INTRODUCTION: Fracture nonunion remains a devastating complication that confers significant morbidity. While it is known that nonunions may occur for several reasons, broadly classified as septic or aseptic, the true extent to which the presence of microbes preclude fracture healing remains unknown. With the increasing availability of metagenomic sequencing techniques, such as next-generation sequencing (NGS), rapid and high-throughput detection of all microbial DNA present within a clinical sample is now possible. It has therefore been proposed that a significant percentage of nonunions actually harbor microbes that escape detection by conventional culture methods. To date, no study has examined the metagenomic profile of fracture nonunions or explored the clinical relevance of this signal. Our aim was to investigate the role of NGS in the diagnosis of nonunion compared to culture, as well as its association with treatment outcomes in terms of fracture union.

METHODS: In this prospective multicenter study, samples were collected from 50 patients undergoing open surgical intervention for long-bone nonunion (femur, tibia, or humerus). Nonunion was defined as a failure to progress toward expected union within an anticipated timeframe, and this judgment was made by the attending surgeon. We excluded patients with pathological fractures at index injury and those on preceding antibiotic therapy. A control cohort of 17 patients undergoing fixation of an acute long bone fracture also were enrolled. Tissue samples and intraoperative swabs were obtained at the time of the surgical procedure and sent for 16s rRNA gene-based microbial profiling. For clinical purposes, positive NGS detections were defined separately as having passed amplification with >2000 classifiable sequence reads. Tissue specimens from concordant sites also were sent to the institutional laboratory for culture. Negative sterile water and sequencing reagent controls also were analyzed. Patients were followed up for a minimum of 6-months (range 6–11) for radiological evidence of fracture union. Chi-squared analysis was used to investigate whether clinical positive NGS detection correlated with nonunion status. Multiple analyses were then conducted to determine whether persistent nonunion and clinical covariates were associated with microbial alpha diversity (ANOVA), composition (PERMANOVA), and differential species abundance (ANCOM).

RESULTS: Positive NGS detection of microbial communities was correlated with persistent nonunion (X2 = 6.1, p = 0.048, Table 1). Following quality control, 82 samples representing 45 patients and two sampling institutions were included to assess differences in microbial composition between patient outcome groups. Here, groups explained significant variation in both overall bacterial diversity (Table 2) and composition (R2 = 0.12, p < 0.001, Figure 1). In particular, increased bacterial diversity was associated with undesirable patient outcomes (R2 = 0.17, p < 0.001). Additional patient covariates were indicated (p < 0.01) to influence microbial composition to a lesser extent including patient age (R2 = 0.017), usage of immune suppressing drugs (R2 = 0.012), sex (R2 = 0.013), and bone fractured (R2 = 0.032). Sampling institution in particular was associated here with significant variation in diversity (R2 = 0.06, p = 0.003) and composition (R2 = 0.028, p < 0.001). Further, 37 species were identified to be non-randomly distributed with respect to patient cohort (Figure 2), where 20 species were specifically associated with persistent nonunion.

DISCUSSION AND CONCLUSION: NGS may be a useful adjunct in identification of the causative organisms in the setting of nonunion. Our findings suggest that the fracture-associated microbiome may be a significant risk factor for nonunion outcomes. Further multicenter work is required to determine the clinical implications of isolated organisms detected on metagenomic sequencing and to identify robust microbial predictors of nonunion outcomes.