H97 Postmortem Microbiome Changes During Thaw for Autopsy: Two Pediatric Case Studies

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After attending this presentation, attendees will better understand how the postmortem microbiome changes as a frozen body thaws before autopsy. While several studies have documented shifts in microbial community structure (composition and abundance) from various locations on a body during the decomposition process, much less is understood regarding how its previous condition (e.g., frozen, buried, burned) affects the microbiome at the time of discovery. This presentation will include microbiome data collected from two pediatric deaths discovered frozen and concealed in a home in urban Detroit, MI.

This presentation will impact the forensic science community by providing the first case studies in which the bodies had been substantially altered and modified after death as part of the process of concealment. As the bodies were completely frozen, data for this presentation also represents new microbiome profiles for two frozen bodies with estimated postmortem intervals that ranged from 22 months to 32 months. Given the dearth of postmortem microbiome studies that have used samples taken during death investigation, this presentation will also impact the forensic science community by detailing the complexities associated with using microbiome information in one of a wide variety of death circumstances. This presentation also provides unique microbiome data that may be useful for establishing long-term postmortem interval estimations that result from concealing a body in ways that slow or limit decomposition and subsequent microbial activity.

Microbial samples were collected from two cases seen in the Wayne County Medical Examiner's Office in Detroit, MI. They consisted of male and female siblings that were hidden in a chest freezer in a residential location. The 9-year-old male was wrapped in a polychromatic bed comforter; his estimated frozen interval was 32 months. The 13-year-old female was loosely wrapped in a black plastic bag and yellow plastic band with a black cloth wrapped around the neck; her estimated frozen interval was 22 months. DNA-free (sterile) cotton-tipped swabs were used to aseptically collect microbial communities at three time points during the thawing process: when completely frozen, partially thawed (ca. 24h post-discovery), and when fully thawed (ca. 48h post-discovery) for autopsy. The microbial communities were sampled from six external anatomic locations at each time period: the external auditory canal, eyes, nares, mouth, umbilicus, and rectum. DNA was extracted using a modified protocol of a commercially available kit; all DNA was quantified to ensure quality samples. The 16S rRNA V4 gene amplicon region was sequenced for each sample using a 2 x 250 base pair, paired-end approach using an amplicon-based high-throughput sequencing platform.

There was a shift in microbial community composition from the external anatomical sampling areas of these bodies throughout the thawing process for autopsy. The mean (± standard deviation) Simpson diversity index increased as thaw occurred: 0.519 ± 0.294 (frozen) to 0.564 ± 0.330 (partially thawed) to 0.768 ± 0.173 (thawed). While the most prevalent increase in microbial diversity during the thawing process was documented in the nares, eyes, and rectum, buccal samples had the highest mean observed taxa, Simpson diversity, and Faith's

phylogenetic diversity detected among sampling areas. Patterns of microbial taxon turnover during the thaw process were also documented. For example, the relative abundance of *Corynebacterium*, *Haemophilus*, *Fusobacterium*, and *Streptococcus* increased by 79.7%, 75.0%, 46.8%, and 31.0%, respectively, from frozen to thawed, while the relative abundance of *Staphylococcus* decreased 33.3% from frozen to thawed, and *Lactobacillus* became nearly undetectable during partially thawed and thawed sampling times (98.3% decrease). Overall, these data demonstrate that the postmortem human microbiome changes during the thawing process of frozen individuals.

In conclusion, this study contributes a unique data set to the studies of the postmortem microbiome; specifically, partnering with a medical examiner's office allowed the opportunity to characterize microbial communities associated with two unusual deaths that involved long-term freezing, with substantial altering of the microbiome profiles and changes in their communities during the thawing process. These data highlight the inherent variability in circumstances of death in which the collected microbiome evidence may be complex in terms of analyses and interpretation, because microbial communities likely change when the cadaver has been moved or preserved for concealment.

Postmortem Microbiome, Forensic Pathology, Frozen