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Mapping of key bacterial species for *Postmortem* interval calculation

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Abstract

Background: Estimation of the *Postmortem* Interval (PMI), the time elapsed since death, is one of the most challenging issues in forensic sciences [1]. Most studies focus on extensive bacterial sequencing, but culture-based experiments for higher taxonomic resolution remain scarce [2]. **Objective:** We aimed to analyze total bacterial counts and map *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* in different organs and timepoints. **Methods:** Male C57BL/6J SPF mice underwent three independent assays during 11 *postmortem* timepoints. Feces and organs (n=10: intestine/stomach/skeletal muscle/liver/spleen/kidney/bladder/lungs/ brain/heart) were collected and resuspended in buffered peptone water, then plated onto enriched nonselective and selective culture media (n=4). Following routine aerobic incubation, Colony Forming Units (CFU) per gram/tissue or per mL/sample were quantified for total/individual bacterial loads. Species were identified by MALDI-TOF MS and statistics were done in GraphPad-Prism v.10.0.1. **Results:** Species (n=44) from 13 families and 3 phyla were identified, with notable consistency in the presence of *Staphylococcus xylosum*, *E. faecalis*, and *E. coli* across all experiments. Particular families were consistently identified across all organs, including Enterococcaceae and Enterobacteriaceae mostly in the later stages of decomposition, and Bacillaceae resisting often until the last timepoint, whereas Staphylococcaceae was variably detected. The early and substantial contamination observed in skeletal muscle, stomach, and intestine, makes them unsuitable for PMI calculations. *E. faecalis* appeared promising as a potential biomarker for kidney, liver, and, possibly, brain invasion at later timepoints, whereas *E. faecalis* and *E. coli* in the bladder, and *E. coli* in the spleen and heart, warrant further investigation for similar biomarker potential. **Conclusions:** This is one of the first quantitative cultural studies assessing how time elapsing *postmortem* affects the growth/evolution of key bacterial species, with *E. faecalis* and *E. coli* emerging as promising traceable biomarkers in real *postmortem* contexts. While recognizing the limitations of not considering the complex microbiota network, our pilot study brings an easy species-specific approach and offers a baseline for future human-oriented investigations.

Keywords: forensic sciences; microbiome; bacteria; taxonomy; *postmortem* interval

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