HHS Public Access

Author manuscript

J Orthop Res. Author manuscript; available in PMC 2015 May 04.

Published in final edited form as:

J Orthop Res. 2014 April ; 32(4): 597–605. doi:10.1002/jor.22578.

Longitudinal culture-independent pilot study of microbiota colonizing open fractures and association with severity, mechanism, location, and complication from presentation to early outpatient follow up

Geoffrey D. Hannigan[1], **Brendan P. Hodkinson**[1], **Kelly McGinnis**[2], **Amanda S. Tyldsley**[1], **Jason B. Anari**[2], **Annamarie D. Horan**[2], **Elizabeth A. Grice**[1,*], and **Samir Mehta**[2,*]

[1]Department of Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

[2]Department of Orthopaedics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Abstract

Precise identification of bacteria associated with post-injury infection, co-morbidities, and outcomes could have a tremendous impact in the management and treatment of open fractures. We characterized microbiota colonizing open fractures using culture-independent, high-throughput DNA sequencing of bacterial 16S ribosomal RNA genes, and analyzed those communities with respect to injury mechanism, severity, anatomical site, and infectious complications. Thirty subjects presenting to the Hospital of the University of Pennsylvania for acute care of open fractures were enrolled in a prospective cohort study. Microbiota was collected from wound center and adjacent skin upon presentation to the emergency department, intraoperatively, and at two outpatient follow-up visits at approximately 25 and 50 days following initial presentation. Bacterial community composition and diversity colonizing open fracture wounds became increasingly similar to adjacent skin microbiota with healing. Mechanism of injury, severity, complication, and location were all associated with various aspects of microbiota diversity and composition. The results of this pilot study demonstrate the diversity and dynamism of the open fracture microbiota, and their relationship to clinical variables. Validation of these preliminary findings in larger cohorts may lead to the identification of microbiome-based biomarkers of complication risk and/or to aid in management and treatment of open fractures.

Keywords

open fracture; microbiome; 16S rRNA; bacteria; infection

[*]To whom correspondence should be addressed. Samir.Mehta@uphs.upenn.edu (SM), Hospital of the University of Pennsylvania, Department of Orthopaedic Surgery, 3400 Spruce Street, 2 Silverstein Pavilion, Philadelphia, PA 19104, (215) 349-8868 (Tel); 215-349-5890 (Fax) and egrice@upenn.edu (EAG), Perelman School of Medicine, Department of Dermatology, 421 Curie Blvd, BRB 1007, Philadelphia, PA 19104, 215-898-3179 (Tel); 215-573-2033 (Fax).

INTRODUCTION

Open fractures are characterized by soft tissue disruption at the fracture site increasing the risk of complications including infection, nonunion/malunion, and amputation. Infection risk increases with increasing injury severity and occurs up to 50% of the time when extensive soft tissue damage is involved, due to compromised vascularity among other factors.[1] Predicting which patients will have an infection remains difficult. Surveillance cultures at the time of presentation (before signs and symptoms) are generally thought to have little predictive value.[1–3] Reliable biomarkers to guide management and treatment of open fractures are needed. We hypothesized that microbiota colonizing open fractures during acute phases of injury, prior to clinical signs of infection, may be an information-rich read-out of the wound environment providing valuable insight into the mechanisms of impending complication.

Our bodies are colonized inside and out with myriad commensal microorganisms (the "microbiome") that have important roles in human health and disease. While many infectious states are seemingly caused by single microorganisms satisfying Koch's postulates, the role of the microbiome in modulating the host immune response and resistance to pathogenic and opportunistic microorganisms is increasingly evident. Microorganisms are exquisitely sensitive to their host environment, and likewise, the host immune response is calibrated to react rapidly and precisely to fluctuations in the microbiota. An intimate relationship between the microbiota and the underlying immune and defense response has been demonstrated in skin and cutaneous wounds.[4–7] In the setting of an open fracture, the skin microbiome is altered as a result of the dramatic change in the local environment and contamination from the injury. Local microbial changes may have significant impact on both local and systemic host defenses, soft tissue healing, and, ultimately, clinical outcome.

Most reported studies characterizing bacteria colonizing and/or infecting open fractures rely on clinical culture-based methodology. Traditional hospital-based culture techniques, however, apply heavy selection pressure in favor of bacteria capable of thriving in restricted artificial growth conditions. The most commonly cultured bacteria in open fractures are *Staphylococcus* and Gram-negative isolates[8–10]. Advances in high-throughput DNA sequencing technology enable the study of the human microbiome via sequencing of the bacteria-specific 16S small subunit ribosomal RNA (rRNA) gene. These genomic approaches are increasingly accessible and provide greater resolution and precision by eliminating biases associated with culturing bacteria.

In this pilot study, the microbiome colonizing the open fracture and adjacent skin during the course of healing was evaluated. Sequencing of bacterial 16S rRNA genes was employed to define the composition and diversity of the microbiota in open fractures as healing progressed. Further analysis was done to assess potential correlations between the open fracture microbiome and clinical factors (location, mechanism, severity) and clinical outcomes.

METHODS

Human subjects protections

Prior to study initiation, this protocol was reviewed and approved by the University of Pennsylvania School of Medicine Institutional Review Board. A modification of the informed consent process was approved for this investigation to enable sample collection under emergent conditions. Informed consent was obtained from all subjects enrolled in this study.

Sample collection

Thirty open fracture patients from the Hospital of the University of Pennsylvania Orthopaedic Trauma and Fracture Service were recruited into the study. Characteristics of the patient population are summarized in Table 1. Using a Catch-All Sample Collection Swab (Epicentre), a microbiota sample was collected from the wound center and adjacent skin (5 cm away from the wound) of each subject at emergency room presentation (ER) prior to debridement, irrigation, and cleansing (DIC), and intraoperatively (OR) after DIC. Additional samples were collected at the first outpatient follow up visit (1st OP) and the outpatient visit closest to 28 days following 1st OP (2nd OP). At 1st OP and 2nd OP, 6/21 and 5/15 samples collected were from open fractures with healed soft tissue, respectively. Sample attrition, from the cohort of 30, occurred due to logistical issues in sample collection and attrition during trauma patient follow-up. Also, some samples did not amplify bacterial DNA in sufficient quantities to include in the analysis (see Supplementary Methods).

Negative control specimens were also collected by exposing swabs to room air and processing them alongside wound samples. Clinical, demographic, and behavioral information was collected for each participant. At initial presentation, each wound was classified according to the Gustilo-Anderson classification system[11], anatomic site, and injury mechanism. Complications were assessed as bivariates with any unplanned intervention in the post-operative period considered positive (i.e., readmission, need for antibiotics, repeat debridement or irrigation, soft tissue procedure).

DNA isolation, amplification, and sequencing of 16S rRNA genes

Detailed DNA extraction methodology is provided in the Supplemental Methods and has been previously described[12]. Sequencing was performed with the Illumina MiSeq system using 150 bp paired-end chemistry at the University of Pennsylvania Next Generation Sequencing Core. A total of 7,708,124 paired-end sequencing reads were included in the analysis, with a mean of 43,796 and a median of 30,048 sequences per sample.

Quantitative PCR (qPCR) of the 16S rRNA gene

DNA from the swab extraction described above was used for qPCR-based bacterial load estimation. A portion of the 16S rRNA bacterial gene was amplified using the primers 533F (GTGCCAGCAGCCGCGGTAA) and 902R (GTCAATTCITTTGAGTTTYARYC)[13] on a ViiA7 platform (Applied Biosystems). Each 10 μL reaction included 1 μL DNA, 5 μL 2× SYBR Green Master Mix (Invitrogen), and 0.1 μL of each 20 μM primer solution. Cycling conditions were 50°C (2 min), 95°C (10 min), and followed by 40 cycles of 95°C (15 sec)

and 60°C (1 min). A standard curve was generated by amplifying serial dilutions of known concentrations of *E. coli* genomic DNA. Estimated 16S rRNA copy number and bacterial load were calculated as described previously[14].

16S rRNA sequence processing and analyses

Details of 16S rRNA dataset processing and analyses are in the Supplemental Methods.

Statistical analyses

The R statistical computing package was used for statistical analyses. Principle coordinates analysis (PCoA) plots were produced for visualizing distances between bacterial communities. ANOSIM tests were run to examine the relationship between sample groupings and overall community composition. *P*-values were calculated using 999 permutations. Wilcoxon rank-sum tests and Benjamini Hochberg false discovery rate (FDR) correction was applied to *P*-values to assess the significance of differences in: bacterial load, alpha diversity, and to test for significant associations in fracture characteristics with alpha diversity. Wilcoxon rank-sum tests were also used to assess the differences between taxon relative abundances of wound center and adjacent skin samples at specific time points and Kruskal-Wallis tests were used to examine taxon relative abundance changes across all time points for the following genera: (a) those with median relative abundances >1% in the entire dataset and (b) those that do not meet the 1% threshold but are designated as clinically relevant taxa of interest by the Department of Defense (i.e., *Propionibacterium*, *Escherichia*, *Enterobacter*, and *Klebsiella*). Because of inherent limitations of 16S rRNA-based taxonomic identification and classification, we could not resolve the genera *Klebsiella* and *Enterobacter* based on 16S rRNA sequence. We therefore include in these analyses the unclassified Enterobacteriaceae, which is the family-level taxon that includes the genera *Klebsiella* and *Enterobacter*.

RESULTS

Composition of microbiota colonizing the open fracture site and adjacent skin

The six bacterial genera present in >1% median relative abundance in the open fracture and adjacent skin were *Staphylococcus*, *Corynebacterium*, *Streptococcus*, *Acinetobacter*, *Anaerococcus*, and *Pseudomonas* (Table 2). We also specifically examined the relative abundance of *Propionibacterium*, *Escherichia*, and unclassified Enterobacteriaceae (family containing the genera *Klebsiella* and *Enterobacter*), due to their known pathogenic potential in traumatic injuries[2; 15–19]. The relative abundance of *Staphylococcus* significantly increased and that of *Pseudomonas* significantly decreased in the wound center versus the adjacent skin during the time course (*P*=0.043 and 0.039, respectively). *Escherichia* relative abundance significantly increased on the adjacent skin, but was unchanged in the wound (*P*=0.012). At the ER time point, the genera *Corynebacterium* and *Anaerococcus* were significantly more abundant in the adjacent skin as compared to the wound, where *Pseudomonas* was significantly more abundant in the wound (*P*=0.004, 0.008, and 0.036, respectively). *Corynebacterium* continued to be significantly higher in relative abundance on the skin compared to the wound even after DIC (*P*=0.030).

Comparison of findings from culture-independent and culture-dependent methodologies

Wound cultures were obtained for 14 of the 30 subjects at the time of presentation to the ER. 2/14 (13%) were culture positive for bacteria, with one being culture positive for *Stenotrophomonas maltophilia* and one was culture positive for *Enterobacter cloacae*. 16S rRNA profiling indicated the presence of *Stenotrophomonas* in the wound from which *Stenotrophomonas maltophilia* was cultured. We did not detect *Enterobacter* in the open fracture that cultured positive for *Enterobacter cloacae*, likely due to limitations of 16S rRNA sequence-based identification and taxonomic classification. However, we did detect unclassified Enterobacteriaceae, which is the family-level taxon that encompasses *Enterobacter* species.

Of the 7 subjects in this study that presented with eventual complication, cultures were obtained as standard of care for 3 of the subjects at the time of complication. Two of the 3 subjects were culture positive for *Staphylococcus* (one coagulase-negative and one MRSA) at the time of surgery for nonunion and multiple debridement surgeries, respectively. We detected *Staphylococcus* by 16S rRNA sequencing in all skin and wound samples at all time points of sampling for these subjects. The third subject developed an infection that was culture positive for *Staphylococcus aureus*, *Peptostreptococcus*, and *Enterococcus*. At the ER time point, we detected *Peptostreptococcus* in skin and wound samples and *Enterococcus* on the skin. *Enterococcus* was detected on the skin at all time points and *Peptostreptococcus* was detected in skin and wound samples at 2nd OP. These findings suggest that the eventual type of bacteria implicated in complication by cultures may be present as early as presentation to the ER, and may result from contamination from skin microbiota or be present in the wound itself.

Dynamic microbial diversity of open fracture and convergence with adjacent skin microbiota

To gain an overall view of bacterial community structures changing over time, the beta diversity of the open fracture wound to the corresponding adjacent skin at each time point was compared. Beta diversity was calculated for each pair of samples using the Bray-Curtis metric, which takes into account the number of shared species-level OTUs and their abundance. PCoA plots were used to visualize the shared diversity of wound and the adjacent skin at presentation to the ER (Fig 1A), at 1st OP (Fig 1B), and at 2nd OP (Fig 1C). Progressively, skin and wound communities converged, becoming increasingly similar to each other at each subsequent time point, as measured by Median Intersample Dissimilarity (MID), where a higher MID value indicates greater dissimilarity. ER, 1st OP, and 2nd OP MID values were 0.690, 0.674, and 0.445, respectively. Significant differences between skin and wound microbiomes only existed at the ER time point (P=0.039; R=0.124; Fig 1). At the latter two time points, wound and skin bacterial community structures are indistinguishable by the metrics employed. Given that 6/21 and 5/15 samples analyzed at 1st and 2nd OP respectively were considered healed at those time points, convergence of wound microbiota with the skin microbiota would be expected.

Alpha diversity of open fracture microbiota was measured by the number of observed species-level OTUs and Faith's Phylogenetic Diversity index (Faith's PD), a metric that

takes into account phylogenetic branch length in addition to the number of OTUs present in a sample. These analyses revealed significantly decreased alpha diversity in the wound compared to the skin at presentation to the ER (P=0.019 and P=0.006 for observed OTUs and Faith's PD, respectively; Fig 2A and 2B). There was also a significant decrease in adjacent skin alpha diversity at the first clinical follow-up compared to when the patient presented to the ER (P=0.011 and P=0.003 for observed OTUs and Faith's PD, respectively). We independently examined total bacterial load by quantitative PCR of the 16S rRNA gene. We did not observe significant differences between ER, OR, 1st OP, and 2nd OP time points, or between the wound center & adjacent skin (Fig 2C).

Because of the synergistic role that Gram-positive and -negative organisms have in forming biofilms in wounds and on orthopaedic devices[17], we compared the relative abundances of Gram-positive and -negative bacteria (Fig 3). In the wound, relative abundances of each type of bacteria were approximately similar at presentation to the ER (Fig 3A; P=0.908), but Gram-positive bacteria were significantly more abundant on the skin than Gram-negative bacteria at the same time point (Fig 3B; $P=1.73\times10^{-11}$). These differences were not detectable following DIC. However, at the 1st and 2nd OP time points, both the skin (P=0.003 and $P=2.58\times10^{-8}$, respectively) and wound (P=0.016 and $P=3.51\times10^{-6}$, respectively) harbored greater relative abundance of Gram-positive bacteria, indicating a return to the original skin-like state.

Injury mechanism, location, severity, and complication are associated with open fracture microbiota

We next analyzed open fracture and adjacent skin microbiomes with respect to clinical factors. We selected four variables noted at time of enrollment or in follow up: mechanism, location, progression to infectious complication, and Gustilo-Anderson classification. When examining mechanism and wound severity with respect to colonizing microbiota, alpha diversity, as measured by Faith's PD (Fig 4A–B) and observed species-level OTUs (data not shown), was not significantly different, nor was beta diversity as measured by the Bray-Curtis metric (data not shown). However, when analyzing the top 6 genera present in >1% total abundance and those genera of interest (Table 3), we found that *Corynebacterium* relative abundance was significantly greater and unclassified Enterobactericeae relative abundance was significantly lesser in penetrating wounds compared to blunt wounds at the 1st OP time point (P=0.006 and P=0.038, respectively). At the 2nd OP time point, *Pseudomonas* relative abundance was significantly greater in penetrating wounds compared to blunt wounds (P=0.048). Regarding severity, Type 1 fractures had increased relative abundance of *Acinetobacter* and decreased relative abundance of *Propionibacterium* compared to Type 3 injuries (P=0.015 and P=0.038, respectively; Table 3).

When analyzing microbiota with respect to development of complications, beta diversity, as measured by the Bray-Curtis metric, revealed significant differences in bacterial community structure (P=0.019, R=0.176) when comparing complicated to uncomplicated outcomes. We did not identify any significant changes in alpha diversity (Fig 4C) nor in the specific genera we selected for analysis, indicating that either rare bacteria present in <1% relative

abundance or other undefined aspects of the microbiota are responsible for the change in community structure we observe when comparing the two groups.

Because skin microbial communities are known to differ by body site [20], we also selected wound location as a variable to analyze with respect to microbiomes. We grouped together open fractures of the upper extremities (humerus and ulna) and the lower extremities (femur, hip, tibia, fibula, foot). Bacterial community structure significantly differed when comparing beta diversity of the two groups using the Bray-Curtis metric (P=0.005, R=0.300). Lower extremity open fractures harbored greater alpha diversity than upper extremity fractures as measured by Faith's PD (Fig 4D; P=0.036) and observed species-level OTUs (data not shown; P=0.019). When analyzing all time points, the genera *Anaerococcus* was significantly enriched in relative abundance in lower extremity compared to upper extremity open fractures (Table 3; P=0.015).

DISCUSSION

The findings from this pilot study using culture-independent, high-throughput sequencing based techniques, suggest that a great diversity of microbiota is present in open fractures. Follow-up studies, in larger cohorts and with more frequent sampling until healing is complete may provide more insight into the dynamic changes in the wound and skin microbiota, the association between the microbiota to clinical outcomes, and the potential predictive nature of colonizing bacteria. Similarly, based on a broader understanding of the microbiota, studies examining the role of early debridement, type and timing of antibiotic administration, and irrigation methods can be better designed. Concurrent molecular profiling of host genomic and expression profiles could further clarify mechanisms of infectious complications and the response to treatment.

Molecular techniques are a powerful tool in detecting bacteria. For example, biofilms, such as those that commonly grow on orthopaedic devices, are recalcitrant to culturing[21], suggesting the utility of DNA-based detection methods where biofilm is suspected. Commonly isolated organisms from orthopaedic devices are *Staphylococcus*, *Pseudomonas*, and *Klebsiella*[22; 23]. It is thought that polymicrobial biofilms, those consisting of both Gram-positive and Gram-negative bacteria, are more severe and recalcitrant to treatment[17]. Our findings reveal that, upon presentation to the ER, traumatic open fractures harbor a nearly equally abundant combination of commensal Gram-positive and -negative bacteria, though the skin is dominated by Gram-positive bacteria. The implications of this finding for biofilm formation are unclear, but it suggests that the substrates to nurture a polymicrobial biofilm are in place at the time of presentation. Early application of internal fixation may be at risk given the diversity of microbiome of an open fracture.

A novel aspect of this study was that we examined microbiomes of both the open fracture and the adjacent skin. The adjacent skin may be a source of contamination for open fractures. It may also provide a baseline for assessing microbiota of the open fracture. Together with our analysis of shared diversity at each time point, our data suggests that traumatic wound bacterial communities are least similar to healthy skin upon presentation to the ER, and as expected become more similar as healing progresses. Furthermore,

mechanism of injury, location, and severity are associated with various aspects defining the colonizing microbiota, suggesting the need for different management techniques depending on the injury pattern, for example the difference between penetrating injuries and blunt force open fractures. The finding that open fractures that proceed to develop complications are associated with different microbial communities than those that are complication-free indicates the potential prognostic value of 16S rRNA profiling for identifying those open fractures at risk for complication.

The limitations of our study are that we are in an urban setting with patients coming from the mid-Atlantic region. The local environmental microbiota may be different when comparing to other parts of the world, areas near open water, or wounds that occur on the battlefield across the world. Furthermore, we did not have a control group, which may have included a second individual not injured but in the vicinity of the injured patient. Hospital length of stay may also impact colonizing microbiota and progression to complication, and future studies in larger cohorts will need to take this potential nosocomial confounder into account. Lastly, some aspects of the analysis focused on those bacteria present in >1% relative abundance across the dataset. By including those species that have a known pathogenic potential and are clinically concerning, we attempted to address this.

Ultimately, this study reveals the complexity of the open fracture wound. The ramifications of improved understanding of the bacterial diversity, load, and noted taxa may have significant relevance to initial treatment, methods of monitoring, and clinical outcomes. Predictive modeling and biomarker panels may be the next step in further developing tools that can be applied clinically to decrease infection after open fractures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Grant sponsors: Orthopedic Trauma Association Grant #29 (SM), NIAMS/NIH R00 AR060873 (EAG), internal funds from the University of Pennsylvania Perelman School of Medicine and the Department of Dermatology (EAG), Department of Defense National Defense Science and Engineering Graduate Fellowship (GH). We thank the Penn Orthopaedic Trauma and Fracture Service faculty, residents, and staff for their assistance with sample collection, and the study participants, without whom this study would not have been possible.

REFERENCES

1. Zalavras CG, Patzakis MJ, Holtom PD, et al. Management of open fractures. Infectious disease clinics of North America. 2005; 19:915–929. [PubMed: 16297739]
2. Burns TC, Stinner DJ, Mack AW, et al. Microbiology and injury characteristics in severe open tibia fractures from combat. The journal of trauma and acute care surgery. 2012; 72:1062–1067. [PubMed: 22491628]
3. Valenziano CP, Chattar-Cora D, O'Neill A, et al. Efficacy of primary wound cultures in long bone open extremity fractures: are they of any value? Archives of orthopaedic and trauma surgery. 2002; 122:259–261. [PubMed: 12070643]
4. Chehoud C, Rafail S, Tyldsley AS, et al. Complement modulates the cutaneous microbiome and inflammatory milieu. Proc Natl Acad Sci U S A. 2013

5. Grice EA, Segre JA. Interaction of the microbiome with the innate immune response in chronic wounds. Adv Exp Med Biol. 2012; 946:55–68. [PubMed: 21948362]
6. Grice EA, Snitkin ES, Yockey LJ, et al. Longitudinal shift in diabetic wound microbiota correlates with prolonged skin defense response. Proc Natl Acad Sci U S A. 2010; 107:14799–14804. [PubMed: 20668241]
7. Naik S, Bouladoux N, Wilhelm C, et al. Compartmentalized control of skin immunity by resident commensals. Science. 2012; 337:1115–1119. [PubMed: 22837383]
8. Dellinger EP, Miller SD, Wertz MJ, et al. Risk of infection after open fracture of the arm or leg. Arch Surg. 1988; 123:1320–1327. [PubMed: 3178479]
9. Johnson EN, Burns TC, Hayda RA, et al. Infectious complications of open type III tibial fractures among combat casualties. Clin Infect Dis. 2007; 45:409–415. [PubMed: 17638186]
10. Chen AF, Schreiber VM, Washington W, et al. What is the Rate of Methicillin-resistant Staphylococcus aureus and Gram-negative Infections in Open Fractures? Clinical orthopaedics and related research. 2013
11. Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. The Journal of bone and joint surgery American volume. 1976; 58:453–458. [PubMed: 773941]
12. Gardner SE, Hillis SL, Heilmann K, et al. The neuropathic diabetic foot ulcer microbiome is associated with clinical factors. Diabetes. 2013; 62:923–930. [PubMed: 23139351]
13. Hodkinson BP, Lutzoni F. A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. Symbiosis. 2009; 49:163–180.
14. Grice EA, Kong HH, Renaud G, et al. A diversity profile of the human skin microbiota. Genome Res. 2008; 18:1043–1050. [PubMed: 18502944]
15. Murray CK, Obremskey WT, Hsu JR, et al. Prevention of infections associated with combat-related extremity injuries. J Trauma. 2011; 71:S235–S257. [PubMed: 21814090]
16. Murray CK, Yun HC, Griffith ME, et al. Recovery of multidrug-resistant bacteria from combat personnel evacuated from Iraq and Afghanistan at a single military treatment facility. Military medicine. 2009; 174:598–604. [PubMed: 19585772]
17. Wolcott R, Costerton JW, Raoult D, et al. The polymicrobial nature of biofilm infection. Clin Microbiol Infect. 2013; 19:107–112. [PubMed: 22925473]
18. Saltzman MD, Marecek GS, Edwards SL, et al. Infection after shoulder surgery. The Journal of the American Academy of Orthopaedic Surgeons. 2011; 19:208–218. [PubMed: 21464214]
19. Athwal GS, Sperling JW, Rispoli DM, et al. Acute deep infection after surgical fixation of proximal humeral fractures. Journal of shoulder and elbow surgery / American Shoulder and Elbow Surgeons [et al]. 2007; 16:408–412.
20. Grice EA, Kong HK, Conlan S, et al. Topographical and temporal diversity of the human skin microbiome. Science. 2009; 324:1190–1192. [PubMed: 19478181]
21. Costerton JW. Biofilm theory can guide the treatment of device-related orthopaedic infections. Clinical orthopaedics and related research. 2005:7–11. [PubMed: 16056019]
22. Fernandes A, Dias M. The Microbiological Profiles of Infected Prosthetic Implants with an Emphasis on the Organisms which Form Biofilms. Journal of clinical and diagnostic research : JCDR. 2013; 7:219–223. [PubMed: 23543034]
23. Schmidt AH, Swiontkowski MF. Pathophysiology of infections after internal fixation of fractures. The Journal of the American Academy of Orthopaedic Surgeons. 2000; 8:285–291. [PubMed: 11029556]

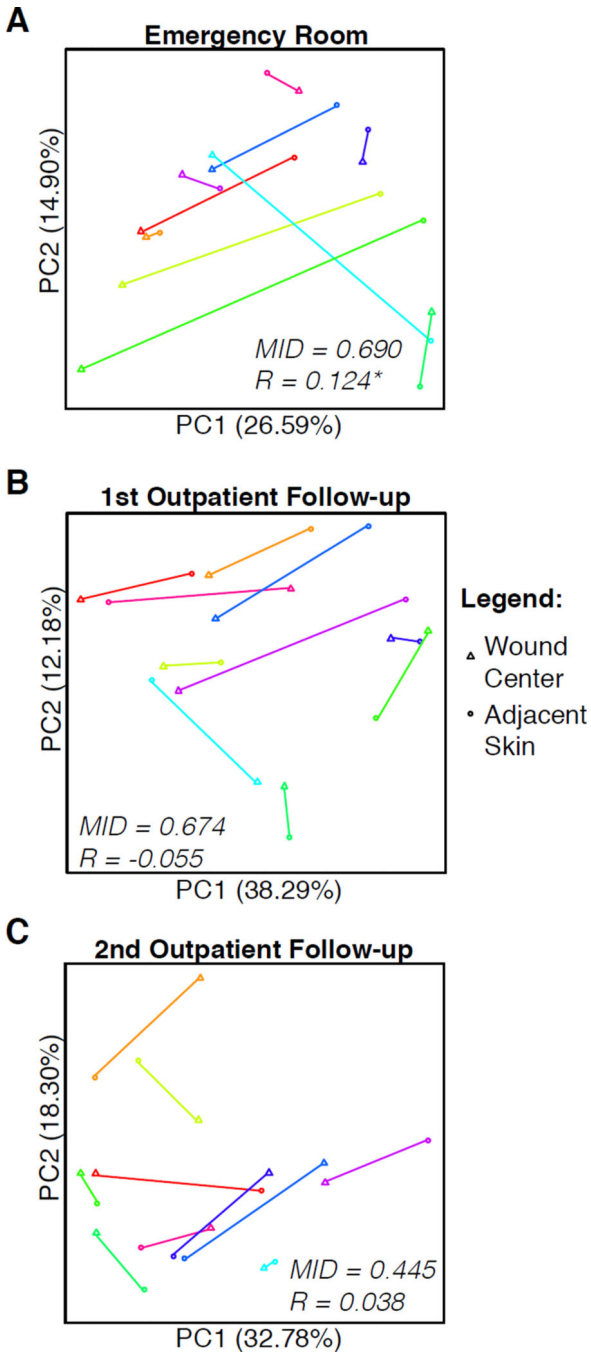

Figure 1. The changing relationships between open fracture wound and adjacent skin microbiota of 10 patients over time

PCoA plots representing the Bray-Curtis metric comparing beta diversity of open fracture and skin microbiota. Each color represents a different patient, while triangles and circles represent wound center and adjacent skin microbiota, respectively. Shown are the first two principle coordinates and the percent variation explained by each principle coordinate is indicated in parentheses by the axis. The two samples (open fracture wound and adjacent skin) for each patient at a given time point are connected by a line. An ANOSIM test was

used to examine the association between swab location and the overall community composition; this association is significant (at $P<0.05$) only for the ER time point.

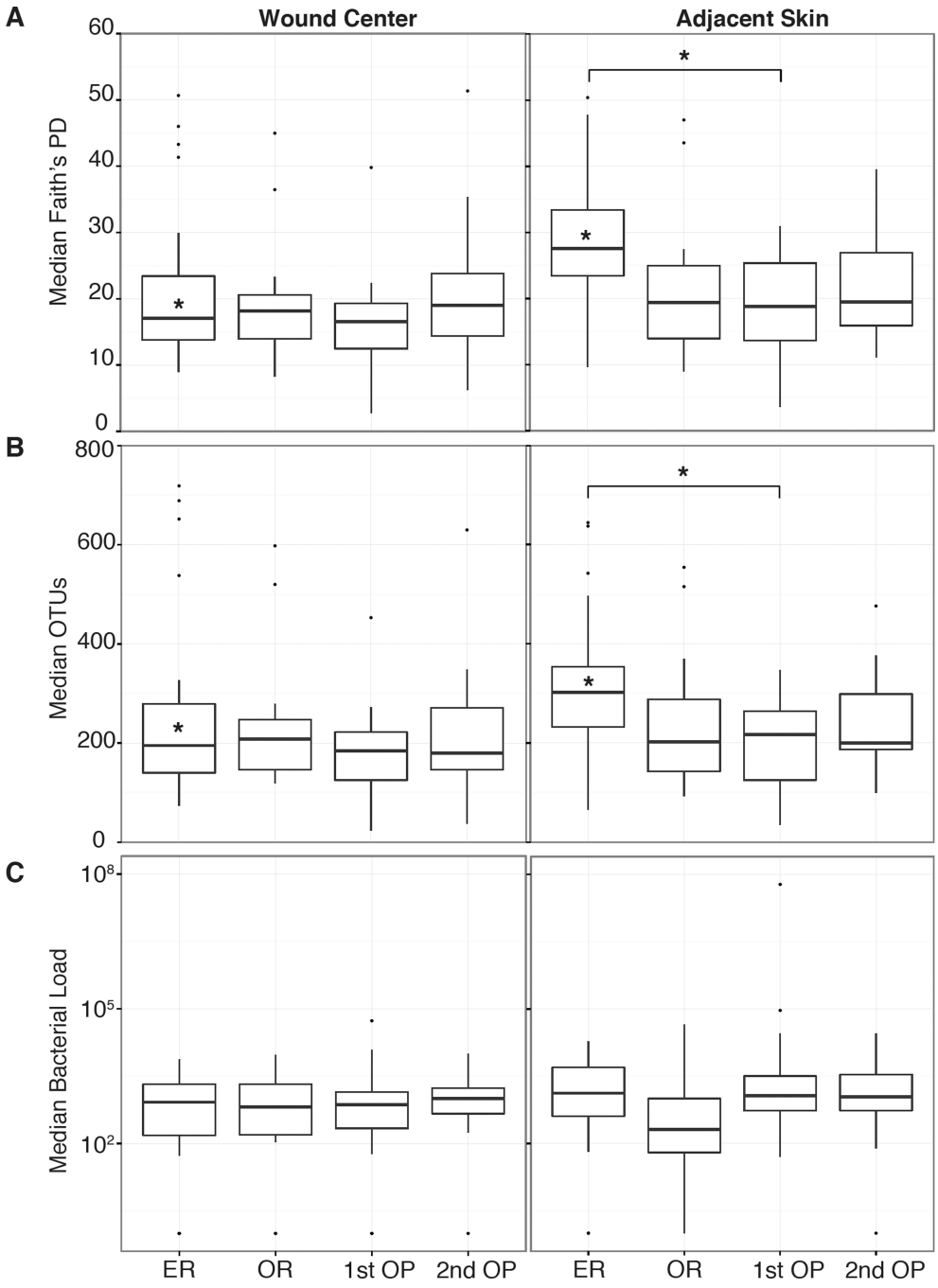

Figure 2. Alpha diversity and bacterial load of open fracture wound and adjacent skin

Alpha diversity is depicted as measured by Faith's PD (A) and observed species-level OTUs (B). Bacterial load (C) is represented as estimates from quantitative PCR of the 16S rRNA gene. The upper and lower box hinges correspond to the first and third quartiles (25% and 75%), and the distance between the first and third quartiles is defined as the inter quartile range (IQR). Lines within the box depict median, and the whiskers extend to the highest and lowest values within 1.5 times the IQR. Outliers of the IQR are depicted with black dots above or below the whiskers. An asterisk (*) inside the box indicates significance of $P<0.05$

(Wilcoxon rank-sum test) between the adjacent skin and open fracture wound at the indicated time point. An asterisk (*) outside of the box indicates significance of $P<0.05$ (Wilcoxon rank-sum test) between the indicated time points.

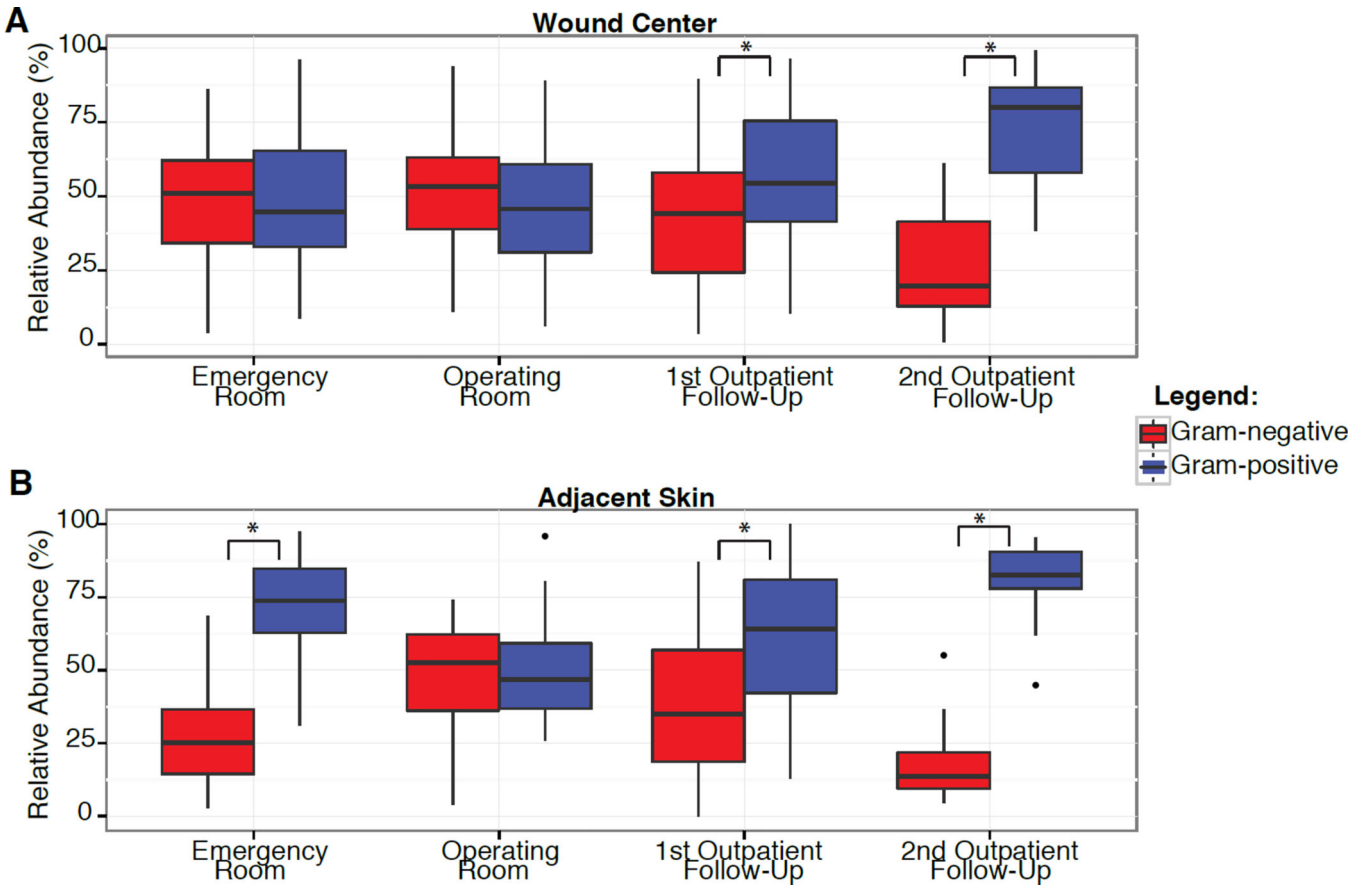

Figure 3. Gram-positive and Gram-negative bacteria in the open fracture wound and on the adjacent skin

Open fracture wound relative abundance is shown in (A) and adjacent skin relative abundance is shown in (B). The upper and lower box hinges correspond to the first and third quartiles. Lines within the box depict median, and the whiskers extend to the highest and lowest values within 1.5 times the IQR. Outliers of the IQR are depicted with black dots above or below the whiskers. *P<0.05 (Wilcoxon rank-sum test).

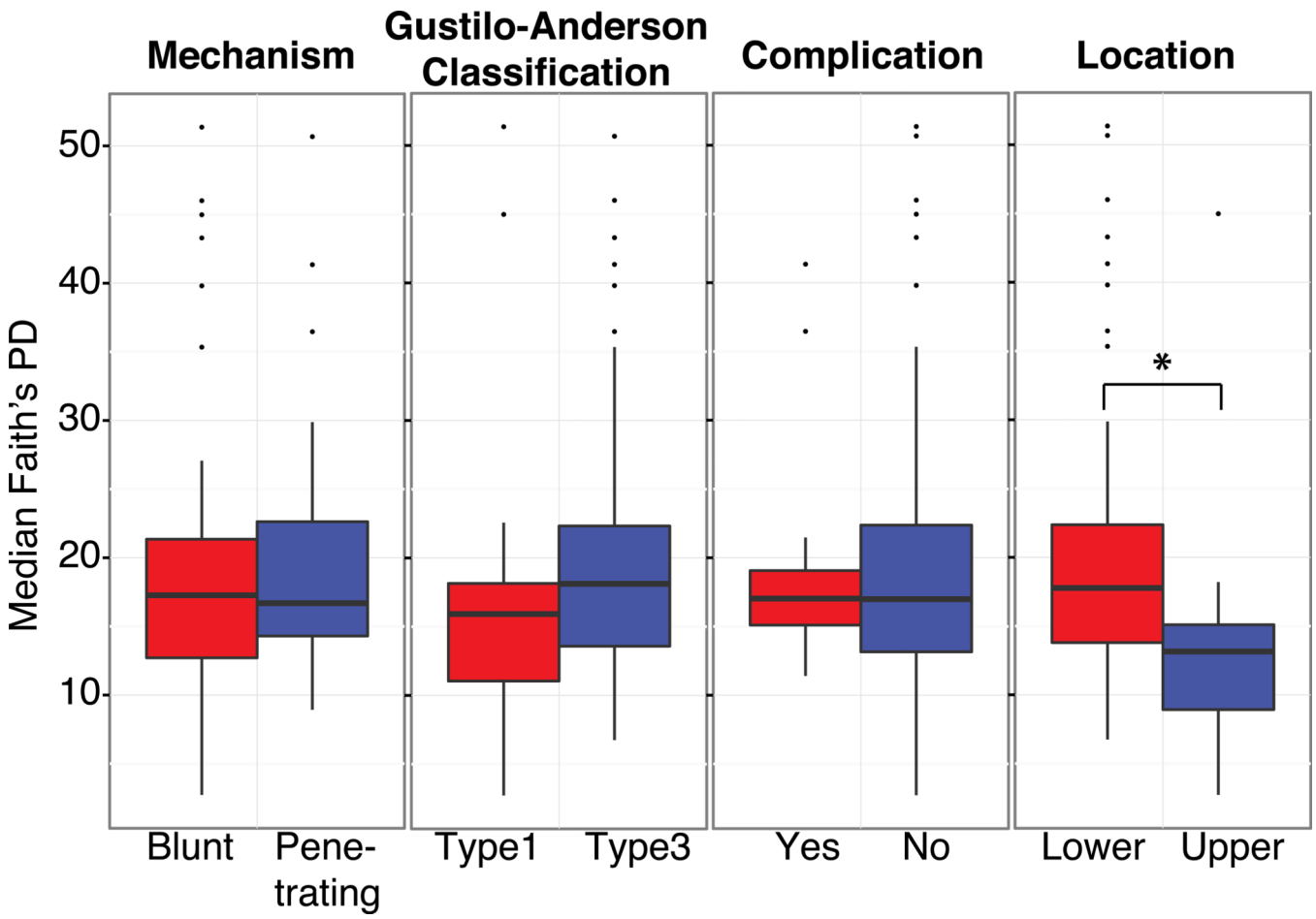

Figure 4. Association of alpha diversity with open fracture characteristics

Faith's PD comparing alpha diversity according to mechanism of injury (A), Gustilo-Anderson classification (B), whether or not the fracture healing process was complicated (C), and the anatomical location of the open fracture (D). The upper and lower box hinges correspond to the first and third quartiles. Lines within the box depict median, and the whiskers extend to the highest and lowest values within 1.5 times the IQR. Outliers of the IQR are depicted with black dots above or below the whiskers. *$P<0.05$ (Wilcoxon rank-sum test).

Table 1

Summary of cohort metadata

Characteristics			Cohort (n=30)
Age			
	Mean, y		43.4
	Range, y		18–82
Female Num (%)			9 (30.0)
Samples Analyzed, Wound Center			
	ER		25
	OR		13
	1st OP		21
	2nd OP		15
Samples Analyzed, Adjacent Skin			
	ER		28
	OR		12
	1st OP		21
	2nd OP		15
Mechanism (%)			
	Blunt		22 (73.3)
	Penetrating		8 (26.7)
Smoker Num (%)			
	yes		7 (23.3)
	no		20 (66.7)
	unknown		3 (10.0)
Gustilo Anderson Classification (%)			
	Type I		5 (16.7)
	Type II		1 (3.3)
	Type III		24 (80.0)
		a	22 (73.3)
		b	1 (3.3)
		c	1 (3.3)
Complication Num (%)			

Characteristics		Cohort (n=30)
	Complication	7 (23.3)
	Non-Complication	23 (76.7)
Location Num (%)		
	Tibia/fibula	12 (40.0)
	Ankle	6 (20.0)
	Femur	7 (23.3)
	Humerus	3 (10.0)
	Calcaneus	1 (3.3)
	Ulna	1 (3.3)
Hospital Stay Length		
	Mean, d	13.3
	Range, d	2–50
Days Between ER and OP1		
	Mean, d	24.3
	Range, d	13–78
	Num Samples	21
Days Between ER and OP2		
	Mean, d	47.8
	Range, d	29–69
	Num Samples	15
Antibiotics Given Num (%)		
	cefazolin	11 (36.7)
	gentamicin	2 (6.67)
	cefazolin + gentamicin	15 (50.0)
	vacomycin + gentamicin	1 (3.3)
	ampicillin + sulbactam	1 (3.3)

Table 2

Median relative abundance of taxa in open fracture wound center and adjacent skin and change over time.

Genus	Wound Center				Adjacent Skin			
	ER	OR	1st OP	2nd OP	ER	OR	1st OP	2nd OP
Staphylococcus[*]	9.643	7.835	9.441	21.014	15.933	11.870	13.974	18.268
Corynebacterium	5.318[a]	2.665[b]	8.707	12.255	17.098[a]	7.454[b]	7.164	21.060
Streptococcus	3.332	2.593	3.270	4.389	2.110	4.106	4.396	3.423
Acinetobacter	3.433	1.945	4.074	3.109	1.927	2.314	1.165	1.362
Anaerococcus	0.406[c]	1.010	0.634	0.858	1.806[c]	1.610	0.826	1.130
Pseudomonas[*]	3.048[d]	2.589	0.990	1.062	0.726[d]	1.769	0.454	0.668
Unclassified Enterobacteriaceae	0.451	0.733	0.627	0.248	0.404	0.949	0.249	0.363
Propionibacterium	0.007	0.000	0.031	0.026	0.018	0.039	0.035	0.018
Escherichia[†]	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000

[*] Changes between time points in wound center and adjacent skin are significant ($p<0.05$; Kruskall-Wallis test)

[†] Changes between time points in adjacent skin are significant ($p<0.05$; Kruskall-Wallis test)

[a,b,c,d] Difference between skin and wound center is significantly different ($p<0.05$; Wilcoxon rank-sum test)

Table 3

Taxa associated with clinical factors

Factor	Taxon	Median Taxon Relative Abundance (%)		Timepoint(s)	P-value*
Mechanism	*Corynebacterium*	Blunt	5.786	1st OP	0.006
		Penetrating	30.656		
	Unclassified Enterobacteriaceae	Blunt	0.877	1st OP	0.038
		Penetrating	0.184		
	Pseudomonas	Blunt	0.944	2nd OP	0.048
		Penetrating	1.966		
Gustilo-Anderson Classification	*Acinetobacter*	Type I	4.732	All	0.015
		Type III	2.028		
	Propionibacterium	Type I	0.000	All	0.038
		Type III	0.017		
Location	*Anaerococcus*	Lower Extremity	0.858	All	0.015
		Upper Extremity	0.115		
		Lower Extremity	0.968	1st OP	0.012
		Upper Extremity	0.000		

*FDR-corrected *P*-values

J Orthop Res. Author manuscript; available in PMC 2015 May 04.