

Δ Culture-Independent Measures of the Traumatic Open Fracture Microbiome to Predict Clinical Outcomes

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Background/Purpose: Predicting which patients with open fractures will have an infection and/or infection-related complication, such as nonunion/malunion or amputation, remains problematic. Surveillance cultures generally have little predictive value, probably because the majority of microbes are not cultivable under standard laboratory conditions. We previously reported pilot results of the open fracture microbiome using culture-independent, high-throughput DNA sequencing of bacterial 16S ribosomal RNA (rRNA) genes, and analyzed those communities with respect to injury mechanism, severity, anatomic site, and infectious complications although the small number of subjects and short-term follow-up at the time of our initial work constrained our understanding. The objective of this current study is to systematically evaluate whether the traumatic open fracture microbiome is a source of clinically relevant markers that are predictive of outcomes.

Methods: 52 subjects with open fractures were enrolled into a prospective longitudinal study. Microbiota were collected using wound swabs at presentation to the emergency department, intraoperatively, at every outpatient follow-up, and in the event that additional surgical intervention was required. 16S rRNA gene sequencing was performed to assess bacterial load, bacterial diversity, and presence and relative abundance of potential pathogens. Outcomes were defined by whether or not surgical intervention was required during the initial 6 months of follow-up. Samples were clustered into microbial community types as defined by Dirichlet mixture modeling. Logistic regression models were used to determine the predictive value of Dirichlet community types, bacterial diversity, bacterial load, and the relative abundance of potential pathogens including *Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Pseudomonas*, *Klebsiella*, and *Enterobacter*. Models were adjusted for covariates: mechanism of injury, fracture severity, and fracture location.

Results: Of the 52 enrolled subjects, median age was 40 ± 17 years; 65% were black, 30% were white; and 76.9% were male. Lower extremity fractures were dominant in our population ($n = 45$, 86%) and no participants had both upper and lower extremity injuries. Outcomes to date include 20 subjects who healed, 12 confirmed nonunions, and the remaining 20 lost to follow-up or still in follow-up with final disposition pending. As illustrated by the example in Figure 1, the open fracture microbiome is dynamic and distinct perturbations were apparent when surgical intervention was required (indicated by arrows). Our preliminary analyses indicate that microbial diversity and composition are associated with infection-related outcomes, and longitudinal assessment of the microbial communities provides greater power for detection.

Conclusion: The open fracture microbiome is dynamic and more complex than standard clinical cultures or clinical presentation can capture. Application of high-throughput genomic

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The FDA has stated that it is the responsibility of the physician to determine the FDA clearance status of each drug or medical device he or she wishes to use in clinical practice.

identification and bioinformatics analysis of microorganisms present in open fractures suggest that the microbiome may be used as a prognostic biomarker for clinically meaningful outcomes.

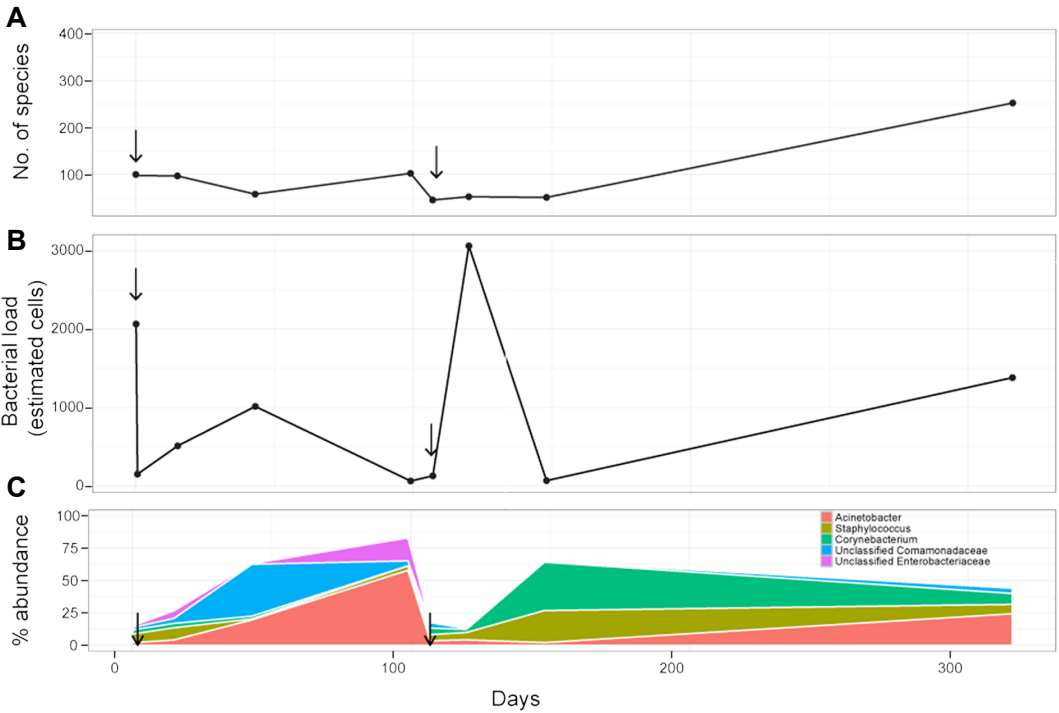


Figure 1
 Example of dynamic microbiome of patient presenting with tibial fracture and developing nonunion in approximately 100 days. A, Bacterial diversity, as measured by the number of species observed (Y-axis) in the open fracture. Diversity decreases with nonunion and increases as healing proceeds. B, Bacterial load (Y-axis) is variable throughout the healing course and offers little predictive value. C, Relative abundance of the top 5 most abundant bacteria (Y-axis). Acinetobacter increases leading up to the nonunion and is replaced by other species following repair.