



### H58 A Global Partnership to Study Geographic Variation in the Human Postmortem Microbiome (HPMM)

*M. Eric Benbow, PhD\**, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824; *Jennifer L. Pechal, PhD*, Michigan State University, 243 Natural Science Bldg, Dept of Entomology, East Lansing, MI 48824; *Heather R. Jordan, PhD*, Mississippi State University, PO Box GY, Mississippi State, MS 39762; *Vadim Mesli, MD*, Institut Medico Legal, Rue Andre Verhaeghe, CHRU Lille, Lille Cedex, Nord 59037, FRANCE; *Erwan Le Garff, MD*, Institut Médico-Légal/Forensic Institute, Rue André Verhaeghe, Lille Cedex, Nord 59037, FRANCE; *Valéry C Hedouin, MD, PhD*, CHRU CS 70001, Lille, Hauts de France 59037, FRANCE; *Carlo P. Campobasso, MD, PhD*, University of Campania, Dept Experimental Medicine, via Santa Maria di Costantinopoli, 16, Napoli 80138, ITALY; *Valentina Bugelli, MD*, Department of Medicine and Health Sciences, via Francesco De Sanctis, Campobasso 86100, ITALY; *Stefan Pittner, PhD*, Salzburg University, Interfaculty Dept Forensic Medicine & Neu, Ignaz Harrer Str. 79, 5020, Salzburg, AUSTRIA; *Fabio Monticelli, Ignaz Harrer str. 79, Salzburg, AUSTRIA*; and *Carl J. Schmidt, MD*, Wayne County MEO/University of Michigan, 1300 Warren, Detroit, MI 48207

In recent years, there has been interest in using microbiomes in the forensic sciences. There are also several publications documenting the excellent potential for this new line of evidence collection; however, many of these studies have either used non-human models (e.g., swine) or donated human remains that are evaluated in controlled environments, limiting our understanding of the variability in the HPMM collected during routine death investigation, especially in widely separated geographic areas. After attending this presentation, attendees will have a better appreciation for the variability of the HPMM at two scales: (1) within a large metropolitan city; and, (2) across three European cities. Further, attendees will be introduced to a developing global partnership to study the HPMM. The mission of the global HPMM partnership is to expand studies of postmortem microbial ecology so scientists can better understand the potential broad application of the HPMM in practice, and the challenges and limitations that will be addressed as this new science emerges into forensic use.

This presentation will impact the forensic science community by providing new data from an ongoing, large-scale (>180 cases) study of the HPMM from samples taken during routine death investigation received at the Wayne County Medical Examiner's Office in Detroit, MI, and compared to new collections from France, Italy, and Austria. Additional impact will be to bring awareness of this global effort and perspective to evaluate the HPMM and discuss some of the challenges and limitations with bringing this new technique into practice.

Microbial samples were collected from 188 cases at the Wayne County Medical Examiner's Office from 2014 to 2016 during routine death investigation, and 25-30 cases received as autopsies or during investigation in Lille, France, Napoli, Italy, and Salzburg, Austria. All samples were collected using standardized kits constructed and packaged in the same location at Michigan State University under aseptic conditions. Each kit contained individually packaged, sterile, DNA-free cotton-tipped swabs and 1.7ml microcentrifuge tubes with 200µl molecular-grade 96% ethanol for each case. Kits were shipped to each international location for standardized collections. For each case, individual swabs were used to collect microbial communities from five anatomic locations: the eyes, external auditory canal, nose, mouth, and rectum. After standardized swabbing, each swab head was placed into an individual tube, then the entire kit was placed at -20°C until DNA extraction and sequencing. DNA was extracted and quantified under aseptic conditions using commercially available kits. 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.

In the Detroit dataset, cases 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 to 88 years. The cases from France represented 28% male and 72% female with an average age of 82 that ranged from 58 to 95 years; cases from Italy and Austria are being processed. Taken together, the HPMM was most highly influenced by anatomic location and then by the Postmortem Interval (PMI), suggesting that anatomic site should be considered before attempting to estimate PMI. Many of the taxa reflected antemortem communities, but this changed with increasing PMI. There was high variation even within body areas from the large sample size from Detroit; however, Proteobacteria was consistently more abundant at longer PMIs. Not surprisingly, much of the variation in the HPMM, even within body area, is likely attributable to antemortem health and lifestyle that is often associated with geographic area and the sociocultural environment.

The enthusiasm for using the HPMM in the forensic sciences is increasing globally; however, there is an urgent need to obtain a more quantified assessment of the local, regional, and global variability in postmortem microbiome communities. Part of this assessment will also require identifying key microbial taxa that are clear forensic indicators and are consistent among different lifestyles, geographic areas, and sociocultural environments. The new global partnership is intended to provide the collaborative network for achieving such inquiry to move this emerging science into future practice.

#### Forensic Microbiology, International Partnerships, Proteobacteria