H31 The Mouse Thanatomicrobiome and Postmortem Interval (PMI) Estimation

Molly B. Still, Emporia, KS 66801; Scott S. Crupper, PhD, Emporia State University, Emporia, KS 66801*

Learning Overview: The goal of this presentation is to offer an alternative method for determining the PMI using the thanatomicrobiome as examining changes in microbial succession patterns may satisfy this objective.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by helping to address current problems in PMI estimation and showing how thanatomicrobiome data may offer an alternative. Although the thanatomicrobiome is a relatively new field of study, data acquired may offer an alternative to PMI determination.

Determining an accurate PMI in a death investigation is a critical piece of information needed to gain a thorough understanding of the circumstances surrounding a death. While different methods are available to forensic investigators, each has its own set of limitations that decrease its accuracy. Microbes are ubiquitous and play a significant role in decomposition. The thanatomicrobiome is a term used to describe the microbes that colonize internal organs postmortem. Since there is dramatic shifting in microbial communities within a deceased host, these unique microbial signatures may be utilized by forensic investigators to establish a PMI.

Previous PMI studies have employed animal models, such as swine and rodents, since it is easier to control the PMI and obtain multiple replicates as compared to human cadavers. To gain a better understanding into the use of the thanatomicrobiome for PMI studies, this study utilized a mouse model system to examine microbial succession patterns in the liver postmortem. Mice were sacrificed by CO_2 asphyxiation and placed at room temperature in sealed containers that allowed air flow. In total, mice were separated into four groups: a control group (0-day postmortem), and 7-, 15-, and 21-day postmortem groups. At the indicated PMI, livers were removed and stored at -80 C. Total DNA was isolated from each liver sample using a Zymo Research Fungal/Bacterial DNA MiniPrep kit according to the manufacturer's recommendations. Subsequently, the V3 hypervariable region of the 16S rRNA gene was amplified and sequenced on an Illumina® MiSeq® platform. The results indicate *Clostridium* species dominated in all three PMI groups, whereas *Lactobacillus* species accounted for only a small proportion of the total thanatomicrobiome. However, in two of the 21-day PMI mice where the total percentage of *Clostridium* species was significantly lower, *Lactobacillus* species accounted for most of the genera identified. Noteworthy is the fact that putrefaction in the 21-day postmortem mice had progressed to a level that significantly liquefied and made the liver difficult to recognize and harvest. Furthermore, the Shannon Species Diversity Index, which measures species richness, significantly increased from approximately .021 in the control groups to 0.78–2.4 in the 7- to 21-day PMI groups, illustrating that decomposition is a process mediated by a wide variety of microbes. In total, results obtained agree with previous human cadaver thanatomicrobiome studies that have noted a "*Clostridium* Postmortem Effect."

Thanatomicrobiome, PMI, Clostridium