various antimicrobial agents (Table) were determined via the broth microdilution method using IA40 MIC-i with Dry Plates Eiken (Eiken Chemical) and then they were interpreted according to the Clinical and Laboratory Standards Institute recommendations (17). The patient was finally diagnosed with a bloodstream infection associated with an implanted port catheter system infection caused by *S. pseudintermedius*. Following treatment, blood cultures obtained on day 5 were negative. The patient received intravenous vancomycin therapy for 2 weeks and was discharged on day 16.

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

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, San Diego, 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.

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 fluoroquinolone 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, Billerica, USA) 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 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-SCC*mec* III after WGS. The clonal complex (CC) 71 clone-carrying SCC*mec* 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-SCC*mec* 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-SCC*mec* III isolated from dogs in Japan (23), and the worldwide-disseminated ST71-SCC*mec* 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-SCC*mec* 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, Brea, USA) and BD Phoenix (BD Biosciences, Franklin Lakes, 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 SCC*mec* III isolated from human clinical specimens is low (31, 32). Therefore, MRSP with SCC*mec* III may be misidentified as MRSA with SCC*mec* 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-SCC*mec* 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.

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

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

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