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Case Report

# Identification of streptococcus dysgalactiae subsp. equisimilis from septic knee by 16S rRNA gene sequencing

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Septic arthritis is the infection of a joint by an infectious agent, leading to arthritis. It is therefore important to identify and treat the correct bacteria in septic arthritis. However, accurate identification of bacteria by conventional methods is difficult because of the distinct biochemical characteristics of individual bacteria. This case report aims at assessing septic arthritis caused by *Streptococcus dysgalactiae* subsp. equisimilis(SDSE) using nucleotide sequences and discusses the associated treatment. Here, *Streptococcus agalactiae* was determined to be the causative bacteria for septic arthritis in a 77 year-old woman using the conventional method of hemolysis pattern interpretation and morphology. However, nucleotide sequence analysis of 16S ribosomal RNA revealed that SDSE was the causative strain. 16S rRNA gene sequencing can correctly identify bacteria strains that are difficult to be identified by traditional method, and this correct identification can provide patients with the opportunity for adequate treatment using the proper antibiotics.

**Key Words**: *Equisimilis*, Infectious arthritis, *Streptococcus dysgalactiae* subsp., 16S rRNA gene sequencing

In septic arthritis, the correct identification of causative agent and proper antibiotics use are important. Septic arthritis comprises 8%–27% of all acute arthritis cases,[1,2] and its clinical manifestations include fever, rash, pressure, and pain, which progress adversely within 2 weeks. The risk factors for septic arthritis are old age ( 〉 80 years), diabetes, rheumatoid arthritis, skin infection, and joint surgery. Early diagnosis and proper treatment of septic arthritis using antibiotics and drainage are very important. A delayed diagnosis increases the possibility of serious complications, including cartilage destruction and joint contracture.[3] Thus, identification of the correct causative bacterium is important, and caution needs to be exercised when choosing a method of identification, to avoid incorrect identification. Conventional biochemical methods are frequently associated with incorrect bacterial identification.[4]

*Staphylococcus aureus* and *Streptococcus pneumoniae* cause more than 60% of all septic

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arthritis-associated infections.[5,6] Recently, incidences of novel infection by *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) from Group C have increased, despite the important consideration of Streptococcus Group A and B. SDSE is present in the normal flora of the skin, nasopharynx, gastrointestinal tract, and urinary tract, and although it causes septic infection in animals, it rarely affects humans; septic infections by SDSE are usually observed only in immunocompromised patients.[7] In 1996, the first case of SDSE infection was reported by Vandamme et al., and some additional cases of SDSE infection have been reported in patients with soft tissue infection, pyothorax causing pneumonia, acute peritonitis, and ovarian tube infection.[8] The prevalence of sepsis due to SDSE infection has been on the rise since 2000.[8] In a recent Japanese study on 263 patients with infiltrative SDSE infection, more than 30% of the patients were diagnosed with septic arthritis and myelitis.[8] In Korea, a case report described septic arthritis caused by SDSE following total knee arthroplasty.[7] Given the inherent limitations of biochemical methods for the identification of SDSE; therefore, novel methods are being investigated to enable accurate identification of the causative organism in septic arthritis. Here, we describe a novel method for the identification of SDSE using 16S rRNA sequencing in primary septic arthritis.

## CASE

A 77 year-old woman presented with a history of fever and pain in the dorsum of her right foot (of 1-day duration) at the hospital via the outpatient service. Tenderness, swelling, and erythema were observed in the right tarsal joint. Prior to this, she had visited the emergency department following a 1-day history of fever and chills, which subsequently subsided. On the second day of admission, pain, edema, and heat sensation in the left knee were observed. The patient did not have any underlying disease or restrictions in ordinary activities. She had undergone hysterectomy because of uterine myoma 40 years ago. At admission, blood pressure of 100/60 mmHg, pulse of 77/min, respiratory rate of 19/min, body temperature of 36.9℃, along with clear conscience, were reported. No abnormal findings were reported in the head, neck, and chest areas. Edema and tenderness were observed in the dorsum of the right foot, but no flare was detected. Heat sensation, flare, and tenderness were observed in left knee, along with restricted movement because of pain. Skin turgor was decreased and dry throughout the body. No other abnormality was observed upon physical

examination.

At admission, laboratory tests revealed the following biochemical parameters: white blood cell, 11,400/mm3 (segmented neutrophil, 81%; lymphocyte, 3%; monocyte, 2%; and band neutrophil, 14%) and hemoglobin, 11.5 g/dL. Electrochemical analyses revealed the following parameters: aspartate transaminase, 28 IU/L; alanine transaminase, 16 IU/L; albumin, 3.6 g/dL; blood urea nitrogen, 23.8 mg/dL; creatinine 1.1 mg/dL; and uric acid, 7.2 mg/dL. Coagulation test revealed a prothrombin time of 14.9 s (international normalized ratio 1.42), and a partial thromboplastin time of 37.2 s. Additional tests revealed the following biochemical levels: C-reactive protein (CRP), 17.47 mg/dL; ESR, 65 mm/hr ; fibrinogen, > 850 mg/dL; D-dimer, 0.3 μg/dL; fibrin degradation product, 9.6 μg/dL; and antithrombin III, 54%. The rheumatoid factor and antinuclear antibody was negative. The electrocardiogram revealed normal sinus rhythm, and no other specific findings in the ST segment or T wave were observed. Chest X-ray showed no cardiomegaly or pulmonary infiltration. The left knee showed marginal osteophytes and asymmetric narrowing of joint space with subchondral sclerosis and intraarticular osteochondral loose bodies, with effusion under simple radiographic tests. Right ankle joint space was narrowed, and marginal osteophytes, along with soft tissue swelling, were observed by simple X-ray analysis. Empirical ceftriaxone (2 mg) was administered once daily following blood culture test sampling. On the second day of admission, improvement in right-foot pain was noted. However, magnetic resonance imaging showed edematous and septic arthritis of the left knee. Enhancement revealed a thick and irregular synovial membrane, while the adjacent muscles were not clearly seen (Fig. 1). Joint fluid showed yellowish and highly turbid 50-cc aspiration, with a white blood cell count of 135,000/mm and a polymorphonuclear leukocyte content of 90%. We performed biochemical identification tests on colonies harvested from 2 pairs of blood-culture bottles (2 for aerobic culture and 2 for anaerobic culture) that were cultured on synovial fluid culture blood agar plate. The result revealed that the isolates were *Streptococcus agalactiae* or SDSE, with a probability of 50% for each strain. The hemolysis pattern and morphological features (e.g. colony size) did not match that of *Streptococcus agalactiae*. Therefore, additional 16S ribosomal RNA nucleotide sequencing was performed, and SDSE was confirmed as the causative bacterium.

Antibiotic tests revealed susceptibility of the isolate to ceftriaxone. Fever subsided, while leukocytosis improved on day 5 of admission through antibiotic treatment and sufficient aspi-

ration, while pain and edema in the left knee continued with increased CRP. The patient was referred to the Department of Orthopedics, where incision and drainage were performed. A Hemovac® drain was applied to the left knee for drainage, and ceftriaxone 2mg was administered for 14 days. On day 15 of admission, the CRP level was 12.4 mg/dL, and 10-20 cc of synovial fluid were drained by using the Hemovac® daily. The antibiotic of choice was changed to amoxicillin 500mg q8hr because of persistence of pain and edema. On day 26 of admission, CRP levels reduced to 0.574 mg/dL. Treatment was terminated after decreased pain and improved inflammatory marker levels were confirmed.

## DISCUSSION

Delayed treatment and incorrect diagnosis of acute septic arthritis can lead to destruction of bone and cartilage, and bone necrosis. In acute septic arthritis, single or multiple joints may be involved, with knee and hip joints comprising more than 60% of reported cases.[5] The most prevalent bacteria in septic arthritis are gram-positive *Staphylococcus aureus* and *Streptococcus spp.*, which are detected in more than 60%-90% of cases. Streptococcus is grouped as Lancefield A, B, C, G, and F, according to the presence of specific antigens. Until recently, group A streptococci (GAS; *Streptococcus pyogens*) and group B streptococci (GBS; *Streptococcus agalactiae*) were considered the major infectious agents. *Streptococcus dysgalactiae*, which belongs to group C, was identified in the 1970s as an infectious agent in horses and cows, while some human infections transmitted via animals or by consumption of unsterilized milk were also detected.[3] In 1996, *Streptococcus dysgalactiae* was further classified into 2 subspecies, SDSE and *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD). SDSE has been isolated from human patients and animals exhibiting strong $\beta$-hemolysis, while SDSD has been isolated only from animals with no $\alpha$- or $\beta$-hemolysis. VITEK®

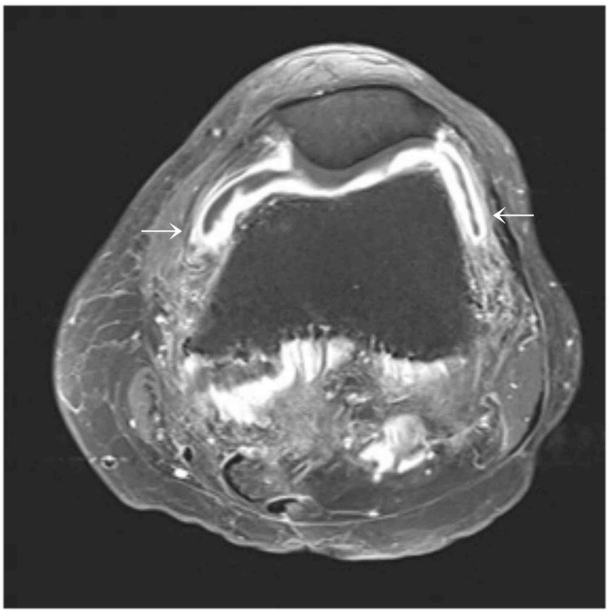

**Fig. 1. Irregular thick enhancement of synovium with joint effusion (arrows) on an Axial T1-enhanced magnetic resonance (MR) image of the left knee.**

2 (bioMérieux Inc., Durham, NC), the biochemical bacterial identification equipment used in our institution, cannot differentiate SDSE from SDSD; however, genetic sequencing of bacteria can differentiate SDSE and SDSD. Since 2003, invasive infections by SDSE, associated with streptococcal toxic shock syndrome, soft tissue infection, and septic arthritis, been reported sporadically in Japan.[4,8] SDSE has rarely been reported in necrotic myositis, meningitis, infectious endocarditis, myelitis, etc. To our knowledge, this case is the first report of septic arthritis caused by SDSE in Korea. The infectious agent was eccentrically identified from a normal host, who had neither had surgery nor was immunocompromised.

Our initial biochemical results revealed that the causative organism might be *Streptococcus agalactiae* or SDSE (with a probability of 50% for each strain) when isolated from 2 pairs of blood-culture bottles (2 aerobic and 2 anaerobic culture bottles) and grown in synovial fluid culture media. However, the morphological features of the causative strain, such as hemolysis pattern and colony size, did not match that of *Streptococcus agalactiae*. Therefore, additional genetic sequencing test using 16S rRNA was performed, and the microorganism was identified as SDSE. Moser et al., have reported 4 cases of SDSE identified by RNA analysis despite no findings from the bacterial identification test and no microscopic observation of *Streptococci sp.*; 3 of 4 cases were from knee joints.[3] In recent time, 16S rRNA nucleotide sequencing is being used increasingly, in addition to blood culture. Misidentification of bacteria is common in the case of clinically rare bacteria or for those exhibiting abnormal phenotypes.[9] In a study by Fenollar et al., bacterial nucleotide sequencing and culture were performed in 525 bone and joint infection patients, and discordant results were observed in 50 patients, accounting for nearly 10% of the cases.[10] Thus, the sequencing method is useful in identifying bacterial strains that are difficult to identify using the conventional method; use of this method can prevent treatment failure associated with incorrect identification.

While penicillin is the primary antibiotic for SDSE, aminoglycoside can also be administered in case of severe infection to prevent failure of penicillin.[11] In a Japanese study conducted in 2006, antibiotic susceptibility tests performed in 231 SDSE patients showed no resistance to penicillin and cephalosporin. Since the first report of fluoroquinolone resistance in North America, the incidence of antibiotic resistance is on the rise; a resistance level of 12% was reported in Portugal for fluoroquinolone.[8]

Empiric ceftriaxone was administered to the

case patient prior to the identification of the causative bacterium, and the patient was maintained on this treatment for 14 days. Despite improvement of fever, pain and edema of the left knee persisted, and a CRP level of 15 mg/dL was noted, suggesting the presence of inflammation. The antibiotic was changed to penicillin subsequently. The authors believed that the use of SDSE-susceptible ceftriaxone improved the fever; however, the use of the primary antibiotic, penicillin, contributed more to the improvement of the patient's inflammatory symptoms.

In septic arthritis, the correct identification of causative agent and proper antibiotics use are important. This case presents a bacteria strain suspicious of SDSE and *Streptococcus agalactiae*, which cannot be differentiated using conventional biochemical identification system, but was identified using the additional 16S rRNA gene sequencing analysis as SDSE. 16S rRNA gene sequencing can correctly identify bacteria strains that are difficult to be identified by traditional method, and this correct identification can provide patients with the opportunity for adequate treatment using the proper antibiotics; thus the use of this method looks promising in the future as the diagnostic tool for infectious disease.

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