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CASE REPORT

Small colony variant of methicillin-resistant *Staphylococcus aureus* isolated from an osteomyelitis case

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* small colony variants may cause soft tissue infections. However, any case presenting with soft tissue abscess after a fracture surgery is not been reported yet. In this report; a case of methicillin-resistant *S. aureus* small colony variant that was isolated from a 68-years-old man who has a fracture surgery history and recurrent abscess developing in his left thigh was reported. This variant was recovered from aspiration material of the open wound. Daptomycin was used successfully in the treatment of the bacterial infection which is resistant to rifampin. *J Microbiol Infect Dis 2013; 3(2): 89-92*

Keywords: Small colony variant, methicillin-resistant *Staphylococcus aureus*, recurrent abscess

Osteomyelit olgusundan izole edilen metisiline dirençli Staphylococcus aureus küçük koloni varyantı

ÖZET

Metisiline dirençli *Staphylococcus aureus*'un küçük koloni varyantları yumuşak doku enfeksiyonlarına sebep olabilmektedir. Ancak kırık ameliyatı sonrası yumuşak doku apsesi ile seyreden bir olgu henüz rapor edilmemiştir. Bu yazıda; kırık öyküsü olan 68 yaşındaki erkek hastanın sol bacağında gelişen ve tekrarlayan apseden izole edilen metisiline dirençli *S. aureus* küçük koloni varyantı olgusu sunulmuştur. Bu varyant açık yaradan alınan aspirasyon materyalinden elde edildi. Rifampine dirençli olan bakteri enfeksiyonunun tedavisinde daptomisin başarıyla kullanıldı.

Anahtar klimeler: Küçük koloni varyantı, metisiline dirençli *Staphylococcus aureus*, tekrarlayan abse

INTRODUCTION

Small colony variants (SCVs) of *S. aureus* are isolated in patients with persistent and recurrent infections such as osteomyelitis, septic arthritis, deep-seated abscesses and respiratory tract infections in patients with cystic fibrosis.[1-4] Small colony variants (SCVs) of *S. aureus* are known as slow growing subpopulations that form small, nonpigmented and non-hemolytic colonies. Deficiencies in electron transport activities leading to auxotrophism for thymidine, menadione, or hemin cause the typical biochemical characteristics of the variants.[5] By routine microbiological methods, these phenotypic variants of *S. aureus* may be misidentified, and they may also respond poorly to some antimicrobial agents.[6] Their isolation and identification remains difficult unless appropriate techniques are used in clinical microbiology laboratories. This problem might lead to diagnostic underestimation, which will cause therapeutic failures, in the clinical settings.

Methicillin-resistant *S. aureus* infections cause higher mortality and morbidity rates especially in intensive care patients. MRSA SCVs are reported to cause more severe infections and mortality rates than those caused by MRSA.[7] However, there are only few case reports of MRSA SCVs in the literature. Here, we describe the first case of a soft tissue abscess caused by MRSA SCV, which occurred after femoral fracture surgery.

CASE REPORT

A 68-year-old man was admitted to the hospital with pain and erythema on his left leg in 29th November 2011. He had been operated for femoral fracture in 1995 and he developed abscess formation 3 years later. He had recurrent abscess formation at inter-

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vals of several years thereafter. On his last admission to orthopedics outpatient department, he had used ciprofloxacin and fusidic acid for 8 days following one-week amoxicillin-clavulanic acid per oral. As his complaints had not been resolved, he had applied to another hospital and MRSA, which was resistant to gentamicin, rifampin and TMP-SXT, was isolated from his abscess culture. Then he referred to the infectious diseases clinic and hospitalized for his abscess. His medical history included diabetes mellitus for 25 years and hypertension for 10 years. He had also benign prostate hypertrophy and unilaterally nephrectomised for nephrolithiasis.

On admission, the patient was cooperated and oriented. On physical examination, a 5x5 cm ulcerated wound with purulent discharge containing necrotic areas in patches was observed on lateral region of his left thigh. His vital signs and the remaining systems were normal on examination. His peripheral blood leukocyte count was $10.40x10^9$/L, C reactive protein 18.8 mg/dl (normal <0.5 mg/dl), and the ESR was 57 mm/hour. Hyperglycemia and slightly increased creatinin levels were found. On superficial USG, there were two loculated collections compatible with the thick-walled abscess formation. The first one was 6.5x2 cm subcutaneous along the superficial muscle groups, and the other was 4.5 x 1.5 cm deep in the posterior region. Although there was bone marrow edema on magnetic resonance imaging (MRI) osteomyelitis was not reported by radiology. Drainage of abscess was not indicated by orthopedicians at that time. The culture of pus aspirate was taken from the wound after skin was decontaminated. Subsequently, treatment with intravenous daptomycin (1 x 350 mg) was initiated.

Wound culture yielded non-pigmented, non-hemolytic small colonies on 5% sheep-blood agar plates (Figure 1). Gram positive cocci were observed. Catalase reaction and tube coagulase results were positive. Colonies were suspected to be *S. aureus* SCVs. Isolates were inoculated onto mannitol salt agar (MSA) (Becton, Dickinson and Company, USA), and mannitol positive yellow colonies were observed after incubation at 35°C overnight (Figure 1). These colonies were inoculated onto sheep blood agar and Schaedler agar (ORBAK, Ankara, Turkey) simultaneously. Sheep blood agar was incubated in normal atmosphere and Schaedler agar in 5-10% CO_2, both at 35°C. The small, non-pigmented, non-hemolytic colonies on sheep blood agar were observed as normal sized, hemolytic and pigmented on Schaedler agar, and they are considered as *S. aureus* SCVs. Antimicrobial susceptibility testing was performed by broth microdilution method according to CLSI guidelines.[8] The isolate identified as MRSA SCV was resistant to penicillin, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, oxacillin, cefoxitin, tetracycline, and rifampin; intermediate for ciprofloxacin; susceptible to gentamicin, imipenem, moxifloxacin, erythromycin, clindamycin, trimetoprim-sulphametoxazole, vancomycin, teicoplanin, linezolid, and tigecycline.

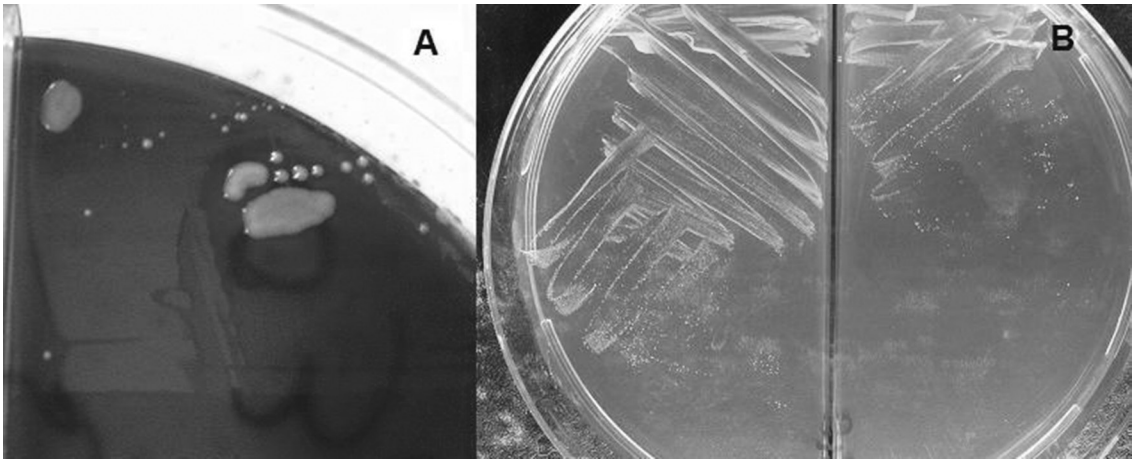

Figure 1. Nonpigmented, non-hemolytic, small colonies suspected as *S. aureus* SCVs on blood agar plates (A), and on mannitol salt agar (MSA) as mannitol positive after subculturing (B)

After re-evaluation of wound region, drainage was performed by orthopedics on the 21st day of treatment and there was no growth on cultures of aspiration material. Wound care including debridement and daily dressing were performed by orthopedics. There was bone marrow edema on MRI and recurrent abscess in his history; so osteomyelitis could not be excluded. Consequently scintigraphic

imaging findings supported ostemyelitis and therefore osteomyelitis treatment was administered to the patient. Daptomycin was stopped on the 60th day of treatment. The patient was recovered, and he was discharged.

DISCUSSION

To our knowledge, this is the first report of soft tissue abscess caused by small colony variant of MRSA in a patient with recurrent abscess history after fracture surgery. However, MRSA SCV was isolated only in the last episode. There are only few case reports of MRSA SCVs in the literature. The first fatal case due to MRSA SCVs was an AIDS patient and the isolate has been recovered from blood cultures and abscess samples after long-term prophylaxis of TMP-SXT for *Pneumocystis jirovecii* pneumonia.[9] In other two reports MRSA SCVs was identified as the cause of recurrent ventriculoperitoneal shunt–related meningitis and brain abscess respectively.[6,10] Proctor et al described a case of septic arthritis arising from hip prosthesis that blood cultures revealed MRSA SCVs and persisted for 38 days.[7]

S. aureus SCVs have the ability of persisting under antibiotic pressure. Exposure to different classes of antibiotics may cause the selection of these variants both in vitro and in vivo, and the diagnosis and treatment becomes difficult consequently.[11] *S. aureus* SCVs may also have decreased susceptibility to cell-wall-active antibiotics.[7] In this case, unfortunately, the antibiotic treatment history of the patient during previous abscess formations was unavailable. Amoxicillin-clavulanic acid was used by orthopedics in the last episode but, abscess formation was not resolved.

S. aureus SCVs may be protected from host defenses and the effect of antibiotics as they could persist intracellularly. Therefore, antimicrobial agents such as rifampin having intracellular activity should be used in the treatment of *S. aureus* SCV infections.[10] It was shown in a tissue culture system that rifampin combined with TMP-SXT was the most active therapeutic regimen, but more research is necessary to clarify the optimal treatment for infections caused by *S. aureus* SCVs.[12] A combination of rifampin and a fluoroquinolone was also proposed, but it was not adequate for MRSA which are often resistant to fluoroquinolones.[13,14] Vancomycin must be added to the regimen if SCV is a MRSA.[15] Thus, treatment with a combination vancomycin and rifampin which has been shown to be intracellularly active is a proper regimen for MRSA SCV. In this patient, *S. aureus* SCV was methicillin resistant and also resistant to some other antibiotics including rifampin. Creatinine levels were increased in the patient thus, vancomycin was not used, and treatment was carried out with intravenous daptomycin for 60 days. Daptomycin was reported to be a potential therapeutic option for infections caused by *S. aureus* SCVs in some experimental studies.[6-19] Also prolonged high doses of daptomycin have been used for a prosthesis joint infection caused by *S. aureus* SCV and the patient have been treated successfully.[20] However additional clinical studies are needed for the antimicrobial activity of daptomycin against *S. aureus* SCVs.

In case of samples sent from patients who received antibiotic treatment for a long period, clinical microbiology laboratories should be on alert of *S. aureus* SCVs, and proper methods should be used to detect SCVs. When there is an infection resistant to treatment, persistent or not respond to proper antimicrobial therapy *S. aureus* SCVs should be considered. In these situations, clinicians should ask the clinical microbiology laboratory to search for S aureus SCVs. Identification of MRSA SCVs might have an impact on the selection of treatment regimens as MRSA SCVs tend to resist intracellular killing and are more resistant to antibiotics.

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