G74 Utility of Bacteria Associated With Human Cadavers in Estimation of Postmortem Interval (PMI)

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After attending this presentation, attendees will gain a better understanding of the role played by bacteria and insects during the human decomposition process and how bacterial succession associated with human cadavers can be used for estimating the human Postmortem Interval (PMI). In addition, attendees will also be updated on recent metagenomics approaches developed for the estimation of bacterial community structure from human cadavers.

This presentation will impact the forensic science community by providing a novel PMI estimation method based on bacterial succession alone. This presentation will also provide detailed information on key bacterial groups whose changes in relative abundance may potentially be modeled for the prediction of time since death.

Like insects, microbes also play a significant role in the carrion decomposition process.[1,2] Studies using pig carcasses in both terrestrial and aquatic environments have shown significant temporal changes in bacterial community structure, but whether the same is true with human cadavers is not clear.[1,3] In this study, six human cadavers were placed in the field as part of three trials (two bodies per trial) at the Forensic Anthropology Research Facility (FARF) of Texas State University, San Marcos, Texas. Out of six carcasses, primary arthropods (e.g., blow flies, flesh flies, staphylinids) that colonize human remains had open access to three carcasses, while the remaining three were excluded from these arthropods. This design permitted the investigation of the impact of arthropods on bacterial community structure as the remains decomposed. Sterile cotton swabs were used for collection of microbe samples from buccal, skin, and anal regions of the cadaver every eight hours for five days. DNA was extracted from these swab samples using organic extraction method.[1] Three variable regions (V1-V3) of 16S rRNA gene were amplified from extracted DNA using primer pairs 28F and 519R.[4] 454-pyrosequencing was performed on amplified Polymerase Chain Reaction (PCR) products using the Bacterial Tag-Encoded FLX Amplicon Pyrosequencing (bTEFAP) method.[5] Sequencing error was minimized using PyroNoise as implemented in Mothur v. 1.29.[6,7] Low-quality regions of the sequences were trimmed using the sliding window (50 bp; Q35) option in Mothur v 1.29.[7] All sequences were checked for chimera formation using Uchime as implemented in Mothur v. 1.29, and using the most abundant sequence as a reference datum.[7,8] Suspected chimeras were deleted and the remaining sequences were utilized for hierarchical classification, α and β-diversity and richness index estimation, multivariate analyses, and for Analysis of Molecular Variance (AMOVA) using Mothur v. 1.29 and R version 2.15.1.[7,9] Indicator species analyses were also performed in Mothur v. 1.29 for determination of temporally informative species.[7]

Preliminary results from the first trial suggest that bacterial community structure changes significantly over time, and primary colonizing arthropods play a significant role in bacterial succession on human cadavers. At the phylum level, an inverse relationship exists between relative sequence abundances of *Proteobacteria* (increases with time) and *Firmicutes*/*Actinobacteria* (decreases with time), and it does not depend on arthropod access or exclusion. However, at the genus level, only human remains that were exposed to insect activity resulted in more accurate estimates of their associated PMI. Significantly different bacterial community structures were observed between 0-3 days and 4-5 days of decomposition. A*cinetobacter*, *Wohlfahrtiimonas*, *Anaerococcus*, *Finegoldia*, and *Ignatzschineria* were the top five indicator genera, whose relative sequence abundances varied with time.

In conclusion, this study provides evidence for the first time that bacteria are a potential forensic indicator for human PMI estimation. There is an influence of primary colonizers on bacterial succession. The data obtained from this research can be used for the development of model-based methods for estimation of human PMI, as was previously done using a porcine model.[1]

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