MAJOR ARTICLE

Staphylococcus aureus Small Colony Variants in Prosthetic Joint Infection

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(See the editorial commentary by Vaudaux et al. on pages 968–70)

Background. Small colony variants of *Staphylococcus aureus* tend to persist despite antimicrobial therapy, especially when involved in implant-associated infections.

Methods. We analyzed 5 cases of hip prosthesis–associated infections due to small colony variants, including their course prior to identification of the pathogen. Biopsy investigations included microbiological examination and, in 1 case, transmission electron microscopy to detect intracellular bacteria in nonprofessional phagocytes. A treatment concept was elaborated on the basis of a published algorithm and patients were managed accordingly.

Results. The patients' mean age was 62.2 years. All patients experienced treatment failures prior to isolation of small colony variants, despite as many as 3 surgical revisions and up to 22 months of antibiotics. Transmission electron microscopy performed on biopsy specimens from periprosthetic tissue revealed intracellular cocci in fibroblasts. All prostheses were removed without implanting a spacer, and antimicrobial agents were administered for 5.5–7 weeks. Reimplantation of the prosthesis was performed for 4 patients. Follow-ups were uneventful in all 5 cases.

Conclusions. In the case of a poor response to adequate antimicrobial and surgical treatment in implant-associated staphylococcal infections, small colony variants should be considered and actively sought. In our case series, a 2-stage exchange without implantation of a spacer combined with antimicrobial therapy for an implant-free interval of 6–8 weeks was associated with successful outcome, with a mean follow-up of 24 months.

Small colony variants (SCVs) are naturally occurring subpopulations of *Staphylococcus aureus* and are associated with persistent or recurrent infection. They differ from normal-phenotype *S. aureus of* not only in their small colony sizes (because of their slow rate of growth) but also by decreased pigmentation and hemolysis. These characteristics are based on auxotrophisms for thymidine, menadione, or hemin, which lead to a deficiency in electron transport [1]. These variants are difficult to treat because of their increased resistance to aminoglycosides and to cell-wall–active antibiotics, as well as their ability to persist. To date, intracellular persistence of SCVs has been demonstrated in vitro but

not ex vivo [2]. Because SCVs are often relatively unstable and can revert to the highly virulent and rapid-growing form, these variants are difficult to identify.

SCVs have been described in cases of sepsis [3, 4], cystic fibrosis [5, 6], soft-tissue infection [2, 3, 7, 8], osteomyelitis [3, 8–11], arthritis [8, 12], brain abscess [13], sinusitis [8], and in some foreign body–associated infections (including infections associated with osteosynthesis [14], pacemaker [15], and ventriculoperitoneal shunt [16]). To our knowledge, there have been no reports of SCVs associated with prosthetic joint infection (PJI); therefore, no treatment concepts have been recommended or tested.

We present 5 cases of PJI caused by SCVs, and we review their clinical courses before and after isolation of SCVs. We also investigated biopsy specimens obtained from the joint capsule of 1 patient by transmission electron microscopy for the intracellular presence of naturally occurring SCVs in nonprofessional phagocytes. The main aims of this study were to present a rational treatment concept for the management of SCVs in PJI using a previously published algorithm [17]

and to control the outcome of this management with regular follow-up visits.

PATIENTS AND METHODS

The 5 cases were identified among 83 PJIs (associated with 66 total hip and 17 total knee arthroplasties) occurring in our institution from January 2002 through December 2005. Our orthopedic surgery clinic is a 48-bed unit that acts as a primary care center for all types of orthopedic surgery of the extremities, as well as a tertiary care center for patients needing revision arthroplasty.

Case history. Patient charts were reviewed to evaluate each patient's first infection period with *S. aureus,* including the location of primary focus, the presence of bacteremia, and the number and location of prosthetic joints and their involvement in the infection. The number of surgical revisions and the length of antibiotic therapy in months prior to the detection of SCVs were documented. The interval between completing antibiotic therapy and relapse of SCV infection was analyzed.

Identification of SCVs. Specimens were isolated from joint aspiration and/or intraoperative tissue and cultured on Columbia blood agar. SCVs were identified by the following phenotypical characteristics: (1) slow growth, (2) small colonies, (3) decreased pigmentation, and (4) weak hemolysis on Columbia blood agar. *S. aureus* species testing included the presence of growth on *S. aureus* ID agar, the use of the ID color catalase test Slidex Staph Plus (bioMérieux), and PCR for the *S. aureus*–specific gene *femA.*

Susceptibility testing. PCR for the *mecA* gene was performed to detect methicillin resistance [18]. MICs were examined using Etest (AB Biodisk) on Muller-Hinton and Muller-Hinton blood agar. The plates were incubated at 35°C and read after 48 and 72 h.

Genotypic analysis. *Sma*I digests of chromosomal DNA of SCVs and of the simultaneously occurring normal phenotype were analyzed by PFGE to prove clonal identity.

Ex vivo investigations for intracellular bacteria in periprosthetic tissue. In patient 5, 7 biopsy samples were obtained during removal of the prosthesis. Each biopsy sample was divided into 2 parts: 1 part for microbiological and 1 part for histological investigation. Three biopsy samples obtained from the joint capsule grew SCV monocultures. Tissue samples were punched from the 3 corresponding histological paraffin blocks and exposed to a temperature of 60°C for 60 min. The specimens were first immersed in xylol (2 × 15 min) and absolute alcohol (2 × 15 min) and then in a descending alcohol series (90%, 70%, and 50% by volume; 15 min each). After tissues were washed in phosphate buffer solution, they were fixed with 1% OsO_4 solution for 60 min. Next, specimens were dehydrated in an ascending alcohol series (50%, 70%, and 90% by volume and absolute alcohol; 15 min each) and acetone (60 min). An infiltration process was performed first with a 1:1 mixture of epon and acetone (60 min) and then with epon alone (240 min). Polymerization was allowed to continue for 24 h at a temperature of 60°C. Samples were then sliced with an ultramicrotome (Reichert-Jung Ultracut E). The 60- to 70-nm–thick slices were analyzed by transmission electron microscopy (FEI, Philips, Morgani 268D).

Treatment. Patient treatment was based on a previously published algorithm for difficult-to-treat microorganisms [17]. In this protocol, the infected prosthesis and all foreign material are completely removed without implanting a spacer, and meticulous debridement of the infected tissue is performed. To prevent soft tissue contraction, hip traction is performed with the aid of a Steinmann pin inserted through the distal end of the femur. During the implant-free interval, antibiotics are administered for 6 weeks, and reimplantation of the prosthesis is performed after 2 further antibiotic-free weeks. After reimplantation, the same antibiotic regimen administered during the implant-free interval is administered again, until newly obtained biopsies from the periprosthetic tissue are reported to be negative (normally after 14 days). In methicillin-susceptible staphylococcal infections, flucloxacillin is administered intravenously after removal of the prosthesis, but it is switched to a combination of rifampin plus levofloxacin within a few days. Rifampin has bactericidal activity against surface-adhering, slow-growing, and biofilm-producing microorganisms, but it should not be administered alone [19, 20]. Quinolones are excellent in combination with rifampin because of their bioavailability, antimicrobial activity, and tolerability [21]. In cases of resistance, decisions about antibiotics are made on an individual basis according to MICs.

Outcomes. Follow-up analyses included clinical examinations, laboratory testing (determination of C-reactive protein levels and WBC counts), and radiological examinations. Cure was defined as treatment failure–free survival for at least 24 months, and a probable cure was defined as treatment failure–free survival with a follow-up of <24 months [22].

RESULTS

Clinical course prior to isolation of SCVs. Data on clinical course prior to isolation of SCVs are presented in table 1. We identified 5 male patients who had total-hip arthroplasty–related infections caused by SCVs. Their mean age was 62.2 years (range, 51–71 years). Patients 2, 4, and 5 received bilateral prostheses and patients 1 and 3 each received a unilateral hip prosthesis. Clinical signs of sepsis and blood cultures positive for *S. aureus* were documented for patients 3, 4, and 5. Hence, patient 4 and 5 had bilateral hip prostheses and documented *S. aureus* bacteremia. In these 2 patients, both prostheses were involved in the infection.

In patient 4, the pathogen had seeded to the contralateral

Table 1. Clinical course prior to isolation of *Staphylococcus aureus* small colony variants.

Patient characteristic and treatment	Patient 1	Patient 2	Patient 3	Patient 4[a]	Patient 5[b]
Age, years	55	70	59	71	51
Side of hip prosthesis	Left	Both	Left	Both	Both
Focus of primary *Staphylococcus aureus* infection	Hip prosthesis	Left hip prosthesis	Hip prosthesis	Right hip prosthesis	Left hip prosthesis
Staphylococcus aureus bacteremia	No	No	Yes	Yes	Yes
Contralateral prosthesis involved	…	No	…	Yes	Yes
No. of surgical revisions performed	2	1	1	Right hip, 3; left hip, 5	0
No. of months receiving antibiotics	4	6	19	22	6
Antimicrobial agents	FLUCLOX, switched to CIP and RIF	FLUCLOX, switched to CIP and RIF	VAN and FEP, switched to CIP and RIF	CIP and RIF	CIP and RIF
Length of relapse-free interval after completing antibiotic therapy	3 months	3 months	Persistent infection	13.5 months	23 months

NOTE.   CIP, ciprofloxacin; FEP, cefepime; FLUCLOX, flucloxacillin; RIF, rifampin; VAN, vancomycin.

[a] Patient 4 suffered from prosthesis-associated infection of the left hip shortly after the primary infection (right hip prosthesis). The left side was surgically revised 5 times but, despite prolonged antimicrobial treatment (22 months), the infection persisted and was secondarily colonized with *Pseudomonas aeruginosa.* After a spacer-free, 2-stage exchange and antibiotic therapy during the implant-free interval, the infection was healed. Relapse of infection with small colony variants occurred at the primary (right) side of infection 13.5 months after completion of treatment for infection in the left side.

[b] Patient 5 was treated with a spacer-free, 2-stage exchange for prosthetic joint infection of the left hip. During the implant-free interval of 6 months, he received ciprofloxacin and rifampin; 23 months later, small colony variants were isolated from synovia of the previously inconspicuous right hip prosthesis.

hip prosthesis during bacteremia. The side on which primary infection occurred was considered to be healed after 3 surgical revisions, but infection persisted on the contralateral side for 22 months, despite 5 surgical revisions and antibiotic therapy. Healing was achieved after a spacer-free, 2-stage exchange, but this was followed by another relapse at the primary infection site 13.5 months later, where SCVs were finally isolated.

Patient 5 suffered from joint pain for several weeks prior to presenting with *S. aureus* sepsis due to PJI of the left hip. It is very likely that SCVs were seeding from the primary focus of infection to the right hip prosthesis during the bacteremia [23]; in the absence of symptoms, however, joint puncture was not performed, and 18 months later, the patient suffered from joint pain of the right hip. Finally, SCVs were isolated from this hip prosthesis, 5 months later and 23 months after completion of therapy for infection in the left hip.

Treatment prior to isolation of SCVs.   Patients experienced up to 3 surgical revisions on the side of SCV isolation and up to 22 months of antimicrobial treatment. All patients ultimately received ciprofloxacin and rifampin; prior to receiving this regimen, 2 patients were treated with flucloxacillin, and 1 received vancomycin plus cefepime.

Relapse-free interval.   In patients 1 and 2, relapse occurred 3 months after completing therapy. Patient 3 experienced persistent infection; several attempts to withdraw antibiotics prompted flare ups, and he was, therefore, treated with suppressive therapy for 19 months. As outlined above, patient 4 experienced relapse after 13.5 months, and patient 5 experienced relapse 23 months after completing therapy for the contralateral hip prosthesis infection.

The characteristics of isolated microorganisms are shown in table 2. Joint puncture was performed prior to surgery in 4 patients; in 3 of these, SCVs were detected in synovial culture.

Culture results.   SCVs grew on Columbia blood agar after 2–3 days, revealing small colonies, decreased pigmentation, and weak hemolysis. Mixed cultures of normal-phenotype *S. aureus* and SCVs were present in patients 1–3. For patients 4 and 5, SCV monocultures grew in synovial cultures, but cultures of biopsy specimens taken during subsequent surgery showed both SCV and normal-phenotype *S. aureus.* On *S. aureus* ID agar, pathogens grew either as SCVs or as the normal phenotype after 24 h of incubation.

Susceptibility testing.   All pathogens were methicillin susceptible and lacked the *mecA* gene. Two strains were resistant to trimethoprim-sulfamethoxazole without previous treatment with this compound (patients 1 and 2). One SCV subculture was resistant to rifampin (patient 5), and 1 was resistant to both ciprofloxacin and rifampin (patient 4).

Genotypic analysis.   PCR for the *femA* gene was positive in all cases. PFGE demonstrated the clonal identity of the normal phenotype and SCVs (figure 1) in all cases. In patient 1, clonal identity was additionally shown for the primary infection; in the other 4 patients, pathogens from primary infections were not available.

Transmission electron microscopy.   Investigations of biopsy specimens obtained from patient 5 showed the presence of intracellular bacteria in nonprofessional phagocytes (figure 2). These cells were identified as fibroblasts.

Treatment.   After removal of the prosthesis, a spacer was implanted in 2 patients, because SCVs either had not been

Table 2. Characteristics of *Staphylococcus aureus* small colony variants (SCVs) and treatment of patients.

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Growth on Columbia blood agar, days	NA[a]	2	2	3	2
Method of detection	Joint puncture	Biopsies	Biopsies[b]	Joint puncture	Joint puncture
Monocultures of SCVs	No	No	No	Yes[c]	Yes[c]
Presence of *femA* gene	Yes	Yes	Yes	Yes	Yes
Clonal identity with normal phenotype by PFGE	Yes	Yes	Yes	Yes	Yes
Presence of *mecA* gene	No	No	No	No	No
Susceptibility testing by Etest, mg/L					
Penicillin	NA	NA	0.03 (S)	0.25 (S)	0.06 (S)
Oxacillin	0.06 (S)	0.25 (S)	0.125 (S)	0.125 (S)	NA
Ciprofloxacin	0.015 (S)	1.0 (S)	0.5 (S)	4.0 (R)	0.5 (S)
Rifampin	0.015 (S)	0.25 (S)	0.25 (S)	>32 (R)	>32 (R)
Trimethoprim-sulfamethoxazole	>32 (R)	>32 (R)	0.06 (S)	0.5 (S)	0.06 (S)
Removal of implant	Yes	Yes	Yes	Yes	Yes
Antimicrobial agents administered during implant-free interval	FLUCLOX and RIF	LVX and RIF	FLUCLOX, switched to LVX and RIF	FLUCLOX	PEN and LVX
Duration of treatment, weeks	6	7	5.5	6	6
Antibiotic-free interval prior to reimplantation, days	3	15	…[d]	10	14
Reimplantation	Yes	Yes	No	Yes	Yes
Follow-up, months	48	13	24	24	12
Outcome	Cured	Probably cured	Cured	Cured	Probably cured

NOTE. FLUCLOX, flucloxacillin; LVX, levofloxacin; NA, not available; PEN, penicillin; R, resistant; RIF, rifampin; S, susceptible.

[a] After thawing the frozen strain, SCVs grew after 2 days.
[b] Joint puncture was not performed.
[c] Monoculture grew in cultures of synovia. Biopsies obtained during subsequent surgery revealed both phenotypes.
[d] Patient refused reimplantation of the prosthesis.

isolated by preoperative joint puncture (patient 2) or were not suspected (patient 3). After detection of SCVs from periprosthetic tissue, the spacers were removed in both cases in a second intervention. Antimicrobial treatment was selected according to susceptibility testing results and, if possible, a combination of levofloxacin plus rifampin was administered. Duration of therapy ranged from 5.5 to 7 weeks. The antibiotic-free interval prior to reimplantation of the prosthesis ranged from 10 to 14 days in 3 patients. In 1 patient, reimplantation of the prosthesis was performed after 3 days. One patient refused reimplantation of the prosthesis because of fear of reinfection. None of the patients showed signs of infection during their postoperative course. Duration of follow-up ranged from 12 to 48 months, with a mean duration of 24 months. According to study definitions, 3 patients were cured, and 2 patients were probably cured.

DISCUSSION

To our knowledge, this is the first report of SCVs associated with PJI. The ratio of SCV infections to all infections in patients with total hip arthroplasty was 5:66 during a 4-year period. The interpretation of this rate is difficult, because 4 of the 5

patients were transferred to our tertiary care center after relapse occurred or when infection persisted. However, it is conceivable that SCVs are underestimated as the causative agent in relapsing or persistent *S. aureus* infections. Several observations that we made with this case series may allow a faster diagnosis and a more efficient treatment of similar cases.

On the basis of the isolation of normal-phenotype *S. aureus* from the primary PJI, all patients were initially administered the correct treatment according to our previously published algorithm [17]. Patients 1–4 experienced a short duration of clinical symptoms (≤3 weeks) and underwent debridement and adequate antimicrobial treatment; patient 5 presented with a long duration of clinical symptoms (>3 weeks) and was, therefore, treated with a 2-stage exchange. Nevertheless, in all cases, staphylococcal infection persisted or relapse occurred—either on the side of primary infection or on the contralateral side—until SCVs were isolated. This emphasizes the hypothesis that SCVs should be considered as difficult-to-treat microorganisms and, therefore, treated differently than normal-phenotype *S. aureus*. Moreover, in the case of persistence or relapse, one must actively look for SCVs; a lack of awareness led to ineffective surgical revisions and antimicrobial treatment in at least
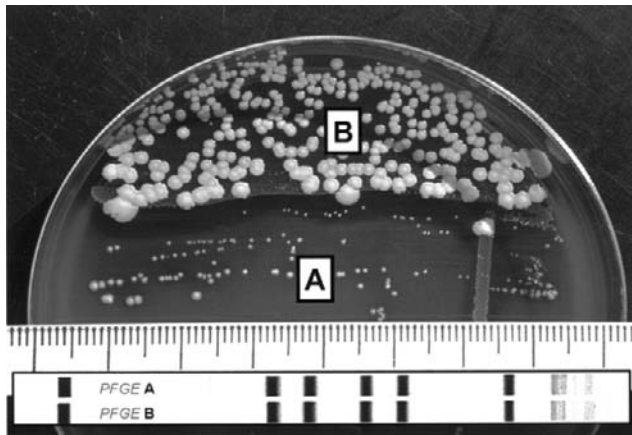
Figure 1. Small colony variant *(A)* and normal-phenotype *Staphylococcus aureus (B)* isolated from patient 1 on Columbia blood agar.

2 of our cases. In patients 1 and 2, unexpected treatment failure prompted a rapid search for SCVs and the cessation of further inadequate therapy.

Because SCVs are difficult to treat, all involved foreign material must be removed. SCV *hemB* mutants have been shown to have a significantly higher rate of adhesion to fibrinogen and fibronectin than their normal-growing parental strains, and they are much more resistant to antibiotics when they adhere to an implant [24]. When SCVs reach a stationary phase after adherence, they are particularly resistant to antibiotic killing [25]. Therefore, and in contrast to PJI due to normal-phenotype *S. aureus,* a spacer should not be implanted. If SCVs are found growing in periprosthetic tissue after a spacer has been implanted, the spacer should be removed. Extensive tissue debridement is, therefore, required, because SCVs have the ability to persist intracellularly and also may adhere to sequesters. To our knowledge, the intracellular persistence of SCVs has been shown only in vitro [2]. A recently published study demonstrated the in vivo intracellular persistence of normal-phenotype *S. aureus* in nasal mucosa cells [26]. Our ex vivo investigation of periprosthetic tissue very likely revealed the intracellular persistence of SCVs, and, therefore, adds significant information to previous in vitro findings. However, because our investigations were conducted with biopsy specimens obtained from just 1 patient and because control biopsy specimens with normal-phenotype *S. aureus* are missing, more studies are needed for in vivo confirmation of published in vitro results [2]. Nevertheless, the intracellular persistence of bacteria in fibroblasts was clearly shown, underlining the importance of meticulous debridement of infected tissue in addition to antimicrobial treatment.

Decisions regarding antimicrobial agents were based on MIC testing, because antibiotic disk diffusion tests are known not to be reliable [27–29]. Although Etests are less reliable than
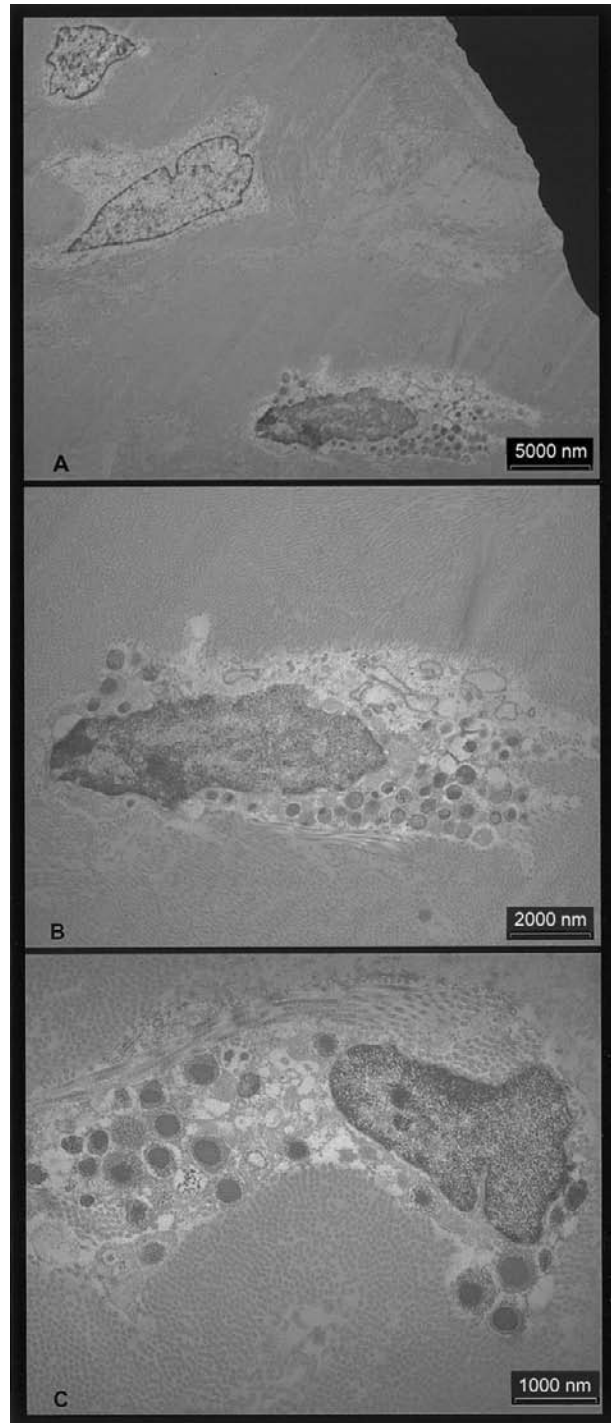

Figure 2. Transmission electron micrography of a sample obtained from the hip joint capsule of patient 5 revealing 3 fibroblasts surrounded by collagen fibers *(A)*. Intracellular cocci are shown in the lower cell of panel *A* and in an enlarged image in panel *B*. Intracellular cocci in a fibroblast are shown in panel *C*.

broth or agar dilution methods in certain cases [1, 27, 30], they have been used by others to examine MICs of SCVs [4, 6, 16]. For methicillin-susceptible strains of SCVs, Etests are a particularly valuable option, because the concentration gradient of the antimicrobial agent remains stable for several days, and comparisons of Etests and agar dilution tests have shown a good correlation for SCVs [6] and other fastidious and slow-growing bacteria, such as *Mycobacterium* species [31, 32]. Interestingly, 2 pathogens were resistant to trimethoprim-sulfamethoxazol without previous exposure. Thymidine-dependent SCVs have been shown to be resistant to this compound [30, 33], but because auxotrophism tests could not be performed in these cases because of reversion, this remains a speculation. One SCV's subculture revealed resistance to rifampin, and 1 revealed resistance to both ciprofloxacin and rifampin. SCV *hemB* mutants are known to acquire resistance to these agents in the same manner as normal phenotypes (i.e., via mutations in certain target genes) [34]. Astonishingly, patient 3 was exposed to ciprofloxacin and rifampin for 19 months, but resistance did not arise. However, the clinical courses in patients 4 and 5 indicate that a rifampin-containing regimen without appropriate surgical treatment does not eradicate SCVs and can result in the emergence of resistance. The optimal treatment for SCV infection is not known, but our decision to administer a combination of rifampin plus levofloxacin relied on the findings that the decreased electron gradient across the cell membrane of SCVs probably does not impair the efficacy of these agents [35]. For the resistant cases, β-lactams were administered, either in combination with levofloxacin or rifampin or alone.

In conclusion, treatment failure in cases of staphylococcal PJI despite correct treatment should prompt an active search for SCVs. This requires a good collaboration between orthopedic surgeons, infectious diseases specialists, microbiologists, and laboratory technicians. A 2-stage exchange without introduction of any foreign material, combined with antimicrobial treatment during an implant-free interval of 6–8 weeks, had a successful outcome, with a mean duration of follow-up of 24 months.

Acknowledgments

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