

## **Culture Positivity in Open Fractures Is an Indicator of Bacterial Bioburden**

**Leah Gitajn, MD;** *Emily A. McClure, PhD, MS; Roman M. Natoli, MD, PhD; Benjamin Ross, PhD; Renan Castillo, PhD; Anthony R. Carlini, MS; Joseph C. Wenke, PhD; Robert V. O'Toole, MD; Michael J. Bosse, MD, FIOTA; METRC*

**Purpose:** Infection following high-energy open fractures remains a major challenge. Open fractures yield positive cultures in 24% to 83% of cases, with culture-positive wounds at significantly higher risk for subsequent infection. Despite this, culture data are rarely used to guide clinical decision-making. Although culture positivity has been thought to reflect overall wound bioburden, this relationship has not been previously validated. The aim of this study was to evaluate the relationship between culture positivity and quantitative bioburden using next generation sequencing (NGS)-based approaches. We hypothesized that higher wound bioburden would be associated with culture positivity.

**Methods:** This is a secondary analysis of the BIOBURDEN Study, including patients age 18 to 74 years with Gustilo type III tibial fractures or traumatic transtibial amputations requiring delayed primary closure or flap coverage with NGS testing (n = 22). At definitive wound closure and follow up, standardized wound samples were collected for culture and banked for future NGS analysis. Patients were followed for 12 months for complications, including deep infection, nonunion, flap failure, amputation, or bone grafting. The primary independent variable was quantitative bioburden, as reflected by the bacteria to human sequencing read ratio by NGS, while culture positivity served as the dependent variable.

**Results:** At definitive wound closure, bioburden was significantly higher in culture-positive specimens compared to culture-negative specimens (mean 28.4 [SD 68.06] vs 0.13 [SD 0.34],  $p = 0.03$ ). When baseline and follow-up time points were analyzed together, culture-positive wounds maintained a significantly higher bioburden (mean 17.7 (43.50) vs 0.16 (0.36),  $p = 0.02$ ).

**Conclusion:** This is the first study to evaluate the relationship between culture positivity and wound bioburden using NGS-based analysis in open fractures. Our findings confirm that culture positivity reflects overall wound bioburden, highlighting its potential role in guiding open fracture management. NGS-based bioburden quantification may offer a more objective and precise method to guide treatment, including decisions regarding additional debridement or local antibiotic therapy. Future research is needed to build on these results and establish bioburden thresholds that predict infection risk, which could enhance clinical decision-making and improve patient outcomes.