



H98 International Associations of the Postmortem Microbiome

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Learning Overview: After attending this presentation, attendees will better understand which metadata has the most impact on structuring a human postmortem microbiome dataset from a multi-country study.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing insight as to how regularly collected case metadata, such as sex, age, body mass index, manner of death, or country, influenced the human postmortem microbiome.

The use of high-throughput sequencing and advanced statistical modeling to characterize the postmortem microbiome during death investigations has experienced a recent surge. This presentation will describe the human microbiome associations from samples collected during routine death investigations across four countries.

Samples were collected during routine death investigations from four medical examiner's offices (or the international equivalent) located in: Salzburg, Austria; Lille, France; Naples Italy; and Wayne County, Detroit, MI, United States. The individuality of the death circumstances and death investigation protocols within each country is key to acquiring a broad survey of samples from non-targeted case demographic or circumstances of death.

Microbial samples were collected in 2014–2017 from a total of 242 cases. Each case needed to meet the following criteria for inclusion in this dataset: (1) adult (≥ 18 years old), and (2) known circumstances of death. To collect the microbial communities, sterile DNA-free cotton-tipped swabs were individually rubbed against the external surfaces of three anatomic locations (mouth, nose, and rectum) as part of the office's established death investigation protocol. Individual swabs were extracted under aseptic conditions using a commercially available kit with a modified protocol, and the resulting DNA was quantified using commercially available kits for a fluorometer and a microchannel-based automated electrophoresis system. Targeted amplicon (16S ribosomal RNA (rRNA) V4 gene region) high-throughput sequencing was performed on individual sample libraries using a 2x250 base pair, paired-end approach. Samples were processed using an open-source bioinformatic pipeline and statistical analyses, including machine learning algorithms, to identify significant associations among the targeted gene amplicon sequences (microbiome) and case metadata from four countries located in North America and Europe.

Microbial analyses results confirmed distinct postmortem microbiome signatures based on anatomic location and estimated postmortem interval, as previously shown.¹ Alpha-diversity differences (e.g., observed species, taxon diversity) were distinguished among anatomic location postmortem microbial communities. Visualization of these differences were clearly seen in principal coordinate analyses based on phylogenetic similarity (abundance weighted UniFrac distances), demonstrating strong intra-anatomical location similarity. Samples were partitioned based on these anatomic location differences for subsequent analyses. Supervised machine learning analyses classified metadata sources based on sequence variant community composition. Bacterial species within Firmicutes were the most important features for all metadata tested structuring this dataset.

In conclusion, this dataset further highlights the spatial and temporal variability of the human postmortem microbiome across an international, large-scale survey of death investigation. Potential pan-microbial signatures of forensic importance, including ubiquitous bacterial species, were identified within this dataset. There is a tremendous need for pathologists and researchers to continue to work together to identify robust microbial signatures that could be routinely used to inform questions of interest during death investigation across the globe. Additional sample collection, processing, and modeling is imperative to validate the models developed from individual datasets, such as this one, to confirm or identify additional postmortem microbial biomarkers.

Reference(s):

1. Pechal, J.L., Schmidt, C.J., Jordan, H.R., and Benbow, M.E. (2018). A Large-Scale Survey of the Postmortem Human Microbiome, and Its Potential to Provide Insight Into the Living Health Condition. *Scientific Reports*, 8(1), 5724.

Forensic Science, Postmortem Microbiome, Forensic Pathology