



Pathology/Biology Section - 2016

H96 Using Microbial Communities to Estimate the Postmortem Interval: A Validation Study

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After attending this presentation, attendees will understand the potential of using microbial community succession to estimate the minimum Postmortem Interval (PMI_{min}). Estimating a PMI_{min} range is an important forensic tool that can assist investigators in establishing the timeline of when a person died and in identifying potential suspects. The PMI_{min} is a significant element of solving crimes, yet is a difficult piece of information to estimate or predict using existing, and often fragmented, evidence collected during a death scene investigation. Current methods employed to narrow the estimated time of death include rigor mortis and patterns of insect succession and development; however, recent advances in molecular technology now offer the possibility of utilizing the postmortem activity of microorganisms, such as bacteria and archaea, in estimating the PMI_{min} .¹

This presentation will impact the forensic science community by offering validation of a novel approach to forensic microbiology. In one of the first studies to explore the potential use of microbial communities and next generation technology in estimating the PMI_{min} , Pechal et al. used swine carcasses as surrogates for human cadavers and high-throughput metagenomic sequencing to characterize the microbial community profiles of the buccal cavity and skin throughout decomposition.¹ Their results determined that there was no statistically significant difference among the replicate carcasses, but there were statistically significant differences among days and sampling regions (buccal vs. skin). By calculating the taxon richness and relative abundance at the phylum and family level for each time point, they were able to identify indicator taxa and develop models for estimating the PMI_{min} using Indicator Species Analysis (ISA) and random forest analysis. Indicator phyla identified were Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes. At both the phylum and family level, there was a negative linear relationship of taxon richness over time. Overall, the most accurate model was produced using ISA at the family level with 94.4% of time since placement of remains explained by the postmortem microbial communities. Utilizing this microbial succession information as a forensic tool will likely enable investigators to devise additional methods for collecting evidence and improve accuracy of estimating the PMI_{min} ; however, the next step in developing this approach as a new microbiological tool is to perform validation studies and estimate error in PMI_{min} estimates.

To validate the microbial succession models of Pechal et al. and better understand community succession at the microscopic level throughout decomposition, a replicate survey study was conducted using six swine (*Sus scrofa* L.) carcasses in an open field surrounded by forest in Indiana. Individual epinecrotic microbial community samples were aseptically collected using sterile cotton-tipped swabs from three regions on each carcass: the buccal cavity, a transect of abdominal skin, and the anal region. Sampling occurred every 12 hours at 7:00 a.m. and 7:00 p.m. for eight days, which was the amount of time for the carcasses to fully decompose and blow fly (Diptera: Calliphoridae) larvae to migrate from the resource to pupate. All samples were stored at -20°C until the microbial DNA could be isolated using a commercially available DNA extraction kit. Using 16S amplicon metagenomic sequencing, the microbial communities were characterized (taxon identification and relative abundance) over decomposition time. Once community profiles were determined, the relative abundance and taxon richness trends were compared to the findings of Pechal et al. The indicator phyla previously identified (Bacteroidetes, Proteobacteria, Actinobacteria, and Firmicutes) were also the most predominant taxa in this replication study, and analysis of the relative abundance over time revealed additional similar trends between studies. Taxon richness also decreased over decomposition at both the phylum and family level for this study.

The concept of postmortem microbial succession as a forensic tool has great potential, but has only recently been realized and made possible by next generation technology, which is why a study like this is so critical. By validating proposed microbial succession models, this study provides an important step toward advancing the field of forensic microbiology and increasing accuracy of estimating the PMI_{min} .



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Reference(s):

1. Pechal J.L., Crippen T.L., Benbow M.E., Tarone A.M., Dowd S., Tomberlin J.K. The potential use of bacterial community succession in forensics as described by high-throughput metagenomic sequencing. *International Journal of Legal Medicine*. 2014 Jan; 128(1):193-205.
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