H163 The Postmortem Clostridium Effect: A Thanatomicrobiome Investigation of Cadaver Brain

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Learning Overview: After attending this presentation, attendees will have learned how to use the Postmortem Clostridium Effect as a biomarker for forensic microbiology in cadaver brains obtained from criminal casework with a postmortem interval of up to 11 days.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on the role of *Clostridium* on the decomposition of human brains from actual criminal cases for forensic science applications.

What happens to the more than 100 trillion bacterial cells and 10 million human cells after a person dies has yet to be determined. It is known that brain cells generally die in as little as three to seven minutes after death. Revealing the diversity of microbial communities located in the human brain using bioinformatics and machine-learning tools have the potential to be a useful biomarker in forensic microbiology. The brain is identified as an intermediate-decomposing body site in the progression of the natural order of decomposition. Human thanatomicrobiome studies have established that putrefactive bacteria have been found in abundance within internal organs of decaying bodies. Of the various types of putrefactive bacteria found in internal body sites, obligate anaerobes of *Clostridium* spp. have been found in highest abundance. According to previous thanatomicrobiome studies, *Clostridium* spp. predominated at both short (4h) and long (up to 10 days) postmortem intervals.

The current study surveyed the postmortem microbiomes of brain tissues from 11 corpses with varying times of death ranging from 29 hours to 11 days. The brains of individuals were collected during autopsy using sterile equipment. Metadata for each cadaver was collected: age, sex, time of death, and cause of death from Official Daily Crime Logs. Total DNA was extracted using the phenol-chloroform method. *Clostridium* genes were amplified and sequenced using primers Clos58-F and Clos530a-R. The influences of case characteristics on microbiome composition, such as beta diversity, were investigated. Following standard demultiplexing and quality filtering using the Quantitative Insights into Microbial Ecology pipeline (QIIME™) and USEARCH global alignment algorithm, *Clostridium* spp. were identified. The output was analyzed using an internally developed Python program that assigned taxonomic information to each sequence. The results demonstrated that age was a significant predictor of the number of observed taxonomic units in brain samples. Furthermore, *Clostridium* and *Escherichia* were the most dominant observed bacterial genera in the study. To date, the results represent the first pilot study to perform *Clostridium* sequencing on postmortem brain tissue from criminal cases and confirm the Postmortem Clostridium Effect in decaying cerebral tissues.

PCE (Postmortem Clostridium Effect), Cadaver Brain, *Clostridium* Sequencing

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