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Cutibacterium subtype distribution on the skin of primary and revision shoulder arthroplasty patients

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Background: The skin of healthy shoulders is known to harbor multiple different subtypes of *Cutibacterium* (formerly *Propionibacterium*) *acnes* at the same time. *C acnes* can often be isolated from deep tissue and explant samples obtained during revision of a failed shoulder arthroplasty, presumably because the shoulder was inoculated with organisms from the patient's skin at the time of the index arthroplasty. It is possible that specific subtypes or distributions of subtypes may be associated with an increased pathogenic potential and that the skin of patients undergoing revision arthroplasty contains different distributions of the subtypes than in patients undergoing primary arthroplasty. We analyzed the subtype distribution of *Cutibacterium* from the skin of shoulders undergoing revision arthroplasty vs. primary arthroplasty.

Methods: Preoperative skin swabs were collected from 25 patients who underwent primary shoulder arthroplasty and 27 patients who underwent revision shoulder arthroplasty. The results of semiquantitative cultures of the skin and deep tissues were reported as specimen *Cutibacterium* values, and scores from all deep tissue samples were added to report the total shoulder *Cutibacterium* score. Single-locus sequence typing (SLST) of *C acnes* from the skin swabs was used to determine the subtype distribution for each patient. The percentage of each subtype for each patient was averaged in patients undergoing revision arthroplasty and then compared with that in patients undergoing primary arthroplasty.

Results: The *C acnes* subtype distribution on the skin of revision arthroplasty patients was different from that of primary shoulder arthroplasty patients, with a significantly higher percentage of SLST subtype A (36.9% vs. 16.0%, $P = .0018$). The distribution of SLST subtypes was similar between revision arthroplasty patients with strongly positive culture findings vs. those with weakly positive or negative culture findings.

Conclusions: Significant differences in the skin *Cutibacterium* subtype distributions were found between shoulders undergoing revision shoulder arthroplasty and those undergoing primary shoulder arthroplasty. Future studies are needed to determine whether certain *Cutibacterium* subtype distributions are associated with an increased risk of arthroplasty revision.

Level of evidence: Level III; Cross-Sectional Design; Epidemiology Study

Keywords: *Cutibacterium*; revision shoulder arthroplasty; periprosthetic shoulder infection; skin microbiome; single-locus subtyping; shoulder PJI

Although *Cutibacterium* (formerly *Propionibacterium*) *acnes* is an important commensal of the normal, healthy skin microbiome,[8-10] it is the most common bacterium isolated from shoulders with periprosthetic infections.[21,23] A substantial proportion of deep tissue

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samples harvested at revision shoulder arthroplasty show positive culture findings for the presence of this bacterium.[23] When the skin is incised at the time of shoulder arthroplasty, leakage of *C acnes* from the dermal sebaceous glands and hair follicles can inoculate the deep tissues, leading to biofilm formation on the implants and deep infection presenting months or years after the index arthroplasty.[6,20]

The variability of *C acnes* types found in both healthy and diseased states reflects the bacterium's substantial genetic diversity. Different subtyping schemes of *Cutibacterium* strains have been described based on phenotypic and phylogenetic analyses centered on possible virulence factors; these include various multi-locus sequence typing schemes (including the ''traditional'' subtyping classifications of type IA1, IA2, IB, IC, II, and III),[12,16-19] single-locus sequence typing (SLST),[24] and ribotyping.[7] On the skin surface of a single shoulder, multiple subtypes of *C acnes* can exist simultaneously.[7] Studying the genetic diversity of *C acnes* on patients' skin surfaces could prove useful given that an imbalance toward certain subtypes may be favored in certain disease states. For example, in acne vulgaris, it has been suggested that the absolute load of *Cutibacterium* itself is not as important as an imbalance toward particular subtypes that have been associated with a pathogenic state.[7]

Because individual shoulders can have different distributions of *Cutibacterium* subtypes on the skin surface, one could hypothesize that an abnormal distribution could be associated with other pathogenic states such as arthroplasty failure leading to surgical revision. Sampling of *Cutibacterium* on the skin can be performed in the clinic prior to an arthroplasty,[11] and performing subtype analysis of these samples has potential in preoperatively risk stratifying individuals for the purposes of preoperative counseling on surgical risk and identifying the potential need for extraordinary prophylaxis. However, there are no data in the literature to support or refute this concept. Therefore, the goal of this study was to use an SLST scheme to determine whether the subtype distribution of *C acnes* on the skin of patients with failed shoulder arthroplasties is enriched with certain subtypes relative to the subtype distribution on the skin of patients prior to primary shoulder arthroplasty.

Methods

Between December 2015 and July 2017, 25 patients undergoing primary shoulder arthroplasty and 34 patients undergoing revision arthroplasty for pain, stiffness, or component loosening provided consent and had preoperative skin swabs taken before surgery. All 25 primary arthroplasty patients were included in the analysis. Seven revision arthroplasty patients who had undergone previous revision arthroplasty surgery for the treatment of infection (eg, irrigation and débridement or antibiotic spacer placement) were excluded. This left 27 patients in the revision arthroplasty group for the final analysis. There was no significant difference in age or sex between the 2 groups (Table I). The 27 patients in the revision arthroplasty group included 13 with failed total shoulder arthroplasties, 10 with failed hemiarthroplasties, 2 with failed reverse arthroplasties, and 2 with failed humeral head resurfacing procedures. None of the revision arthroplasty patients had any obvious preoperative signs of infection (skin erythema, drainage, or systemic signs of infection). All patients underwent a single-stage exchange followed by postoperative antibiotic therapy until cultures were finalized.

Sample collection

Patients were instructed to use home chlorhexidine washes the night before and the morning of surgery. On the day of surgery, 2 standardized swab cultures (ESwab 480C; Copan Diagnostics, Murrieta, CA, USA) of the unprepared, unshaved skin in the area of the planned incision were obtained as previously described.[11,14] Four passes with each swab were taken by a surgical assistant wearing sterile gloves, turning the swab 90° for each pass. One sample was sent to the laboratory for DNA extraction and subtype analysis, and one sample was sent to the microbiology laboratory for semiquantitative culturing.[4,15] In patients undergoing revision arthroplasty, multiple deep surgical specimens were also taken from the shoulder at various locations including the collar membrane, humeral membrane, periglenoid tissue, humeral explant, and glenoid explant. These cultures were taken prior to administration of perioperative antibiotics. An average of 6.0 ± 1.8 deep tissue samples were submitted for analysis in each revision case. Patients undergoing primary arthroplasty did not have deep specimens taken.

DNA extraction, sequencing, and subtyping

The skin swabs were transferred to a 1.5-mL Eppendorf tube (Eppendorf, Hauppauge, NY, USA) containing 750 μL of PowerLyzer PowerSoil Bed Solution (MO-BIO, Carlsbad, CA, USA). After the samples were vortexed for 2 minutes to release bacteria, the suspension was transferred to a PowerLyzer Glass Bead Tube (MO-BIO). The PowerLyzer PowerSoil DNA Isolation KIT (MO-BIO) was used to extract DNA. Polymerase chain reaction (PCR) reaction mixtures were composed of 5 μL of the DNA sample, 2.5 μL of AccuPrime PCR Buffer II (Invitrogen, Carlsbad, CA, USA), 0.15 μL of AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen), and 14.35 μL of PCR-grade water. The following cycle protocol was used: a cycle of 50°C for 2 minutes, a cycle of 95°C for 10 minutes, and 40 cycles of 95°C for 15 seconds, followed by 60°C for 1 minute. The resulting PCR products were subsequently tagged with sequencing adapters and sample barcodes following the standard Illumina amplicon sequencing protocols (Illumina, San Diego, CA, USA). These amplicons were sequenced (2× 300–base pair reads) on an Illumina MiSeq next-generation sequencing platform. This allowed us to determine the relative amounts of each subtype present in each sample by comparison to the subtyping database.

A single-locus subtyping region (using SLST) in *C acnes* has been previously described by Scholz et al.[24] PCR across this region combined with next-generation sequencing was used to determine the distribution of subtypes in a mixture. We used the

Table I Patient demographic characteristics of primary and revision arthroplasty groups

	All	Primary arthroplasty	Revision arthroplasty	*P* value
No. of patients	52	25	27	—
Age, yr	63.2 ± 10.5	66.0 ± 11.5	60.5 ± 8.9	.062
Sex, n				.548
Male	37	19	18	
Female	15	6	9	
Type of surgery, n				—
Primary arthroplasty	25	25		
Revision of total shoulder arthroplasty	13		13	
Revision of hemiarthroplasty	10		10	
Revision of reverse shoulder arthroplasty	2		2	
Revision of HemiCAP	2		2	.341

reverse primer as described by Scholz et al but modified the forward primer by shifting 61 nucleotides from the primer described.

Culturing and reporting

The laboratory processed all specimens in a class 2 laminar-flow biological safety cabinet within 1 hour after surgery. Specimens were inoculated onto the following microbiological media: blood agar (trypticase soy agar with 5% sheep blood), chocolate agar, *Brucella* agar (with blood, hemin, and vitamin K), and brain-heart infusion broth. All media, with the exception of *Brucella* agar, were incubated at 37°C with 5% carbon dioxide for 21 days. *Brucella* agar plates were incubated anaerobically at 37°C for 21 days. Plates were sealed in a manner that allowed sterile aeration without desiccation. Media were examined daily for growth visually but were opened only if growth was noted.

Culture results were recorded in a semiquantitative manner, categorized in terms of the specimen *Cutibacterium* value (SpCuV), as described previously[11,14,15]: 0, no growth; 0.1, one colony; 0.2, growth in broth only; and 1, 2, 3, and 4 for culture reports of 1+, 2+, 3+, and 4+ growth, respectively. The culture results were reported as SpCuVs.[1] For the deep specimens, individual SpCuVs were summed and reported as the total shoulder *Cutibacterium* score (ShCuS).

Statistical analysis

The percentage of each SLST subtype (A-H, K, and L) for each patient was averaged in patients undergoing revision arthroplasty and then compared with that in patients undergoing primary arthroplasty by a 2-tailed unequal-variance *t* test. A subgroup analysis of the 27 patients who underwent revision arthroplasty was also performed. We compared shoulders with strongly positive culture findings (total ShCuS $>$ 1.1) with those with weakly positive culture findings (total ShCuS $\leq$ 1.1) to determine whether the subtype distribution was different between the 2 groups. We selected a total ShCuS of 1.1 as the threshold because this represents 2 positive culture results, at least one of which has a substantial bacterial load (1+ or more). The level of statistical significance was set at $P < .05$.

Results

Although no significant difference in the overall skin-surface *Cutibacterium* load was found between primary and revision arthroplasty patients (SpCuV, 1.1 ± 1.1 for primary vs. 0.9 ± 0.9 for revision, $P = .512$), the *C acnes* skin subtype distribution was different between revision and primary shoulder arthroplasty patients. The rate of *Cutibacterium* SLST subtype A was higher in revision shoulder arthroplasty patients (36.9% in revision group vs. 16.0% in primary group, $P = .002$) (Table II).

Of the 27 shoulders undergoing revision procedures, 11 (41%) showed strongly positive culture findings (total ShCuS $\leq$ 1.1) whereas 16 (59%) showed weakly positive culture findings (total ShCuS $>$ 1.1) or had no positive culture findings. In this group, 12 shoulders (44%) had $\geq$2 positive culture results for *Cutibacterium* whereas 15 (56%) had $<$2 positive culture results for *Cutibacterium*. None of the shoulders had $\geq$2 positive culture results for any other bacteria; 15 shoulders (56%) had 1 positive culture result for a bacterium other than *Cutibacterium*, with coagulase-negative *Staphylococcus* in 6 of these.

Male patients were more likely than female patients to show strongly positive culture findings on the shoulder skin ($P = .009$). The skin SpCuV was significantly higher in revision arthroplasties with strongly positive culture findings than in those with weakly positive culture findings (1.6 ± 0.9 vs. 0.4 ± 0.6, $P = .003$). However, the subtype distribution was not statistically different between revision arthroplasties with strongly positive culture findings (n = 10) and those with weakly positive culture findings (n = 17) ($P > .267$) (Table III).

Discussion

This study found differences in the *C acnes* skin subtype distribution in patients undergoing revision arthroplasty compared with patients undergoing primary arthroplasty. This finding could suggest that patients with skin enriched with certain *C acnes* subtypes may be at increased risk of

Table II Comparison of bacterial load and SLST subtypes on skin of primary and revision arthroplasty patients

	Primary arthroplasty	Revision arthroplasty	*P* value
Skin SpCuV	0.9 ± 0.9	1.1 ± 1.1	.512
SLST subtype, %			
A	16	37	.002*
B	1	2	.268
C	4	4	.978
D	14	11	.622
E	0	1	.133
F	6	5	.889
G	0	1	.736
H	30	26	.554
K	23	13	.109
L	0	0	.099

SLST, single-locus sequence typing; *SpCuV*, specimen *Cutibacterium* value.
* Statistically significant ($P < .05$).

Table III Comparison of revised shoulders with strongly positive and weakly positive or negative culture findings

	ShCuS ≤ 1.1	ShCuS > 1.1	*P* value
Age, yr	61.8 ± 8.4	58.4 ± 9.7	.380
Sex, n			
Male	8	10	.009*
Female	9	0	
Skin SpCuV	0.4 ± 0.6	1.6 ± 0.9	.003*
SLST subtype, %			
A	41	30	.340
B	2	1	.503
C	5	4	.886
D	12	9	.698
E	1	1	.593
F	5	5	.977
G	0	1	.344
H	25	27	.871
K	8	20	.267
L	0	0	—

ShCuS, shoulder *Cutibacterium* score; *SpCuV*, specimen *Cutibacterium* value; *SLST*, single-locus sequence typing.
* Statistically significant ($P < .05$).

arthroplasty revision or that the skin subtype distribution may somehow change after arthroplasty. Subtype analysis of a simple skin swab taken during the preoperative clinic visit may provide additional information in identifying patients who might be at higher risk of arthroplasty revision.

The concept that certain bacterial distributions of subtypes can be associated with an increased risk of disease has been suggested in other organ systems. Fitz-Gibbon et al[7] compared *Cutibacterium* strains in 49 patients with acne and 52 healthy patients and found that strain population structures were significantly different between the 2 groups despite similar relative abundances of the bacterium. Other studies have suggested a causal role of this imbalance, sometimes referred to as "dysbiosis," in skin disorders such as eczema.[2] Disruption in homeostasis of the gut microbiota has also been associated with a number of intestinal disorders.[5,13] Certain bacteria can potentially exploit intestinal dysbiosis, and reconstitution of normobiosis can be protective against pathologic bacteria.[3]

It is unclear whether the differences in subtype distribution seen between primary and revision arthroplasty patients are a result of innate individualistic skin microbiome differences between these 2 sets of patients or whether the skin microbiome changes after shoulder arthroplasty. Oh et al[22] collected longitudinal sequence data of 12 individuals over months and years and found that microbial communities are generally stable despite external exposures. However, to our knowledge, no study has investigated whether a major perturbation such as shoulder arthroplasty could potentially lead to microbiome changes over time.

A few limitations to this study should be considered. First, our results suggest an association between the distribution of bacterial subtypes and failed shoulder arthroplasty, but our study does not prove a causative effect. Second, we did not perform subtyping of the deep tissue specimens. Third, for patients undergoing revision, we had data on the *Cutibacterium* subtype distribution on the skin at the time of revision but not at the time of the index arthroplasty. It is possible that the subtype distribution at the time of revision may be different from that at the time of the index procedure. Fourth, we obtained the skin swabs before skin preparation in the operating room, not in the clinic. However, the skin microbiome remains stable over a period of a few months,[22] particularly in the absence of surgery and antibiotics. Fifth, we only reported the culture results of the revision arthroplasty patients and did not use any definitions for periprosthetic shoulder infection. Sixth, the ages of the patients were similar between the 2 groups but, as a result, the patients in the revision group were likely significantly younger at the time of index arthroplasty.

Conclusion

Significant differences in the skin *Cutibacterium* subtype distributions were found between shoulders undergoing revision shoulder arthroplasty and those undergoing primary shoulder arthroplasty. Further studies are needed to determine whether disruption of normal subtype distributions could potentially be predictive of an increased risk of arthroplasty revision.

Disclaimer

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