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Poster 25

Bacterial load and dynamics: exploring their potential as biomarkers for *postmortem* interval estimation

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Abstract

Background: Establishing the *postmortem* interval (PMI), the time since death, is vital in forensic investigations but remains challenging due to multiple factors like the cause of death, body location, and environmental conditions [1]. Traditional PMI estimation methods include *Algor Mortis*, *Rigor Mortis,* and *Livor Mortis*, along with complementary approaches from entomology, botany, and microbiology, though all carry some degree of inaccuracy [2]. Recently, microbiology and *postmortem* biochemistry (thanatochemistry) have gained attention, as microbial succession and biochemical changes offer promising, though still limited, insights into PMI estimation [3]. **Objective:** This study aimed to quantify the total bacterial load and map two bacterial species, *Escherichia coli* (Ec) and *Enterococcus faecalis* (Efs), two gut microbiota species, across various organs (lungs, heart, kidneys, liver, and brain) at varying PMIs under controlled, pathogen-free conditions. **Methods:** Male C57BL/6J SPF mice were analyzed at six *postmortem* timepoints (0, 12, 24, 48, 72, and 96h). Feces and organs (n=3 animals/assay) were collected, thoroughly resuspended in buffered peptone water, and then seeded onto non-selective (Blood agar) and selective (MacConkey-MC for Ec and Slanetz-Bartley-SB for Efs) media. After routine aerobic incubation, colony-forming units (CFU) were quantified per gram of tissue to assess total and species-specific bacterial loads. **Results:** The total bacterial load increases at later *postmortem* timepoints. The heart and lungs (highly vascularized organs) showed a detectable bacterial load at the initial timepoint (0 h). Organs typically considered more sterile, such as the kidneys and brain, showed low or undetectable bacterial loads initially, although with a gradual increase over time. In SB, used for the quantitative detection of Efs, bacterial growth emerged at later timepoints. On MC, selective for Enterobacteriaceae, and used for the detection of Ec, bacterial growth was organ-dependent and detectable only after 24 h. Notably, *E. coli* was absent from liver samples on this medium. Biochemical analyses are ongoing to complement microbiological analysis. **Conclusions:** This culture-based quantitative study shows how PMI impacts the growth and dynamics of *E. faecalis* and *E. coli*, suggesting their potential as traceable biomarkers for PMI in forensic contexts. Nonetheless, further studies are required to validate and extend these findings to more complex scenarios.

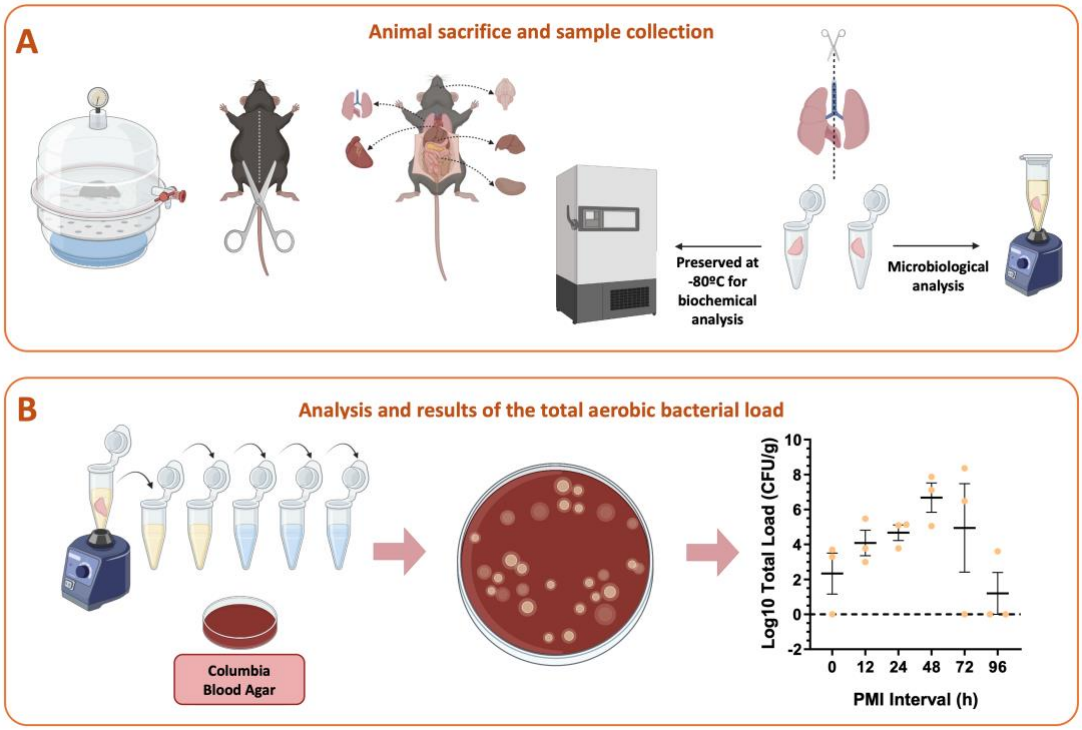

Figure 1. (A) Schematic representation of animal sacrifice using an isoflurane chamber followed by cervical dislocation and sample collection. Tissues were preserved at -80 ºC for biochemical and microbiological analysis. (B) Workflow for microbiological quantification of collected organ samples, including homogenization, culture on Columbia Blood Agar, and total bacterial load assessment. The graph on the right shows total aerobic bacterial load (Log10 CFU/g) for lungs across different *postmortem* intervals.

Keywords: *postmortem* interval; thanatomicrobiome; bacterial translocation

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