RESEARCH ARTICLE

Clinical Characteristics and Outcomes of Prosthetic Joint Infection Caused by Small Colony Variant Staphylococci

Aaron J. Tande,[a,b] Douglas R. Osmon,[a] Kerryl E. Greenwood-Quaintance,[b] Tad M. Mabry,[c] Arlen D. Hanssen,[c] Robin Patel[a,b,d]

Division of Infectious Diseases, Department of Internal Medicine,[a] Infectious Diseases Research Laboratory,[b] Department of Orthopedic Surgery,[c] and Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,[d] Mayo Clinic, Rochester, Minnesota, USA

ABSTRACT **Small colony variants (SCVs) are naturally occurring subpopulations of bacteria. The clinical characteristics and treatment outcomes of patients with prosthetic joint infection (PJI) caused by staphylococcal SCVs are unknown. This study was a retrospective series of 113 patients with staphylococcal PJI, with prospective testing of archived sonicate fluid samples. SCVs were defined using two-investigator review. Treatment failure was defined as (i) subsequent revision surgery for any reason, (ii) PJI after the index surgery, (iii) prosthesis nonreimplantation due to ongoing infection, or (iv) amputation of the affected limb. There were 38 subjects (34%) with SCVs and 75 (66%) with only normal-phenotype (NP) bacteria. Subjects with SCVs were more likely to have been on chronic antimicrobials prior to surgery ($P = 0.048$), have had prior surgery for PJI ($P = 0.03$), have had a longer duration of symptoms ($P = 0.0003$), and have had a longer time since joint implantation ($P = 0.007$), compared to those with only NP bacteria. Over a median follow-up of 30.6 months, 9 subjects (24%) with SCVs and 23 (32%) with only NP bacteria experienced treatment failure ($P = 0.51$). Subjects infected with *Staphylococcus aureus* were more likely to fail than were those infected with *Staphylococcus epidermidis* (hazard ratio [HR], 4.03; 95% confidence interval [CI], 1.80 to 9.04). While frequently identified in subjects with PJI and associated with several potential predisposing factors, SCVs were not associated with excess treatment failure compared to NP infections in this study, where they were primarily managed with two-stage arthroplasty exchange.**

IMPORTANCE **Bacteria with the small colony variant (SCV) phenotype are described in small case series as causing persistent or relapsing infection, but there are insufficient data to suggest that they should be managed differently than infection with normal-phenotype bacteria. In an effort to investigate the clinical importance of this phenotype, we determined whether SCVs were present in biofilms dislodged from the surfaces of arthroplasties of patients with staphylococcal prosthetic joint infection and assessed the clinical outcomes associated with detection of SCVs. We found that prosthetic joint infection caused by SCV staphylococci was associated with a longer duration of symptoms and more prior treatment for infection but not with an increased rate of treatment failure, compared to infection caused by normal-phenotype staphylococci.**

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Address correspondence to Aaron J. Tande, tande.aaron@mayo.edu.

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While prosthetic joint infection (PJI) occurs in <1.5% of cases (1), its associated morbidity, mortality, and economic cost render its successful treatment a priority. Staphylococci are the most frequent cause of PJI (2). While the likelihood of treatment success is dependent on many variables, it may be as low as 82% even with two-stage arthroplasty exchange (3).

Small colony variants (SCVs) are naturally occurring subpopulations of bacteria that differ from phenotypically normal bacteria in their small colony size and low growth rate (4). *In vitro* studies of laboratory-derived mutant staphylococci with an SCV phenotype have demonstrated increased intracellular persistence (5), attachment to fibrinogen or fibronectin (6), and expression of genes involved in biofilm formation (7). Biofilm formation is increased when SCVs are exposed to subinhibitory concentrations of aminoglycosides (8, 9). These data suggest that bacteria with the SCV phenotype may be adapted for chronic infections in which surface adherence and biofilm formation are important. However, it is not clear whether this applies to clinically isolated SCVs or what impact this might have on the management of patients with these infections.

Prior work suggests that SCVs are frequently identified in staphylococcal PJI (10–12) and osteomyelitis (13). While the clinical implications of this finding in terms of patient management have not been well defined, some experts suggest that infections with SCVs be managed differently than infections with normal-phenotype (NP) staphylococci (11, 14, 15). However, no study has adequately described the comparative treatment outcomes for these two groups of patients. Furthermore, most clinical laboratories do not routinely report the presence of SCVs.

The purpose of this study was to describe the clinical charac-

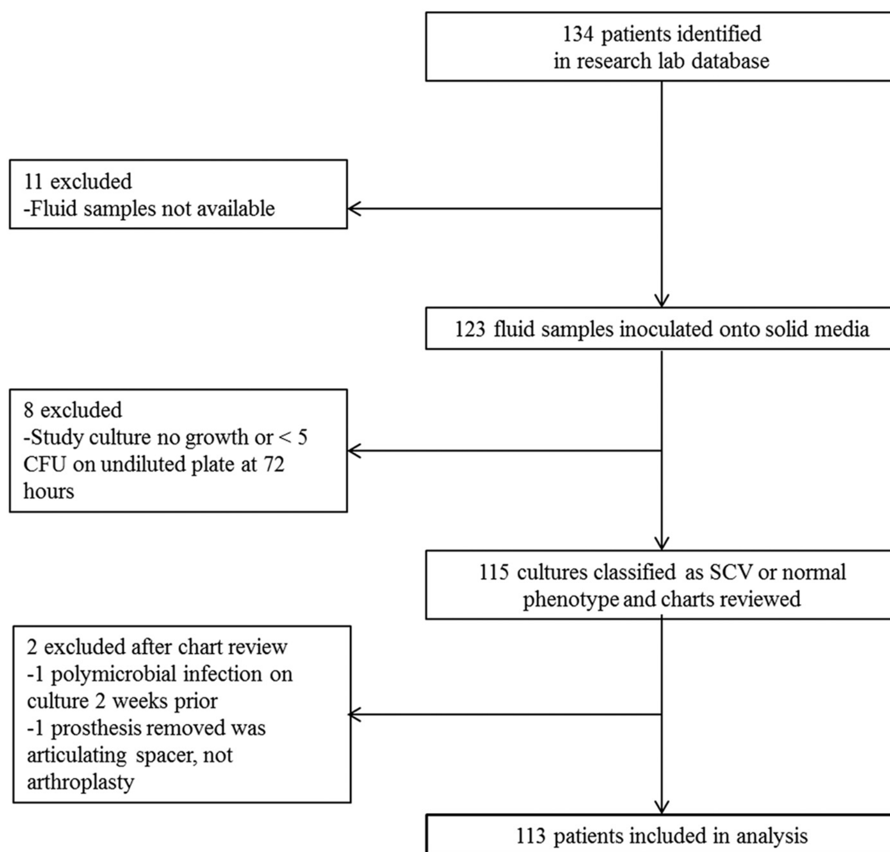

FIG 1 Flow chart of subjects evaluated for inclusion in this study.

teristics and outcomes of patients with PJI caused by staphylococcal SCVs, compared to those caused by only NP staphylococci. We also determined the frequency of isolation, antimicrobial susceptibilities, and auxotrophy (growth supplementation) requirements of SCVs. A full understanding of this could have a significant impact on current methods of detection of SCVs and clinical management of patients with infection caused by SCVs.

RESULTS

Description of the study participants. A total of 134 subjects with monomicrobial infection were identified from the sonicate fluid database. Twenty-one were excluded, as detailed in Fig. 1. The remaining 113 comprised the study population.

Medical history and PJI presentation. SCVs were found in 38 (33.6%) subjects, while only NP staphylococci were found in 75 (66.4%). There were no differences in the baseline medical characteristics of the two groups (Table 1). The majority of the PJI cases involved knee arthroplasties. Relative to the date that the isolates had been obtained, the median time elapsed since the initial joint implantation (1,295 versus 646 days, $P = 0.007$) and since last joint surgery (743 days versus 306 days, $P < 0.0001$) was longer in those with than without SCVs. Most PJI cases were chronic (>4 weeks), with subjects with SCVs having a longer median duration of symptoms than those without SCVs (491 versus 165 days, $P = 0.0003$). While there was no difference in history of prior revision for any reason, subjects with SCVs were more likely to have had a prior surgery for the current PJI episode (60.5 versus 37.3%, $P = 0.03$). Subjects with SCVs were also more likely to be on chronic antimicrobial agents before surgery than were those without SCVs (42.1 versus 22.7%, $P = 0.048$). Among the 33 subjects receiving chronic antimicrobial agents prior to surgery, the most frequently used classes of agents were tetracyclines ($n = 11$), β-lactams ($n = 7$), trimethoprim-sulfamethoxazole ($n = 4$), and fluoroquinolones ($n = 3$). Five subjects were receiving multiple antimicrobials. A numerically higher number of subjects with SCVs had an aminoglycoside in the cement, among the 59 subjects for whom full records were available.

PJI diagnosis. Diagnosis of PJI was based on identification of the same organism from two or more synovial fluid aspirates and/or operative tissue samples in 104 (92.0%) subjects, a single positive culture in combination with at least three supportive Musculoskeletal Infection Society (MSIS) criteria (16) in four (3.5%), and presence of a sinus tract in two (1.8%). The three remaining subjects had a non-sonicate-fluid single culture with coagulase-negative staphylococci with significant growth of an identical organism from sonicate fluid (>50 CFU/10 ml). These subjects also had acute inflammation on histopathology (two subjects) or were receiving chronic oral antimicrobials up until the time of surgery for infection with an identical organism (one subject).

Microbiologic identification. The most frequently identified species in both groups was *Staphylococcus epidermidis*, followed by *Staphylococcus aureus* (Table 2). The Staphaurex test (Thermo Scientific, Waltham, MA, USA) for *S. aureus* was falsely negative in

TABLE 1 Medical and orthopedic surgical history and PJI presentation[c]

Characteristic[d]	SCV[b]		P value
	Yes (*n* = 38)	No (*n* = 75)	
Demographic factors			
Age in yr, median (range)	64 (25–85)	63 (36–84)	0.49
Female sex	16 (42.1)	31 (41.3)	1
Diabetes mellitus	10 (26.3)	20 (26.7)	0.97
Rheumatoid arthritis by ACR criteria	1 (2.6)	5 (6.7)	0.66
Rheumatoid or inflammatory arthritis	3 (7.9)	11 (14.7)	0.38
CKD	2 (5.3)	6 (8.0)	0.72
Immunosuppressive therapy	4 (10.5)	12 (16.0)	0.57
Joint infected			0.98
Knee	25 (65.8)	46 (61.3)	
Hip	8 (21.1)	16 (21.3)	
Shoulder	3 (7.9)	8 (10.7)	
Elbow	2 (5.3)	5 (6.7)	
Orthopedic history			
Joint age in days, median (range)	1,295 (216–13,712)	646 (23–11,883)	0.007
Prior arthroplasty revision	32 (84.2)	52 (70.3)	0.17
Time since last surgery in days, median (range)	743 (31–10,030)	306 (20–8,686)	<0.0001
Cemented arthroplasty	33 (86.8)	60 (80.0)	0.44
Antibiotic-loaded cement in place[a]	8 (44.4)	16 (39.0)	0.78
Aminoglycoside in cement[a]	7 (38.9)	8 (19.5)	0.19
PJI history			
Sinus tract	11 (28.9)	19 (25.3)	0.82
Duration of PJI symptoms in days, median (range)	491 (14–2,306)	165 (2–1,656)	0.0003
Prior surgery for this PJI	23 (60.5)	28 (37.3)	0.03
Cumulative antibiotic days in prior 6 mo, median (range)	66 (0–180)	13 (0–180)	0.37
Receiving 120 or more days of antibiotics in prior 6 mo	16 (42.1)	17 (22.7)	0.048
Serum WBC in 10^9 cells/liter, median (range)	7.4 (4.5–11.7)	7.9 (3–23.3)	0.13
Serum ESR in mm/h, median (range)	46 (5–111)	43 (3–123)	0.54
Serum CRP in mg/liter, median (range)	23 (5–222)	44 (3–269)	0.2
Preoperative SF aspirate	26 (68.4)	51 (68.0)	
SF WBC in cells/μl, median (range)	28,574 (8,175–155,000)	44,275 (629–1,071,472)	0.13
SF neutrophil %, median (range)	88 (79–98)	91 (51–100)	0.84

[a] Excluding 54 subjects for whom the presence or absence of antimicrobials in the cement could not be ascertained.
[b] All values indicate number (%) unless otherwise specified.
[c] Continuous variables were compared using Wilcoxon rank sum test, and categorical variables were compared using Fisher's exact test.
[d] Abbreviations: ACR, American College of Rheumatology; CKD, chronic kidney disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SF, synovial fluid; WBC, white blood cell count.

two SCVs and one NP isolate; all were identified as *S. aureus* using matrix-assisted light desorption ionization–time of flight (MALDI-TOF) mass spectrometry.

In total, there were 76 isolates found among the 38 subjects in whom at least one SCV was observed; 42 isolates (55.3%) were SCVs and 34 (44.7%) were NPs. Five (13%) of 38 subjects had only SCVs isolated without a coisolated NP strain, while one subject had an SCV *S. aureus* strain and an NP *Staphylococcus capitis/caprae* strain. An SCV strain and an NP strain of the same species were identified in 32 subjects. Of these, there were 19 subjects with *S. epidermidis*, 8 with *S. aureus*, 3 with *Staphylococcus lugdunensis*, and 2 with *S. capitis/caprae*. Most of these pairs were indistinguishable (16 pairs) or closely related (3 pairs), based on pulsed-field gel electrophoresis (PFGE) analysis. One pair was possibly related, and one pair was different. One subject had one SCV and two NP isolates, all of which were *S. epidermidis*; the SCV was indistinguishable from one NP isolate and closely related to the other. Despite multiple attempts, PFGE analysis failed in 10 subjects.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing performed in the clinical microbiology laboratory found both oxacillin-susceptible and -resistant strains in the same subject in 5 (13.2%) and 7 (9.3%) subjects with and without SCVs, respectively. Among the 12 subjects with mixed-oxacillin-susceptibility infections, 10 were infected with *S. epidermidis*, one with *Staphylococcus capitis/caprae*, and one with NP oxacillin-

TABLE 2 Microbiologic identification and oxacillin susceptibility results for all subjects

Identification or susceptibility result	No. (%) for SCV status:	
	Yes (n = 38)	No (n = 75)
Species identification		
S. aureus	10 (26.3)	25 (33.3)
S. epidermidis	22 (57.9)	40 (53.3)
S. capitis/caprae	2 (5.3)	3 (4)
S. lugdunensis	3 (7.9)	2 (2.7)
Staphylococcus warneri	0	1 (1.3)
Multiple *Staphylococcus* species[a]	1 (2.6)	4 (5.3)
Oxacillin susceptibility result determined in clinical microbiology laboratory		
Susceptible	16 (42.1)	28 (37.3)
Resistant	17 (44.7)	40 (53.3)
Both susceptible and resistant isolated in clinical laboratory	5 (13.2)	7 (9.3)

[a] The multiple organisms identified included *S. epidermidis* and *S. warneri* (n = 2), *S. aureus* and *S. epidermidis* (n = 1), *S. aureus* and *S. capitis/caprae* (n = 1), and *Staphylococcus simulans* and *S. capitis/caprae* (n = 1).

resistant *S. epidermidis* and NP oxacillin-susceptible *S. aureus*. No subjects with only *S. aureus* infection had both oxacillin-susceptible and -resistant isolates.

Table 3 shows the results of susceptibility testing performed as part of this study on the isolates recovered from subjects with SCVs. Due to poor growth of SCVs on Mueller-Hinton agar, 5% sheep blood was added when performing susceptibility testing for 11 subjects. There were 12 (31.6%) subjects in whom antimicrobial susceptibilities for one of the isolates differed from those reported in the clinical laboratory. Interpretation of the susceptibility results for trimethoprim-sulfamethoxazole using Etest was difficult or impossible for some of the SCVs due to poor growth and indistinct boundaries. Among subjects in whom both SCV and NP isolates of the same species were found, susceptibilities to at least one antimicrobial agent differed in 11 (32.4%) of 32 (see Table S1 in the supplemental material). Oxacillin was the most frequent antimicrobial agent with discordant susceptibilities. We observed inconsistent oxacillin susceptibility results in two subjects when using medium containing blood, where the cefoxitin disc diameter fluctuated on repeat testing, resulting in a change in interpretation. Both isolates with inconsistent results tested positive for *mecA*.

TABLE 3 Antimicrobial susceptibility and auxotrophy testing results for isolates recovered from subjects with SCVs

Susceptibility or auxotrophy result	No. (%) for SCV status:	
	Yes (n = 42)	No (n = 34)
Antimicrobial susceptibility testing		
Oxacillin susceptible[a]	20 (47.6)	21 (61.8)
Gentamicin susceptible	36 (85.7)	30 (88.2)
Rifampin susceptible	37 (88.1)	33 (97.1)
Minocycline susceptible	42 (100)	33 (97.1)
Vancomycin susceptible	42 (100)	34 (100)
Trimethoprim-sulfamethoxazole susceptible[b]	34 (85.0)	31 (91.2)
Auxotrophy observed		
CO_2	8 (19.0)	
CO_2 and menadione	1 (2.4)	
Hemin	1 (2.4)	
No CO_2, menadione, thymidine, or hemin auxotrophy detected	32 (76.2)	

[a] Oxacillin susceptibilities were determined using cefoxitin disc diffusion testing.
[b] Trimethoprim-sulfamethoxazole susceptibility could not be determined for 2 SCV isolates due to poor growth, even with blood-containing medium.

Medical and surgical management. Resection was the initial surgery performed in the majority of subjects in both groups (Table 4). Debridement with prosthesis retention (n = 9), debridement with revision of a single component (n = 2), and one-stage arthroplasty exchange (n = 1) were performed in a minority of patients in both groups. All 38 subjects with SCVs and 69 (92.0%) of the 75 subjects without SCVs were initially managed in accordance with previously published algorithms (17) that recommend resection as the initial surgery for subjects with a sinus tract or symptoms for more than 3 weeks (P = 0.10). Among the subjects who underwent resection, the majority had an antimicrobial-loaded cement spacer implanted and ultimately underwent reimplantation of a new prosthesis.

Intravenous antimicrobials were given to all subjects after surgery, with a median duration of therapy of 42 days in both groups. The most frequently used antimicrobial agents after initial surgery were vancomycin (n = 68), cefazolin (n = 26), daptomycin (n = 9), and ceftriaxone (n = 6).

Treatment outcome. Treatment failure was observed in 9 (23.7%) subjects with SCVs and 23 (30.7%) without SCVs (P = 0.51). There was no difference in the risk of treatment failure among subjects with or without SCVs (Fig. 2; hazard ratio [HR], 0.78; 95% confidence interval [CI], 0.36 to 1.69). Among patients treated with resection and antimicrobial-loaded cement spacer placement, treatment failure occurred in 9 (25.7%) of 35 patients with SCVs and 19 (30.6%) of 62 patients without SCVs. The median duration of follow-up was 871 days (range, 5 to 3,795 days) in subjects with SCVs and 922 days (range, 1 to 37,78 days) in subjects without SCVs. Failure occurred a median of 142 days after the initial surgery in subjects with, compared to 219 days in subjects without, SCVs (P = 0.6). The most common reason for initial treatment failure in subjects with SCVs was revision surgery for noninfection (n = 5), followed by PJI (n = 4). In contrast, subjects without SCVs more frequently failed due to PJI (n = 15), compared to revision surgery for noninfection (n = 7). One subject without SCVs failed due to amputation. Characteristics of patients experiencing initial treatment failure due to infection or amputation are shown in Table S2 in the supplemental material. The median C-reactive protein (CRP) level prior to surgery was 64.2 mg/liter and 23.2 mg/liter in subjects with and without subsequent treatment failure, respectively (P = 0.003). The joint infected was associated with treatment failure on univariate analysis (P = 0.01 for any difference). Treatment failure was observed in 24 of 71 (33.8%) subjects with knee PJI, compared to 8 of 42 (19.0%) subjects with PJI of other joints (P = 0.09). Subjects infected with *S. aureus* were more likely to fail than were those infected with *S. epidermidis* (HR, 4.03; 95% CI, 1.80 to 9.04). At the most recent follow-up, 31 (81.6%) subjects with and 64 (85.3%) subjects without SCVs had a functional arthroplasty (P = 0.60).

DISCUSSION

While SCVs were found in one-third of patients in this study, there was no difference in the likelihood of treatment failure between patients with PJI caused by SCVs and those with PJI caused by only NP staphylococci. However, patients infected with *S. aureus* were four times more likely to experience treatment failure

TABLE 4 Treatment and outcomes for subjects with staphylococcal PJI[c]

Treatment or outcome parameter	SCV[b]		P value
	Yes (n = 38)	No (n = 75)	
Treatment[d]			
Antimicrobial used for initial i.v. therapy			0.66
Vancomycin	23 (60.5)	45 (60.0)	
β-Lactam/cephalosporin	10 (26.3)	24 (32.0)	
Daptomycin/linezolid/other[a]	5 (13.2)	6 (8.0)	
Duration of i.v. therapy in days, median (range)	42 (29–48)	42 (1–63)	0.23
Treatment according to Zimmerli/IDSA algorithm	38 (100)	69 (92.0)	0.1
Initial surgery performed was resection	37 (97.4)	64 (85.3)	0.06
Antimicrobial-loaded cement spacer used among subjects undergoing resection	35 (92.1)	62 (82.7)	0.25
No. of subjects reimplanted, n (% of those resected)	31 (84)	55 (86)	
Duration of time in days between resection and reimplantation, median (range)	71 (39–429)	64 (35–274)	0.21
Follow-up and outcomes			
Duration of follow-up in days, median (range)	871 (5–3,795)	922 (1–3,778)	0.48
Treatment failure	9 (23.7)	23 (30.7)	0.51
Duration to failure in days, median (range)	142 (65–2,675)	219 (2–1,608)	0.63
Reason for treatment failure			
PJI	4 (10.5)	15 (20.0)	
Further revision for noninfection	5 (13.2)	7 (9.3)	
Amputation	0	1 (1.3)	
Final joint status			
Functional arthroplasty in place	31 (81.6)	64 (85.3)	0.71
Resected	6 (15.8)	7 (9.3)	
Arthrodesis	1 (2.6)	2 (2.7)	
Amputated	0	2 (2.7)	
No. of surgeries performed, median (range)	2 (1–4)	2 (1–8)	1

[a] One subject received both vancomycin and ceftriaxone due to a single positive culture for *Enterobacter cloacae*, which, based on retrospective review, was a likely contaminant.
[b] All values indicate number (%) unless otherwise specified.
[c] Continuous variables were compared using Wilcoxon rank sum test; categorical variables were compared using Fisher's exact test.
[d] Abbreviations: IDSA, Infectious Diseases Society of America; i.v., intravenous.

than were those infected with *S. epidermidis*, independent of the presence of SCVs. Other investigators have suggested that SCVs causing PJI should be managed as "difficult-to-treat microorganisms" and that an antimicrobial-loaded cement spacer not be used during a two-stage arthroplasty exchange, in order to avoid microbial adherence to the spacer (11, 14, 15). These recommendations are largely based on studies showing that *S. aureus* isolates with an SCV phenotype exhibit increased adhesion to fibronectin-coated surfaces (6) and antimicrobial resistance when adhered (18). However, these studies were performed using laboratory-derived bacteria with an SCV phenotype and may not be reflective of clinical SCVs. Two prior studies of *S. epidermidis* SCVs isolated from patients with PJI found no consistent increase in biofilm formation in SCVs compared to NP staphylococci (10, 12). Among patients in the current study who were treated with resection and antimicrobial-loaded cement spacer placement, there was no difference in treatment success between patients with and those without SCVs. Antimicrobial-loaded cement spacers provide mechanical support, maintain proper joint position, increase patient comfort, and allow local antimicrobial delivery between the first and second stages of surgery for PJI (2). Given these benefits, we consider that there are insufficient data at this point to recommend against using cement spacers in the treatment of PJI caused by SCVs. Further, based on our data, there is no reason for clinical microbiology laboratories to specifically call out SCVs. However, these results cannot be applied to other treatment strategies, such as debridement with prosthesis retention or one-stage exchange. Antimicrobial therapy may play a greater role when using those treatment strategies, and SCVs may therefore be important in that setting.

Our data support several prior case series suggesting that SCVs causing bone and joint infection are found in patients with a prolonged duration of symptoms who have undergone prior treatment attempts (11, 13, 19). In this study, subjects infected with SCV staphylococci were more likely to have received prior surgical and chronic antimicrobial therapy for their infection and had a nearly 3-fold-longer duration of symptoms, compared to subjects infected with only NP staphylococci. There is little literature systematically evaluating clinical factors associated with the identification of SCVs. Two cohort studies in cystic fibrosis subjects found a correlation between prior systemic antimicrobials and isolation of *S. aureus* SCVs (20, 21). Our study assessed antimicrobial treatment during the 6 months prior to surgery, compared to one of the cystic fibrosis studies that included antimicrobial use over the preceding 3 years (20). It is possible that the greater duration of symptoms and greater time since arthroplasty placement observed in the subjects with SCVs in this study are simply surrogate markers for prior antimicrobial exposure not specifically assessed by our data collection. Local antimicrobial therapy with aminoglycosides has been suggested as a factor contributing to the development of SCVs in osteomyelitis (13), a finding supported by the ability of subinhibitory concentrations of aminoglycosides

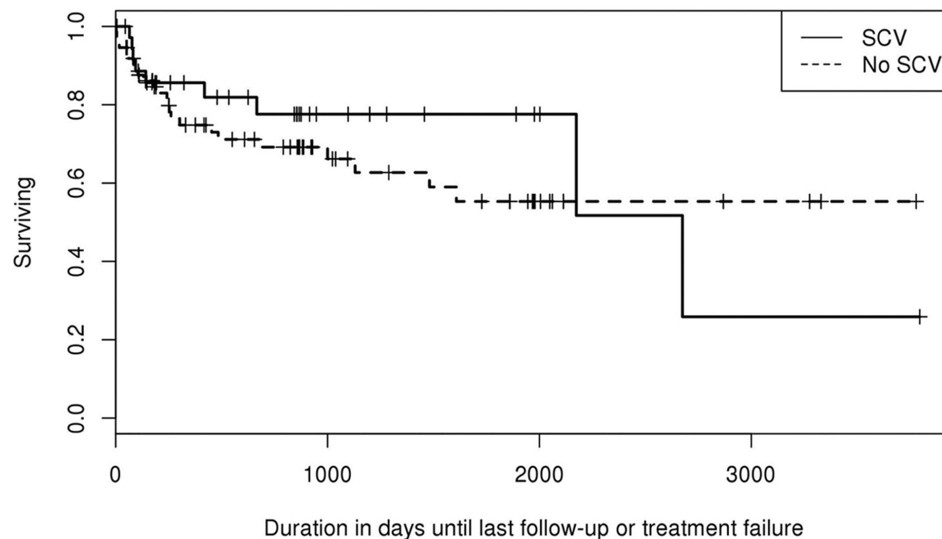

FIG 2 Survival free from treatment failure among subjects with and without SCV staphylococci. There was no difference in likelihood of experiencing treatment failure between subjects infected with and those without SCVs ($P = 0.45$). Solid line, subjects with SCV staphylococci ($n = 38$); dashed line, subjects without SCV staphylococci ($n = 75$).

to select for bacteria with an SCV phenotype *in vitro* (22). Among the 59 subjects with full joint information in this study, 7 of 18 subjects with SCVs (38.8%) had aminoglycoside-loaded cement securing their arthroplasty, compared to 8 of 41 (20.0%) without SCVs ($P = 0.19$). Although not statistically significant, this suggests that local antimicrobial therapy may be important in selecting for SCVs.

We observed SCVs in similar frequency among *S. aureus* strains and various species of coagulase-negative staphylococci. Prior studies have found a wide range in the frequency with which staphylococcal SCVs have been identified, from 6 to 47% in PJI (10–12) and from 17 to 49% in cystic fibrosis (20, 21, 23). The variability in frequency reported in the literature may be due to a difference in the definition of SCVs that is used and whether species other than *S. aureus* were evaluated. We used a rigorous definition of SCVs and included assessments by two independent investigators, in an attempt to standardize this definition as much as possible. We believe that this is critical when SCVs are studied in clinical settings, given that this is a phenotypic description, with inherent subjectivity.

While the majority of SCVs were clonally related to the coisolated normal phenotype bacteria, up to one-third had discordant susceptibilities using Etest or disc diffusion testing. In most cases, these susceptibilities were identified in the clinical laboratory; there were no subjects treated with an antimicrobial for which our research-based testing identified resistance. This highlights the importance of performing susceptibility testing on all observed isolates of variant colony morphotype, as is currently practiced in our clinical laboratory. We observed five subjects, all of whom had infection caused by *S. epidermidis*, with SCV and NP isolates with discordant oxacillin susceptibilities by cefoxitin disc diffusion testing. *mecA* PCR did not consistently correlate with phenotypic susceptibility testing. This is compatible with data suggesting that phenotypic oxacillin resistance in *S. epidermidis* may depend on factors other than the presence or absence of *mecA* (24, 25).

Our study has several limitations. First, as a retrospective study, uniform data were not collected for each subject at the time of his or her hospitalization or in follow-up. There were 11 subjects who did not experience treatment failure for whom fewer than 6 months of follow-up data were available; it is possible that some of these subjects were incorrectly classified as having been successfully treated. Our overall treatment success rate of 71% is lower than that in other studies of two-stage arthroplasty exchange for PJI treatment (3, 26), which may question the generalizability of our finding. The higher rate of failure that we observed is likely related to the definition of treatment failure applied. We included revision surgery for any reason in our definition of failure, in order to assess the hypothesis that SCVs may contribute to subsequent apparent noninfectious failure. In order to perform antimicrobial susceptibility testing, we added sheep blood to the agar for some of the SCVs. While this limits the direct application of the susceptibility trends seen here, it highlights the challenge that these organisms can pose in the clinical microbiology laboratory.

A final limitation is that previous investigators have suggested that sessile bacteria dislodged from biofilm using sonication may resemble SCVs when cultured on solid medium (14) or that the process of freezing bacteria may generate SCVs. We examined only stored sonicate fluid for SCVs, rather than tissue or synovial fluid, raising the possibility that the isolates that we observed did not actually represent SCVs. In order to investigate this possibility, we froze and then subsequently grew a biofilm-forming strain of *S. epidermidis* (ATCC 35984) on sterile Teflon coupons and performed sonication for various durations. On culture of the resulting sonicate fluid, no SCVs were observed, suggesting that sonication or freezing alone is insufficient to produce bacteria with this phenotype.

In summary, in this large cohort of patients with staphylococcal PJI, there was no association between the isolation of SCVs and subsequent treatment failure when primarily using a two-stage exchange treatment protocol.

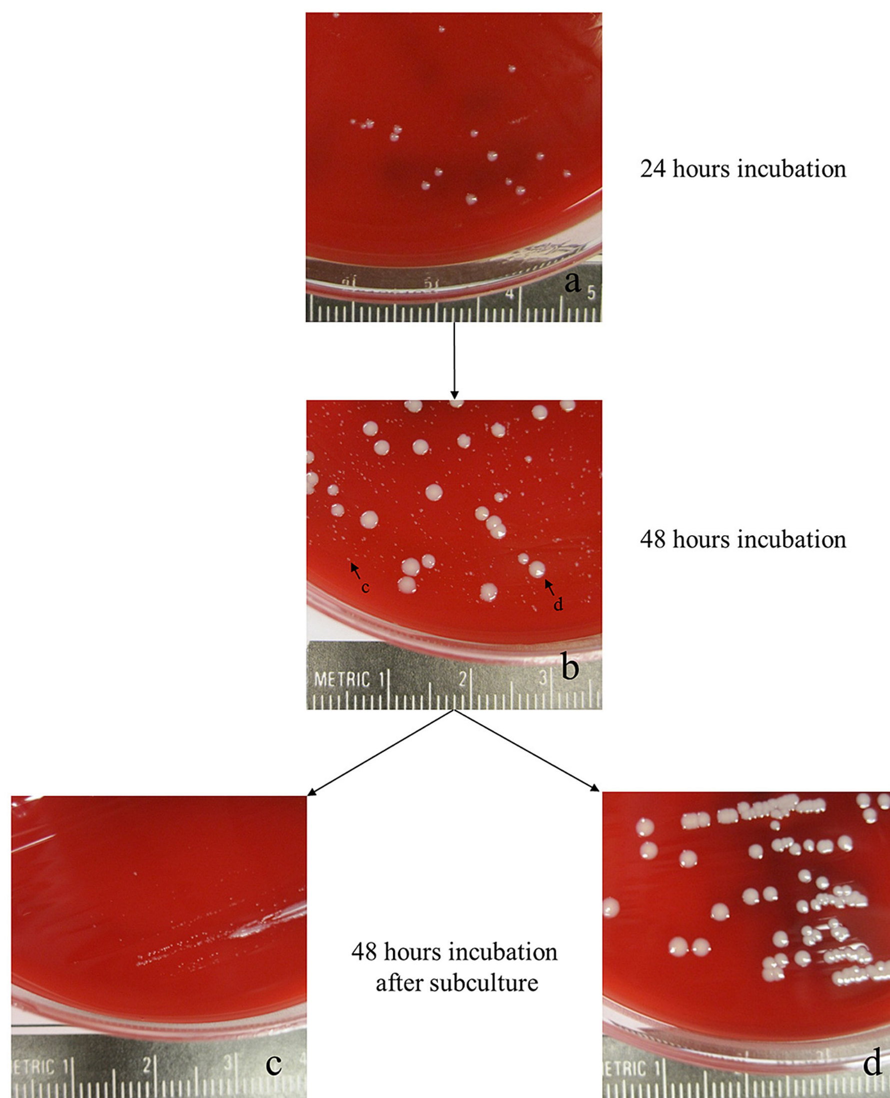

FIG 3 *S. lugdunensis* SCV and normal-phenotype bacteria isolated from a single subject. (a) Appearance of sonicate fluid culture on sheep blood agar after 24 h of incubation, showing only normal-phenotype colonies. (b) At 48 h, both SCV and normal-phenotype colonies are present. (c and d) Subculture of a single colony of the normal and SCV phenotype from the plate in panel b yielded a uniform SCV phenotype (c) and a uniform normal phenotype (d), respectively, after 48 h of incubation.

MATERIALS AND METHODS

Study population. Since 2001, the Mayo Clinic Infectious Diseases Research Laboratory has collected and stored fluid containing bacteria removed by vortexing/sonication from arthroplasty components of patients undergoing surgery for PJI. The sonication protocol is described elsewhere (27, 28). All subjects with sonicate fluid available from June 2001 through July 2012 were identified and evaluated for inclusion. Subjects were included if they had staphylococcal PJI with ≥20 CFU of staphylococci/10 ml sonicate fluid isolated, without growth of other organisms. PJI was defined using Musculoskeletal Infection Society (MSIS) criteria (16) or by two-investigator review (A.J.T. and D.R.O.). Clinical findings of subjects not meeting MSIS criteria for PJI are described in the Results. All subjects had consented to participate in research studies at the Mayo Clinic; the study was approved by the Mayo Clinic Institutional Review Board (IRB).

Identification of small colony variants. After being brought to room temperature, 100 μl each of undiluted and 10^{-1} and 10^{-2} diluted sonicate fluid was inoculated onto Trypticase soy agar (TSA) with 5% sheep blood (BD Diagnostic Systems, Franklin Lakes, NJ, USA) using a bent glass rod. Cultures were incubated at 37°C in air. At 24 h, plates were examined and photographed using high-resolution digital photography. A single colony of each morphology observed was subcultured, and the original plate and subculture plate were returned to the air incubator and incubated for at least 48 h. If no growth was observed on the original plates at 48 h, incubation was continued for at least another 24 h. At the end of 24 to 48 h, each subcultured isolate of different morphotype was classified as provisional SCV or NP. Gram staining and coagulase testing using the Staphaurex assay (Thermo Scientific, Waltham, MA, USA) were performed on all isolates. Each isolate was identified to species level using matrix-assisted light desorption ionization–time of flight mass spectrometry (Bruker Corporation, Billerica, MA, USA). Alternately, if only NP isolates were observed, Gram staining with Gram-positive cocci resembling *Staphylococcus* species and a positive coagulase test using the Staphaurex assay were used for identification of *Staphylococcus aureus*. Following species identification, all isolates were individually frozen at −70°C in Microbank vials (Pro-Lab Diagnostics, Round Rock, TX, USA).

For definitive identification of SCVs, the isolates that had been frozen in Microbank vials were evaluated by two investigators. After being brought to room temperature, 1 μl of fluid from each Microbank vial was inoculated onto TSA with 5% sheep blood and incubated at 37°C in air. Control strains of *S. aureus* and *S. epidermidis* (ATCC 29213 and ATCC 35984, respectively) were frozen, thawed, and inoculated using the same methods. Each isolate was deidentified using a random number generator to blind the investigator who performed the initial isolation (A.J.T.). Two investigators (A.J.T. and K.E.G.-Q.) independently evaluated each isolate at 24 and 48 h and classified them as SCV or NP (Fig. 3). The definition of SCV was either of the following: (i) appearance of pinpoint colonies or colonies with 1/10 of the diameter of coisolated NP colonies (or control strain colonies if no NP colonies were identified) at 24 or 48 h or (ii) appearance of new small colonies at 48 h where none existed at 24 h, in the presence of NP colonies on the same plate.

A third investigator (R.P.) served as a tiebreaker when necessary.

Antimicrobial susceptibility testing. In subjects with only NP bacteria identified, antimicrobial susceptibilities determined in the clinical microbiology laboratory were used. In subjects in whom SCVs were found, antimicrobial susceptibilities were determined for all isolates (i.e., SCV and NP). A 0.5 McFarland concentration of bacteria in 0.9% normal saline was inoculated onto Mueller-Hinton agar (MHA) using a sterile cotton swab. Etest strips (bioMérieux, Marcy l'Etoile, France) containing vancomycin, rifampin, minocycline, gentamicin, or trimethoprim-sulfamethoxazole (TMP-SMX) were applied; cefoxitin discs (BD Diagnostic Systems, Franklin Lakes, NJ, USA) were utilized for oxacillin resistance testing. *S. aureus* strain ATCC 29213 was used as a control strain. MHA was incubated at 37°C in air for 24 h, and susceptibilities were interpreted according to CLSI guidelines. Susceptibility testing was evaluated again at 48 h (19). If no growth or insufficient growth was observed after 48 h, testing was repeated using the same procedures on MHA agar with 5% sheep blood added. If differences between susceptibilities of an SCV and NP were observed and the results were not considered to be reliable, repeat testing was performed. PCR for the presence of *mecA* was performed on all coisolated SCV and NP isolates with discrepant oxacillin susceptibilities, using previously described methods (29).

Auxotrophy testing. All SCVs were tested for auxotrophy with discs containing hemin, menadione, or thymidine using a modification of previously published methods (12). Auxotrophy was defined as increased growth surrounding the disc at 24 and/or 48 h. *S. aureus menD* and *hemB* mutants (30), kindly provided by Christof von Eiff (Department of Medical Microbiology, University of Muenster Medical School, Muenster, Germany), were used as positive controls for menadione and hemin auxotrophy, respectively. A previously identified thymidine auxotrophic strain from our laboratory (IDRL-9149) was used as a positive control for thymidine auxotrophy.

For CO_2 auxotrophy testing, a single colony was streaked onto two sheep blood agar plates. These plates were incubated simultaneously at 37°C in air and in 5% CO_2 for 48 h. The cultures were evaluated at 24 and 48 h. CO_2 auxotrophy was defined as increased colony size or hemolysis with CO_2 (compared to that without CO_2) at 24 and/or 48 h.

PFGE. SCVs and NPs isolated from the same joint were assessed for relatedness by pulsed-field gel electrophoresis (PFGE) with the SmaI restriction enzyme. SCV-NP pairs were considered clonally related if they were indistinguishable (no band difference) or closely related (2- to 3-band difference) by PFGE (31). Pairs were also classified as possibly related (4- to 6-band difference) or different (7-band difference or more).

Clinical characteristics and outcomes. Clinical data were obtained by retrospective chart review of electronic medical records. Immunosuppression was defined as a prior stem cell or organ transplant, ingestion of corticosteroids or disease-modifying antirheumatic drugs for more than 30 days, or any tumor necrosis factor alpha (TNF-α) inhibitor or chemotherapy in the antecedent 6 months. The orthopedic surgical history of each joint was obtained, and original surgical reports where reviewed where possible. The dates and reasons for primary arthroplasty, prior revision surgeries, type of prosthesis, and presence of plain or antimicrobial-loaded cement were recorded. Possible or definite prior surgery for the current PJI episode was defined as previous operative treatment for PJI caused by the same species category (coagulase-negative staphylococci or *S. aureus*) with the same oxacillin susceptibility. Chronic preoperative antimicrobial treatment was defined as receiving antibiotics on at least 120 days over 180 days prior to surgery. The antibiotic given for the largest amount of time was recorded. The operative report(s), pathology record(s), and microbiology results for the current PJI episode were reviewed in all cases. Partial revision was defined as replacement of at least one but not all arthroplasty components. The electronic medical record for all subjects was reviewed through the last known date of follow-up.

The primary endpoint was treatment failure, which was defined as (i) further revision surgery for any reason, (ii) PJI after the first surgery for infection, (iii) nonreimplantation of a prosthesis due to ongoing infection, or (iv) amputation of the affected limb. The secondary endpoint was a functional arthroplasty at the most recent follow-up visit.

Statistical analysis. All information was collected and entered into a RedCap database (Vanderbilt University, Nashville, TN, USA). Continuous variables were compared using the Wilcoxon rank sum test; categorical variables were compared using Fisher's exact test. Times to treatment failure among subjects with and without SCV staphylococci were displayed using Kaplan-Meier survival curves and compared using the log rank test. The Cox proportional hazards model was used for survival analysis. All tests were 2 sided, and *P* values less than 0.05 were considered statistically significant. Statistical analysis was performed using SAS (SAS Institute, Cary, NC, USA).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01910-14/-/DCSupplemental.

Table S1, DOCX file, 0.02 MB.

Table S2, DOCX file, 0.02 MB.

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