Fatal Case Due to Methicillin-Resistant *Staphylococcus aureus* Small Colony Variants in an AIDS Patient

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We describe the first known case of a fatal infection with small colony variants of methicillin-resistant *Staphylococcus aureus* in a patient with AIDS. Recovered from three blood cultures as well as from a deep hip abscess, these variants may have resulted from long-term antimicrobial therapy with trimethoprim/sulfamethoxazole for prophylaxis of *Pneumocystis carinii* pneumonia.

Staphylococcus aureus causes acute and often fatal infections. Small colony variants (SCVs), which are subpopulations of *S. aureus,* are implicated in persistent and recurrent infections (in particular osteomyelitis, septic arthritis, respiratory tract infections in patients with cystic fibrosis, and deep-seated abscesses) (1-4). These phenotypic variants produce small, slow-growing, nonpigmented, nonhemolytic colonies on routine culture media, making correct identification difficult for clinical laboratories. Biochemical characterization of these variants suggests that they are deficient in electron transport activity (5).

We report a fatal case of a persistent deep-seated hip abscess due to methicillin-resistant *S. aureus* SCVs that led to osteomyelitis and bloodstream infection in a patient with AIDS.

Case Report

A 36-year-old man with AIDS came to the Cologne University Hospital, Cologne, Germany, in June 1997 with fever and progressive pain (of 6 weeks duration) in his right hip. HIV infection had been diagnosed in 1986. In 1994, his CD4 cell count was 250/µL, and oral zidovudine therapy was started. His medical history included *Pneumocystis carinii* pneumonia, pulmonary tuberculosis, and recurrent oral thrush; his medication included zidovudine, lamivudine, fluconazole, and trimethoprim/sulfamethoxazole. In September 1996, he was in a traffic accident and had severe cerebral trauma resulting in spastic hemiparesis with occasional seizures. After an intramuscular injection 2 months before admission, pus was surgically drained to treat recurrent abscesses of his right hip. Specimens for culture were not obtained.

Physical examination found limited mobility of his right thigh and a tender, nondraining scar at the site of surgical drainage. Neither warmth nor swelling was observed over his right hip. Vital signs were temperature, 38.2°C; respiration rate, 28; and heart rate, 108. He was awake and alert and had spastic paresis in his right arm.

Laboratory studies performed on admission showed hemoglobin, 10.8 g/dL; leukocyte count, 3,000/µL with a normal differential; CD4 cell count, 20/µL; platelet count, 131,000/µL; C-reactive protein, 184 mg/L; and alkaline phosphatase, 1490 U/L. Radiographs of the chest and a plain film of the pelvis were normal. A triple-phase bone scan showed an area of minor tracer accumulation in the acetabulum region of the right hip. Blood cultures were drawn, but antimicrobial therapy was withheld until culture results became available.

On hospital day 2, one of two blood cultures drawn on admission yielded nonhemolytic staphylococci that were clumping factor–negative. The organisms were initially misidentified as coagulase-negative staphylococci and were considered contaminants.

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Empiric antistaphylococcal therapy with clindamycin (600 mg q8hr) was instituted. On hospital day 4, two sets of blood cultures obtained on hospital day 2 yielded phenotypically identical organisms, which on the basis of a positive tube coagulase test were identified as oxacillin-resistant *S. aureus*. The colony morphology was suggestive of an SCV of *S. aureus*. The patient was started on parenteral vancomycin treatment (1 g q12hr). However, his condition deteriorated rapidly, and he died of refractory septic shock 6 days after admission.

Autopsy showed a large (12 x 10 x 8 cm), deep-seated abscess of the right hip and osteomyelitis of the ischial tuberosity. Both SCVs and typical large colony forms of *S. aureus* were cultured from postmortem specimens of the abscess and the bone.

Findings

S. aureus SCVs were recovered from one of two blood culture sets obtained on admission and from two of four blood culture sets obtained on hospital day 2. Growth was not detected until the blood culture bottles had been incubated 24 hours. *S. aureus* with a normal phenotype was recovered from nose and throat specimens but not from blood cultures, whereas both SCVs and typical *S. aureus* phenotypes were isolated from the deep hip abscess (Figure 1) before death, as well as from a postmortem specimen. All isolates were clumping factor–negative but showed a delayed positive reaction in the tube-coagulase test at 24 hours. The results of the ID 32 staph test did not unambiguously identify SCVs as *S. aureus* because the tests for urease and trehalose were negative. Both the *nuc* gene and the *coa* gene were identified by polymerase chain reaction (PCR) amplification. Methicillin resistance was confirmed for both small and large colony forms by PCR amplification of the *mecA* gene.

When cultured without supplementation, all SCVs were nonpigmented and nonhemolytic. Supplementation with hemin, thymidine, or menadione identified two SCVs showing thymidine auxotrophy and a combined thymidine and menadione auxotrophy, respectively. All SCVs were stable on repeated subculturing.

Epidemiologic typing by PCR analysis of inter-IS256 spacer length polymorphisms (Figure 2) and pulsed-field gel electrophoresis of genomic DNA (data not shown) showed identical banding patterns for both SCVs and large colony forms, which indicates that the phenotypically different *S. aureus* isolates represented a single strain. Antimicrobial susceptibility testing was performed by microbroth dilution, according to the National Committee for Clinical Laboratory Standards guidelines. Susceptibility to trimethoprim/sulfamethoxazole was tested with Etest (AB Biodisk, Solna, Sweden). In contrast to current standards, the MICs for SCVs were determined after 48 hours of incubation at 35°C. Susceptibility testing showed that all *S. aureus* isolates were resistant to penicillin (MIC, >8 µg/mL), ampicillin (MIC, >32 µg/mL), oxacillin (MIC, >8 µg/mL), erythromycin (MIC, >32 µg/mL), clindamycin (MIC, >32 µg/mL), ciprofloxacin (MIC, >8 µg/mL), gentamicin (MIC, >500 µg/mL), and trimethoprim/sulfamethoxazole (MIC, >32 µg/mL) and susceptible to vancomycin

Figure 1. *Staphylococcus aureus* small colony variants (A) and *S. aureus* with a normal phenotype (B) cultured on sheep blood agar after 24 hours of incubation at 35°C. Staphylococci were identified by conventional methods (6) and with the ID 32 Staph system (bioMérieux, Marcy-L'Etoile, France) following the instructions of the manufacturer. The tube-coagulase test was read after 24 hours. *S. aureus* isolates were characterized as small colony variants as described before (7-8). Auxotrophic requirements were evaluated with 10-µg hemin disks, 1.5-µg menadione disks, and 1.5-µg thymidine disks on Mueller-Hinton agar and on chemically defined medium (CDM) agar as well as on CDM agar supplemented with 1 µg/mL hemin, 100 µg/mL thymidine, and 1 µg/mL menadione, respectively.

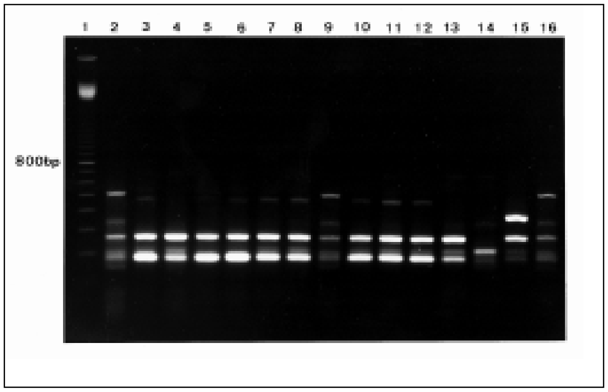

Figure 2. Fingerprint patterns obtained for *Staphylococcus aureus* small colony variants (lanes 3-5, bloodculture isolates; lanes 6 and 7, isolates from hip abscess; lane 8, postmortem specimen) and *S. aureus* isolates with a normal phenotype (lanes 10 and 11, isolates from nose and throat; lanes 12 and 13, isolates from hip abscess and postmortem specimen) after polymerase chain reaction (PCR) analysis of inter-IS256 spacer length showing identical strains. Lane 1, 100-bp ladder; lanes 2, 9, and 16, methicillin-resistant *S. aureus (*MRSA) reference strain; lanes 14 and 15, epidemiologically unrelated MRSA strains. Strain relatedness of all isolates with different colony morphologies and from different sources was analyzed by PCR analysis of inter-IS256 spacer length polymorphisms (9) and pulsed-field gel electrophoresis after *Sma*I restriction (8). Minor modifications included the use of brain heart infusion broth instead of trypticase soy broth to obtain sufficient growth of *S. aureus* small colony variants.

(MICs, 1-2 µg/mL), teicoplanin (MICs, 0.5-1 µg/mL), and quinupristin/dalfopristin (MICs, 0.5-1 µg/mL). No differences in MICs were observed between *S. aureus* SCVs and *S. aureus* isolates with normal phenotype.

To our knowledge, this case represents the first of a serious *S. aureus* infection in an AIDS patient in which all blood cultures yielded SCVs. The SCVs' unusual morphologic appearance and slow growth delayed the correct identification of these organisms as *S. aureus*. The empiric antimicrobial regimen in our patient did not include a glycopeptide, because of the low rate of methicillin resistance in community-acquired *S. aureus* infection in Germany. Appropriate antistaphylococcal therapy was, therefore, not started until hospital day 4. Delayed antimicrobial therapy on day 4 rather than on day 2 may have contributed to the patient's death.

Proctor and colleagues recently reported five cases in which SCVs of *S. aureus* were implicated in persistent and relapsing infections. They identified only a single case reported in the previous 17 years and ascribed this to insufficient ability of laboratories to identify these organisms (8). In most cases, patients had received antibiotics. Aminoglycoside treatment may have selected for *S. aureus* SCVs (10), and in cases of osteomyelitis or deep-seated abscesses, persistence of these variants in the intracellular milieu may have permitted evasion of host defenses and allowed for the development of resistance to antimicrobial therapy (7,11). Von Eiff and colleagues recently reported four cases of chronic osteomyelitis due to SCVs of *S. aureus* in patients who had received gentamicin beads as an adjunct to surgical therapy for osteomyelitis (2). Kahl et al. described persistent infection with *S. aureus* SCVs in patients with cystic fibrosis (4). All these patients had received long-term trimethoprim/sulfamethoxazole prophylaxis. It may be tempting to speculate that administration of trimethoprim/sulfamethoxazole for prophylaxis against *P. carinii* pneumonia may have selected for SCVs within the patient's large hip abscess. Further prospective studies are needed to assess the role of *S. aureus* SCVs in HIV-infected patients on long-term antimicrobial therapy.

Dr. Seifert is assistant professor at the Institute of Medical Microbiology and Hygiene, University of Cologne, Germany. His research interests include the molecular epidemiology of nosocomial pathogens, in particular *Acinetobacter* species, catheter-related infections, and antimicrobial resistance.

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