

Case Report

A case report of Small Colony variant of *Staphylococcus aureus* isolated from a patient with chronic osteomyelitis in a tertiary care hospital of eastern India

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Abstract Small colony variants (SCVs) of *Staphylococcus aureus* often cause persistent and relapsing infections. SCVs are characterized by a strong reduction in growth rate, atypical colony morphology and unusual biochemical characteristics. We here report a case of chronic osteomyelitis caused by SCV of *Staphylococcus aureus* in a middle aged male patient.

Key words: Chronic osteomyelitis, small colony variants, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus aureus small colony variants (SCVs) have been associated with relapsing infections in bone, heart valves, lung and soft tissues.^[1] SCVs are characterized by low metabolism and almost no cell wall synthesis due to a defect of electron transport system. They are usually resistant to aminoglycosides and cell wall active antibiotics. Because of atypical colony morphology and unusual biochemical profile diagnosis is often delayed.^[2] Infections SCVs of *S.aureus* is an emerging problem owing to diagnostic difficulty and refractoriness to therapy. We here report a case of post traumatic chronic osteomyelitis due to SCVs form of *S.aureus* in eastern India.

CASE REPORT

A 46 years old man had a closed proximal left humeral fracture following a road traffic accident one and half year back. After that he was referred to our hospital where fixation of fractured fragments was done by internal screw fixation followed by pre-emptive treatment with cefoperazone-sulbactam combination and ceftazidime for 10 days. The patient was initially fine for two weeks after that he suffered from persistent pain over affected region and six months later a sinus tract developed along with purulent discharge. [Figure 1] The patient received various combinations of antimicrobials (mainly second and third generation cephalosporins) to control the disease. Conventional radiographs showed subperiosteal thickening in earlier stage. Bone scintigraphy showed increased uptake in the humeral diaphysis. Laboratory investigations revealed hemoglobin 12 mg/dl, leucocyte count $12 \times 10^3/\text{cmm}$, erythrocytic sedimentation rate 30 mm/1st hr, random plasma glucose level 130 mg/dl and CRP 180 ug/ml. The expressed pus from the sinus tract was aseptically collected. The direct gram stained smear

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of collected pus showed gram positive cocci (GPC) arranged in small clusters. The other part of collected sample seeded on blood agar (BA) and McConkey's agar (MA) plate and both the plates were incubated aerobically at 37°C for 48 hours. After 48 hours of incubation non-pigmented small 0.1 mm diameter non haemolytic colonies grew on BA plate. [Figure 2] MA plate showed no growth. The colonies were further sub cultured on Nutrient Agar (NA) and grew as small non-pigmented colonies. Gram stained smear from colony of BA and NA revealed GPC arranged in small clusters. The colonies were catalase positive and oxidase negative. Slide coagulase test from the isolated colonies was negative and tube coagulase test was positive after 18 hours. Antibiotic susceptibility test result showed the isolated strain was only susceptible to vancomycin and resistant to oxacillin, azithromycin, co-trimoxazole, and cefpodoxime. [Figure 3] A lawn culture was put on NA plate along with 20 ug and 40 ug menadione impregnated discs over it. The colonies around menadione discs grew as large colonies thereby confirming menadione auxotrophy. Subculture from this large colony revealed yellow pigmented normal *Staphylococcal* colony. [Figure 2b] These large colonies were catalase, coagulase (slide and tube) tests positive and oxidase negative.

DISCUSSION

SCVs constitute a slow growing subpopulation of bacteria with distinctive phenotypic and pathogenic characteristics. They cause persistent infections with long standing predisposing conditions cystic fibrosis and osteomyelitis. SCVs have a slow growth rate, atypical colony morphology, and unusual biochemical characteristics.^[2] They are usually auxotrophic for Vitamin K, Thymidine, and Haemin and usually lacking in essential component of electron transport chain.^[3] Hence they are resistant to aminoglycosides and other cell wall active antimicrobials. In addition *S. aureus* SCVs have been found to present inside of cultured endothelial cell, probably because these variant produce very little α -toxin.^[3] The intracellular location may shield SCVs from phagocytosis. Diagnosis usually achieved by reversal to wild type *Staphylococcus aureus* colonies in presence of vitamin K, resistance to co-trimoxazole (auxotrophocity for thymidine) and increased size and growth around haemin or factor X discs.^[4]

There are a few reports of SCVs from clinical specimens. One report mentions isolation of SCVs from pacemaker related endocarditis from Germany.^[5] Von Eiff and colleagues recently reported few cases of chronic osteomyelitis due to SCVs of *Staphylococcus aureus* in patient who had received gentamicin beads as an



Figure 1: Discharging sinus of chronic osteomyelitis



Figure 2: Blood agar plate showing (a) small colony variant (b) normal variant of *Staphylococcus aureus*

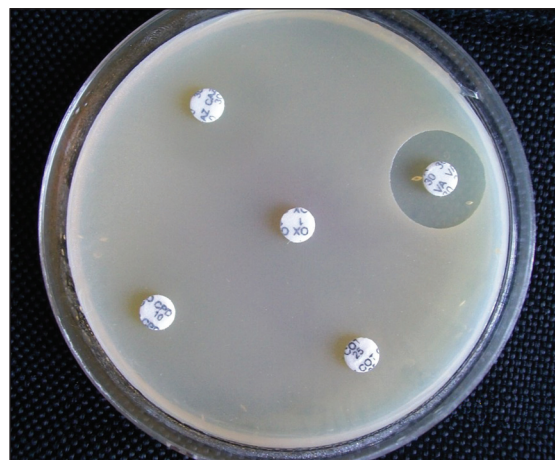


Figure 3: Antibiotic susceptibility test result of small colony variant of *S. aureus*

adjunct to surgical therapy for osteomyelitis.^[6] Sendi *et al.* describes few cases of prosthetic joint infection with small colony variants (SCVs) of *Staphylococcus aureus*.^[7] But in our best knowledge there is no such

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reported case in eastern part of India. One interesting finding in our case that the patient had not received any form of gentamicin therapy either systemic form or local gentamicin bead therapy. Laboratories should be particularly cautious for *S. aureus* small colony variants when samples are submitted from patients who have received long-term antimicrobial therapy, especially if the infection is persistent or recurrent. In our case, findings suggest that *S. aureus* small colony variants might have been selected from the parent strain population with a normal phenotype after prolonged use of antimicrobial particularly cell surface active one.

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