

## H57 The Ecology of the Human Postmortem Microbiome: Insights From a Large-Scale Study

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After attending this presentation, attendees will better understand the human postmortem microbiome. The majority of research related to the postmortem microbiome involves the use of surrogate animal models (e.g., swine or rodent) or donated bodies placed in anthropological research facilities.

This presentation will impact the forensic science community and practitioners by providing data from a large-scale (185+ cases) characterization of the human postmortem microbiome.

Studies using these methodological approaches, while scientifically valid, result from financial, legal, and/or resource availability limitations. Few published studies have used real-world cases to investigate microbial community changes during the postmortem interval. This presentation will describe the human microbiome characteristics from samples collected during routine death investigation in Detroit, MI.

Since samples were collected on a daily basis, and not limited to the number of donors to a facility, this presentation will offer a unique perspective to the microbial community constituents, composition, and function across a variety of death circumstances. Further, there is a lack of established, long-term collaborations between basic researchers and forensic pathologists to reliably and consistently collect data during death investigation to study the ecology of microbial communities after death. Therefore, this presentation will provide unique insight into the largest known repository of postmortem microbiome samples and the considerations needed for collecting during death investigations.

Microbial samples were collected from 188 cases seen in the Wayne County Medical Examiner's Office in Detroit, MI. Sterile, DNA-free cotton-tipped swabs were used to collect microbial communities from five individual, external anatomic locations: the eyes, external auditory canal, nose, mouth, and rectum. For this study, a variety of cases were desirable to characterize postmortem microbial community stability and variation; thus, no demographic or manner of death was selected *a priori* to collections to ensure samples were obtained from a diverse set of death circumstances. DNA was extracted under aseptic conditions using a commercially available kit with a modified protocol; DNA was quantified using commercially available kits for a fluorometer and a microchannel-based automated electrophoresis system to ensure adequate sample quality and yield for next generation sequencing. The 16S ribosomal RNA (rRNA) V4 gene amplicon region was sequenced for each sample using a 2 x 250 base pair, paired-end approach using a high-throughput sequencing platform. Samples were processed using bioinformatic pipelines to analyze 16S rRNA gene sequences and to predict metagenome functional content from marker (amplicon) genes.

The dataset consisted of samples collected in 2014–2016. There were a balanced proportion of cases between male (56%) and female (44%), and Black (48%) and White (52%). The average ( $\pm$ Standard Deviation (SD)) age was 44 $\pm$ 15 years with a range from 18-88 years. Deaths from homicide had a lower average age (35 $\pm$ 13 years), while natural deaths had a higher average age (53 $\pm$ 11 years). Accidents and suicides were in between (40 $\pm$ 14 years and 49 $\pm$ 17 years, respectively). Microbial analysis results revealed distinct postmortem microbiome signatures based on anatomic location and time since death. Microbial taxa characterized in the postmortem communities were consistent with communities previously detected in antemortem studies (e.g., *Alloiococcus otitis* in the external auditory canal samples). There were clear microbiome differences among sampling areas as decomposition time increased. Proteobacteria had a statistically significant increase (p<0.05) in abundance two days after death. A statistically significant increase in predicted cellular motility was detected after two days postmortem across sampling locations.

In conclusion, this dataset reveals the spatial and temporal variability of the human postmortem microbiome across the largest-scale survey of death investigation in a major, metropolitan city known to date. While traditional techniques for estimating the postmortem interval are important during death investigations, there is potential for using microbial communities as additional biomarkers to corroborate time-since-death estimates. As a community, we need to build the foundational datasets derived from real-world cases to test the validity of microbial communities as postmortem interval indicators. The collaboration between researchers and practitioners to improve non-traditional datasets, such as this one, will ultimately enhance science with practical application to the broader forensic community.

## Forensic Science, Forensic Pathology, Postmortem Microbiome

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