H95 Investigating Italian Criminal Cases' Postmortem Microbiome Signatures

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Learning Overview: After attending this presentation, attendees will understand how to use postmortem microbial diversity of reproductive organs, spleen, etc. from Italian cadavers with times of death up to 15 days to aid in the correlation of time and manner of death.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing statistically significant next generation sequencing data that may realistically support crime scene investigation findings (homicide, suicide, etc.).

There is active microbial life in the human body after death throughout the stages of human decomposition, and reproductive organs are among the last internal organs to deteriorate. The role that microbes play during decomposition may provide critical data that is potentially useful for the determination of the time or manner of death. Previous thanatomicrobiome studies using corpses from the United States and Finland established statistically significant differences in microbial signatures between two geographical locations. However, the impact of microorganisms on human decomposition in Italian corpses has yet to be fully elucidated for death investigations. In the present study, 16S ribosomal RNA (rRNA) gene amplicon sequencing of DNA extracts was performed using 40 Italian cadavers' internal organs. The samples were obtained from the University of Pavia's Department of Public Health, Experimental and Forensic Medicine. The tissues were collected from between 1-5 internal body sites, namely brain, heart, liver, spleen, and reproductive organs. It was hypothesized that the microbiome profiles associated with decaying reproductive organs maintain signatures that are indicative of the time and manner of death. This study tested the hypothesis by surveying Italian cadavers with postmortem intervals between 24 hours and 15 days with causes of death of homicide, suicide, natural, and accident. All cases were Caucasian with the exception of two, who were of South American nationality. The ages of the cases at the time of death ranged from 16 to 89 years old.

Results of these studies showed that 16S rRNA gene amplicons demonstrated alpha diversity of microbial communities and were significantly different between organ types. For example, the alpha diversity of prostate- and uterus-associated microbial communities was significantly greater than ($p < 0.005$; Kruskal-Wallis) heart and liver. Microbial beta diversity was weakly, but significantly, correlated ($p < 0.001$; ADONIS) between gender ($R^2=0.031$), Body Mass Index (BMI) ($R^2=0.053$), manner of death ($R^2=0.073$), and postmortem interval ($R^2=0.04$). Spleen, liver, heart, and brain microbial diversities were significantly enriched in the cyanobacterial class 4C0d-2. Furthermore, prostate and uterus samples were depauperate for this class, but were dominated by *Bacilli*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Clostridia*.

In conclusion, the postmortem microbial diversity in cadaver organ tissues was analyzed by high-throughput next generation, 16S rRNA amplicon sequencing techniques. This rapidly developing technique is likely to provide several discoveries in DNA casework. This study confirmed that the alpha and beta diversities varied significantly among internal organ tissues. The significant enrichment of specific bacteria show correlations to several factors, particularly gender, BMI, and postmortem interval, which may provide insight into several aspects of medicolegal death investigations. This data will be used to build statistical models to generate a predictive environment whereby the microbiome of these internal tissue samples can be used to predict the time and manner of death for a given corpse.

Cadaver Internal Organs, 16S rRNA, Thanatomicrobiome