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- **Summary of the project**
    - *Major goals and objectives*

During life, the microbiome serves important health-related functions including nutrient acquisition, pathogen defense, energy salvage, and immune defense training (1). The microbiome has also been linked to cardiovascular, metabolic and immune disease, as well as mental health disorders via the gut-brain-axis (2). Upon death, microbial communities present within and on the body are exposed to radical environmental changes, and recent studies have shown that microbial succession among mammalian cadavers follows a metabolically predictable progression (3, 4).

    - *Research questions*

This study aimed to investigate the extent to which microbial associations among different organs in human cadaver can be used to predict manner of death (MOD), postmortem interval, and geographic locality of origin. By sampling human cadavers from three disparate geographic origins (Finland, Italy, and the United States), we were able to ascertain that geographic locality has a significant influence on microbial community composition of postmortem tissues, and that despite these differences, commonalities may still be identified both among tissues, and individuals that died due to varying causes of death (e.g. natural, accidental, homicidal, and suicidal deaths). We were unable to detect significant correlations between various samples and the postmortem interval, likely due to the fact that the sampling regimen was optimized to capture variation among geographic locality, organ type, and manner of death. Significant patterns were observed associated with geography and manner of death, which warrant reinforcement from additional investigations to elucidate the origin of these associations.

- *Research design, methods, analytical and data analysis techniques*

The microbiome serves important functions in human health, but post-mortem the microbial signatures of colonized organ tissue could also be useful in helping to predict the manner of death of cases where this is not known. We surveyed the microbiota (16S rRNA V4 amplicon sequencing) of 280 organ tissue samples including liver, blood, brain, heart, prostate, spleen and uterus from cadavers in Italy, Finland and the United States with confirmed manner of death comprising either accidental death, natural death, homicide, and suicide. Unweighted UniFrac (but not weighted UniFrac) was significantly different by cadaver ethnicity and age, which suggests that taxa with very low proportions may account for the differences observed between these groups. Weighted UniFrac beta diversity (but not unweighted UniFrac) was significantly different by manner of death, post-mortem interval and the body-mass index of the cadaver, suggesting that these characteristics affect the proportions of abundant micro-organisms. Different tissues exhibit differential associations with bacteria, with the prostate and uterus tissues maintaining highly unique microbial composition compared to other organs. For example, in Italian cadavers, MLE1-12 was abundant in nearly all tissues, except the prostate and uterus. We identified specific bacterial ASVs as biomarkers of either natural or accidental death and suicide, but not homicide. While the manner of death may have an impact on microbial associations, further investigation under more controlled conditions will be needed to validate whether these associations are predictive.

- *Expected applicability of the research*

Forensic microbiology represents a potential emerging discipline in which microorganisms serve as forensic tools or trace evidence. Advances in DNA sequencing technologies paired with increased understanding of the human microbiome have hinted at the possibility that the microbiome could be used as a biomarker of decay (3) and as

trace evidence to link individual people to objects they have previously interacted with (5-9). Recent studies have also shown that the microbiome can be used to estimate the amount of time that has elapsed since death, referred to as the post-mortem interval (PMI), allowing investigators to establish a potential timeline of death (3, 10-16).

The microbial composition and abundance associated with internal organ tissues are dependent on temperature, manner/cause of death, and PMI, since bacteria have different growth optima based on the physicochemical constraints of their environment (17-19). Also microbial abundance associated with the body pre-mortem can play a role in decay, as a cadaver of an aged adult human, with approximately 40 trillion microbial cells, decays more rapidly than a deceased fetus or newborn, which usually have reduced microbial colonization density (20). Of course, these trends are contingent upon the medications and disease state of the individual.

- *Participants and other collaborating organizations*

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- *Outcomes*

We collected 276 samples of multiple organs from corpses derived from Finland, Italy, and the United States (Table 1; Table S1). Sampling spanned postmortem intervals (PMIs) of 3.5 to 432 hours (avg = 87.6 hours) and included tissues from cadavers corresponding to different manners of death grouped into four categories: accidental death (n = 88), natural death (n = 106), homicide (n = 23), and suicide (n = 45) (Table 2). In total, 4,337,301 16S rRNA V4-5 amplicon sequencing reads were generated from 276 samples, comprising 2,204 Absolute Sequence Variants (ASVs). Following sequence deblurring and rarefaction analysis (5,000 read cut-off), we identified 1,855 ASVs across 163 samples, with a range of 239 to 1,413 ASVs across different organs.

Table 1. Sampling by geographic location and organ, post-rarefaction

	Organ						
Location	Blood	Brain	Heart	Liver	Prostate	Spleen	Uterus
Finland	0	0	0	20	0	0	0
Italy	0	10	9	13	13	10	5
USA	6	9	11	47	0	10	0
Total	**6**	**19**	**20**	**80**	**13**	**20**	**5**

Table 2. Sampling by manner of death and geographic locality, post-rarefaction, with PMI statistics; undetermined MOD (n=2) excluded from analyses.

	Manner of Death				Postmortem Interval			
Location	Accident	Natural	Homicide (n)	Suicide	PMI_{min}	PMI_{max} (hrs)	PMI_{avg}	PMI_{SD}

	(n)	(n)		(n)	(hrs)		(hrs)	(hrs)
Finland	6	12	0	2	48	192	108	42.3
Italy	20	24	3	13	24	432	112	96.4
USA	31	29	9	12	3.5	240	37.8	47.8
Total	**57**	**65**	**12**	**27**				

Alpha diversity, calculated as observed number (richness) of ASVs and the Shannon diversity index, differed significantly between some but not all organs and varied by locality (both, $p < 0.05$, Kruskal-Wallis). Post-hoc tests (corrected for multiple comparisons using the Benjamini-Hochberg method) revealed that among Italian subjects, the prostate and uterus differed significantly from all other organs (brain, heart, liver, and spleen) in both observed richness ($p < 0.05$, Dunn's Test) and Shannon diversity ($p < 0.05$, Dunn's Test), but they did not differ significantly from each other (Fig. 1A and 1B). Among subjects from the United States (USA), the only organs that differed significantly by Shannon diversity were heart and liver ($p = 0.032$, Dunn's Test), and no organs differed significantly by observed richness (Fig.1A). A comparison of alpha diversity measures for liver samples from all three localities (Finland, Italy, USA) identified significant differences in both observed richness and Shannon diversity between liver tissue from Finland and the USA ($p < 0.05$, Dunn's Test), and Finland and Italy ($p < 0.05$, Dunn's Test), but not between Italian and US livers (Fig. 1C).

Figure 1

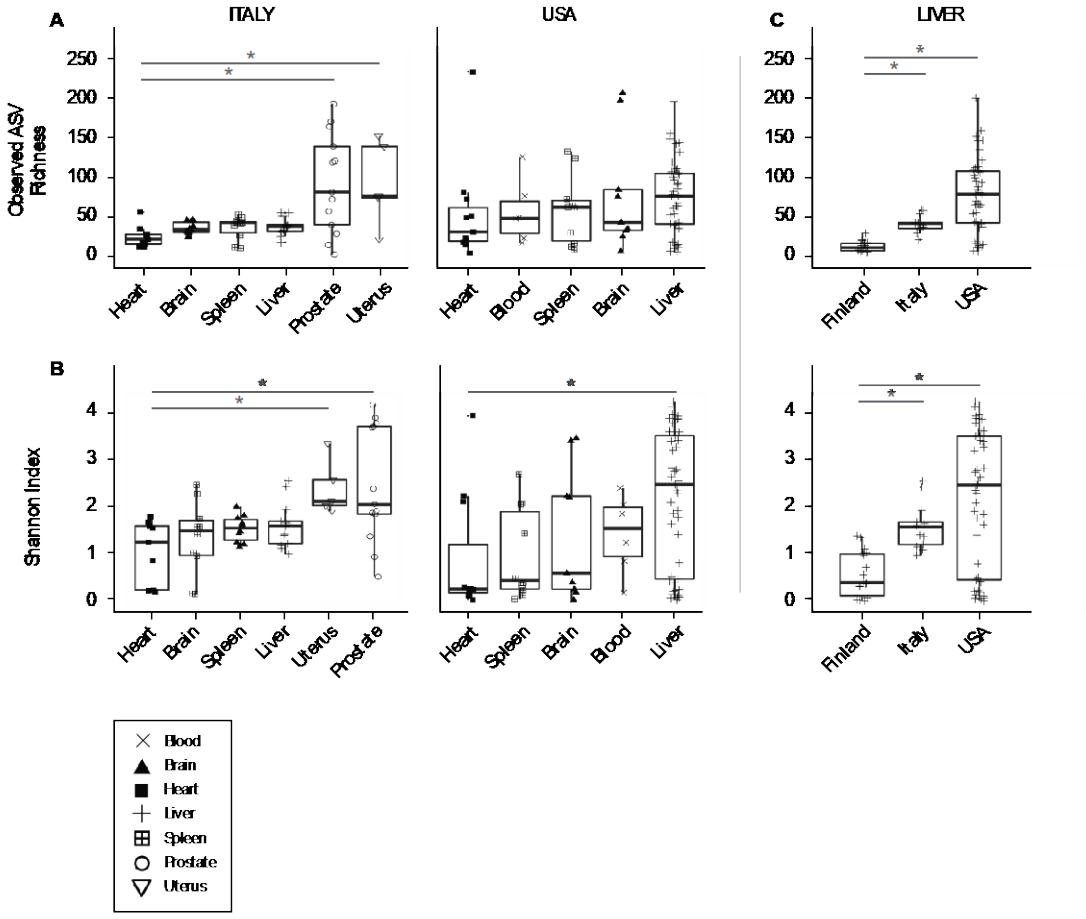

Figure 1. Variation in alpha diversity by organ type, A) comparing observed ASV richness between organs from different localities, B) comparing Shannon diversity index between organs from different localities. Asterisks indicate significant difference between groups based on post-hoc Dunn's Tests, $p < 0.05$.

Alpha diversity differed significantly by manner of death among USA organs ($p < 0.05$, Kruskal-Wallis), but not among Italian or Finnish organs. Among USA organs, observed richness differed significantly between accidental deaths and homicides ($p < 0.0177$, Dunn's Test), accidental deaths and suicides ($p < 0.0002$, Dunn's Test), and natural

deaths and suicides ($p < 0.0005$, Dunn's Test), but not between homicides and suicides, natural deaths and accidents, or natural deaths and homicides (Fig. 2A). Also among USA organs, Shannon diversity differed significantly between accidental deaths and suicides ($p < 0.0001$, Dunn's Test), natural deaths and homicides ($p < 0.008$, Dunn's Test), and natural deaths and suicides ($p = 0.000$, Dunn's Test), but not between homicides and suicides, natural deaths and accidents, or accidental deaths and homicides (Fig. 2B).

Figure 2

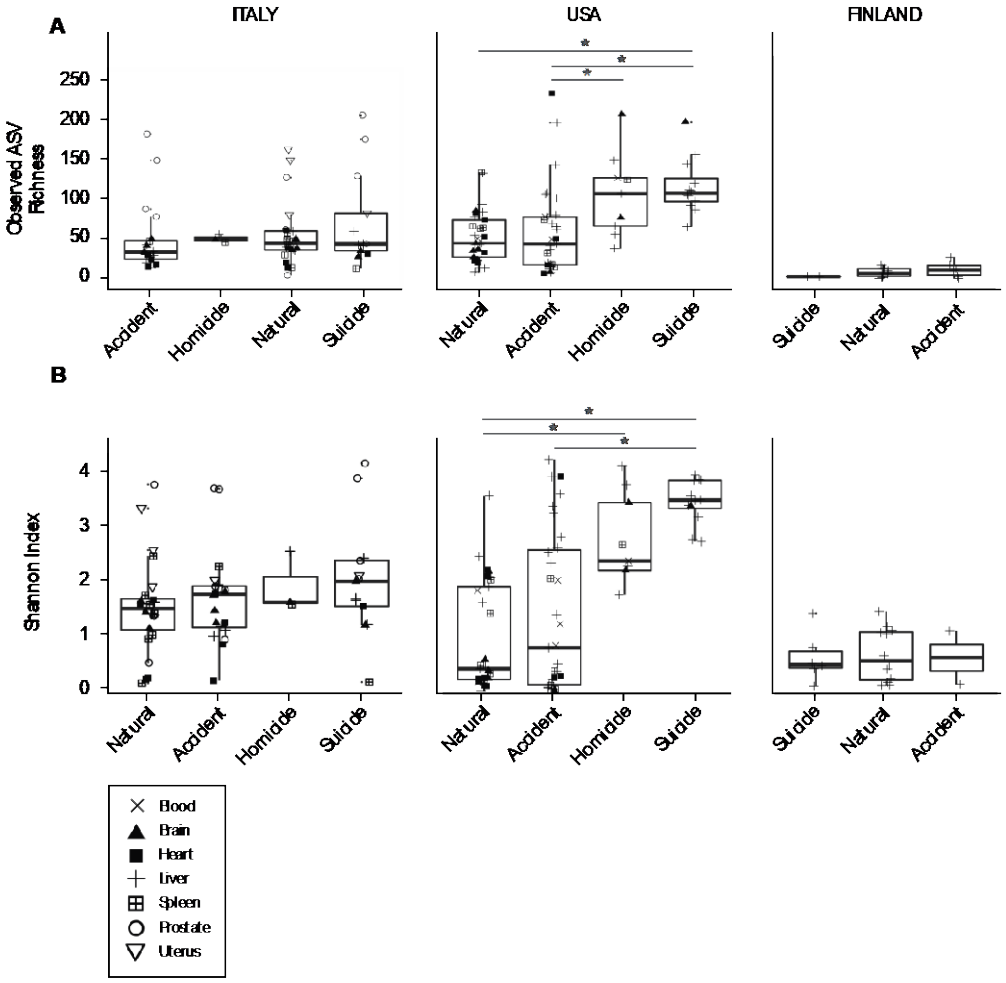

Figure 2. Variation in alpha diversity by manner of death, A) comparing observed ASV richness between manners of death from different localities, B) comparing Shannon

diversity index between manners of death from different localities. Asterisks indicate significant difference between groups based on post-hoc Dunn's Tests, $p < 0.05$.

Using linear regression of alpha diversity against PMI, the only significant associations observed were among Italian spleens (observed richness: $p = 0.016$, $R^2 = 0.48$) and Finnish livers (Shannon Index: $p = 0.021$, $R^2 = 0.22$). Similarly, we found little evidence for a correlation between BMI and bacterial alpha diversity among organs, with the exception of the Italian prostate (observed richness: $p = 0.019$, $R^2 = 0.35$; Shannon Index: $p = 3.58$ e -05, $R^2 = 0.62$) and the US spleen (Shannon Index: $p = 0.017$, $R^2 = 0.47$).

Analysis of beta diversity, using unweighted UniFrac, found a strong effect of geographic locality on postmortem bacterial community composition (Fig. 3A), whereby the microbial composition and compositional proportion were significantly different between each country (PERMANOVA: unweighted UniFrac, $p = 0.001$, $R^2 = 0.18$; weighted UniFrac, $p = 0.001$, $R^2 = 0.12$). No clear differences in beta diversity were visible by organ type (Fig. 3B) or organ type within each country, except for the uterus and prostate differing from all other organs in Italy (Fig. 3C), although, organ was technically a significant predictor of beta diversity (PERMANOVA: unweighted UniFrac, $p = 0.001$, $R^2 = 0.08$; weighted UniFrac, $p = 0.001$, $R^2 = 0.06$). Controlling for locality as a confounding variable, PERMANOVA analyses of weighted and unweighted UniFrac diversity metrics identified several variables significantly associated with microbial beta diversity, though these variables differed between the two metrics (Table 3). For unweighted UniFrac, significant variables included ethnicity and age ($p < 0.05$, PERMANOVA). For weighted UniFrac, significant variables included manner of death, PMI, and BMI ($p < 0.05$, PERMANOVA).

Figure 3

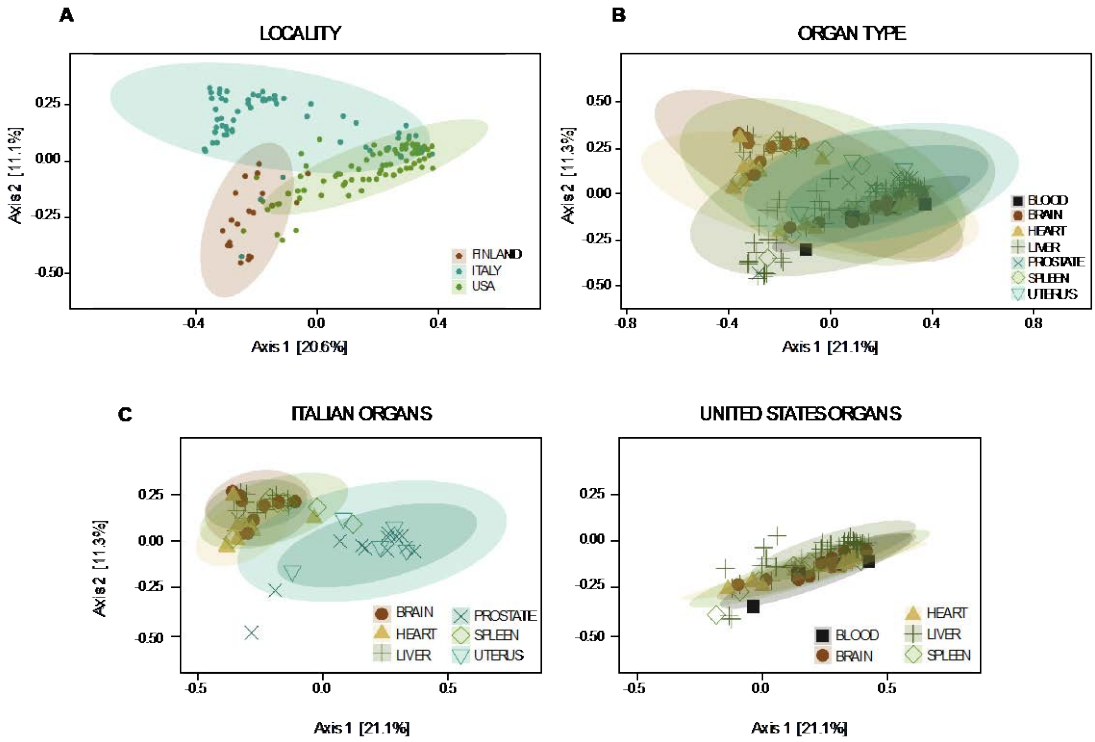

Figure 3. PCoA plots of unweighted UniFrac beta diversity, A) labeled by geographic locality, B) labeled by organ, and C) labeled by organ and faceted by geographic locality (Finland not included, as only liver was sampled).

Table 3. PERMANOVA analysis assessing marginal effects of variables on weighted and unweighted UniFrac beta diversity, controlling for geographic locality (ADONIS, strata = locality); asterisk indicates Bonferroni adjust *p*-value < 0.05.

	Df	SumOfSqs	R^2	F	p-value

Unweighted					
UniFrac					
Sex	1	0.41	0.01	1.7	0.36
Ethnicity	3	4.03	0.09	5.46	0.006*
Age	-	0.77	0.02	2.95	0.012*
Manner of death	3	1.2	0.03	1.57	0.096
PMI	-	0.37	0.01	1.49	0.56
BMI	-	0.41	0.01	1.65	0.26
Weighted UniFrac					
Organ	6				
Sex	1	0.07	0.01	2.32	0.35
Ethnicity	3	0.37	0.06	3.85	0.73
Age	-	0.1	0.02	3.23	0.14
Manner of death	3	0.23	0.04	2.33	0.006*
PMI	-	0.11	0.02	3.41	0.018*
BMI	-	0.14	0.02	4.21	0.006*

Analysis of composition of microbiomes (ANCOM) between different localities, organs, and manners of death identified significant differences in relative abundance (measured as the log2fold change in 16S rRNA ASV read counts) of multiple bacterial taxa. Assessing differences between localities (controlling for age, sex, BMI, PMI, ethnicity, and organ), we found that Finnish cadavers exhibited enrichment of two ASVs in the class Bacilli, as well ASVs belonging to the Alphaproteobacteria and Gammaproteobacteria, relative to cadavers from Italy and the United States ($p < 0.05$, ANCOM). Among Italian cadavers we observed enrichment for ASVs in the classes Saprospirae, 4C0d-2 (phylum Cyanobacteria), Betaproteobacteria,

Gammaproteobacteria, and Gemmatimonadetes ($p < 0.05$, ANCOM). And among US cadavers, we observed a significant enrichment in ASVs annotated to the class Clostridia, as well as enrichment of several ASVs belonging to the classes Alphaproteobacteria, Bacilli and Bacteroidia ($p < 0.05$, ANCOM) (Fig. 4).

Figure 4

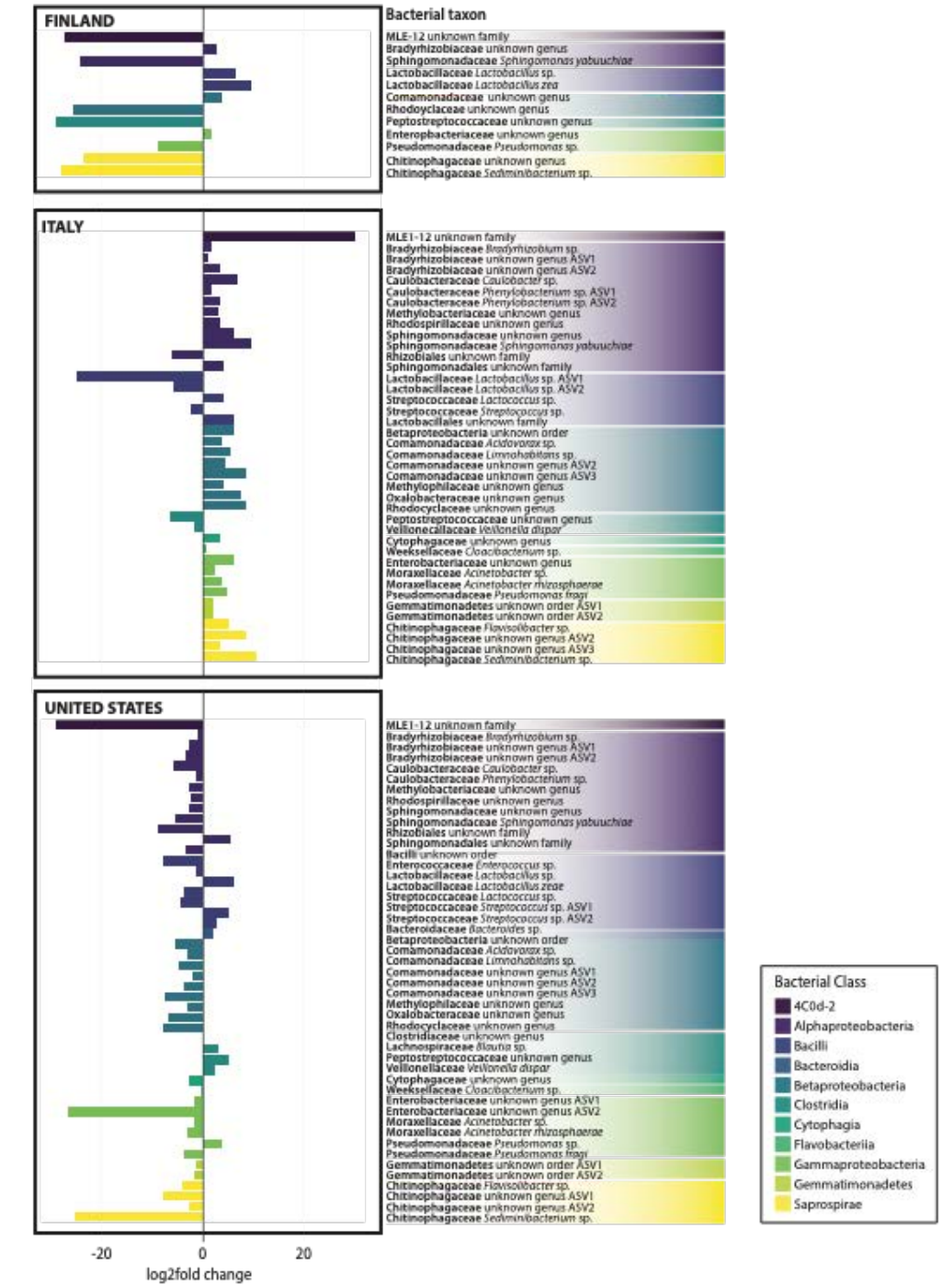

Figure 4. ANCOM – log2fold change in relative abundance between different cadaver localities, controlling for age, sex, ethnicity, BMI, PMI, and organ as covariates. ASVs are colored by bacterial class.

Analysis of differences in bacterial relative abundance between organs (controlling for age, sex, BMI, PMI, ethnicity, and locality) found increased proportion of a single Clostridia ASV (family Peptostreptococcaceae) in the blood, and a single Gammaproteobacteria in the heart (family Pseudomonadaceae, *Pseudomonas* sp.). Among brain tissue, several bacterial taxa were found to be underrepresented relative to all other organs, and none were found to be significantly enriched. Both liver and spleen exhibited an increased relative abundance of a bacterial ASVs in the class 4C0d-2 (order MLE1-12, unknown family), as well as *Sphingomonas yabuuchiae* (family Sphingomonadaceae). Other bacterial ASVs enriched in both the liver and spleen included those from classes Betaproteobacteria (specifically a single ASV in the family Rhodocyclaceae), Clostridia (specifically a single ASV in the family Peptostreptococcaceae), and Saprospirae (specifically two ASVs in the family Chitinophagaceae, and one ASV in the genus *Sediminibacterium*). The liver and prostate were both enriched for two ASVs in the class Bacteroidia, one in the family Comamonadaceae (genus *Limnohabitans*) and another in the family Oxalobacteraceae (unknown genus). The liver alone was enriched for several bacterial taxa not seen in other organs, including a Clostridia ASV in the family Lachnospiraceae (genus *Blautia*), an Alphaproteobacteria ASV in the order Rhizobiales (unknown family), and a Gammaproteobacteria in the family Enterobacteriaceae (genus *Salmonella*). Uterine tissues were enriched for only two ASVs, which were not found to be enriched in any other organs, including a single ASV in the class Bacilli (family Lactobacillaceae, genus *Lactobacillus*) and a single ASV in the class Gammaproteobacteria (family Enterobacteriaceae, unknown genus). Lastly, among prostate tissues we found a

significant underrepresentation of the same 4C0d-2 ASV (order MLE1-12) observed in both liver and spleen, and a single Clostridia ASV (family Lachnospiraceae, unknown genus) relative to all other organs (except for brain, which was also depauperate with respect to the 4C0d-2 ASV) (Fig. 5).

Figure 5

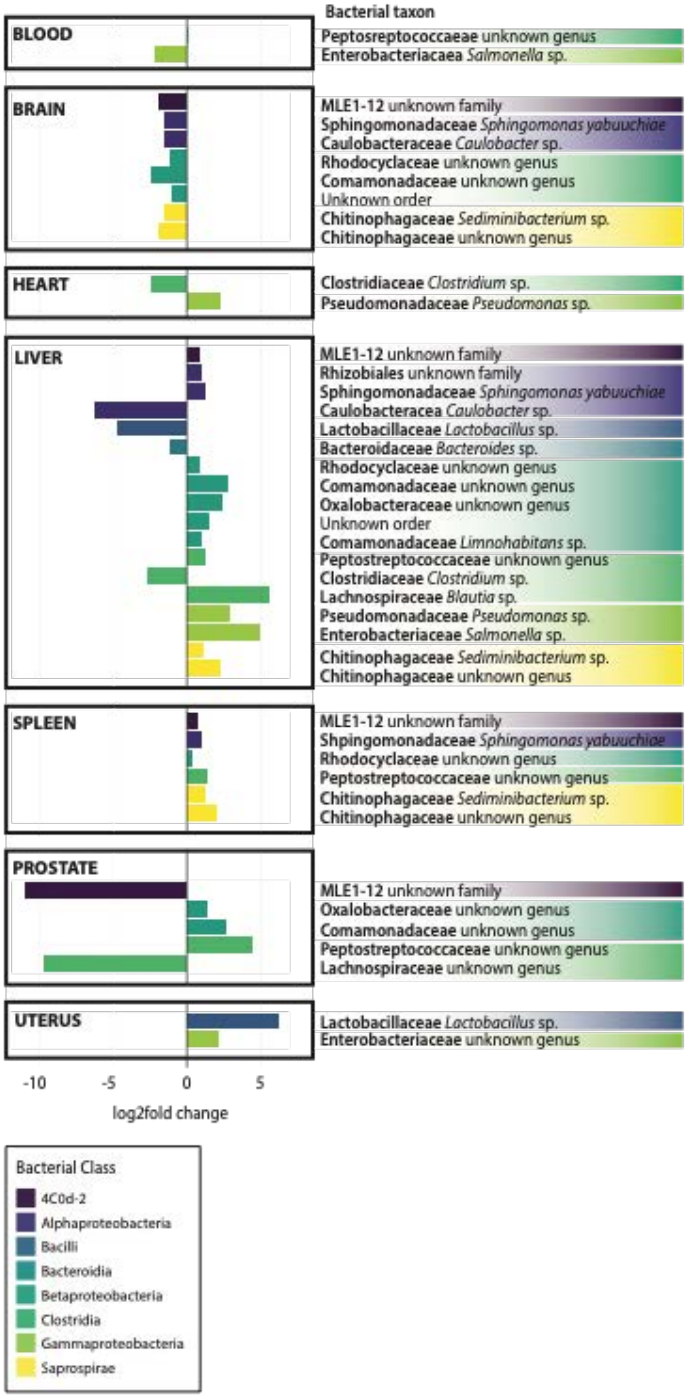

Figure 5. ANCOM – log2fold change in relative abundance between different organs, controlling for age, sex, ethnicity, BMI, PMI, and locality as covariates. ASVs are colored by bacterial class.

Several unique associations between ASVs and manner of death (controlling for age, sex, BMI, PMI, ethnicity, locality, and organ) were observed. For natural deaths, this included an enrichment of the same ASV in class 4C0d-2 (order MLE1-12) mentioned previously, as well as enrichment for single ASVs in the classes Bacilli (family Lactobacillaceae, *Lactobacillus zeae*), Gammaproteobacteria (family Enterobacteriaceae, unknown genus), and Saprospirae (family Chitinophagaceae, genus *Sediminibacterium*). Among victims of accidental death, a single Bacilli ASV (order Lactobacillales, unknown family) and Gammaproteobacteria (family Enterobacteriaceae, unknown genus) were enriched. Homicide victims did not exhibit enrichment of any bacterial taxa but exhibited a decreased abundance of ten different ASVs belonging to the class Bacilli, as well as ASVs in the classes Bacteroidia (family Prevotellaceae, *Prevotella melaninogenica*), Clostridia (family Veillonelliceae, *Veillonella dispar*), and Gammaproteobacteria (family Enterobacteriaceae, genus *Salmonella*) relative to other samples. Lastly, victims of suicide showed a similar decrease in the same Gammaproteobacteria ASV (family Enterobacteriaceae, *Salmonella*) as homicide victims, as well as decreases in another gammaproteobacterium ASV (family Pseudomonadaceae, genus *Pseudomonas*), and two ASVs in the class Clostridia (family Peptostreptococcaceae, unknown genus, and family Ruminococcaceae, *Faecalibacterium prausnitzii*). Other Clostridia ASVs were enriched in suicide victims, including two ASVs in the family Lachnospiraceae (genus *Blautia*), and one in the family Clostridiaceae (genus *Clostridium*). The only ASV belonging to class Alphaproteobacteria (order Rhizobiales) with significantly different relative abundance among manner of death categories was found to be enriched in suicide victims as well (Fig. 6).

Figure 6

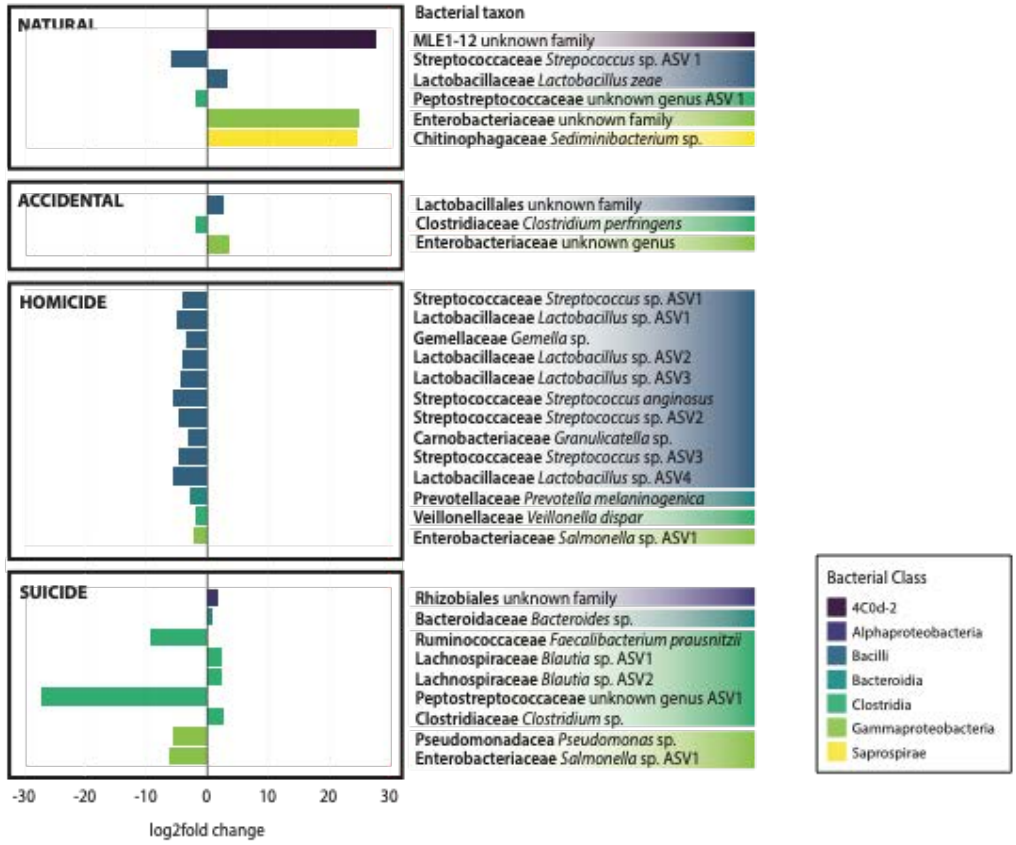

Figure 6. ANCOM – log2fold change in relative abundance between different manners of death, controlling for age, sex, ethnicity, BMI, PMI, organ, and locality as covariates. ASVs are colored by bacterial class.

A previous microbial survey of internal organ tissues (e.g., brain, heart, liver, and spleen) of four cadavers, associated with a homicide, suicide, over-dose, and accidental death cases, demonstrated that the obligate anaerobe, *Clostridium* was found in cadavers of varying PMIs, while the facultative anaerobe, *Lactobacillus*, was more abundant in cadavers with shorter PMIs (Javan *et al*., 2016b). Other investigations performed exploratory analyses of bacteria present in mouth and rectal scrapings taken at the onset and end of the bloat stage of corpses decomposing in a natural setting (32). However,

internal organs were not sampled across time points in this study. Another postmortem microbiome study of 33 bodies was conducted using bacterial culturing and reverse transcriptase quantitative PCR (RT-qPCR) techniques to profile the microbes in blood, liver, portal vein, mesenteric lymph node, and pericardial fluid, and identified 21 genera, with the most abundant being *Staphylococcus* sp., *Streptococcus* sp., *Clostridium* sp., *Enterococcus* sp., and *Escherichia* sp. (33)

We identified many different taxa as being associated with manner of death, including *Lactobacillus,* Enterobacteriaceae, *Sediminibacterium, Blautia,* Rhizobiales, and *Clostridium.* In several recent postmortem microbiome studies, the clostridia were observed to proliferate post-mortem (11, 12), potentially in part due to an increase in available nutrients and energy obtained from fermentation reactions (34). Most *Clostridium* spp. grow strictly in the absence of oxygen and a doubling time of 7.4 minutes (35) which may explain why they so easily colonize the still anaerobic body cavity post-mortem. The presence of species of *Lactobacillus,* Enterobacteriaceae, and *Blautia* may be similarly explained. However, the enrichment of *Sediminibacterium* and Rhizobiales in natural deaths and suicides respectively, which are traditionally associated with soil, is harder to understand but may represent colonization by environmental bacteria.

- **Artifacts**

List of products:

- The Postmortem Clostridium Effect in Human Decomposition https://emerging-researchers.org/projects/123-5/
- Editorial. Life and Death: New Perspectives and Applications in Forensic Science - https://www.frontiersin.org/articles/10.3389/fevo.2021.725046/full
- Professional presentations at professional conferences and online webinar series.

- Effects of Extended Postmortem Interval on Microbial Communities in Organs of the Human Cadaver - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7752770/
- The roles of medical examiners in the COVID-19 era: a comparison between the United States and Italy - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7882048/
- Identification of cadaveric liver tissues using thanatotranscriptome biomarkers https://www.nature.com/articles/s41598-020-63727-9
- Life and death: A systematic comparison of antemortem and postmortem gene expression https://www.sciencedirect.com/science/article/abs/pii/S0378111920300184

Data sets generated (broad descriptions will suffice)

The genetic sequencing datasets presented in this study can be found in the European Bioinformatics Institute - The EBI Accession Number is PRJEB41511. This dataset includes all the 16S rRNA sequencing data acquired during this study.

Dissemination activities

Results of this project have provided a framework for the Forensic Technology Center of Excellence Webinar Series, which Dr. Lutz led. Results were also prepared for publication in peer-reviewed forensic science journals and were presented to the forensic scientific community by Dr. Javan at the annual scientific meeting of the American Academy of Forensic Sciences.

- https://www.rti.org/rti-press-publication/2020-nij-rd-symposium/fulltext.pdf
- http://hbcunetwork.com/content/340334/alabama-state-university-forensic-majors-win-national-science-foundation-travel-awards
- Dr. Javan presented at the 73rd annual scientific meeting of the American Academy of Forensic Sciences (AAFS) in 2021. We have also published 5 papers.

- Javan GT. The Thanatomicrobiome. Microbial Life After Death. 2021 Virtual Forensic Science & Investigation Day, Delaware State University, April 10, 2021.
- Javan GT. Human Decomposition and Postmortem Microbiology. 2021 Virtual Forensic Science Lecture Series, Delaware State University, October 11, 2021.

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Cited References

1. **Gilbert JA, Quinn RA, Debelius J, Xu ZZ, Morton J, Garg N, Jansson JK, Dorrestein PC, Knight R.** 2016. Microbiome-wide association studies link dynamic microbial consortia to disease. Nature **535:**94-103.
2. **Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R.** 2018. Current understanding of the human microbiome. Nat Med **24:**392-400.
3. **Metcalf CJE, Xu ZZ, Weiss S, Lax S, Van Treuren W, Hyde ER, Song SJ, Amir A, Larsen P, Sangwan N, Haarmann D, Humphrey GC, Ackermann G, Thompson LR, Lauber CL, Bibat A, Nicholas C, Gebert MJ, Petrosino JF, Reed SC, Gilbert JA, Lynne AM, Bucheli SR, Carter DO, Knight R.** 2016. Microbial community assembly and metabolic function during mammaliann corpse decomposition. Science **351:**158-162.
4. **Javan GT FS, Tuomisto S, Hall A, Benbow ME, Mills D. .** 2019. An interdisciplinary review of the thanatomicrobiome in human decomposition. Forensic Science, Medicine, and Pathology **15:**75-83.
5. **Lax S, Hampton-Marcell JT, Gibbons SM, Colares GB, Smith D, Eisen JA, Gilbert JA.** 2015. Forensic analysis of the microbiome of phones and shoes. Microbiome **3:**21.
6. **Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R.** 2010. Forensic identification using skin bacterial communities. Proc Natl Acad Sci U S A **107:**6477-6481.
7. **Park J, Kim SJ, Lee J-A, Kim JW, Kim SB.** 2017. Microbial forensic analysis of human-associated bacteria inhabiting hand surface. Forensic Science International: Genetics Supplement Series **6:**e510-e512.
8. **Schmedes SE, Woerner AE, Budowle B.** 2017. Forensic human identification using skin microbiomes. Appl Environ Microbiol **83**.

9. **Kodama WA, Xu Z, Metcalf JL, Song SJ, Harrison N, Knight R, Carter DO, Happy CB.** 2018. Trace Evidence Potential in Postmortem Skin Microbiomes: From Death Scene to Morgue. J Forensic Sci doi:10.1111/1556-4029.13949.

10. **Burcham ZM HJ, Pechal JL, Krausz KL, Bose JL, Schmidt CJ, Benbow ME, Jordan HR.** 2016. Fluorescently labeled bacteria provide insight on postmortem microbial transmigration. Forensic Science International **264:**63-69.

11. **Javan GT FS, Smith T, Miller J, Wilkinson JE.** 2017. Cadaver thanatomicrobiome signatures: the ubiquitous nature of Clostridium species in human decomposition. Front Microbiol **8**.

12. **Javan GT FS, Can I, Wilkinson JE, Hanson JD, Tarone AM.** 2016. Human thanatomicrobiome succession and time since death. Sci Rep **6:**195-298.

13. **Cobaugh KL SS, DeBruyn JM. .** 2015. Functional and structural succession of soil microbial communities below decomposing human cadavers. PLoS ONE **10**.

14. **Hauther KA CK, Jantz LM, Sparer TE, DeBruyn JM.** 2015. Estimating time since death from postmortem human gut microbial communities. J Forensic Sci **60:**1234-1240.

15. **Pechal JL CT, Benbow ME, Tarone AM, Dowd S, Tomberlin JK. .** 2014. The potential use of bacterial community succession in forensics as described by high throughput metagenomic sequencing. Int J Legal Med **128:**193-205.

16. **Maujean G GT, Fanton L, Malicier D.** 2013. The interest of postmortem bacteriology in putrefied bodies. J Forensic Sci **58:**1069-1070.

17. **Ercolini D, Russo F, Nasi A, Ferranti P, Villani F.** 2009. Mesophilic and psychrotrophic bacteria from meat and their spoilage potential in vitro and in beef. Appl Environ Microbiol **75:**1990-2001.

18. **Deutscher J.** 2008. The mechanisms of carbon catabolite repression in bacteria. Curr Opin Microbiol **11:**87-93.

19. **Rakoff-Nahoum S, Foster KR, Comstock LE.** 2016. The evolution of cooperation within the gut microbiota. Nature **533:**255-259.

20. **Campobasso C, Di Vella G, Introna F.** 2001. Factors affecting decomposition and Diptera colonization. Forensic Science International **120:**18-27.

21. **Amendt J. KR, Zehner R, Bratzke, H. .** 2003. Practice of forensic entomology--usability of insect fragments in the estimation of the time of death. Archiv für Kriminologie **214:**11-18.

22. **Caporaso JG, Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., Knight, R.** 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS **108:**4516-4522.

23. **Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R.** 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J **6:**1621-1624.

24. **Kozich JJ, Westcott, S. L., Baxter, N. T., Highlander, S. K., Schloss, P. D.** 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on teh MiSeq Illumina sequencing platform. Applied and Environmental Microbiology **79:**5122-5120.

25. **Caporaso JG, Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Gonzalez Peña, A., Goodrich, E. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R.** 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods **7:**335 - 336.

26. **Rognes T, Flouri T, Nichols B, Quince C, Mahe F.** 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ **4:**e2584.

27. **Amir A, McDonald D, Navas-Molina JA, Kopylova E, Morton JT, Zech Xu Z, Kightley EP, Thompson LR, Hyde ER, Gonzalez A, Knight R.** 2017. Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. mSystems **2**.

28. **McMurdie PJ, Holmes S.** 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One **8:**e61217.

29. **Oksanen J., Blanchet F. G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P., Henry M., Stevens H., Szoecs E., H. W.** 2018. vegan: Community Ecology Package, vR package version 2.5-2. https://CRAN.R-project.org/package=vegan.

30. **Wickham H.** 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, NY.

31. **Wickham H, François R, Henry L, Müller K.** 2018. dplyr: A Grammar of Data Manipulation, vR package version 0.7.6. https://CRAN.R-project.org/package=dplyr.

32. **Hyde ER, Haarmann DP, Lynne AM, Bucheli SR, Petrosino JF.** 2013. The living dead: bacterial community structure of a cadaver at the onset and end of the bloat stage of decomposition. PLoS One **8:**e77733.

33. **Tuomisto S, Karhunen PJ, Vuento R, Aittoniemi J, Pessi T.** 2013. Evaluation of postmortem bacterial migration using culturing and real-time quantitative PCR. J Forensic Sci **58:**910-916.

34. **DeBruyn JM, Hauther KA.** 2017. Postmortem succession of gut microbial communities in deceased human subjects. PeerJ **5:**e3437.

35. **Willardsen RR, Busta, F. F., and Allen, C. E.** 1979. Growth of Clostridium perfringens in three different beef media and fluid thioglycollate medium at static and constantly rising temperatures. J Food Prot **42:**144-148.