

# Quantitation and Composition of Cutaneous Microbiota in Diabetic and Nondiabetic Men

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**Background.** Diabetic foot infections are a leading cause of lower extremity amputations. Our study examines the microbiota of diabetic skin prior to ulcer development or infection.

**Methods.** In a case-control study, outpatient males were recruited at a veterans hospital. Subjects were swabbed at 4 cutaneous sites, 1 on the forearm and 3 on the foot. Quantitative polymerase chain reaction (qPCR) with primers and probes specific for bacteria, *Staphylococcus* species, *Staphylococcus aureus*, and fungi were performed on all samples. High-throughput 16S ribosomal RNA (rRNA) sequencing was performed on samples from the forearm and the plantar aspect of the foot.

**Results.** qPCR analysis of swab specimens from 30 diabetic subjects and 30 control subjects showed no differences in total numbers of bacteria or fungi at any sampled site. Increased log<sub>10</sub> concentrations of *Staphylococcus aureus*, quantified by the number of *nuc* gene copies, were present in diabetic men on the plantar aspect of the foot. High-throughput 16S rRNA sequencing found that, on the foot, the microbiota in controls (n = 24) was dominated by *Staphylococcus* species, whereas the microbiota in diabetics (n = 23) was more diverse at the genus level. The forearm microbiota had similar diversity in diabetic and control groups.

**Conclusions.** The feet of diabetic men had decreased populations of *Staphylococcus* species, increased populations of *S. aureus*, and increased bacterial diversity, compared with the feet of controls. These ecologic changes may affect the risk for wound infections.

**Keywords.** microbiota; microbiome; diabetic foot; cutaneous; *Staphylococcus*; *Staphylococcus aureus*.

Diabetic foot ulcers are an important clinical problem, leading to infection and loss of limb [1]. Diabetic foot ulcers occur in 15% of patients with diabetes mellitus, with a yearly incidence of 1%–2% [2]. Diabetic patients have higher rates of hospitalization for skin and soft-tissue infections than patients without diabetes [3]. Most studies based on culture have shown that the infections are polymicrobial, with *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus* species

(especially *Staphylococcus aureus*) being the most prevalent isolates [4, 5]. Traditionally, the pathogenesis of these wounds has been attributed to factors such as neuropathy (a functional disturbance in peripheral nerves), repeated trauma, and peripheral vascular disease [6]. More recently, the roles of wound bioburden and functionally equivalent pathogroups in diabetic wound infections have been studied [7, 8]. Data on the composition of the cutaneous microbiota of diabetic patients is lacking. One of the few studies to address this question was performed in 1975, in the era prior to high-throughput 16S rRNA sequencing (HTS) technology [9]. No differences in culturable organisms between control and diabetic skin were found, but the study also did not examine the feet.

*Staphylococcus* species account for 70% of the normal skin microbiome on the plantar aspect of the foot [10, 11]; most are coagulase-negative *Staphylococcus* species that are of low virulence and may compete

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with the more virulent *S. aureus* [12]. In contrast, on intact skin on the contralateral (opposite) limb of patients with unilateral diabetic ulcers, only 7% of the microbiota was represented by *Staphylococcus* species [13], suggesting a loss of these protective organisms or their replacement by other species. HTS identification based on deep sequencing is currently being used to better characterize the human microbiome [14, 15]. We sought to use HTS along with quantitative polymerase chain reaction (qPCR) to better characterize the microbiota of the diabetic foot. The aim of this study was to determine whether there are differences in surface microbiota of the arm and foot between nondiabetic men and diabetic men without a history of diabetic foot ulcer.

## METHODS

### Study Participants

This was a single-center, case-control, observational study conducted from 2011 through 2012. Seventy men aged 18–70 years, with or without diabetes, were enrolled. All participants gave informed consent, and the institutional review boards of the VA NY Harbor Healthcare System (VANYHHS) and New York University approved the study. The subjects were recruited from the outpatient clinic of the VANYHHS (Manhattan campus). Exclusion criteria included any history of a diabetic foot ulcer, a body mass index (BMI; defined as the weight in kilograms divided by the height in square meters) of > 40, active dermatologic conditions at sampled anatomical sites, corticosteroid or antibiotic use within the prior month, known active infection at any site, and inability to take daily showers. Enrollment was stopped when we obtained 30 evaluable subjects in the diabetic group and 30 in the nondiabetic (ie, control) group. A survey was administered that included questions about medical history, recent antibiotic use, and bathing habits. A chart review based on the VA electronic medical record was performed to obtain clinical data and to quantify the subjects' glycemic control.

### Skin Sampling

To study the skin microbiota, samples were collected with cotton swabs (Fisher Scientific) soaked in 0.15 M NaCl and 0.1% Tween 20 (Fisher Scientific), as described elsewhere [16]. The following sites were swabbed: the right forearm (midway between the wrist and the antecubital fossa, the triangular area anterior to the elbow) and the dorsum (upper portion), plantar arch, and first interdigital space of the right foot. Each site was swabbed back and forth 30 times, and the procedure was repeated with a second swab.

### Specimen Processing

Specimens were obtained under sterile conditions, placed in 1.5-mL Eppendorf tubes, and stored at  $-80^{\circ}\text{C}$ . The samples were thawed for extraction, performed with the MoBio

PowerLyzer Power Soil DNA Isolation Kit (MoBio Laboratories), according to the manufacturer's protocol. Samples were divided into aliquots for qPCR and 454 pyrosequencing.

### Preparation of qPCR DNA Standards

qPCR standards specific to fungi were created using the transcribed spacer (ITS) sequence. Standards for *Staphylococcus* species and *S. aureus* were prepared from the 16S rRNA gene and the *nuc* gene, respectively, which were amplified from *S. aureus* USA 300 strain SA11–41 by specific primers for these 2 genes (Supplementary Table 1). PCR products were cloned into the pGEMT easy vector (Promega) and confirmed by sequencing. DNA from the purified recombinant plasmids was quantified by the Nanodrop-1000 spectrophotometer (Thermo Scientific) and then serially diluted in TE buffer (Qiagen) to obtain standards ranging from  $3 \times 10^1$  to  $3 \times 10^6$  copies/ $\mu\text{L}$ . These reagents were used for qPCR in duplicate to create standard curves for calibration of assays.

### Quantitative PCR Analysis

qPCR was used to analyze cutaneous samples from the 60 participants. Quantitation of total bacteria, total fungi, *Staphylococcus* species, and *S. aureus* was performed using the Roche LightCycler 480 II PCR system (Roche). Each assay was run in duplicate in wells, with a 20- $\mu\text{L}$  final reaction volume. As described elsewhere [16], the TaqMan assay with fluorogenic probe was used for quantitation. Primers and probes are shown in Supplementary Table 1.

### Pyrosequencing of 16S rRNA Gene Amplicons

DNA amplification and sequencing followed the protocol developed by the Human Microbiome Project [17]. The V1–V3 hypervariable region of 16S rRNA genes was amplified from the skin samples to be used for metagenomic analysis, using primers 27F and 534R (27F: 5'-AGAGTTTGATCCTGGCTCAG-3'; and 534R: 5'-ATTACCGCGGCTGCTGG-3'). The oligonucleotides containing the 16S primer sequences also contained a 454 adaptor sequence, as well as one of 94 tag sequences unique to each sample. A total of 2  $\mu\text{L}$  of each metagenomic sample was amplified under the following conditions: initial denaturation at  $95^{\circ}\text{C}$  for 2 minutes, followed by 30 cycles of annealing at  $56^{\circ}\text{C}$  for 0.5 minutes, elongation at  $72^{\circ}\text{C}$  for 5 minutes, and final elongation at  $72^{\circ}\text{C}$  for 5 minutes. A negative control without a template was included. After excess primers and nucleotides were removed, the tagged amplicon products were pooled and sequenced on the 454 Titanium pyrosequencing platform at The Genomics Institute at Washington University School of Medicine at St. Louis.

Samples were deconvoluted by tag sequences. We allowed 1 mismatch in the barcode and up to 2 mismatches in the primer. Barcode, forward, and reverse primer sequences were

removed from the reads. We further filtered out the reads of >200 bp. Chimera-slayer with default settings was used for chimera filtering before any further processing [18].

The obtained reads were further analyzed using QIIME scripts [19]. We first clustered the sequences into 97% similarity operational taxonomic units (OTUs), using the UCLUST program [20]; these were used as the finest level of taxonomic resolution. A representative sequence from each OTU cluster, automatically selected by UCLUST, was used to assign taxonomy to the cluster by using RDP Classifier [21] at 80% bootstrap confidence. These representative sequences were further aligned using PyNAST [22] with the Greengenes core set alignment template, and the alignment was used to reconstruct an approximate phylogenetic tree, using FASTTREE [23], for the purpose of calculating UniFrac beta-diversity indices [24]. The OTU absolute abundance table and UniFrac beta-diversity matrices [25] were extracted from the pipeline for further analysis in the R statistical programming environment [26, 27]. The rarefactions for richness and Shannon diversity indices were calculated using scripts based on Community Ecology Package vegan. PCoA analysis was performed using the ade4 package in R. Intergroup and intra-group beta-diversity comparisons were performed using 1-way

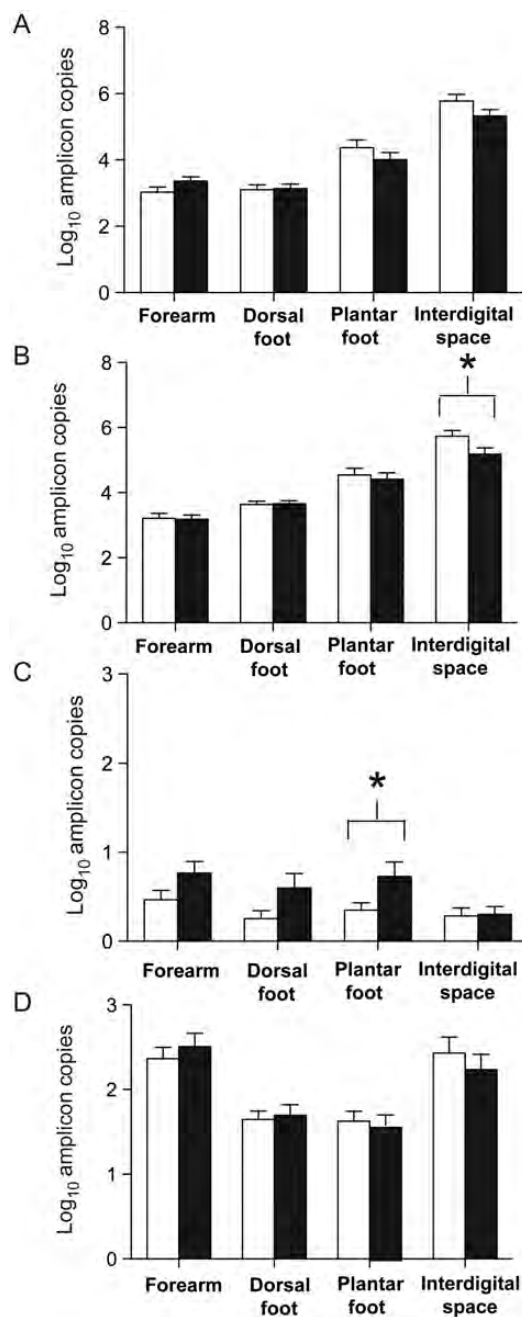
analysis of variance with the Tukey honestly significant difference multiple comparison correction procedure. The OTU relative abundances were calculated by dividing the absolute abundances by the total sequence count per sample analyzed. Differential relative abundance of specific taxa and OTU was calculated using the Kruskal–Wallis test, for simultaneous diabetes status and site comparison, and the Mann–Whitney *U* test,

**Table 1. Demographic and Clinical Characteristics of 30 Subjects Without Diabetes (Control) and 30 Subjects With Diabetes**

Characteristic	Control Group	Diabetes Group	<i>P</i>
Age, y	59.2 ± 7.1	61.2 ± 5.1	.23
Race/ethnicity			
White	40	33	
Hispanic	23	20	.73
African American	37	47	
Height, cm	177.2 ± 6.3	175.2 ± 5.9	.44
Weight, kg	86.0 ± 16.8	92.9 ± 16.8	.12
BMI	27.6 ± 4.8	30.3 ± 4.9	.04
Most recent serum glucose level, mg/dL	95 ± 12	163 ± 98	<.01
Hemoglobin A1c level, %	5.5 ± 0.3	7.9 ± 1.6	<.01
Active smoking	17	23	.75
Antibiotic use in prior 12 mo	30	30	1.00
Known skin condition	23	23	1.00
History of malignancy	20	13	.73
Vascular disease	10	23	.3
Statin use	30	77	<.01
NSAID use	30	57	.12
Time since last shower, h	12.2 ± 10.8	8.4 ± 10.2	.17
High temperature on sampling day, °C	15.8 ± 5.2	18.2 ± 7.6	.16

Data are no. of subjects or mean ± SD.

Abbreviations: BMI, body mass index (defined as the weight in kilograms divided by the height in square meters); NSAID, nonsteroidal antiinflammatory drug.



**Figure 1.** Quantitation of bacteria from four sites. Bars: open, control subjects (*n* = 30); black, diabetic subjects (*n* = 30). Panels: log<sub>10</sub> amplicons per site of, *A*, total bacteria, *B*, total *Staphylococcus* species, *C*, total *Staphylococcus aureus* per site and, *D*, total fungi per site. \**P* < .05.

for within-site comparisons. The *P* values were adjusted for false discovery, using the Benjamini-Hochberg procedure [28].

### Statistical Analysis

Wilcoxon and Student *t* tests were performed using SAS 9 to compare the mean similarities between the diabetic and non-diabetic groups. ANOVA test was performed to compare the mean similarities among sites. All the data analyses were done in SAS 9.2.

## RESULTS

### Subject Characteristics

From September 2011 to February 2012, a total of 70 subjects were enrolled in the study. Ten subjects were excluded (3 had an undocumented diagnosis of diabetes, 2 had diabetic foot ulcers observed on examination, and 1 each had tinea pedis, human immunodeficiency virus infection, severe neuropathy, history of burns on feet, and incomplete sampling). The demographic characteristics of the final group of 60 subjects are shown in Table 1. Baseline characteristics were generally similar for the 2 groups. As expected, the diabetic group had a significantly higher BMI and rate of statin use ( $P < .05$ ) and higher levels of hemoglobin A1c and serum glucose.

### Detection of Total Bacteria and Total Fungi by qPCR

qPCR analyses were performed to enumerate the bacteria in the skin samples obtained from the 4 studied anatomical sites (the forearm, the dorsal aspect of the foot, the plantar aspect of the foot, and first interdigital space of the foot). Each sample was run in duplicate along with the standards described in the Methods section. In total, for the 240 samples studied, the number of bacterial 16S rRNA genes varied widely, with a mean of  $9 \times 10^5$  amplicons/ $\mu\text{L}$  and median of  $5 \times 10^3$  amplicons/ $\mu\text{L}$ . The total number bacterial amplicons per microliter was at least 1.35  $\log_{10}$  higher in the interdigital space than for all other studied sites ( $P < .0001$ ; Figure 1A). Total bacterial counts also were higher on the plantar aspect of the foot, compared with the dorsal aspect of the foot and the forearm ( $P < .0001$ ; Figure 1A). There were no significant differences in total bacterial levels between men with diabetes and controls (Figure 1A). The fungal ITS gene had a mean of  $2 \times 10^3$  amplicons/ $\mu\text{L}$  and a median of  $9 \times 10^1$  amplicons/ $\mu\text{L}$ . Total fungal counts were higher in the interdigital space and on the forearms than on the plantar and dorsal aspects of the foot. There were no significant differences between fungal counts at any site between men with diabetes and controls (Figure 1D).

### qPCR Detection of *Staphylococcus* Species

qPCR analyses were performed for the genus *Staphylococcus* on samples obtained from the same 4 anatomical sites. The number of *Staphylococcus* copies present in the control group at the first interdigital space of the foot was greater in the

control group, compared with the diabetes group (5.74 vs 5.18  $\log_{10}$  16S copies/ $\mu\text{L}$ ;  $P = .04$ ; Figure 1B). The samples from the 3 other sites showed no significant differences between the groups. Diabetic men had an increased number of *S. aureus* at the plantar aspect of the foot (0.73 vs 0.35  $\log_{10}$  *nuc* copies/ $\mu\text{L}$ ;  $P = .04$ ; Figure 1C) and at all sites combined (0.60 vs 0.34  $\log_{10}$  *nuc* copies/ $\mu\text{L}$ ;  $P = .002$ ), compared with controls.

### *S. aureus* Carriage Rates

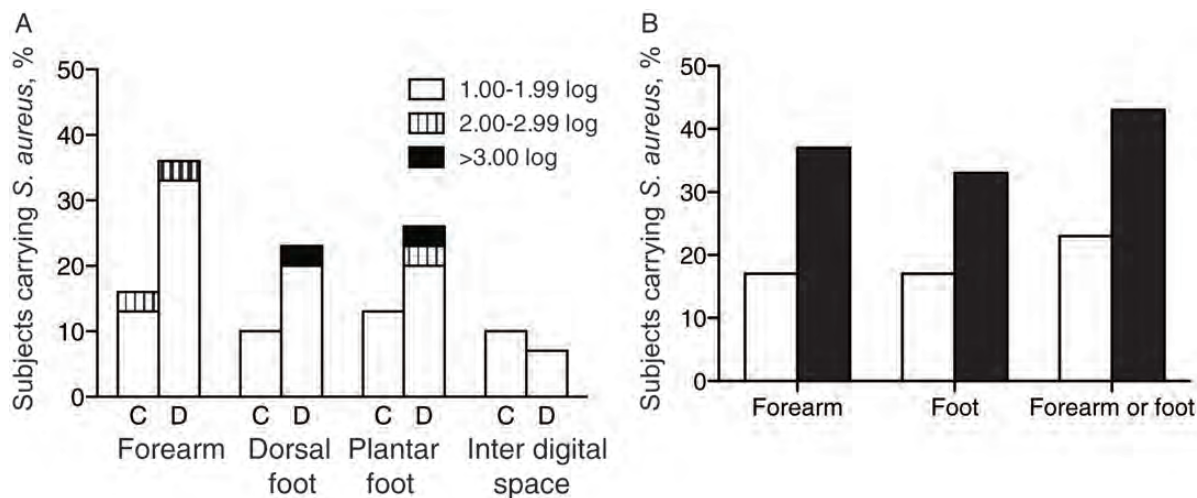
By qPCR, the rate of *S. aureus* carriage at each of the 4 sites did not significantly differ between the control and the diabetic groups (Figure 2); the *S. aureus* carriage rate at any site on the foot was 17% in controls and 33% in the diabetic group ( $P = .23$ ). However, at the plantar aspect of the foot, more men in the diabetic group than the control group had *S. aureus* detected at levels  $>0.01\%$  of the total bacterial population (Table 2). The combined *S. aureus* carriage rate on the forearm, including both controls and diabetic subjects, was higher than the carriage rate at the interdigital space (28% vs 8%;  $P = .008$ ). The rates of carriage on the dorsal and plantar aspects of the foot were 17% and 20%, respectively.

### Composition of Cutaneous Bacterial Communities in Diabetes

We analyzed 1 sample from the forearm and 1 from the plantar aspect of the foot for 47 subjects (24 controls and 23 men with diabetes; 94 total samples). The minimal sequence depth was 1775, and the median depth was 5274. The plantar aspect of the feet of diabetic subjects had greater taxonomic richness and Shannon diversity at all taxonomic levels, compared with the plantar aspect of the feet of controls (Figure 3). Forearm samples from both controls and diabetic men had similar alpha diversity to each other and to the diabetic foot samples (Figure 3). The bacterial composition, as determined by HTS and stratified by anatomical site and study group, is shown at the phylum and genus level in Figure 4A and 4B. We examined the taxa that composed  $\geq 1\%$  of the total microbiota of the foot (Figure 4C). The relative abundance of *Staphylococcus* species in the control group was similar to prior reports, at approximately 70% [9, 10].

Focusing only on highly abundant taxa (defined as those with mean relative abundance of  $>1\%$  across all samples), we analyzed bacterial community compositions in terms of differential relative abundance at anatomical sites (arm and foot) and according to diabetes status (Supplementary Table 2). As expected, most of the differences occurred between arm and foot specimens. Among arm samples, no significant differences in relative abundances were found between diabetic and nondiabetic subjects. Among foot specimens, the relative abundance of Firmicutes was greater in controls, compared with diabetic subjects (78.8% vs 33.0%;  $P < .01$  after adjustment for false discovery rate [FDR]), while Actinobacteria had a greater relative abundance in foot specimens from diabetic





**Figure 2.** Rate of *Staphylococcus aureus* carriage. Panel A: Percent of subjects carrying *Staphylococcus aureus* on skin at four sites, stratified by log<sub>10</sub>amplicon copy number. Subjects are controls (C, n = 30) or diabetics (D, n = 30). Panel B: Percent of 60 subjects carrying *S. aureus* by part of body (Open, control; Black, diabetes).

men, compared with foot specimens from controls (31.1% vs 16.2%;  $P < .03$  after adjustment for FDR). At the genus level, *Staphylococcus* had the greatest relative abundance in foot specimens from both controls and diabetic patients (76.0% and 30.5%); however, because of the intersubject variability, the difference was not statistically significant ( $P = .08$  after adjustment for FDR). On the other hand, *Corynebacterium* abundance was greater in the feet of diabetic subjects (18.2%) than in the feet of controls (5.1%;  $P < .03$  after adjustment for FDR).

By using weighted UniFrac analysis, the first plane of the principal coordinate analysis (PCoA) shows that arm specimens are more tightly clustered than the foot specimens (Figure 5A) and that the diabetic and control arm ellipses virtually overlap. These data suggest that there were no significant differences in the composition of the microbiota at the forearm (Figure 5A). Foot communities split according to

diabetes status (Figure 5B and 5D;  $P = .015$ ). There was increased intragroup beta-diversity in foot samples of diabetic men (Figure 5E). In arm specimens, the trend was reversed (Figure 5F); the arms of diabetic men had lower intragroup beta-diversity than the controls. The intergroup beta-diversity in the arm samples had intermediate values, suggesting that diabetic samples form a subcluster with the controls, which was evident from the PCoA plot (Figure 5C). Such findings are also illustrated in Supplementary Figure 1.

## DISCUSSION

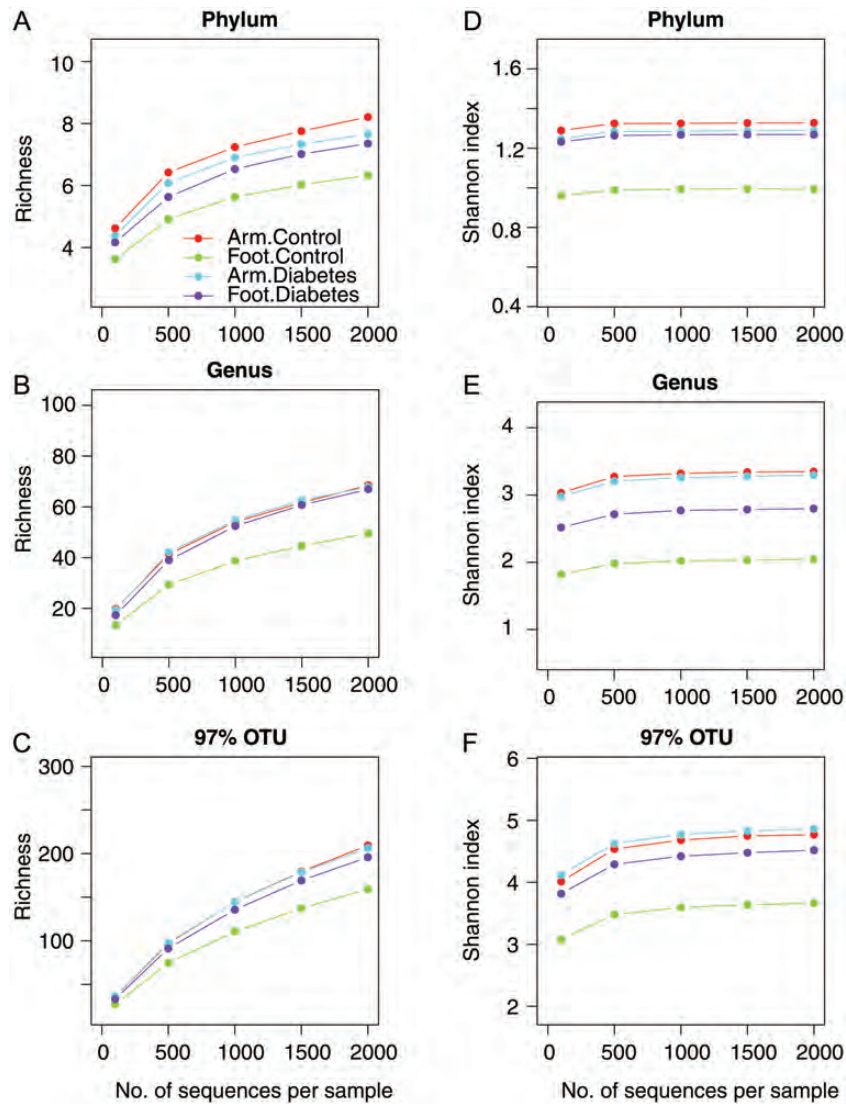
The goals of our study were to compare the bacterial community structure of the foot in diabetic and control (nondiabetic) subjects and to quantitate clinically relevant species. We found that bacterial populations at the plantar aspect of the feet of

**Table 2. Representation of *Staphylococcus aureus* in Relation to Other Bacterial Taxa, by Body Site**

Total Bacterial Population Consisting of <i>S. aureus</i> , %	Subjects, %, by Anatomical Site and Study Group							
	Forearm		Dorsal Aspect of Foot		Plantar Aspect of Foot		Interdigital Space of Foot	
	Control	Diabetes	Control	Diabetes	Control	Diabetes	Control	Diabetes
>10.0	3.3	0.0	3.3	3.3	0.0	0.0	0.0	0.0
>1.0	30.0	16.7	10.0	3.3	3.3	3.3	0.0	0.0
>0.1	63.3	70.0	66.7	73.3	23.3	43.3	0.0	10.0
>0.01	96.7	100.0	93.3	96.7	63.3	93.3 <sup>a</sup>	26.7	26.7

Data were determined by comparing quantitative polymerase chain reaction results for *nuc* with those for the universal bacterial 16S ribosomal RNA.

<sup>a</sup>  $P = .01$ , by the  $\chi^2$  test, compared with controls.

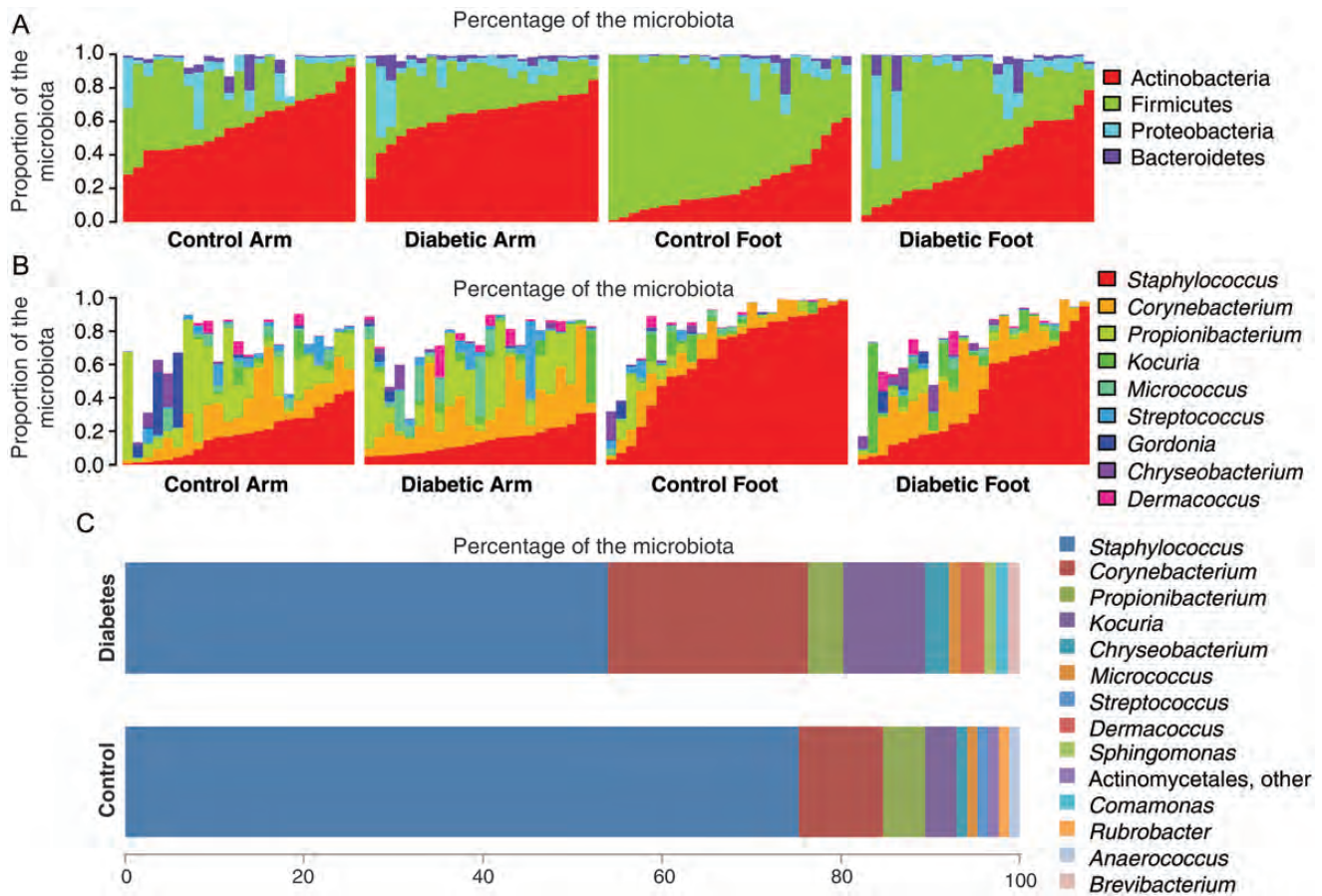


**Figure 3.** Alpha diversity rarefaction plots by site and diabetes status. From each of 94 specimens, 454-pyrosequencing results are normalized to 2000 reads/specimen. Panels: *A*, Taxonomical richness at phylum, Panel *B*, genus and, Panel *C*, 97% similarity OTU levels; and Shannon evenness index at, Panel *D*, phylum, Panel *E*, genus and Panel *F*, 97% similarity OTU levels are shown as rarefaction curves with colors representing site and diabetes status. Color codes are the same in each panel. Abbreviation: OTU, operational taxonomic unit.

diabetic men were more diverse and had different composition, compared with values for nondiabetic men. In particular, the quantity of *Staphylococcus* organisms was reduced on the diabetic plantar aspect of the foot, whereas the more virulent *S. aureus* was present in greater quantities; these changes may be precursors to diabetic foot infection and ulcer development. The quantity of *Staphylococcus* species at the interdigital space of the feet of diabetic men was significantly lower than that for controls, by qPCR. This finding, along with pyrosequencing data for the plantar aspect, which showed a trend toward a higher relative abundance of *Staphylococcus* species in control subjects (76.0% vs 30.5%;  $P = .08$ ), suggest that the diabetic foot has a lower population of *Staphylococcus* species present.

Since both groups had low total levels of *S. aureus*, most *Staphylococcus* species present were coagulase negative and less virulent.

To our knowledge, this is the first study to use qPCR and metagenomics to study the bacterial populations of intact skin in diabetic subjects prior to clinical foot infections. The increased diversity and loss of possibly protective coagulase-negative staphylococcal species [29–31] and the enrichment of *S. aureus* are findings that correlate with the elevated risk of skin and soft-tissue infections that occur in diabetic patients [1]. This risk is also supported by the trend among diabetic subjects toward higher *S. aureus* carriage rates on the foot (Figure 2). As such, the differing populations of the plantar



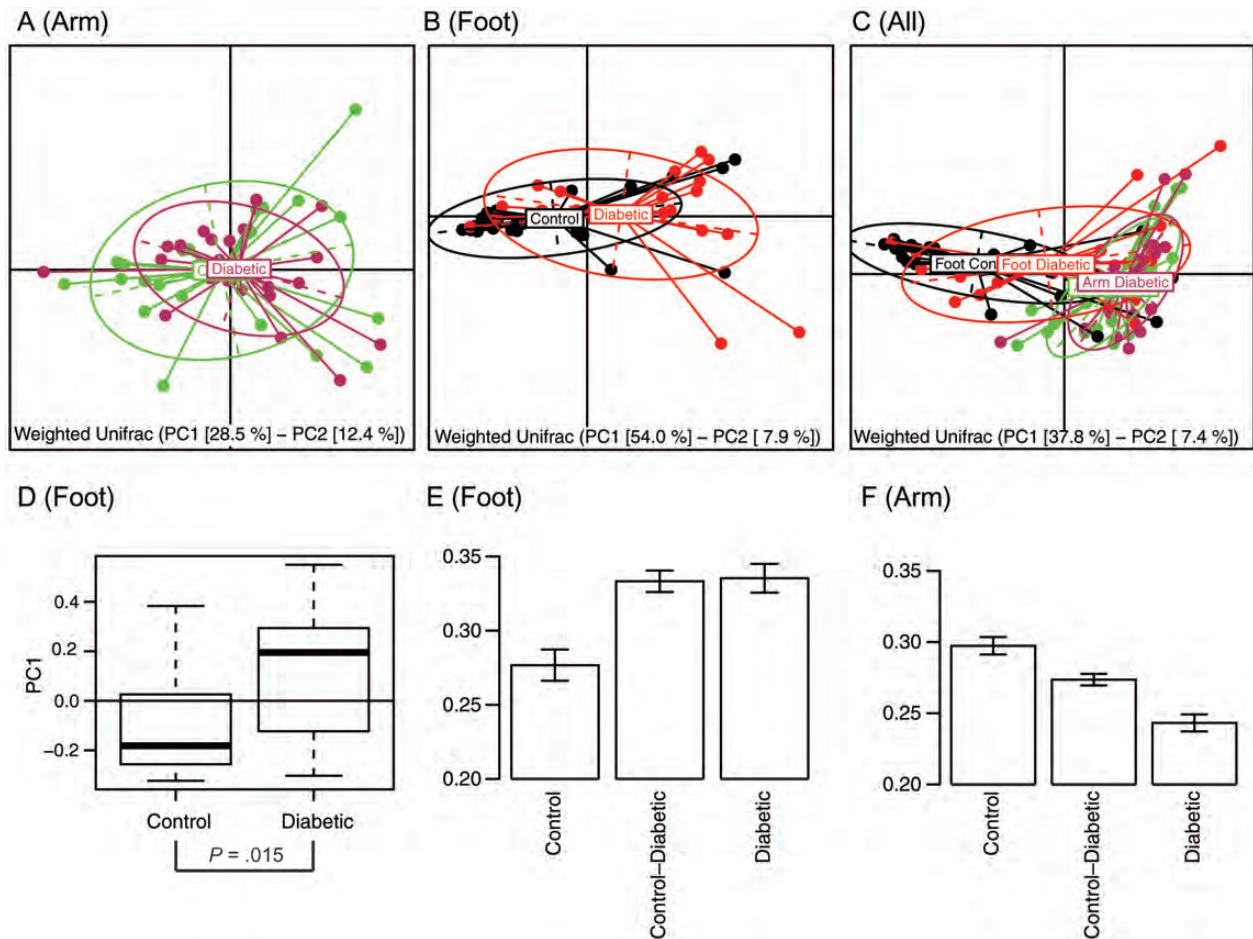
aspect of the diabetic foot, vis-a-vis the controls, may reflect a dysbiosis [32] that enhances disease risk. To further understand the role of the relevant microbial populations in disease, longitudinal study of high-risk patients is needed.

The difference in bacterial diversity on the skin of the foot between diabetic subjects and controls could be partially explained by the ambient outdoor temperature on the day of sampling, which was lower when control specimens were obtained by HTS ( $17 \pm 5^\circ\text{C}$  vs  $21 \pm 6^\circ\text{C}$ ;  $P = .01$ ). No other factors in our study, including glycosylated hemoglobin levels, correlated with bacterial diversity. However, there were no differences in the arms between the diabetic group and controls, suggesting that the ambient temperature did not play a major role in the differences observed. Since diabetes status also correlated with diversity, we speculate that diabetes mellitus alters the microenvironment of the skin. Sweat glands, sebaceous glands, and hair follicles all contribute to specific microenvironments and each have their own microbiota [33]. Diabetic patients are known to have altered sweat and thermoregulatory

responses, even prior to the development of clinical neuropathy [34, 35]. Similarly, the glucose concentration of sweat is elevated during diabetes [36]. These factors together may explain why bacterial diversity in our study correlated with ambient temperature and diabetes status. Altered thermoregulation of the skin on the foot also could help explain why the microbiota is more diverse. Sweat gland output of the forearms of diabetic patients and controls was similar, while in the lower limb, patients with diabetes had less sweat output [37]. The data indicating that forearm diversity is similar in the diabetic subjects and controls but increased in the plantar aspect of the feet of diabetic individuals are consistent with the differential propensity for skin ulceration at the 2 sites.

Sweat glands provide a role in innate immunity in their secretion of antimicrobial peptides, among other factors [38]. Antimicrobial peptides, such as dermcidin [39], secreted from eccrine sweat glands, have activity against *S. aureus* and other cutaneous microbes. Since these glands are less prevalent in the lower extremities of diabetic patients, this mechanism may





**Figure 5.** Principal Coordinate Analysis (PCoA) of weighted Unifrac beta-diversity. Diabetic and control specimens from Panel A: Arm specimens (maroon – diabetes, green – control), Panel B: Foot specimens (red – diabetes, black – control), Panel C: both sites are represented on the first principal plane of corresponding PCoA. The ellipses show the amount of variation along major axes (dashed lines) within each group. Percentage of variability represented by the axes are shown below each panel. Panel D: Foot specimens differ along the first principal axis according to  $t$ -test ( $P = .015$ ). Panels E and F: Weighted Unifrac beta-diversity analysis of (E) feet and (F) arm samples separately. There is increased intragroup diversity in diabetic foot specimens compared with controls ( $P < .0002$ ) matched by a similar intergroup increase ( $P < .00002$ ). Intragroup diversity of diabetic arms is less than that of controls, while intergroup diversity takes an intermediate value (all differences significant at  $P < .005$ ).

contribute to the differences in the microbiota observed in our study. Defective sweat glands and low levels of dermcidin may contribute to atopic dermatitis [38, 40] and may be part of the pathogenesis of diabetic skin infections. Further investigation of the pathogenesis of the cutaneous microbial composition may provide a link between the functions of antimicrobial peptides and the resident microbiota in diseases such as diabetes.

Microbial diversity on the skin is influenced by many factors [41], including sex [42]. A few of the strengths of our study were that we analyzed a cohort of single-sex (ie, male) veterans, who were well matched, that all samples were taken by the same researcher who followed a consistent protocol, and that our control population was similar to previously reported controls in the literature in terms of microbial

composition and quantitation [10, 11, 43]. The sampling occurred over a number of months, and there was a trend toward more cases being recruited during warmer months of the study. This limitation in the study may curtail interpretation of the correlations that we observe. However, the overall diversity at the forearm of the diabetic subjects and controls remained similar, which indicates that the differences may indeed reflect the effects of diabetes on pedal microbial populations, which may contribute to the pathogenesis of wound infections.

### Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of



data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

**Acknowledgments.** H. R. and M. J. B. conceived and designed the study and wrote the manuscript. M. J. B. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Z. G., G. W., and E. S. contributed to the experiment. H. L. provided support in statistical analysis. A. V. A. and Y. Z. provided statistical analysis of the metagenomic data. G. P. P. contributed to the design of the study.

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**Potential conflict of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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