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Microbial succession after death: genomic and culture-based insights from external sampling sites in forensic science

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ABSTRACT

Background: Post-mortem microbial communities (microbiota and microbiome) have emerged as promising tools for forensic investigations, particularly in estimating the post-mortem interval (PMI). However, experimental variability in sampling protocols, analytical methods, and reporting standards has limited the comparability and reproducibility of findings across studies.

Methods: A systematic review was conducted in accordance with PRISMA guidelines to evaluate the current literature on human post-mortem microbiota and microbiome. Inclusion criteria focused on studies that examined microbial communities in human cadavers using culture-based techniques, next-generation sequencing (NGS), or both. Data were extracted regarding sample types, microbial targets, analytical methods, decomposition stages, insect activity, and study objectives.

Results: A total of 24 studies were included, revealing substantial heterogeneity in methodological approaches. NGS techniques dominated recent literature, targeting bacterial 16S rRNA gene sequences to characterize microbial succession during decomposition. While some studies have shown promising correlations between microbial taxa and PMI, the inconsistent use of controls and variable decomposition conditions impeded cross-study comparisons. Culture-based approaches were generally limited to early investigations and provided narrower taxonomic resolution.

Conclusions: Despite encouraging results, the forensic application of post-mortem microbiome and microbiota remains hindered by methodological inconsistencies and a lack of standardization. Establishing unified protocols and adopting interdisciplinary approaches will be essential for validating microbial signatures as reliable forensic tools.

1. Introduction

In recent years, the post-mortem microbiota has assumed a pivotal role in forensic medicine, owing to its potential in estimating the post-mortem interval (PMI) and reconstructing the circumstances of death [1–4]. The term *microbiota* refers to the community of living microorganisms (bacteria, archaea, fungi, viruses) that inhabit the human body, whereas the *microbiome* denotes their genetic material, which can be analyzed using modern sequencing techniques [4–6].

Following death, the loss of homeostasis promotes the proliferation of resident microbes and the infiltration of exogenous microorganisms, leading to a relatively predictable microbial succession. This process, known as the *thanatomicrobiome*, unfolds across five stages of decomposition, with significant variations in the composition of microbial

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communities [1,2,7,8].

The post-mortem microbiota undergoes a series of changes that are temporally consistent and reflect the progression of physiological and environmental alterations that occur after death. This dynamic renders the microbiota — both bacterial and fungal — a promising biological marker for PMI estimation, due to its capacity to vary reproducibly in relation to the stage of decomposition, anatomical site, and individual factors such as sex and body mass index. Despite the paucity of studies conducted on the subject, the fungal microbiota (i.e. *thanatomycobiome*) also demonstrates a coherent temporal progression post-mortem, thus suggesting its complementary use in estimating time of death and characterizing environmental conditions [9,10].

Recent studies have shown that the microbiota retains useful biological signatures even after the freezing and thawing of the body, a common practice in human taphonomic research facilities [4,5,11,12].

The advancement of molecular techniques — from 16S rRNA sequencing to metagenomics — has led to the expansion of analytical capabilities, allowing for the taxonomic and functional identification of microbial communities even in degraded samples [6,13].

In this context, the present systematic review aims to critically and comparatively examine the available evidence on the application of microbiological techniques to the study of the human post-mortem microbiota. Particular focus is placed on the use of genomic methodologies, such as 16S rRNA sequencing and metagenomics, in contrast to conventional culture-based approaches, including aerobic, anaerobic, and fungal techniques. These methods have been primarily applied to external anatomical sampling sites such as the oral cavity, rectum, and cornea.

The objective is to assess the extent to which these approaches differ in terms of accuracy, sensitivity, reproducibility, and predictive value in reconstructing post-mortem microbial succession and estimating the post-mortem interval (PMI) across the various stages of decomposition. Particular emphasis is placed on the influence of methodological and environmental variables — including sampling protocols, storage conditions, climate, and diagnostic or bioinformatic tools — with the aim of outlining the operational strengths and limitations of each approach and contributing to the development of standardized guidelines for the application of post-mortem microbiota analysis in forensic practice.

2. Materials and methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines. It has been registered with Prospero (registration number: CRD420251002151).

The PRISMA checklist is provided as supplementary material.

A systematic literature search was conducted in the PubMed, Scopus, and Web of Science databases to identify English-language studies on post-mortem variations in the microbiota and microbiome of the oral cavity, cornea, and rectum. To account for the heterogeneity of terminology used in the literature, a broad free-text search strategy was adopted using the following terms:

—**PubMed**: ((((post-mortem microbiome[Title/Abstract]) OR (cadaveric microbiome[Title/Abstract])) OR (microbiota decomposition[Title/Abstract])) OR (bacteria forensic analysis[Title/Abstract])) OR (microbiome post-mortem interval[Title/Abstract]).

—**Scopus**: TITLE-ABS-KEY("post-mortem microbiome" OR "cadaveric microbiome" OR "microbiota decomposition" OR "bacteria forensic analysis" OR "microbiome post-mortem interval").

—**Web of Science**: TS=("post-mortem microbiome" OR "cadaveric microbiome" OR "microbiota decomposition" OR "bacteria forensic analysis" OR "microbiome post-mortem interval").

No date restrictions were applied; filters were set to "Humans" and "English." Additional relevant articles were identified by screening the references of the included studies. Although controlled vocabularies (e.g., MeSH terms) could have increased specificity, a broad search was preferred to ensure inclusiveness. Irrelevant articles were subsequently excluded through an independent screening process.

Studies were selected based on predefined inclusion and exclusion criteria.

2.1. Inclusion criteria

- Articles published in English
- Studies conducted on human subjects
- Research investigating microbial composition in deceased individuals
- Studies focused on external anatomical sites (oral cavity, rectum, and cornea)
- Both original research articles and systematic literature reviews
- Studies employing any type of investigative technique, including microbiological and genetic analyses

2.2. Exclusion criteria

- Studies conducted on living subjects
- Studies addressing nosocomial infections in cadavers
- Studies addressing sepsis or septic shock in cadavers
- Methodological studies not involving the oral cavity, rectum, or cornea
- Studies on ocular microbiota that focus on the vitreous humor rather than the cornea
- Narrative review
- Systematic review

This systematic review adhered to PRISMA guidelines.

The selection process was carried out using **Rayyan**, a free web and mobile application that facilitates the initial screening of titles and abstracts through a semi-automated process (http://rayyan.qcri.org) [14,15]. Four researchers independently screened the titles and abstracts of retrieved articles based on the predefined inclusion criteria. Discrepancies were resolved by consensus.

The resulting documents underwent a second round of screening based on titles, abstracts, methods, and keywords. Additional eligible studies were identified through manual screening of the reference lists of the initially retrieved articles.

The selection process encompassed both the identification and extraction of relevant data. Data extraction was performed by two researchers and subsequently reviewed and confirmed by two additional pairs of investigators.

From the selected studies, the following data were recorded: authors and year of publication, country of affiliation, type of article, type of study, research question, object of study, sampling site, study population, type of population, sampling time, sampling method, analytical technique used, and statistical analysis.

The selected studies were analyzed to investigate how the microbial composition of the oral cavity, rectum, and cornea evolves after death. Particular attention was given to the influence of post-mortem interval, environmental conditions, and individual characteristics of the deceased—such as age, cause of death, and nutritional status—on the detectable microbial communities.

The evaluation of the quality of evidence and strength of recommendations in this review was performed in accordance with the updated methodology of the **Scottish Intercollegiate Guidelines Network (SIGN 2019)**[16]. Rather than applying a rigid numerical hierarchy, this framework adopts a more nuanced and context-sensitive approach, emphasizing the transparency of the decision-making process and the applicability of evidence to clinical practice.

The quality of evidence is assessed using the **GRADE** (Grading of Recommendations, Assessment, Development and Evaluations) system, which classifies the certainty of evidence into four levels:

—**High**: Further research is very unlikely to change confidence in the

estimate of effect.

—**Moderate**: Further research may have an important impact on confidence in the estimate and may change the estimate.

—**Low**: Further research is likely to have an important impact on confidence in the estimate and is likely to change the estimate.

—**Very low**: The estimate is very uncertain.

The strength of recommendations is expressed as either:

—**Strong**: Indicating that the guideline panel is confident that the benefits of the intervention clearly outweigh the risks (or vice versa).

—**Conditional (or Weak)**: Suggesting that the balance between benefits and risks is less certain or may vary depending on individual circumstances or values.

In forming recommendations, SIGN 2019 considers not only the quality of the evidence, but also factors such as clinical relevance, consistency across studies, potential harms and benefits, patient values and preferences, and resource implications.

Study quality was assessed using CASP checklists, yielding an overall rigor score. The methodological quality of each study was assessed using the CASP checklists [17]. Each review received an overall score summarizing the methodological rigor of the studies discussed. Responses to checklist items were categorized as "Yes," "No," or "Can't tell," with each item scored as 1 or 0 depending on whether the specified criteria were met. The review table presents a final quality score for each study, ranging from 0 to 14 for cohort studies ($\geq$9 = high quality; 6–8 = moderate quality; $\leq$5 = low quality), and from 0 to 11 for cross-sectional studies ($\geq$8 = high quality; 6–7 = moderate quality; $\leq$5 = low quality). Checklist items were evaluated using the categories "Yes," "No," or "Can't tell," with each response assigned a score of 1 or 0 based on whether the predefined criteria were satisfied. Studies concerning the microbe were considered separately from those on the microbiota.

The studies concerning the microbe have been considered separately from those on the microbiota.

3. Results

3.1. In total, 3526 publications met the search criteria

A total of 908 duplicate articles were excluded. After assessing the relevance of the studies to the aims of our research, an additional 2551 publications were excluded, while one work was not retrieved, leaving 66 full-text articles. Upon reviewing the full-text articles, an additional 56 studies were excluded for not adhering to the inclusion criteria. The remaining 10 full-text articles therefore fully met the inclusion criteria for the review. Furthermore, from the bibliographies of the included articles, 4 more publications were selected, which also respected the established inclusion and exclusion criteria, for a total of 14 articles. The article selection process is summarized in Fig. 1.

Based on the first author's affiliation, the selected studies originated from the following countries: seven from the USA, two from Italy, and one each from China, the UK, Spain, Denmark and Poland.

Regarding the type of article, the selection included 14 original articles.

Of the articles reviewed, 11 focused on the study of the microbiome, while only three addressed the investigation of the microbiota.

Of the 14 original articles included in the systematic review, all were classified as observational studies, as none involved direct intervention or control over exposure or treatment by the research teams.

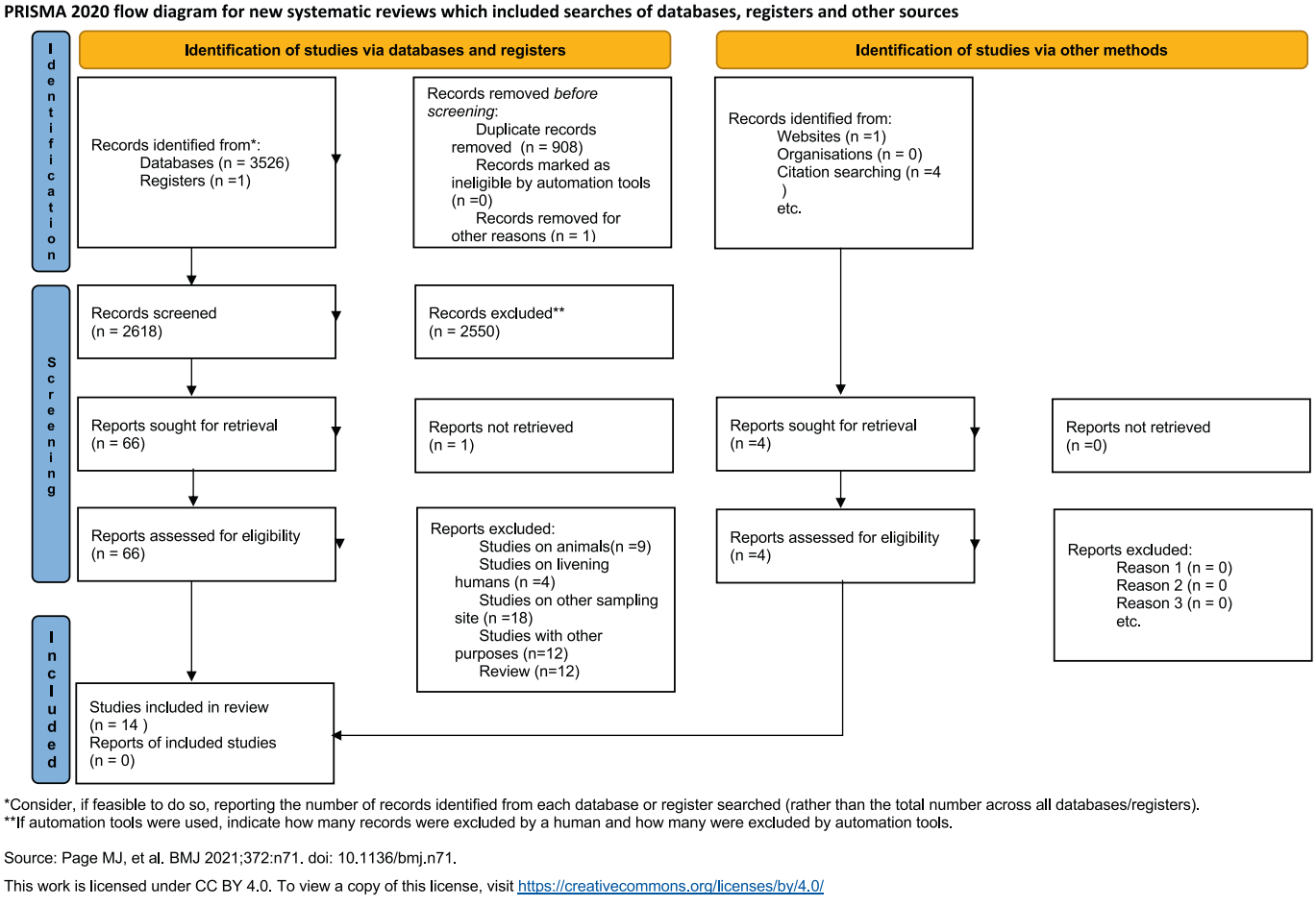

Fig. 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources.

3.2. Microbiome

Eight studies were classified as cohort observational studies. These followed the temporal evolution of the microbiome in one or more anatomical sites, documenting microbial succession through sequential sampling and omics-based approaches such as 16S rRNA or metagenomic sequencing. Their shared objective was to characterize the time-dependent shifts in post-mortem microbial communities and assess their potential relevance for estimating the PMI. Six studies were classified as cross-sectional observational studies.

With regard to original studies on the microbiome, all 11 works included sampling from the oral cavity (noting that in one case, the swab was taken specifically from the throat). Six of these studies also included rectal sampling (with one study specifying that fecal material was collected), and three of them involved external sampling from the ocular globe. The results are summarized in Table 1.

Concerning the anatomical sites sampled, Hyde et al. (2013) [7] performed sampling from both the oral cavity and the rectum. Hyde et al. (2015) [8] conducted sampling exclusively from the oral cavity and rectum, also including swabs from the right and left cheeks, biceps, and torso. Pechal et al. (2017) [18] included sampling from the ocular globe, oral cavity, rectum, external auditory canal, nostrils, and umbilicus, while Pechal et al. (2018) [19] included the ocular globe, oral cavity, and rectum. Sguazzi et al. (2022) [10] analyzed samples from the oral cavity, heart, blood, spleen, and quadriceps muscle. Ashe et al. (2021) [20] performed exclusive sampling from the oral cavity, specifically the hard palate. Ogbanga et al. (2023) [21] collected samples from the oral cavity, rectum, hand, foot, and neck. Xi Huang et al. (2024) [5] obtained samples from the oral cavity, nasal cavity, heart, liver, spleen, lungs, kidneys, muscles, and intestines. Pittner et al. (2020) [22] included the ocular globe, oral cavity, rectum, ear, nose, and thigh skin in their sampling sites. Garriga et al. (2017) [2] performed sampling exclusively from the oral cavity. Gulnaz T. Javan et al. (2016) [1] analyzed samples from the oral cavity, brain, heart, liver, spleen, and blood.

Among the eleven original microbiome studies, seven explicitly reported the cause of death of the subjects analyzed, whereas in four cases, this information was not provided.

Among those that did report cause of death, Ashe et al. (2021) [20] examined three cadavers who had died from gastric cancer, heart disease, and pneumonia, respectively. Huang et al. (2024) [5] provided a detailed list of causes of death for each of the eight cadavers analyzed, including head trauma, coronary artery disease, severe anemia, tumors, and infections. Hyde et al. (2013) [7] distinguished two subjects: one who died of carbon monoxide poisoning and another with a complex clinical history that included diabetes, chronic alcoholism, and myocardial infarction. Similarly, Pittner et al. (2020) [22] reported two specific causes of death: cardiac arrest and metastatic neoplasia. Pechal et al. (2017) [18] investigated two homicide victims with severe cranial injuries, while in a subsequent study, Pechal et al. (2018) [19], analyzed a much larger sample and categorized causes of death as accidental (38 %), natural (30 %), homicide (20 %), and suicide (12 %). Lastly, Javan et al. (2016) [1], examined 27 cadavers from criminal cases, specifying causes of death such as homicide, suicide, and overdose.

The sampling timelines adopted in various post-mortem microbiome studies vary considerably, depending on the research question, logistical constraints, and the availability of cadavers. In some cases, the experimental design incorporated extended temporal follow-up, while in others, sampling was conducted at one or two key stages of decomposition. Certain studies employed a longitudinal approach with sequential sampling. For example, Ashe et al. (2021) [20] monitored three cadavers, collecting between five and seven samples per subject from the fresh stage through to skeletonization, thereby enabling observation of microbial succession over time. Similarly, Garriga et al. (2017) [2] monitored three individuals over several consecutive days (up to 12 days post-mortem), recording microbial shifts that aligned with decomposition phases (fresh, bloat, advanced). Hyde et al. (2015) [8], although not reporting the exact interval between death and sampling, provided details regarding the storage conditions of the two cases examined. Specifically, the cadavers were stored frozen for 22 days and 14 days, respectively (at − 17 to − 12 °C), with five samples collected over a 20-day period following exposure to ambient temperature. Conversely, Hyde et al. (2013) [7] compared two distinct timepoints (“pre-bloat” and “end-bloat”) for each of the two cadavers, which had been frozen for 102 and 149 days, respectively, at temperatures ranging from − 17 °C to 3.33 °C. Pechal et al. (2018) [19], although not performing repeated sampling on the same individuals, analyzed a large cohort (n = 188) with PMIs ranging from 1 to 73 h, offering a cross-sectional analysis of short post-mortem intervals. Similarly, Pittner et al. (2020) [22] ha effettuato due esumazioni: una a 28 giorni e l’altra a 105 giorni post-sepoltura. Pechal et al. (2017) conducted two exhumations at 28 and 105 days post-burial. Pechal et al. (2017) investigated two pediatric cases significantly affected by prolonged freezing (22 and 31 months, respectively). Sampling was carried out at different thawing stages (frozen, partially thawed, fully thawed), allowing for monitoring of microbial community changes during re-equilibration to ambient temperature.

Other studies adopted a more temporally limited but focused approach. Sguazzi et al. (2022) [10] analyzed three samples from only two cadavers, with sampling at 2, 10, and 16 days post-mortem, focusing on the influence of − 20 °C storage and comparability across tissue types

Table 1
Summarisation of the information of Microbiome obtained from the articles under analysis. PMI: Postmortem interval at sampling (days) SD (State of decomposition): F(Fresh); A(Active decay); AD(Advanced decay); DS: Dry remains / Skeletonization; M: Mummification FZ: Frozen; ADD (Accumulated Degree Days): A measure combining time and temperature to standardize decomposition rate by summing daily average temperatures above a baseline threshold.

Authors, Year	Typed regions	Primers	Typing method	Platform
G. Sguazzi et al., 2022 [10]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq
E.C. Ashe et al., 2021 [20]	V6 and V8, 16S rRNA	B969F and BA1406R	NGS	Illumina NextSeq 550
J. Adserias-Garriga et al., 2017 [2]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq
X. Huang et al., 2024 [5]	V3 and V4, 16S rRNA	343F and 798R	NGS	Illumina NovaSeq 6000
	2bRAD-M sequencing		NGS	Illumina NovaSeq 6000
E.R. Hyde et al., 2013 [7]	V3 and V5, 16S rRNA	357F and 926R	Pyrosequencing	454-FLX-Titanium
E.R. Hyde et al, 2014 [8]	V3 and V5, 16S rRNA	357F and 926R	Pyrosequencing	454-FLX-Titanium
N. Ogbanga et al., 2023 [19]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq
S. Pittner et al., 2020 [22]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq
J.L. Pechal et al., 2017 [18]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq
J.L. Pechal et al., 2018 [19]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq
G.T. Javan et al., 2016 [1]	V4, 16S rRNA	515F and 806R	NGS	Illumina MiSeq

and preservation intervals. Ogbanga et al. (2023) [21] compared pre- and post-freezing samples, with a second collection occurring between 11 and 40 days after the first. Javan et al. (2016) [1] also employed a narrow but variable temporal range (3.5 to 240 h post-mortem) for sampling internal tissues.

Notably, the study by Huang et al. (2024) [5] featured broad temporal coverage: eight cadavers were analyzed with PMIs ranging from 1 to 71 days, including both frozen and non-frozen bodies. This allowed for comparison of different decomposition stages across individuals.

Regarding temporal classification of data, eight studies used the Post-Mortem Interval (PMI), measured in days or hours, while four studies employed Accumulated Degree Days (ADD) as the reference unit. ADD denotes a thermal measurement intended to quantify elapsed time by accounting for ambient temperature conditions [23].

With regard to the conditions under which the bodies were stored, seven studies did not provide any information on the storage temperature and/or other relevant storage parameters, or reported only generic information without specific values; in contrast, four studies provided precise thermal ranges or other physical parameters related to the storage conditions of the bodies.

As for the technical methods of microbiome sampling at the sites considered, one study utilized buccal and dental swabs, immediately frozen on dry ice after collection using Puritan Hydraflock swabs. Another study allowed the oral swabs to air dry before storing them at − 20 °C pending DNA extraction.

A separate investigation transported samples under refrigerated conditions and processed them within one hour of collection to minimize potential contamination. Two studies stored swabs in sterile phosphate-buffered saline (PBS) tubes, with immediate transfer and subsequent storage at either − 80 °C or − 20 °C. In one study, samples collected from both the oral cavity and rectum were stored at − 20 °C immediately after collection. Another study reported the use of sterile cotton swabs inserted into tubes containing 50 μL of 100 % ethanol and stored at − 20 °C. Two studies simply stated that samples were preserved at − 20 °C until analysis. One additional investigation employed sterile, DNA-free cotton-tipped applicators, again with storage at − 20 °C. Finally, one study performed internal tissue sampling using sterile scalpels, storing the specimens at − 80 °C.

In studies focusing on the microbiome, the following findings emerged regarding the genetic analysis and methods of execution.

With respect to the gene regions analyzed, all eleven original microbiome studies employed portions of the 16S rRNA gene, although with some variation in the specific target segments. Seven studies amplified only the V4 region (Sguazzi et al., 2022; Adserias-Garriga et al., 2017; Ogbanga et al., 2023; Pittner et al., 2020; Pechal et al., 2017; Pechal et al., 2018; Javan et al., 2016) [1,2,10,18,19,21,22], while two studies (Hyde et al., 2013; Hyde et al., 2015) [7,8] jointly analyzed the V3 and V5 regions. The study by Ashe et al. (2021) [20] focused on the V6 and V8 regions, whereas Huang et al. (2024) [5] targeted the V3 and V4 regions.

Regarding the analytical methodologies, it should be noted that PCR was used exclusively to confirm the presence of microbial DNA prior to genotyping via next-generation sequencing (NGS). This preliminary step was generally adopted across the reviewed studies and is therefore not further highlighted.

As for the genotyping approach, nine of the studies utilized NGS based on sequencing of the 16S rRNA gene. Two studies (Hyde et al., 2013; Hyde et al., 2015) [7,8] employed a pyrosequencing approach, which, although now less commonly used, was widely adopted at the time. In addition to conventional NGS methods, the study by Huang et al. (2024) [5] also integrated a 2bRAD-M sequencing strategy, which enables higher-resolution microbial identification by sequencing fragments generated by type IIB restriction enzymes, thereby extending the analysis beyond the 16S rRNA gene alone [24].

Regarding the sequencing platforms used, Illumina MiSeq was by far the most represented, having been employed in seven studies (Sguazzi et al., 2022; Adserias-Garriga et al., 2017; Ogbanga et al., 2023; Pittner et al., 2020; Pechal et al., 2017; Pechal et al., 2018; Javan et al., 2016) [1,2,10,18,19,21,22]. The 454-FLX-Titanium platform was used in the two studies that adopted pyrosequencing (Hyde et al., 2013; Hyde et al., 2014) [7,8] [7,8]. The Illumina NextSeq 550 and the Illumina NovaSeq 6000 instruments were adopted in one study each, namely by Ashe et al. (2021) and Huang et al. (2024), respectively. The results pertaining to the genetic methodologies employed are summarized in Table 2.

3.3. Oral cavity

Among the studies included in this systematic review, the analysis of post-mortem microbial populations in the oral cavity revealed a clear pattern of bacterial succession over time, albeit with variations influenced by individual, environmental, and methodological factors. The oral cavity emerged as one of the most frequently sampled anatomical sites for post-mortem microbiome studies.

In the study by Sguazzi et al. [10], the oral cavity was examined under controlled conditions, with a specific focus on comparisons among fresh, preserved, and skeletonized samples. The results showed that the phylum *Firmicutes* was dominant across all conditions, though it experienced a marked reduction during the early stages of decomposition. The analysis also revealed temporal shifts in specific genera: for example, *Fusobacteriota* were exclusive to fresh samples, while *Proteobacteria* progressively increased with advancing decomposition. Notably, microbial composition remained relatively stable in samples stored at − 20 °C.

Ashe et al. (2021) [20] adopted a longitudinal approach, monitoring microbial succession in the oral cavity of three cadavers throughout the full decomposition process, from the fresh stage to skeletonization. Their findings indicated that the structure of the bacterial community evolved systematically over time: phyla such as *Fusobacteriota* were present only in early-stage samples, while *Firmicutes* and *Proteobacteria* remained the dominant taxonomic groups. Specifically, *Firmicutes* showed a gradual decline as decomposition progressed, while *Proteobacteria* increased in later stages. Additionally, samples subjected to charring exhibited a significant reduction in microbial diversity, likely due to heat-induced selection of thermotolerant bacteria and loss of viability in more sensitive species.

Garriga et al. (2017) [2] also documented oral microbial succession. In the early stages, typical oral flora such as *Streptococcus* and *Veillonella*predominated, while with progression of decomposition, intestinal and environmental anaerobes such as *Clostridium* and *Plano coccaceae* emerged. The onset of the bloat stage was particularly associated with the appearance of *Tenericutes* and insect-associated bacteria such as *Ignatzschineria*, suggesting a correlation between entomological activity and microbiome composition.

Through a comparative analysis using different sequencing platforms, Huang et al. [2024) [5] confirmed that the oral microbiome exhibits distinct diversity compared to other body sites. *Firmicutes* and *Bacteroidota* were the most represented phyla, with genera such as *Streptococcus* and *Prevotella* forming the core microbiota. Temporal differences in microbial composition were observed even in samples with extended PMIs, notably showing a progressive increase in *Proteobacteria* during decomposition. The use of high-resolution techniques such as 2bRAD-M enabled the detection of stable microbial profiles even in highly degraded tissues.

The studies by Hyde et al. (2013 and 2014) [7,8] focused on the behavior of the oral microbiome during the bloat stage. Specifically, Hyde et al. (2013) [7] observed a clear transition from communities dominated by aerobic bacteria such as *Staphylococcus* and *Enterobacteriaceae* to anaerobic communities typically represented by *Clostridia* and *Bacteroides*. The subsequent 2014 study reinforced these findings by examining the evolution of the microbial communities of two subjects over the course of a 20-day period. During this interval, a peak in *Ignatzschineria* was associated with larval infestation, followed

Table 2
Summary of the genetic analysis techniques used. PCR was employed solely to confirm the presence of microbial DNA prior to genotyping via NGS. This approach is common in other studies as well and is therefore not specifically highlighted. In the "Primers" column, F: Forward; R: Reverse.

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time (PMI), SD	Sampling mode	Statistical analysis	Results
E. C. Ashe et al. (2021)[20]	Cohort Study	Western Carolina University, Cullowhee, NC, USA	The aim of the study is to analyse the dynamics of bacterial communities in the human oral cavity during the decomposition process using metagenomic, metatranscriptomic, and culturing techniques	Oral cavity (taken from the hard palate)	3 Cadavers: −C1 (M, 65Y) deceased for gastric cancer (date of death 4/03/2018) –C2 (F, 77Y), heart disease (early stage of decomposition, 2 days prior to discovery, date of death 4/23/2018) –C3 (F, 84Y) deceased for pneumonia (date of death 5/11/2018) * The facility recorded temperatures from –1.7 to 32.2 °C and 12.12 in of precipitation during the study period. ADD were used to standardize the decomposition process.	Each donor was sampled 5–7 times throughout Decomposition: C1, C1.1: 6d, F C1.2: 10d, E C1.3: 13d, E C1.4: 17d, E C1.5: 20d, AD C1.6: 24d, AD C1.7: 27d, AD C2, C2.1: 8d, E C2.2: 11d, E C2.3: 14d, E C2.4: 18d, AD C2.5: 21d, AD C3, C3.1: 6d, F C3.2: 10d, E C3.3: 13d, AD C3.4: 17d, DS C3.5: 21d, DS	Puritan Hydraflock flocked swabs. The swabs intended for nucleic acid extraction were placed back in their tubes and immediately frozen on dry ice.	Main Statistical Analyses – Summary ANOVA (One-way): To compare microbial and environmental differences across treatments −Bonferroni Post-Hoc Test: To identify which groups differed significantly after ANOVA −Shannon Index (H) & Evenness (EH): To assess microbial diversity and distribution uniformity −PCA (Principal Components Analysis): To visualize patterns and variation in microbial communities −t-Test: To compare means between two groups (e.g., treated vs. control) for statistical significance	A total of 69 bacterial isolates were recovered, representing 46 unique species across a range of Accumulated Degree Days (ADD). Proteobacteria dominated (43.5 %), followed by Firmicutes (32.6 %), Actinobacteria (19.6 %), and Bacteroidetes (4.3 %). Four species (e.g., Bacillus cereus, Proteus vulgaris) were isolated from the same donor at multiple time points. Eight other species (e.g., Staphylococcus, Morganella) were found in multiple donors.

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time (PMI), SD	Sampling mode	Statistical analysis	Results
J.Adserias-Garriga et al. (2017) [2]	Cohort Study	Fundació Universitat de Girona, Universitat de Girona, Girona, SPAIN	This study aimed to identify specific oral bacteria that change during decomposition to help more accurately estimate time since death	Oral Cavity	3 corpses: –C1: M, 80Y –C2: F, 81Y –C3: F, 27Y (None of them died from infectious disease and none of them were embalmed) * Air temperature 21–27 °C (avg. 24.5 °C); relative humidity 71–95% (avg. 81.2%).	**–C1:** C1.1: 2d, F C1.2: 3d, F C1.3: 5d, E C1.4: 6d, E C1.5: 7d, E C1.6: 8d, A C1.7: 10d, AD **–C2:** C2.1: 2d, F C2.2: 3d, F C2.3: 4d, F C2.4: 6d, A C2.5: 8d, A C2.6: 10d, AD C2.7: 11d, AD **–C3:** C3.1: 1d, F C3.2: 2d, F C3.3: 3d, F C3.4: 6d, A C3.5: 7d, A C3.6: 10d, AD C3.7: 11d, AD C3.8: 12d, AD	Oral swab. The swabs of the same site were grouped in storage containers, where further drying occurred followed by storage at −20°C before DNA extraction.	The statistical analysis was performed using QIIME to assess alpha and beta diversity, with differences between samples evaluated through weighted UniFrac-based Principal Component Analysis and visualized using R software packages	Microbial Succession by Decomposition Stage: −Fresh Stage (1–5 days): Dominated by Firmicutes and Actinobacteria → Families: Lactobacillaceae, Staphylococcaceae, Gemellaceae, Carnobacteriaceae, Aerococcaceae, Veillonellaceae, Streptococcaceae, Campylobacteraceae, Micrococcaceae, Bifidobacteriaceae, Actinomycetaceae, Corynebacteriaceae −Bloat Stage (5–7 days): Characterized by the appearance of Tenericutes, consistently associated with this phase → Families: Peptostreptococcaceae, Pseudomonadaceae, Alcaligenaceae, Planococcaceae −Days 6–12: Increase in Firmicutes, but with different taxa than those in the fresh stage −Dry Remains: Presence of Bacilli and Clostridia

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time (PMI), SD	Sampling mode	Statistical analysis	Results
X. Huang et al. (2024) [5]	Cross-sectional study	Institute of Forensic Science, Fudan University, Shanghai, CHINA	In this study, the microbiota of eight cadavers at different stages of decomposition were detected using 16S rRNA, metagenomic sequencing and 2bRAD-M sequencing.	−Oral cavity (nasal cavities, hearts, livers, spleens, lungs, kidneys, muscles and guts	8 cadavers: −C1 (M, 54Y), Craniocerebral injury (Frozen) −C2 (M, 64Y), Sudden cardiac death (Frozen) −C3 (F, 69Y), Severe anemia (Unfrozen (limb corpse green) −C4 (F, 87Y), Coronary heart disease (Frozen) −C5 (M, 80Y), Stomach tumor and lung infection (Unfrozen (Thoracic abdominal corpse green) −C6 (M, 41Y), Craniocerebral injury (Unfrozen) −C7 (F, 65Y), Coronary heart disease (Frozen) −C8 (M, 47Y), Coronary heart disease (Unfrozen) * Environmental conditions not reported	−C1: 9d, N/A –C2: 15d, N/A –C3: 71d, AD −C4: 22d, N/A −C5: 5d, E/A −C6: 1d, F −C7: 22d, N/A −C8: 1d, F	Sterile cotton swabs. The samples were transported to the laboratory under refrigerated conditions to prevent the growth of any incidental microbial contaminants and processed within one hours of collection to preserve the microbial integrity of the samples.	Alpha Diversity: Assessed using Chao1, Shannon, and Simpson indices to measure species richness and evenness. Beta Diversity: Calculated via Euclidean distance matrices, visualized with PCoA to compare microbial community differences between groups. Kruskal-Wallis Test: Used to identify statistically significant differences in microbial composition across sample groups.	The microbiome clustered mainly into five phyla: Firmicutes (most dominant), Bacteroidetes, Actinobacteria, Fusobacteria, and Proteobacteria — confirmed by both 16S rRNA and metagenomic analyses. The ORAL CAVITY showed a distinct composition, with Firmicutes and Bacteroidota most prevalent. Metagenomics identified key genera: Streptococcus and Prevotella 16S rRNA had low taxonomic resolution at the genus level. The NASAL CAVITY was dominated by Bacillus and Klebsiella, with 2bRAD-M revealing widespread Klebsiella across groups. 2bRAD-M data highlighted inter-group differences, particularly in Proteobacteria abundance.

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E. R. Hyde et al. (2013) [7]	Cohort Study	Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas, USA	This study aimed at the characterization of the microbiome of decomposition, particularly at the onset and end of the bloat stage, in two cadavers placed aboveground to mimic terrestrial decomposition scenarios under natural environmental conditions.	−Mouth	Two cadavers were placed sequentially in this outdoor facility to decompose under natural conditions:	Two time points: at placement (i.e. pre-	Sterile cotton applicator, placed in a collection tube of sterile phosphate	−PCoA (Principal Coordinates Analysis):	FECAL/RECTAL SAMPLES (pre-bloat):
				−Rectum	−C1 (M, 52Y), no	bloat) and conclusion (i.e. end-bloat)	buffered saline (PBS)	Used with weighted and unweighted UniFrac distances to visualize differences in microbial communities across samples.	C2: Dominated by Firmicutes, low Bacteroidetes — consistent with a living human profile
					medical history beyond carbon monoxide poisoning as the cause			−Rarefaction Curves:	C1: Dominated by Proteobacteria, indicating a different microbial state
				Pre-bloat C1 and C2,	of death	**−C1**(143d FZ):	Oral and rectal samples were obtained by gently scraping the inner surface of the mouth or rectum with a sterile single-use plastic spatula.	Assessed species richness (OTUs) relative to sequencing depth, comparing microbial diversity across body sites.	ORAL CAVITY (pre-bloat):
				−Mouth swab	–C2 (M, 68Y), medical history of diabetes mellitus, chronic alcoholism,,cardiovascular disease with acute myocardial infarction as the cause of death	C1.1: 149d, F		−Standard Error of the Mean (SEM):	C2: More similar to a healthy oral microbiome, with Streptococcus, Prevotella, and Veillonella
				−Mouth scrape	*	C1.2: 156d, E		Calculated to represent variability in microbial diversity among body sites.	C1: Less representative of healthy profiles
				−Rectal scrap	Study at Southeast Texas Applied Forensic Science (STAFS) (humid subtropical climate). Two cadavers placed outdoors: in Sept, warmer temperatures and low humidity; in Nov, cooler temperatures and higher humidity slowed decomposition.	**–C2** (89d FZ):		These analyses revealed:	MOUTH SAMPLES – swab vs. scrape, pre-bloat (C2):
				End-bloat C1	ADD were used to standardize the decomposition process.	C2.1: 102d, F		−A shift from aerobic to anaerobic bacteria during decomposition	Swab: Dominated by Firmicutes
				−Mouth scrape		C2.2: 116d, E		−Differences in microbial composition	Scrape: Dominated by Actinobacteria

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time (PMI), SD	Sampling mode	Statistical analysis	Results
								between cadavers, between body sites, and between decomposition stages	
				−Small intestine swab, −Transverse colon swab, −Sigmoidal colon swab −General body cavity swab C2 −Mouth scrape −Small intestine swab −Transverse colon swab −Stomach scrape					MOUTH SAMPLES – end-bloat (C2): Scrape: Dominated by Firmicutes, followed by Proteobacteria One scrape resembled the pre-bloat swab (Firmicutes-dominated)

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time (PMI), SD	Sampling mode	Statistical analysis	Results
E.R. Hyde et al. (2015) [8]	Cohort Study	Alkek Center for Metagenomics and Microbiome Research, Baylor College of Medicine, Houston, TX, USA	This study aimed at assessing how the composition and function of the human microbiome change during states of health and disease, and describe how these variations are associated with the host's molecular profiles.	− Oral cavity −Fecal (rectal) [external left/right cheeks −left/right bicep region, torso]	2 cadavers (The two cadavers were placed in tandem in the outdoor facility to decompose under natural conditionsincluding weather, insects, and vertebrate scavengers) −C1: F, 85Y, with an unknown medical history and unknown cause of death –C2: M, 55Y, with unknown cause of death. *	C1 was stored frozen for 22 days, with 0 days in the cooler C2 was stored frozen for 14 days, with 0 days in the cooler MOUTH SAMPLES −1d, F −5d, E	−Steril cotton swab. The cotton tip was removed and placed in a collection tube containing 0.5 ml sterile phosphate-buffered saline, pH 7.4, and stored at −80 °C until processing for bacterial genomic DNA. −Collection of fecal sample.	Statistical analysis used QIIME to assess alpha and beta diversity, with significance tested by Monte Carlo t-tests and visualized via PCoA of UniFrac distances.	−MOUTH SAMPLES: high abundance of Proteobacteria and Firmicutes. −FECAL ~~RECTAL~~ SAMPLES: Firmicutes (70%) and −Bacteroides (20%) were the most abundant phyla before purge −Proteobacteria: after purge −Firmicutes drier phases −Actinobacteria also increased in the drier phases of decomposition,

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time (PMI), SD	Sampling mode	Statistical analysis	Results
					STAFS (humid subtropical climate, 2-acre vegetated area with sandy, acidic soil). Cadavers placed outdoors (exposed to rain, insects, scavengers). Aug: warmer, lower RH; Sept: cooler, higher RH. Rainfall negligible. ADD were used to standardize the decomposition process.	−9d, A −12d, AD −20d, AD RECTAL SAMPES −1d, F −5d, E −9d, A −12d, AD −20d, AD			

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
N. Ogbanga et al. (2023) [21]	Cohort Study	Forensic Anthropology Center, Texas State University, San Marcos, TX, USA	The aim of the study is to assess the effects of freezing on the post-mortem human microbiome by analysing the microbial diversity and abundance of seven human cadavers before and after freezing.	−Oral cavity −Rectum (hand, foot, neck)	9 cadavers (the study was conducted on only 7, C1-7, of these donors) Sex, age, date, and cause of death are not specified. (Donor bodies were frozen and defrosted within a closed and controlled indoor laboratory (21∘C) to prevent insect access) *	Refrigeration duration before swabbing (days) C1: 8d, N/A C2: 2d, N/A C3: 6d, N/A C4: 4d, N/A C5: 7d, N/A C6: 5d, N/A	Sterile swabs −For the oral cavity, samples were collected from the left cheek, right cheek, and oral vestibule, including any teeth if available −Rectal samples were obtained by inserting the swab into the anus to about 5 – 10 cm into the rectum (All swabs were immediately stored frozen at −20∘C)	Present: statistical analyses revealed that although there were variations in the relative abundance of certain bacterial phyla, these differences were not statistically significant at the overall microbial community level.	Freezing had no significant impact on overall phylum-level composition. Proteobacteria increased, while Bacteroidota and Firmicutes decreased post-thaw. Five genera showed significant changes between fresh and thawed samples. The RECTUM had a distinct and more stable microbiome, minimally affected by freezing. Donor C6 showed unique microbial profiles across all sites.

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
					Study conducted on frozen cadavers.	C7: 0d, N/A Refrigeration duration after swabbing (days) C1: 0d, N/A C2: 0d, N/A C3: 9d, N/A C4: 1d, N/A C5: 3d, N/A C6: 0d, N/A C7: 0d, N/A Second sampling: after freezing duration (days) min 11, max 40			

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
S.Pittner (2020) [22]	Cohort Study	Dept. of Medicine and Health	This study	−eyes	2 body exhumed, or partially exhumed 105 days after burial (121/122 days after death)	−C1:	Sterile cotton swabs	there was not sufficient statistical power to perform regression models	−Firmicutes and Proteobacteria were dominant. Proteobacteria reached 62% of prevalence in the eyes
		Sciences, University of Florence, Florence,	aimed to investigate the applicability and challenges of using a multidisciplinary approach	−ears, −mouth −nose	Both cadavers went down two complete exhumations (day 28 and day 105)	C1.1: 17d, F	were placed into tubes containing 50 µL of 100% ethanol, broken off, and stored in cooling units at –20 °C		−Firmicutes reached 65% of prevalence in the rectum.
		ITALY	to PMI estimation for advanced decomposition stages in buried human bodies.	−rectum	2 cadavers	C1.2: 45d, A			− Actinobacteria decreased by 26–31% from August to November
				−thigh skin	C1: M, 70Y, cause of death: cardiac arrest	C1.3: 122d, AD/ M			−Proteobacteria and Firmicutes both increased by 1–6% in EAR and NOSE communities
					C2: M, 60 Y				−Firmicutes increased in the MOUTH by 7% and decreased in the RECTUM by 7%.
					cause of death: metastatic malignacy	–C2: C2.1: 16d, F			
					*	C2.2: 44d, A C2.3: 121d, AD			

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
					Burial study: Aug–Nov. Air 1.4–34.6 °C (avg. 1–2 °C above normal), low rainfall. Grave/ body temps stayed above freezing (min: 4.1–6.8 °C in graves; 3.9–4.3 °C in bodies). After probe adjustment, a double-buffer effect was observed: soil and bodies showed delayed, reduced temperature fluctuations vs. air. ADD were used to standardize the decomposition process.				

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
J.L. Pechal et al. (2017) (18)	Cohort Study	Department of Entomology, Michigan State University, USA	The present study reports two unusual pediatric cases in which the bodies were found substantially altered due to long-term freezing and concealment, one for 22 months and the other for 31 months. Given the dearth of postmortem microbiome studies conducted during routine death investigation, these data provide the first insights into the complexities associated with using microbiome information in one of a wide variety of death circumstances.	−eyes, −mouth, −rectum (external auditory canal, nares, umbilicus	2 homicide victims (Both suffered extensive blunt force, mainly to the head, and both had prominent swelling of the brain; this was the cause of death. The 9-year-old male also had thermal burns, mainly on the abdomen, buttocks, and genitalia). C1: (M, 9Y) wrapped in a polychromatic bed comforter; his estimated frozen interval was c. 31 months C2: (F, 13Y) loosely wrapped in a black	Three time points samples during the thawing process: −**C1** (31m FZ) C1.1: 0d, F C1.2: 1d, F C1.3: 2d, F –**C2** (22m FZ) C2.1: 0d, F C2.2: 1d, F	Sterile cotton-tipp Samples were stored at −20°C until further processing Swabbing (rectum, fecal collection not specified): The sampling method is not described.	The article does not provide specific details about the statistical techniques used in data analysis. Since it is a case study with a very small sample size (two subjects), it is likely that the analysis focused on qualitative and descriptive observations rather than formal statistical analyses.	During thawing, microbial diversity increased most in the nares, eyes, and rectum, with the buccal cavity showing the highest overall diversity (Simpson, Faith's PD, and observed taxa). OTU richness increased in most sites (ears, eyes, nares, buccal), while the rectum decreased. Corynebacterium, Haemophilus, Fusobacterium, Streptococcus all increased in abundance; Staphylococcus decreased (–33.3%) and Lactobacillus nearly disappeared (–98.3%). Each anatomical site developed a distinct microbial profile during thawing. Average species per site:

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
					plastic bag and yellow plastic band with a black cloth wrapped around the neck; her estimated frozen. * Bodies placed 30–40 cm apart under autopsy room AC vent, thawed ~48 h. Covered with water-dampened sheets to prevent drying/ mummification.	C2.3: 2d, F			−BUCCAL CAVITY: 12 −NARES AND EARS: 7 −RECTUM: 6 −EYES AND UMBILICUS: 5

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
J.L. Pechal et al. 2018 (19)	Cross-sectional study	Department of Entomology, Michigan State University, USA	Here the authors reveal distinct postmortem microbiomes of human hosts from a large-scale survey of death cases representing a predominantly urban population, and demonstrate how these microbiomes reflected antemortem health conditions within 24–48 h of death	−Eyes, −Mouth,	188 (90 Black, 98 White)	PMI 1-73h	−DNA-Free sterile cotton-tipped applicators (Samples were stored at −20 °C until further processing)	The study employed a combination of descriptive, predictive, and inferential methods to investigate the human postmortem microbiome: −Microbial Diversity Indices assessed species richness and evenness across samples.	Phylogenetic diversity decreased with decomposition across most anatomical sites, except the rectum. Actinobacteria and Bacteroidetes declined, while Proteobacteria increased.
				−Rectum (external auditory canal, nares, umbilicus)	The dataset consisted of samples collected in 2014–2016. Accidental and natural deaths comprised a majority of the sampled cases at 38% and 30%, respectively, while homicides (20%) and suicides (12%) accounted for the remaining deaths.		−Rectal swabbing: Swab rubbed while rotating for 3–5 s.	Correlation analysis explored links with age, sex, health status, and postmortem interval (PMI). −Predictive Modeling (via PICRUSt) inferred potential metabolic functions of microbial communities from 16S rRNA data. Multivariate analysis revealed associations between microbial profiles and demographic/clinical variables.	Less than 10% of OTUs overlapped across postmortem intervals. Among typical host-associated taxa, only Streptococcus, Haemophilus parainfluenzae, and Staphylococcus were consistently present.
					Median age 43 years (18–88 years)			−Spatial and Temporal Analyses examined how microbiome composition varied across anatomical	

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
								sites and changed over time after death.	
					The average (± SD) age of the cases at			−Inferential Statistical Tests were used to compare groups and link specific microbes to antemortem health conditions, supporting the microbiome's potential as a postmortem biomarker.	Streptococcus was most abundant in the eyes and mouth during early PMI (<48h).
					the time of death in this dataset was 44 ± 15 years.				
									H. parainfluenzae was prevalent in the oral cavity during the same time frames.
					*				
					Environmental conditions not reported				

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
G.T. Javan et al.	Cross-sectional study	Physical Sciences Department, Alabama State University, Montgomery,	Time-dependent changes in the thanatomicrobiome within internal organs can estimate the time of death as a human body decays	−Buccal cavities, (brain, heart, liver, and spleen, blood)	27 human corpses,	PMI:	Sterile scalpels	The study applied alpha and beta diversity metrics to assess microbial richness and composition across samples, along with multivariate and regression analyses to explore correlations with demographic factors and postmortem interval (PMI).	Bacterial genera were similar across organs within each sex but differed between males and females.
(2016) (1)		USA			from criminal cases (homicide, suicide, and overdose)	3.5– 240 hrs	(Specimens were placed in a freezer at − 80 °C until further analysis)	Inferential tests were used to detect group differences and associations with antemortem conditions.	
					−15 males				Females: Dominated by Pseudomonas (aerobic, Gram-negative) and Clostridiales
					−12 females,				Males: Enriched in Clostridium, Streptococcus, and uniquely in Rothia (facultative anaerobe, Gram-positive)
					*				The BUCCAL CAVITY showed the most distinct microbial profile, with significant fold changes and

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Statistical analysis	Results
									broad phylum representation. Firmicutes from the mouth were also detected in internal organs and blood.
					Corpses were kept in the morgues at 1 °C until the time of tissue collection				

Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time (PMI), (SD)	Sampling mode	Statistical analysis	Results
G. Sguazzi et al. (2022) [10]	Cohort Study and Cross-sectional study	Northumbria University, Newcastle upon Tyne,	The study aims to assess whether microbial profiles in human DNA extracts:	Oral cavity (buccal swab, tooth)	8 human cadavers. Of the 8 cadavers, samples were taken from only two:	−C1:	Tooth and buccal swab.	The study used descriptive statistical analysis based on 16S rRNA sequencing to explore microbial community composition. Key focuses included:	Firmicutes and Proteobacteria were detected in all samples, representing the dominant phyla, and together accounting for 79.8% of the bacterial population..
		UK	−Reflect the true postmortem microbiome or are affected by contamination	(heart, blood, spleen, quadriceps)	−C1, F 91y	1) C1.1: 10d, F 2) C1.2: 16d, A	Swabs	−Relative abundance of genera and species	Actinobacteriota and Bacteroidota (~8%) were also present, while Chloroflexi and Acidobacteriota appeared in lower proportions (~1%).
			−Are preserved after long-term storage at –20 °C		–C2, F 67y		were stored frozen at − 20 ∘C. DNA extractions were carried out specifically for the present study, and the extracts were processed immediately, avoiding storage at −20°C of the DNA extracts.	−Taxa distribution by decomposition stage, tissue type, and environment	Some phyla were stage-specific:
			−Retain decomposition-related microbial signatures over time			–C2:		−Qualitative comparisons between recent and archived samples	
			−Vary with burial conditions, geography, tissue		Both C1 and C2 died of unknown causes	1)C2.1: 2d, A		No inferential tests (e.g., t-tests, ANOVA) were used. The analysis was exploratory, aimed at	−Actinobacteriota in fresh and skeletonized remains

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Article	Type of study	Place where the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time (PMI), (SD)	Sampling mode	Statistical analysis	Results
			type, and cause of death					identifying microbial patterns and shifts.	
					*				–Cyanobacteria in skeletonized remains only
					Exact storage conditions not reported, except for two donated bodies placed outdoors at Forensic Anthropology Research Facility (FARF), fully exposed to weather conditions.				–Asgardarchaeota exclusive to charred bodies
									Notably, microbial profiles can still be obtained from old human DNA extracts, showing comparable results to recently collected decomposition samples.

by an increase in *Corynebacterium* and *Clostridium* during the final desiccation stage [8].

Although focused on skeletal remains exposed in tropical environments, the study by Ogbanga et al. (2023) [21] demonstrated that the oral microbiome still retains significant taxonomic signals even under extreme degradation conditions. The most abundant groups were - Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes.

In the study by Pittner et al. (2020) [22], a random forest predictive model was applied to oral microbiome data. The results demonstrated a reasonable capacity to estimate the PMI, with a prediction error of 5.6 %. Genera such as *Enterococcus*, *Clostridium*, *Lactobacillus*, and *Bacteroides* were found to be informative across various post-mortem stages, suggesting a microbial progression consistent with elapsed time.

Pechal et al. (2018) [19], in a cross-sectional study of 188 cases, also reported microbiological patterns compatible with temporal succession. However, the predictive accuracy of PMI based solely on the oral microbiome was lower compared to other sites, such as the rectum. Nevertheless, well-established genera including *Streptococcus*, *Clostridium*, *Prevotella*, and *Lactobacillus* remained consistently present in oral samples, confirming inter-study reproducibility.

The study by Pechal et al. (2017) [18] investigated microbial dynamics in two previously frozen cadavers, revealing a progressive increase in oral microbial diversity during thawing. *Corynebacterium*, *Haemophilus*, and *Fusobacterium* increased, whereas *Lactobacillus* showed a drastic decline.

Finally, Javan et al. (2016) [1] confirmed that the oral microbiome undergoes measurable changes within the first few post-mortem hours. Specifically, *Streptococcus*, *Veillonella*, and *Prevotella* were consistently detected in early samples, while the genus *Clostridium*—particularly the species *C. novyi*—increased in more advanced PMIs. A statistically significant relationship between oral microbiota composition and PMI was also demonstrated.

Regarding the analysis of individual cases involving the microbiome, extracted from the original studies (excluding the work by Pechal et al. (2018), which investigates 188 cases without focusing on individual-level data), the following findings emerged.

The descriptive analysis of age-related data concerning oral cavity samples—comprising a total of 24 individuals—reveals a heterogeneous distribution, with ages ranging from a minimum of 9 years to a maximum of 91 years, for an overall range of 82 years. The mean age was 62.1 years, while the median age was 66 years, indicating slight asymmetry in the distribution, likely influenced by the presence of pediatric subjects. In terms of gender distribution, there was a slight predominance of male subjects: 13 individuals (54.2 %) were male (M), and 11 (45,8%) were female (F).

In the analyzed sample, the average number of oral cavity samplings per case was 3.17, with a median of 2.5. This suggests that in half of the cases, the number of samples collected per subject ranged between two and three. However, the mode was 1, indicating that in a considerable number of cases, only a single oral sample was obtained per individual. The standard deviation, approximately 2.30, reflects a moderate degree of variability, with the number of samples per case ranging from 1 to 8, resulting in an overall range of 7. The first quartile (Q1) was 1, the third quartile (Q3) was 5, and the interquartile range (IQR) was 4.

Analysis of post-mortem preservation states across the collected samples indicates a predominance of early decomposition stages. Out of a total of 72 valid samples (excluding unclassifiable or unavailable cases), the most frequently observed condition was “Fresh” (F), accounting for 36.1 % of cases (n = 26). This was followed by the “Advanced Decay” (AD) stage, representing 26.4 % of cases (n = 19), highlighting a particular research interest in the advanced stages of decomposition, where microbial community composition undergoes substantial changes. The “Early Decomposition” (E) stage was also well represented, comprising 19.4 % of samples (n = 14).

The “Active Decay” (A) phase—an intermediate and dynamic stage of decomposition—was observed in 11 samples (15.3 %), while the “Dry

Remains/Skeletonization" (DS) stage was far less frequent, with only 2 samples (2.8 %), suggesting lower availability or experimental focus on severely degraded tissues.

A few cases with mixed classifications (e.g., E/A, AD/M) were disaggregated for statistical purposes, while unassigned values (N/A) were excluded from the count.

Of the 72 samples analyzed, entomological activity was not available (N/A) in 39 cases, representing 54 % of the total. Among the remaining 33 samples with valid data, 14 (37.8 %) were classified as score 0 (no visible entomological activity), 7 (18.9 %) as score 1 (presence of adult flies only), 13 (35.1 %) as score 2 (adult flies and small larvae), and 3 (8.1 %) as score 3 (abundant presence of both adults and larvae).

Rectum.

Hyde et al. (2013) [7] employed 16S rRNA gene pyrosequencing to study rectal samples collected during the pre-bloat and end-bloat stages. In the initial stage, the samples were predominantly composed of *Firmicutes*, whereas in the later stage there was a marked increase in obligate anaerobes such as *Clostridium* and *Lactobacillus*. The temporal succession was accompanied by an increase in microbial richness and diversity, consistent with progressive tissue degradation. The study also highlighted considerable inter-individual variability in the initial microbial composition, although a common evolutionary trajectory of microbial communities was maintained across cases.

In the subsequent study by Hyde et al. (2014) [8], samples from the intestine and pelvic skin of two naturally decomposing cadavers were analyzed across five time points. Initially, the predominant phyla included *Clostridiaceae*, *Bacteroidetes*, and *Porphyromonas*. After the purge phase, following bloat, a peak in the genus - *Ignatzschineria*—associated with insect colonization—was observed, followed by a return to *Clostridium* dominance during the dry stage. Although the microbial succession dynamics were generally consistent between individuals, variations in the timing and intensity of transitions were noted.

Ogbanga et al. (2023) [21] found that rectal samples were dominated by *Firmicutes*, followed by *Proteobacteria*, *Bacteroidota*, and *Actinobacteriota*. Genera such as *Clostridium*, *Peptostreptococcus*, *Escherichia*, and *Enterococcus* were common and stable, even after freezing. Rectal microbial communities demonstrated good preservation of taxonomic signals.

Pittner et al. (2020) [22] analyzed rectal samples from exhumed cadavers at 28 and 105 days post-burial, identifying a predominance of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, with consistent presence of *Enterococcus*, *Clostridium*, *Lactobacillus*, and *Bacteroides*. The data revealed stable microbial patterns with measurable temporal variations. Among the sampled body sites, the rectum proved to be the most informative for PMI estimation, showing high performance in machine learning prediction models and low inter-individual variability.

In Pechal et al. (2018) [19], rectal swabs from 188 cadavers with PMIs ranging from 1 to 73 h were analyzed. The rectal microbiome was initially dominated by *Firmicutes*, evolving over time toward decomposer genera such as *Clostridium*, *Bacteroides*, and *Enterococcus*. Predictive models confirmed the rectum as the most accurate site for PMI estimation compared to the oral cavity and ocular bulb.

Finally, in Pechal et al. (2017) [18], based on the aforementioned pediatric cases that had undergone extended freezing, a decrease in *Firmicutes* and an increase in *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were observed at the rectal level. Microbial richness declined, with selective emergence of genera such as *Corynebacterium*, *Haemophilus*, and *Fusobacterium*. The rectal microbiome was shown to be sensitive to storage and thawing conditions, underscoring the need for cautious interpretation in forensic contexts involving refrigerated or frozen remains.

The descriptive analysis of age-related data for rectal samples—comprising a total of 8 individuals—revealed a heterogeneous distribution, with ages ranging from 9 to 85 years (a range of 76 years). The mean age was 51.5 years. Regarding gender, the distribution was skewed toward males: 6 individuals (75 %) were male (M), while 2 individuals (25 %) were female (F).

In the analyzed sample, the average number of rectal samplings per case was 3.25, with a median of 3, indicating that in 50 % of the cases, each cadaver underwent at least three samplings. Notably, the mode also matched the median. From a quartile perspective, the first quartile (Q1) was 2.75, the third quartile (Q3) was 3.5, and the interquartile range (IQR) was 0.75.

Analysis of post-mortem preservation states for the rectal samples indicated a predominance of early decomposition stages. Out of 26 valid samples (excluding unclassifiable or unavailable cases), the most frequently observed condition was "Fresh" (F), representing 46.1 % (n = 12). This was followed equally by the "Advanced Decay" (AD), "Active Decay" (A), and "Early Decomposition" (E) stages, each accounting for 15.4 % (n = 4) of the cases. The "Dry Remains/Skeletonization" (DS) stage was the least represented, with only 2 samples (7.7 %), suggesting limited availability or experimental focus on extensively degraded tissues.

One case with a mixed classification (AD/M) was disaggregated for statistical analysis, and unassigned values (N/A) were excluded from the dataset.

Out of 26 rectal samples, entomological activity was not available (N/A) for 6 cases (23.1 %). Among the 20 samples with valid data, 9 (45 %) were scored as 0 (no visible entomological activity), 9 (45 %) as score 2 (presence of adult flies and small larvae), 1 (5 %) as score 1 (adult flies only), and 1 (5 %) as score 3 (abundant presence of both adults and larvae).

Regarding rectal sampling, five microbiome studies reported the use of swabbing; however, only one explicitly mentioned fecal collection. The remaining four described swab sampling—sometimes involving rubbing—without clarifying whether fecal material was included. Additionally, one study reported the use of scraping, but it did not specify whether the sampling targeted only the mucosa, only feces, or both.

3.4. External eye

Among the studies focusing on the external eye as an anatomical site for post-mortem microbiome analysis, a clear correlation emerged between microbial community structure and the stage of decomposition. Although the dynamics observed were less pronounced than in other body sites, they remained informative and forensically relevant.

In the study by Pittner et al. (2020), ocular samples collected from two exhumed cadavers at 28 and 105 days post-burial revealed significant shifts in microbial composition. In both cases, *Firmicutes* and *Proteobacteria* remained the predominant phyla. However, in the samples with longer PMIs, there was a notable increase in environmental anaerobes such as *Clostridium* and *Enterococcus*, as compared to the oral and skin flora observed in fresher samples. Microbial diversity also increased over time, indicating a progressive recolonization of the ocular site by environmental bacteria as the tissues degraded.

Pechal et al. (2017) [18] showed that during thawing phases, the external eye underwent a taxonomic shift, characterized by a significant increase in *Actinobacteria*, *Fusobacteria*, and *Gammaproteobacteria*, along with a concurrent decline in *Firmicutes*. Genera such as *Corynebacterium*, *Haemophilus*, and *Fusobacterium* increased during thawing, while *Staphylococcus* showed a marked reduction.

Finally, in the large-scale study by Pechal et al. (2018) [19], the ocular microbiome was analyzed in 188 cadavers with PMIs ranging from a few hours to over 48 days. This study also confirmed a temporal bacterial succession, with a transition from genera such as *Streptococcus* and *Veillonella*—typical of the human microbiota during life—to decomposer and environmental genera such as *Clostridium*. Microbial composition varied according to the decomposition stage: aerobic and commensal bacteria dominated in fresher samples, while anaerobic and environmental taxa emerged in advanced PMIs, consistent with the

onset of extensive putrefactive processes.

The descriptive analysis of age-related data for ocular samples—comprising a total of 4 individuals—revealed a heterogeneous distribution, with ages ranging from 9 to 70 years (a total range of 61 years). The mean age was 38 years. With regard to gender, the distribution was skewed toward male subjects: 3 individuals (75 %) were male (M), and 1 individual (25 %) was female (F).

As for the number of samplings performed on this subgroup, the mean, median, and mode were all equal to 3. This indicates that all cases underwent the same number of samplings, reflecting consistency across subjects for this anatomical site.

Analysis of the post-mortem preservation states of the collected samples showed a predominance of early decomposition stages. Out of 12 valid samplings (excluding unclassifiable or unavailable cases), the most frequently observed condition was "Fresh" (F), accounting for 66.6 % (n = 8). This was followed equally by "Advanced Decay" (n = 2) and "Active Decay" (n = 2), each representing 16.6 % of the cases. No samples were collected during the "Early" or "Dry Remains/Skeletonization" stages.

Of the 12 ocular samples analyzed, entomological activity was not available (N/A) for 6 cases (50 %). Among the 6 remaining samples with valid data, 3 (50 %) were classified as score 0 (no visible entomological activity), 1 (16.6 %) as score 2 (presence of adult flies and small larvae), 1 (16.6 %) as score 1 (adult flies only), and 1 (16.6 %) as score 3 (abundant presence of both adults and larvae).

The results pertaining to the individual cases extracted from each study are summarized in Table 3.

3.5. Microbiota

Regarding the original studies on the post-mortem microbiota, all three works addressing this topic included sampling from the oral cavity (noting that in one case the swab was collected from the throat), as well as from the rectum; only one study included sampling from the external eye.

In terms of anatomical sampling sites, Sidrim et al. (2010) [9] extended sampling beyond traditional mucosal sites to include the skin, scalp, and both main and peripheral bronchi. In the advanced stages of decomposition, they also sampled clothing and environmental materials, such as soil from the burial pit and fragments of the coffin. Christoffersen et al. (2015) [25] collected samples from the abdominal cavity, specifically targeting decomposing soft tissues. Campobasso et al. (2022) [26] included in their sampling protocol additional sites such as the nose, ears, brain, spleen, liver, and heart, thereby demonstrating interest in both epinecrotic surfaces and deep tissues.

Of the three studies, two explicitly reported the causes of death of the analyzed individuals. Campobasso et al. (2022) [26] provided detailed information, distinguishing between deaths of natural origin (e.g., cardiac or respiratory failure, septic shock, hepatic cirrhosis) and violent causes (e.g., blunt trauma, gunshot wounds, suicides). Christoffersen et al. (2015) [25] also reported causes of death, noting a predominance of natural causes. Conversely, Sidrim et al. (2010) [9] did not provide specific information regarding the causes of death of the sampled individuals.

The timing and methods of sampling across the three studies varied significantly, depending on research objectives and logistical or judicial availability of the bodies, particularly in relation to the oral cavity, rectum, and eye.

In the study by Campobasso et al. (2022) [26], samples were collected from 18 bodies with post-mortem intervals (PMIs) ranging from 24 h to 15 days. The subjects were divided into three groups (<72 h, 72–168 h, and > 168 h), and samples were taken from five external sites, including the eye, oral cavity, and rectum. Although sampling was performed only once per individual, the wide temporal distribution enabled a cross-sectional analysis of epinecrotic bacterial communities.

In contrast, Sidrim et al. (2010) [9] employed a more comprehensive approach based on 60 cadavers distributed across three decomposition stages: bloated (n = 34), putrefaction (n = 6), and skeletonization (n = 20). Oral and rectal samples were collected using sterile swabs in at least two replicates per site. Although the study design did not provide a true temporal sequence for each individual, the inclusion of bodies at various decomposition stages offered a comparative overview of fungal evolution across anatomical sites. Recurrent identification of *Aspergillus*, *Penicillium*, and *Candida* was reported in the oral and rectal samples.

The study by Christoffersen et al. (2015) [25], while primarily focused on infectious causes of death rather than microbial succession, nonetheless reported post-mortem microbiome data from the oral cavity and lungs. Samples were collected during single autopsy events, typically within a few hours of death (in forensic settings), and often included pharyngeal swabs, though no further methodological details were provided.

With regard to the environmental storage conditions of the bodies in the microbiota studies, two out of three studies did not report these conditions; only one study provided information on storage within the mortuary, specifying the temperature.

As for the microbiological methods employed, the following was observed:

Campobasso et al. (2022) [26] cultured samples on various agar plates, including selective Columbia CNA with 5 % sheep blood, MacConkey, Chocolate, and Sabouraud agars. Cultures were incubated at 37 °C for 24 h under aerobic conditions, except for Sabouraud agar (30 °C for 48 h) and Chocolate agar (incubated anaerobically). Culture plates were analyzed using MALDI-TOF mass spectrometry.

Sidrim et al. (2010) [9] cultured samples on Sabouraud media supplemented with 2 % glucose, Sabouraud enriched with vancomycin and polymyxin B to suppress Gram-positive and Gram-negative bacteria, and Sabouraud with added cycloheximide to inhibit environmental fungi. Cultures were incubated in the dark at 25–28 °C for up to 20 days, with daily monitoring for fungal growth. Microbial identification was performed through micromorphology, fermentation assays, and biochemical testing.

Christoffersen et al. (2015) [25] also utilized agar culture methods, though no further details were provided regarding the specific techniques used.

3.6. Oral cavity

A review of the results reveals that the study by Christoffersen et al. (2015) [25] demonstrated the absence of microbial growth on throat samples for both known and unknown causes of death.

As reported by Campobasso et al. (2022) [26], it appears that for periods of PMIs lasting less than 72 h, 70 % of the bacteria were classified as Firmicutes, 10 % as Proteobacteria, 10 % as Ascomycota, and 10 % as Actinobacteria. From 72 to 168 h after death, the percentage of Firmicutes decreased to 40 %, while the percentages for Proteobacteria, Ascomycota, and Actinobacteria increased to 30 %, 20 %, and 10 %, respectively. Beyond 168 h post-mortem, the predominant bacterial phyla were Proteobacteria (60 %), Firmicutes (20 %), and Ascomycota (20 %).

The study by Sidrim et al. (2010) [9] demonstrated that in the oral cavity, as well as in the other mucous membranes analysed (including the rectum), the predominant fungal species were *A. flavus*, *A. niger*, *P. rugulosum*, *P. piceum*, *C. albicans*, *C. parapsilosis*, *Trichosporon* sp., *Acremonium* sp., *Mucor* sp. and *Fusarium* sp. In the "putrefaction stage", the presence of *P. piceum*, *C. albicans* and *C. guilliermondii* was observed. It is important to note that sampling from the oral cavity during the skeletonization stage was not performed in the latter study.

3.7. Rectum

With regard to the findings, the study by Christoffersen et al. (2015) [25] reported no microbial growth in ~~rectal~~ fecal samples, with the

exception of one cadaver (for which neither the post-mortem interval nor the preservation state were specified), who had died of sepsis and from whom *Escherichia coli* and *Clostridium* species were isolated.

Sidrim et al. (2010) [9] identified essentially the same microbial species previously reported in oral cavity samples.

Campobasso et al. (2022) [26] observed the following rectal microbiota composition within 72 h post-mortem: 40 % Proteobacteria, 30 % Actinobacteria, 20 % Firmicutes, and 10 % Ascomycota. Between 72 and 168 h, the composition shifted to 50 % Proteobacteria and 50 % Firmicutes. Beyond 168 h post-mortem, Proteobacteria increased to 75 %, while Firmicutes decreased to 25 %.

It should be noted that in both the study by Sidrim and that by Campobasso, rectal samples were collected using swabbing, without specifying whether the collected material consisted of fecal matter, mucosal material, or both.

3.8. External eye

Regarding the only study that included external eye sampling, Campobasso et al. (2022) [26] reported that within 72 h post-mortem, 66.7 % of the microbiota belonged to Proteobacteria and 33.3 % to Actinobacteria. Between 72 and 168 h, the microbial composition consisted of 44.4 % Firmicutes, 33.3 % Proteobacteria, 11.1 % Actinobacteria, and 11.1 % Ascomycota.

The results pertaining to microbiota are summarized in Table 4.

As described in the Materials and Methods section, study quality was assessed using the appropriate CASP checklists, yielding an overall rigor score for each included study. Responses to checklist items were categorized as "Yes," "No," or "Can't tell," and scored as 1 or 0 depending on whether the specified criteria were met. The results are summarized in the review table, which presents a final quality score for each study, ranging from 0 to 14 for cohort studies and from 0 to 11 for cross-sectional studies.

A total of 8 cohort studies were assessed. Of these, two were classified as high quality, four as moderate quality, and two as low quality. Of the six cross-sectional studies, three were of high quality, three were of moderate quality, and none were of low quality.

The results about quality assessment are summarized in Table 5.

In line with the SIGN 2019 methodology, conclusions were weighted according to the quality of evidence assessed via the GRADE system. High-throughput sequencing studies (especially 16S rRNA and metagenomics) from oral and rectal sites provided moderate to high certainty, supporting strong recommendations. In contrast, evidence from fungal or corneal sampling was weaker, limiting recommendation strength. The formulation of recommendations also considered clinical relevance, reproducibility, and feasibility. While some findings support confident application, others remain conditional, underscoring the need for more standardized and validated research.

4. Discussion

The first noteworthy observation emerging from the collected data is the numerical predominance of scientific literature focused on the study of the microbiome over that on the microbiota in post-mortem contexts. Of the fourteen original studies included in this systematic review, eleven focus on microbiome analysis, while only three investigate the microbiota. This disproportion likely reflects an established trend in recent research, favoring high-resolution molecular approaches—particularly 16S rRNA gene sequencing—over traditional culture-based methods [27–29]. Microbiome analyses indeed offer a broader, more sensitive, and detailed view of microbial community composition, including non-cultivable or low-abundance species [30,31], which can be particularly advantageous in forensic investigations.

Conversely, culture-based techniques are generally more accessible, less costly, and more easily implemented in regional or under-resourced laboratories that lack advanced genomic infrastructure [4]. Additionally, unlike molecular techniques, they provide direct information about the viability and metabolic activity of microorganisms [32,33].

It is important to acknowledge, however, that the observed quantitative imbalance may not solely reflect the actual distribution of scientific output, but may also be partially influenced by the bibliographic search strategy employed. The search string used in this systematic review was designed to prioritize terms more commonly associated with genomic literature in order to ensure high sensitivity in a field characterized by significant terminological variability. While this methodological choice is justified, it may have led to an underrepresentation of literature on the microbiota, particularly studies using traditional culture-based approaches described with less standardized terminology. This may be considered a partial limitation of the present study.

One of the most salient findings from the methodological analysis is the considerable heterogeneity in protocols adopted across studies, beginning with the experimental design. Eight microbiome studies were conducted as cohort studies, while six followed a descriptive cross-sectional approach. Cohort designs—such as those by Pittner et al. (2020) [22] and Sguazzi et al. (2022) [10] —enabled observation of microbial succession over time through sequential sampling during cadaver decomposition. Although this design allows for the detection of dynamic patterns, it is logistically more complex and often limited by small sample sizes. In contrast, cross-sectional studies, while offering broader case coverage—as exemplified by Pechal et al. (2018) with 188 cadavers [19] —are less informative regarding temporal sequences at the individual level.

Another critical issue concerns the variability in sample preservation conditions: ranging from cases in which tissues were frozen for over 100 days at temperatures between –17 and 3.33 °C (Hyde et al., 2013) [7], to protocols involving immediate freezing at –20 or –80 °C, and even to more variable conditions where samples were processed within one hour of collection or exposed to ambient conditions (e.g., Ashe et al., 2021) [20].

An additional relevant source of inter-study variability concerns the reporting of environmental parameters, particularly those related to the storage conditions of bodies prior to sampling. With regard to the microbiome, seven of the included studies did not provide any information on storage temperature or other relevant physical parameters, whereas only four studies reported specific thermal ranges or details on storage conditions. Similarly, for the microbiota, two out of three studies failed to report such information, with only one study specifying mortuary storage temperature. Although four studies attempted to integrate environmental variables by using ADD as a combined measure of time and temperature, the majority of the studies—both genomic and culture-based—provided only limited or generic details, typically referring to broad intervals of morgue storage. It should also be noted that, as summarized in Table 3, ADD values corresponding to the specific sampling time are not always directly extrapolatable in some studies. This lack of systematic reporting represents a limitation since temperature and other physical factors could, at least theoretically, have a significant influence on microbial succession across the different anatomical sites examined. Therefore, future research would benefit from the adoption of more standardized protocols in this regard, preferably conducted under controlled environmental conditions, and from ensuring comprehensive reporting of key environmental variables in order to improve reproducibility and facilitate inter-study comparisons.

In addition to highlighting methodological discrepancies, it is worth noting that many authors addressed the variation in cadaver storage environments—whether in climate-controlled facilities or under natural ambient conditions, the latter being more common in forensic practice. It is well known that preservation methods and timing can significantly impact observed microbial composition, especially concerning microbial viability and DNA integrity [21,34,35]. In forensic contexts, such variability can act as a confounding factor in PMI estimation and affect the comparability of results, which must be presented in court settings.

Regarding genetic analytical techniques, the studies employed a relatively narrow range of methods. All microbiome studies utilized 16S rRNA gene sequencing, though they differed in target regions (V3–V4, V4, V6–V8) and sequencing platforms (MiSeq, NovaSeq, 454). While the V4 region was most commonly analyzed, the use of alternative regions in some studies complicates direct comparison of microbial profiles. Only one study (Huang et al., 2024) [5] incorporated a more innovative sequencing method, 2bRAD-M, which enables higher taxonomic resolution [24].

In contrast, the three studies focusing on microbiota through culture-based methods exhibited even greater variability—not only in incubation conditions and media used but also in identification criteria. For example, Sidrim et al. (2010) [9] relied on morphological and biochemical identification, whereas Campobasso et al. (2022) [26] integrated MALDI-TOF, enhancing accuracy. However, the absence of standardized protocols limits result comparability and generalizability. Notably, in the study by Christoffersen et al. (2015) [25], no microbial growth was observed.

In terms of results, the comparative analysis of the included studies highlights both converging findings and methodological heterogeneity. One of the most significant observations is the reproducibility of certain post-mortem microbial successions, especially in more protected anatomical regions such as the gastrointestinal tract. Across multiple studies—regardless of experimental design or analytical platform—a consistent pattern emerges: a decline in aerobic bacteria (mainly *Firmicutes* and *Bacteroidetes*) and a relative increase in facultative anaerobes and *Proteobacteria* as decomposition progresses. Despite environmental or preservation-related variations, this pattern suggests a relatively stable temporal microbial dynamic that may serve as a foundation for PMI prediction models with forensic applicability.

Specifically, the rectal microbiome has emerged as the most stable and informative site. Several studies (e.g., Ogbanga et al., 2023; Pechal et al., 2018) [19,21] demonstrate that intestinal content retains taxonomic signals days or even weeks after death, offering higher reliability compared to more exposed regions like the oral or ocular cavities, where environmental contamination can significantly alter bacterial composition.

Regarding rectal sampling, our review found that only two studies explicitly mentioned the collection of fecal material. In the remaining studies, rectal samples were primarily obtained through swabbing, with the methodology either unspecified or described in general terms (e.g., swab insertion or rubbing), without clarifying whether the collected material included feces, rectal mucosa, or both. Only one study employed the scraping technique, but even in this case, the exact target of the sampling was not specified. This lack of detail reflects a broader trend in the literature, in which most studies do not explicitly address this distinction.

It is important to note that, from a technical and practical perspective, strictly differentiating between mucosal and fecal material in post-mortem rectal sampling can be challenging. For this reason, the use of generic terminology referring solely to the sampling site (e.g., "rectal sampling") may represent a deliberate choice by the authors to avoid excessive specification that cannot be consistently ensured in practice.

Nevertheless, this distinction may be biologically relevant. Fecal material may better reflect intestinal microbial communities, whereas mucosal swabs may be more influenced by host-associated microbes and less susceptible to post-mortem environmental contamination. These potential differences in microbial composition and their post-mortem stability highlight the importance of future research aimed at systematically comparing these two sampling approaches.

It should also be noted that, based on the available results, no substantial differences in microbial community composition were observed between samples explicitly identified as fecal and those generically referred to as rectal. This suggests that both approaches may provide comparable information for forensic purposes, although greater methodological clarity in future studies would be desirable.

Nonetheless, despite the recurrence of some key indicators, substantial interindividual and interstudy variability persists, attributable not only to biological or environmental differences but also to methodological inconsistencies and the inherent differences between genomic and culture-based techniques. Bacterial succession patterns are not always directly comparable across studies, and in some cases, predictive models built from one dataset are not generalizable to others. This limitation currently restricts the use of the microbiome as a universally reliable tool for PMI estimation, making it more suitable for controlled experimental contexts than for complex real-world forensic scenarios.

Interestingly, some studies have identified microbial genera associated with insect colonization, such as *Ignatzschineria*and *Wohlfahrtiimonas*—bacteria typically found in necrophagous insect larvae and systematically emerging during specific decomposition stages [2,8,20]. If validated in future research, these microorganisms could serve as highly informative secondary markers, reflecting the overlap between microbial activity and entomological colonization.

Finally, the few studies focusing on the microbiota (culture-based) yielded more fragmented but still valuable results. Culture methods confirmed the presence of key genera during early and intermediate decomposition phases. While limited by media selectivity and residual microbial viability, they showed general consistency with findings from molecular methods.

In this regard, recent systematic reviews by Moitas (2024) [3] and Tozzo (2022) [36] —focused exclusively on the microbiome—highlight that nearly all recent studies rely on 16S rRNA sequencing. Both reviews emphasize the microbiome's capacity to reflect temporal dynamics of human decomposition, presenting it as a potential "biological clock" for PMI estimation. However, both also agree that methodological heterogeneity, environmental variability, and the lack of standardized protocols currently hinder the practical forensic translation of these findings. This aligns with the conclusions of the present review, despite methodological differences, broader anatomical coverage, and the inclusion of studies on culture-based microbiota.

In conclusion, current evidence suggests the existence of recognizable post-mortem microbial patterns with potential forensic utility, particularly for PMI estimation. However, the significant variability—driven by methodological, biological, and environmental factors—demands caution when extrapolating results to casework. To enhance the reliability of these approaches, future efforts should focus on increasing sample sizes, encouraging multicenter studies, and, crucially, standardizing protocols for sample collection, preservation, and analysis. Only through such efforts can the microbiome evolve from an exploratory research tool into a practical operational asset in forensic medicine.

5. Conclusion

This systematic review highlights the ongoing expansion of research into post-mortem microbiome and microbiota, with growing interest in the forensic potential of microbial communities for estimating the post-mortem interval (PMI). Among the fourteen studies included, the majority focused on the microbiome, reflecting a methodological

Table 3
Overview of individual cases in which microbiome analysis was performed. Insect activity is recorded as follows: 0 = no visible insect activity; 1 = only adult flies present; 2 = adult flies and small maggots present; 3 = large quantities of both adult flies and maggots present[1].

ORAL MICROBIOME SAMPLING						
RECTAL MICROBIOME SAMPLING						
AUTHOR DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
AUTHOR DATE	**AGE**	**GENDER**	**PMI (DAYS) * (ADD = Accumulated Degree Days, if used)**	**POST-MORTEM PRESERVATION STATUS**	**INSECT ACTIVITY**	**RESULTS**
G. Sguazzi et al. 2022 [10]	91	F	10	F	N/A	−Dominance of **Firmicutes**, particularly **Streptococcus**. −Presence of **Neisseria** and **Rothia**, which are commonly found in the oral microbiota during life. −Low bacterial diversity, indicative of a stable microbiota not yet affected by significant decomposition.
C1			16	A	N/A	−Significant increase in **Proteobacteria** and **Bacteroidetes**. −Marked decrease in **Streptococcus**, **Neisseria**, and other oral commensals. −Growing presence of putrefactive taxa such as **Pseudomonas** and **Clostridium**, associated with the early stages of decomposition.
G. Sguazzi et al. 2022 [10] C2	67	F	2	F	N/A	−Predominance of **Firmicutes**, particularly **Streptococcus**, is a hallmark of the vital oral microbiota. −High abundance of **Rothia** and **Neisseria**, both typical indicators of a healthy and unaltered microbial community. Absence or low detection of decompositional taxa such as **Clostridium** or **Pseudomonas**. Low alpha diversity, characteristic of fresh and vital samples. Microbial profile highly similar to that observed in a living individual, as also confirmed by comparison with living subjects included in the control dataset.
E.C. Ashe et al. 2021 [20] C1	65	M	6 ADD: 0	F	0	≈75 % **Actinobacteria** (mostly **Rothia mucilaginosa**), ≈25 % **Firmicutes** (mainly **Lactobacillus crispatus**)
			10 ADD: 49	E	2	−Dominant **Firmicutes: Vagococcus** (~38 %), **Lactobacillus**, and **Streptococcus**; **Rothia** decreases to ~ 13 %. **−Candida** spp. peak (~50 % of transcripts: C. albicans, C. dubliniensis, C. glabrata).
			13 ADD: 89	E	1	**Lysinibacillus:** > 85 %
			17 ADD: 138	E	2	**Firmicutes** still abundant (**Streptococcus, Bacillales/Planococcaceae**), but the first **Proteobacteria** associated with flies (Ignatzschineria) appear
			20 ADD: 168	AD	0	**Proteobacteria (Ignatzschineria, Escherichia**) rapidly increasing, **Firmicutes** declining. **Oblitimonas** makes its first appearance.
			24 ADD: 222	AD	1	**Proteobacteria** predominant, with **Oblitimonas** now dominant; **Pseudomonas** and **Bacillus** also present.
			27 ADD: 253	AD	0	Stably proteobacterial profile: **Oblitimonas**, **Proteus**, and **Acinetobacter albensis** dominate; **Firmicutes** (**Bacillus, Staphylococcus**) are residual. Reads from insects (Drosophila) and environmental Streptophyta are increasing
E.C. Ashe et al.	77	F	8	E	1	Community dominated by **Firmicutes** (**Lactobacillus, Streptococcus, C2 Staphylococcus**) with a minor presence of **Actinobacteria** (**Rothia** < 5 %), but two distinctive signals stand out: −**Fusobacteria** (**Fusobacterium**, **Leptotrichia**), exclusive to this first sample

(*continued on next page*)

Table 3 (*continued*)

ORAL MICROBIOME SAMPLING RECTAL MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS) POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
2021 [20]			ADD: 41		−Traces of **Epsilonbacteraeota** (ASV related to **Campylobacter**)
C2			11	E	3 −Clear increase in **Proteobacteria** −Indicative genera in MetaG data: **Pseudomonas** already rising sharply
			ADD: 106		
			14	E	2 Mixed community of **Firmicutes** and **Proteobacteria**: **Firmicute**s (**Lactobacillus**, **Streptococcus**) remain relevant, but **Proteobacteria** associated with insects and soil are increasing: −**Ignatzschineria** rising in mid-decomposition samples −**Providencia**
			ADD: 155		
			18	AD	1 Shift toward **Proteobacteria** – **Firmicutes** decline, and **Proteobacteria** become predominant: −**Oblitimonas** (typical of advanced remains) appears among the most abundant genera −**Bacillus** mycoides
			ADD: 223		
			21	AD	1 −Strongly proteobacterial profile: **Oblitimonas**, **Escherichia**, and **Ignatzschineria** remain dominant; **Firmicutes** are residual (**Staphylococcus, Bacillus**). **—Bacillus paranthracis** and **Bacillus simplex** were isolated/identified in this sample
			ADD: 292		
E.C. Ashe et al. 2021[20]	84	F	6	F	0 −Typically oral community dominated by **Firmicutes** and **Actinobacteria**: **Streptococcus parasanguinis** (~10 % of ASVs) and traces of **Rothia mucilaginosa** (<5%). −Also present: **Corynebacterium striatum** and **Curtobacterium citreum**, isolated in culture.
			ADD: 0		
C3			10	E	3 **Firmicutes** still in the forefront, but the first "bloom" of **Kurthia zopfi**i is observed, along with early signs of insect-associated **Proteobacteria** (**Ignatzschineria** among the characteristic genera of intermediate stages)
			ADD: 84		
			13	AD	2 Shift toward **Proteobacteria** with a **mixed community**; isolates of *Corynebacterium xerosis* and *Kocuria rhizophila* still suggest a significant **actinobacterial component**. −Opportunistic **Firmicutes** (*Staphylococcus*, *Lactobacillus*) are steadily declining; the share of **soil/insect-associated bacteria** is increasing
			ADD: 169		
			17	DS	1 **Proteobacteria** predominant, with *Acinetobacter gerneri* isolated as an indicator species. −Fungal peak of *Yarrowia* spp
			ADD: 291		
			21	DS	0 Strongly **proteobacterial** profile with *Comamonas terrigena*, *Flavobacterium saccharophilum*, and *Janthinobacterium lividum* cultured from the sample. −*Oligoflexus*, *Undibacterium*, *Flavobacterium*
			ADD: 392		−*Arcobacter* (**Epsilonbacteraeota**) −*Yarrowia* remains abundant **—Firmicutes** are residual
J. Adserias Garriga et al. 2017 [2]	80	M	2	F	N/A **Oral Firmicutes**: *Lactobacillaceae*, *Staphylococcaceae*, *Gemellaceae*, *Carnobacteriaceae*, *Aerococcaceae*, *Veillonellaceae*, *Streptococcaceae* **—Oral Actinobacteria**: *Micrococcaceae*, *Bifidobacteriaceae*, *Actinomycetaceae*, *Corynebacteriaceae*
			3	F	Comparable to day 2, with a predominance of **Firmicutes** and **Actinobacteria**
C1			5	E	The **sporadic appearance of Tenericutes** (class **Mollicutes**) begins to be recorded
			6	E	**Peak of Tenericutes**; families characteristic of this stage: **Peptostreptococcaceae**, **Bacteroidaceae** (oral origin), and **Enterococcaceae** (intestinal origin) −Massive appearance of **Clostridiales/Bacillaceae** (spore-forming)
			7	E	−**Clostridiales** and **Bacillaceae** become dominant −**Tenericutes** still present −Marked increase in **Ignatzschineria**
			8	A	−**Clostridiales** and **Bacillaceae** (spore-forming **Firmicutes**) −**Peptostreptococcaceae** persist
			10	AD	Transition toward an "environmental" microbiota: **Gammaproteobacteria** predominate, including **Pseudomonadaceae** and **Alcaligenaceae**,

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Table 3 (*continued*)

ORAL MICROBIOME SAMPLING / RECTAL MICROBIOME SAMPLING AUTHOR / DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
						along with **Planococcaceae** (soil-associated **Firmicutes**). Spores **of Bacilli** and **Clostridia** remain well represented
J. Adserias Garriga et al. 2017 [2]	81	F	2	F	N/A	—**Firmicutes** (*Lactobacillaceae*, *Staphylococcaceae*, *Gemellaceae*, *Carnobacteriaceae*, *Aerococcaceae*, *Veillonellaceae*, *Streptococcaceae*) —**Actinobacteria** (*Micrococcaceae*, *Bifidobacteriaceae*, *Actinomycetaceae*, *Corynebacteriaceae*) —**Campylobacteraceae** also present
C2			3	F		Profile nearly identical to day 2, with a slight reduction in **Actinobacteria** and a slight increase in **Firmicutes**.
			4	F		Further decline in **Actinobacteria** and the first sporadic reads of **Tenericutes** (class **Mollicutes**), which foreshadow the **bloat stage**
			6	A		—**Peak of Tenericutes**; characteristic families: **Peptostreptococcaceae**, **Bacteroidaceae** (oral origin) —**Enterococcaceae** (intestinal origin) –Consistent growth of **Clostridiales/Bacillaceae** begins, while the opportunistic anaerobe **Ignatzschineria**, associated with **Sarcophagidae larvae**, appears.
			8	A		–Dominance of spore-forming **Firmicutes** (*Clostridiales*, *Bacillaceae*); —**Ignatzschineria** remains abundant; —**Peptostreptococcaceae** persist
			10	AD		—**Gammaproteobacteria** (*Pseudomonadaceae*, *Alcaligenaceae*) —**Planococcaceae**; —**Spores of Bacillaceae/Clostridiales** remain elevated
			11	AD		—**Pseudomonadaceae** —**Alcaligenaceae** —**Planococcaceae**; increase in spore-forming **Bacilli** and **Clostridia**, which will characterize the **dry remains** stage
J. Adserias Garriga et al. 2017 [2]	27	F	1	F	N/A	—**Firmicutes**: *Lactobacillaceae*, *Staphylococcaceae*, *Gemellaceae*, *Carnobacteriaceae*, *Aerococcaceae*, *Veillonellaceae*, *Streptococcaceae* —**Actinobacteria**: *Micrococcaceae*, *Bifidobacteriaceae*, *Actinomycetaceae*, *Corynebacteriaceae*; **Campylobacteraceae** also present
C3			2	F		Slight decline in **Actinobacteria** and initial establishment of **oral Firmicutes**.
			3	F		Sporadic appearance of **Peptostreptococcaceae** and **Bacteroidaceae** (indicators of early bloat), while **Firmicutes** and **Actinobacteria** continue to decline
			6	A		—**Peak of Clostridiales** and **Bacillaceae** (spore-forming **Firmicutes**); —**Peptostreptococcaceae** and **Bacteroidaceae** persist; —**Tenericutes** (class **Mollicutes**) still detectable, indicative of the previous **bloat stage**; –Strong presence of **Ignatzschineria** (associated with **Sarcophagidae larvae**).
			7	A		—**Clostridiales/Bacillaceae** dominant with abundant **Ignatzschineria**; —**Tenericutes** tend to disappear by the end of this stage
			10	AD		—**Environmental Gammaproteobacteria**: *Pseudomonadaceae*, *Alcaligenaceae*; —**Soil-associated Firmicutes** appear; —**Spores of Bacillaceae and Clostridia** remain elevated
			11	AD		Further increase in **Planococcaceae** and **environmental Proteobacteria**; the **oral cavity** is now almost completely devoid of **strictly oral taxa**
			12	AD		**Final transition**: spore-forming **Bacilli** and **Clostridia** (resistant to desiccation) persist, while **environmental Proteobacteria** (*Pseudomonadaceae / Alcaligenaceae*) remain present but begin to decline with the **loss of soft tissues**
X. Huang et al. 2024 [5]	54	M	9	N/A	N/A	—**Firmicutes** (*Streptococcus*, *Prevotella*) —**Bacteroidota** –Slight increase in **Klebsiella** (*Proteobacteria*)
C1						

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Table 3 (*continued*)

ORAL MICROBIOME SAMPLING RECTAL MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS) POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
X. Huang et al. 2024 [5] C2	64	M	15	N/A	**Firmicutes** (*Streptococcus* and *Prevotella*) still abundant, but the presence of **Klebsiella pneumoniae** is noted.
X. Huang et al. 2024 [5] C3	69	F	71	AD	Marked increase in **Proteobacteria** (*Klebsiella pneumoniae*)
X. Huang et al. 2024 [5] C4	87	F	22	N/A	Increase in **Proteobacteria** (*Klebsiella* spp., *Pseudomonas*) and reduction of typical oral commensals (**Firmicutes**)
X. Huang et al. 2024 [5] C5	80	M	5	E/A	−Predominance of **Firmicutes** (*Streptococcus / Prevotella*) −Increase in opportunistic anaerobes (**Fusobacteria**) −First appearance of **Proteobacteria** (*Enterobacteriaceae*) is observed
X. Huang et al. 2024 [5] C6	41	M	1	F	−Vital oral flora: dominant **Streptococcus** and **Prevotella** (**Firmicutes**) **—Bacteroidota** −Lesser amounts of **Actinobacteria** and **Fusobacteria**
X. Huang, et al. 2024 [5] C7	65	F	22	N/A	Increase in **Proteobacteria** (*Klebsiella* spp., *Pseudomonas*) and reduction of typical oral commensals (**Firmicutes**)
X. Huang et al. 2024 [5] C8	47	M	1	F	−Vital oral flora: **Streptococcus** and **Prevotella** dominant (**Firmicutes**) **—Bacteroidota** −Lower levels of **Actinobacteria** and **Fusobacteria**
E. Hyde et al. 2013 [7] C1	52	M	150 (143 FZ)	F	0	**Pre-Bloat Swab**: **—Firmicutes** ≈ 70 % **—Bacteroidetes** ≈ 15 % **—Actinobacteria** & **Proteobacteria** < 10 % **Genera**: *Streptococcus* (strong), *Veillonella*, *Gemella* **Pre-Bloat Scrap**: **—Proteobacteria** ≈ 55 % **—Firmicutes** ≈ 25 % **—Bacteroidetes** & **Actinobacteria** lower **Genera**: *Pseudomonas*, *Streptococcus*
			157 ADD: **197.14** (ADD appear to have been calculated at the end of the study, likely around the end-bloat stage)	E	2	**End-Bloat Scrape**: **—Firmicutes** ≈ 65 % **—Proteobacteria** ≈ 20 % **—Bacteroidetes** ≈ 10 % (**Genera**: *Streptococcus* predominant, *Clostridium* secondary)
E. Hyde et al. 2013 [7] C2	68	M	102 (89 FZ)	F	0	**Pre-bloat swab**: − Firmicutes ≈ 50 % − Bacteroidetes ≈ 25 % − Actinobacteria ≈ 15 % − Proteobacteria ≈ 10 % Genera: − Streptococcus, prevotella, veilonella

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Table 3 (*continued*)

ORAL MICROBIOME SAMPLING RECTAL MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
						Pre-bloat scrape: – Actinobacteria ≈45 % – Firmicutes ≈30 % – Bacteroidetes ≈15 % Genera: – Corynebacterium, Streptococcus, Actinomyces
			116 ADD: 199,97 (ADD appear to have been calculated at the end of the study, likely around the end-bloat stage)	E	2	**End-bloat scrape**: – Firmicutes ≈60 % – Proteobacteria ≈25 % – Bacteroidetes ≈ 10 % Genera: – Streptococcus (dominante), Chlostridium, Lactobacillus
E. Hyde et al. 2015 [8] C1	85	F	1	F	0	–Proteobacteria ≈ 80 % (Pseudomonas 92 %) – Firmicutes ≈ 15 %
			5	E	2	–Proteobacteria ≈ 60 % (Pseudomonas 68 %) – Firmicutes ≈35 % – Actinobacteria ≈2%
			9	A	2	–Proteobacteria ≈ 45 % (Ignatzschineria ≈ 95 %) – Firmicutes ≈50 %
			12	AD	2	–Proteobacteria ≈35 % (Ignatzschineria ≈ 7 %) – Firmicutes ≈70 % (Clostridium/Clostridiaceae) – Actinobacteria ≈5% – Bacteroidetes
			20	AD	0	–Proteobacteria ≈25 % – Firmicutes ≈50 % – Actinobacteria ≈20 % (Corynebacterium 32 %)
E. Hyde et al. 2015 [8] C2	55	M	1	F	0	–Proteobacteria ≈ 80 % – Firmicutes ≈ 15 % (Enterococcaceae ≈44 %)
			5	E	2	–Proteobacteria ≈ 60 % (Pseudomonas 68 %) – Firmicutes ≈35 % – Actinobacteria ≈2%
			9	A	2	–Proteobacteria ≈ 45 % (Ignatzschineria ≈ 95 %) – Firmicutes ≈50 % (Planococcaceae): 45 %
			12	AD	2	–Proteobacteria ≈35 % (Ignatzschineria ≈7 %) – Firmicutes ≈70 % (Clostridium/Clostridiaceae) – Actinobacteria ≈5% – Bacteroidetes
			20	AD	0	–Proteobacteria ≈25 % – Firmicutes ≈50 % – Actinobacteria ≈20 % (Corynebacterium 32 %)
S. Pittner et al. 2020 [22] C1	70	M	17 ADD: 68	F	0	**Firmicutes**≈40 % (*Anaerococcus, Peptoniphilus, Finegoldia, Parvimonas, Veillonella*) **Proteobacteria** ≈ 38 % **Actinobacteria** ≈ 20 % **Bacteroidetes** < 2 % **Relatively stable proportion of:** *Neisseria, Haemophilus, Acinetobacter*
			45 ADD: 710	A	0	**Firmicutes** ≈ 45 % **Proteobacteria** ≈ 37 % **Actinobacteria** ≈ 12 %

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Table 3 (*continued*)

ORAL MICROBIOME SAMPLING RECTAL MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
						Bacteroidetes ≈ 4–6 % **Relatively stable proportion of:** *Neisseria, Haemophilus, Acinetobacter*
			122 ADD: 2049	AD/M	0	**Firmicutes** ≈ 47 % **Proteobacteria** ≈ 38 % **Actinobacteria** < 5 % **Bacteroidetes** ≈ 8–10 % (*peak of Prevotella, Porphyromonas*) **Relatively stable proportion of:** *Neisseria, Haemophilus, Acinetobacter*
S.Pittner et al. 2020 [22] C2	60	M	16 ADD: 64	F	1	**Firmicutes** ≈ 38 % **Proteobacteria** ≈ 55 % **Actinobacteria** ≈ 7 % **Bacteroidetes** < 1 % **Predominant genera:** *Acinetobacter, Pseudomonas* (Proteobacteria), few *Streptococcus*
			44 ADD: 706	A	2	**Firmicutes** ≈ 48 % **Proteobacteria** ≈ 40 % **Actinobacteria** ≈ 5 % **Bacteroidetes** ≈ 4 % **Predominant genera:** increase in *Streptococcus* and *Veillonella* (Firmicutes), decrease in *Acinetobacter*
			121 ADD: 2027	AD	3	**Firmicutes** ≈ 60 % **Proteobacteria** ≈ 32 % **Actinobacteria** < 3 % **Bacteroidetes** ≈ 5 % **Predominant genera:** *Peptostreptococcus, Veillonella, Granulicatella*; minor presence of **Proteobacteria**
J.L. Pechal et al. 2017 [18] C1	9	M	0 (31 Months FZ) 1 2	F F F	N/A	**Phyla (Δ% thawed vs. frozen):** −*Actinobacteria* + 68.2 % −*Fusobacteria* + 49.3 % −*Gammaproteobacteria* + 49.5 % −*Firmicutes* –18.3 % −*Bacteroidetes* ≈ 0 %
J.L. Pechal et al. 2017 [18] C2	13	F	0 (22 Months FZ) 1 2	F F F	N/A	**Genera (Δ% thawed vs. frozen):** −*Corynebacterium* + 79.7 %
E. Hyde et al. 2013 [7] C1	52	M	150 (143 FZ) 157 ADD: 197.14	F E	0 2	**Proteobacteria** > 70 % **Firmicutes** < 20 % **Bacteroidetes** negligible **Genera:** −*Pseudomonas* (dominant) −*Escherichia coli* −*Klebsiella* Not sampled due to anatomical absence of the rectum
E. Hyde et al. 2013 [7] C2	68	M	102 (89 FZ)	F	0	**Firmicutes** clearly dominant (>75 % of reads) **Bacteroidetes** low abundance (<10 %) **Proteobacteria** present in moderate proportion **Genera:** −*Clostridium* −*Lactobacillus* −*Bacteroides*

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Table 3 (*continued*)

ORAL MICROBIOME SAMPLING RECTAL MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
						–*Escherichia coli*
			116	E	2	Not sampled due to anatomical absence of the rectum
			ADD: 199,97			
E. Hyde et al. 2015 [8]	85	F	1	F	0	**Clostridiaceae** 55 % **Bacteroidetes** 20 % **Proteobacteria** < 10 %
C1			3	E	2	Comparable to day 1
			5	A	2	**Ignatzschineria** (*Xanthomonadaceae*, *Proteobacteria*) 90 % **Clostridiaceae** ≤ 5 %
			9	AD	2	**Clostridiaceae** 64 % **Actinobacteria** 10–12 % **Ignatzschineria** 6 %
			25	DS	0	Sampling was not possible
E. Hyde et al. 2015 [8]	55	M	1	F	0	– Firmicutes 70 % – Bacteroidetes 20 % (Bacteroides 33 %) – Proteobacteria
C2			3	E	2	Comparable to day 1
			5	A	2	Sample not available due to rectal liquefaction
			7	AD	2	– Proteopacteria dominant Ignatzshineria 60 %)
			10	DS	0	**Firmicutes dominant** **Bacteroidetes and Proteobacteria** present in residual amounts
S.Pittner et al. 2020 [22] C1	70	M	17	F	0	**Firmicutes** ≈ 65 % (peak) **Proteobacteria** ≈ 25–30 % **Actinobacteria** ≈ 8 % **Bacteroidota** < 3 % **Predominant genera:** *Peptoniphilus* ~ 20 %
			45	A	0	**Firmicutes** ≈ 60 % **Proteobacteria** ≈ 30–35 %
			ADD: 710			**Decrease in Actinobacteria**, **Bacteroidota stable** **Predominant genera:** *Peptoniphilus* > 15 %, *Clostridium* spp. (<10 %)
			122	AD/M	0	**Firmicutes** ≈ 58 % **Proteobacteria** ~ 32 %
			ADD: 2049			**Actinobacteria** reduced by ~ 25–30 % compared to August **Bacteroidota** ≈ 5–8 % **Predominant genera:** –No genera > 20 % (great diversity) –*Peptoniphilus* < 10 %
S.Pittner et al. 2020 [22] C2	60	M	16	F	1	**Firmicutes** ≈ 65 % **Proteobacteria** ≈ 25 % **Actinobacteriota** ≈ 8 % **Bacteroidota** ≈ 2 % **Predominant genera:** –*Peptoniphilus* 20 % –*Escherichia* ~ 7 %
			44	A	2	**Firmicutes** ≈ **60 %** **Proteobacteria** ≈ **30–32 %**
			ADD: 706			**Actinobacteriota** ≈ **6 %Bacteroidota** ≈ **4 %** **Predominant genera:** –***Peptoniphilus* ~ 15 %**

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Table 3 (*continued*)

ORAL MICROBIOME SAMPLING RECTAL MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
						−***Clostridium*** ≈ **12 %**
			121	AD/M	3	**Firmicutes** ≈ 58 %
						Proteobacteria ≈ 32 %
			ADD: 2027			**Bacteroidota** ≈ 6–8 %
						Actinobacteriota ≤ 3 %
						Predominant genera:
						No genera > 20 % (great diversity)
						−*Peptoniphilus* < 10 %
J.L. Pechal et al. 2017 [18] C1	9	M	0 (31 Months FZ)	F	N/A	**Phyla (Δ% thawed vs. frozen):** −**Actinobacteria**: +68.2 % −**Fusobacteria**: +49.3 % −**Gammaproteobacteria**: +49.5 % −**Firmicutes**: –18.3 % −**Bacteroidetes**: ≈ 0 % **Genera (Δ% thawed vs. frozen):** −*Corynebacterium*: +79.7 %
			1	F		−*Haemophilus*: +75.0 %
			2	F		−*Fusobacterium*: +46.8 %
J.L. Pechal et al. 2017 [18] C2	13	F	0 (22 Months FZ)	F	N/A	−*Streptococcus*: +31.0 % −*Staphylococcus*: –33.3 % −*Lactobacillus*: –98.3 %
			1			
			2	F		

EYE MICROBIOME SAMPLING AUTHOR DATE	AGE	GENDER	PMI (DAYS)	POST-MORTEM PRESERVATION STATUS	INSECT ACTIVITY	RESULTS
S.Pittner et al. 2020 [22] C1	70	M	17	F	0	−**Proteobacteria** – ~62% −**Firmicutes –** ~15% −**Actinobacteria** – ~13% −**Bacteroidetes** – ~8% −**Others** – ~2% **Staphylococcus epidermidis** (a baseline microbiota showing minimal alteration due to decomposition)
			ADD: 68			
			45	A	0	−**Proteobacteria** – ~48% −**Firmicutes** – ~22%

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Table 3 (*continued*)

EYE MICROBIOME SAMPLING						
AUTHOR DATE	**AGE**	**GENDER**	**PMI (DAYS)**	**POST-MORTEM PRESERVATION STATUS**	**INSECT ACTIVITY**	**RESULTS**
			ADD: 710			–**Actinobacteria** – ~16% –**Bacteroidetes** – ~12% –**Others** – ~2% Pseudomonas fluorescens –Detected Clostridium perfringens – Present Staphylococcus epidermidis –Sporadic Escherichia coli – Low abundance Enterococcus faecalis – Detected Acinetobacter baumannii – Rare
			122 ADD: 2049	AD/M	0	**Proteobacteria** – ~45% **Firmicutes** – ~25% **Bacteroidetes** – ~20% **Actinobacteria** – ~8% **Others** – ~2% Pseudomonas fluorescens – Occasional Clostridium perfringens – Present Staphylococcus epidermidis – Absent Escherichia coli – Detected Enterococcus faecalis – Absent Acinetobacter baumannii – Detected
S.Pittner et al. 2020 [22] C2	60	M	16 ADD: 64	F	1	**Phyla:** —**Proteobacteria** – ~60% —**Firmicutes** – ~18% —**Actinobacteria** – ~15% —**Bacteroidetes** – ~5% —**Others** – ~2% **Notable Species:** —**Staphylococcus epidermidis** – **Present**
			44 ADD: 706	A	2	**Proteobacteria ~50%** **Firmicutes~24%** **Actinobacteria ~13%** **Bacteroidetes~11%** Others– ~2% Pseudomonas fluorescens– De tected Clostridium perfringens– Present Staphylococcus epidermidis– Present Escherichiacoli– Low abundance Enterococcus faecalis– Detected Acinetobacter baumannii– Rare
			121 ADD: 2027	AD/M	3	**Proteobacteria– ~42%** **Firmicutes– ~28%** **Bacteroidetes– ~22%** **Actinobacteria– ~6%** **Others– ~2%** Pseudomonas fluorescens-Occasional Clostridium perfringens– Present Staphylococcus epidermidis– Absent Escherichia coli– Detected Enterococcus faecalis– Absent Acinetobacter baumannii– Detected
J.L.	9	M	0	F	N/A	—**Staphylococcus epidermidis –** Present

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Table 3 (*continued*)

EYE MICROBIOME SAMPLING						
AUTHOR DATE	**AGE**	**GENDER**	**PMI (DAYS)**	**POST-MORTEM PRESERVATION STATUS**	**INSECT ACTIVITY**	**RESULTS**
Pechal et al. 2017 [18] C1			(31 Months FZ)			—**Rothia dentocariosa –** Present —**Corynebacterium spp.** – Low abundance —**Haemophilus spp. –** Detected —**Prevotella melaninogenica –** Detected
			1	F		—**Staphylococcus epidermidis** – Reduced —**Rothia dentocariosa** – Present —**Corynebacterium spp.** – Increased —**Haemophilus spp.** – Detected —**Prevotella melaninogenica** – Detected —**Fusobacterium spp.** – Newly detected
			2	F		—**Staphylococcus epidermidis –** Greatly reduced —**Rothia dentocariosa** – Present —**Corynebacterium spp. –** Increased —**Haemophilus spp. –** Detected —**Prevotella melaninogenica –** Detected —**Fusobacterium spp. –** Detected —**Streptococcus spp. –** Newly detected
J.L. Pechal et al. 2017 [18] C2	13	F	0 (22 Months FZ)	F	N/A	–**Staphylococcus epidermidis** – Present –**Rothia dentocariosa** – Present –**Corynebacterium spp.** – Low abundance –**Haemophilus spp.** – Detected –**Prevotella melaninogenica** – Detected –**Fusobacterium spp.** – Not detected –**Streptococcus spp.** – Not detected
			1	F		–**Staphylococcus epidermidis** – Reduced –**Rothia dentocariosa** – Present –**Corynebacterium spp.** – Increased –**Haemophilus spp.** – Detected –**Prevotella melaninogenica** – Detected –**Fusobacterium spp.** – Detected –**Streptococcus spp.** – Not detected
			2	F		–**Staphylococcus epidermidis** – Greatly reduced –**Rothia dentocariosa** – Present –**Corynebacterium spp**. – Increased –**Haemophilus spp**. – Detected –**Prevotella melaninogenica** –Detected –**Fusobacterium spp**. – Detected –**Streptococcus spp**. – Newly detected

Article	**Type of study**	**Place the study was performed**	**Main goal**	**Sampling site**	**Case-specific information and body storage/environmental conditions**	**Sampling time**	**Sampling mode**	**Microbiological technique applied**	**Statistical analysis**	**Results**
S. Christoffersen et al.	Descriptive/ Cross-Sectional Study	Institute of Forensic Medicine, University of Southern Denmark, Odense DENMARK	The aim of this study was to review the use of	– Nose and throat,	In a retrospective study including all autopsy cases from our	N/A	nose and throat swab, feces	Agar culturing for bacterias and fungi, PCR for viruses	The study employed descriptive statistical methods, including frequencies, percentages, and cross-tabulations, to report microbiological findings and explore associations with known	Of the 42 microbiologically tested cases, only 5 included the throat, nose, and feces among the sampling sites, and the microbiological analysis for these was negative.

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Table 3 (*continued*)

Article	Type of study	Place the study was performed	Main goal	Sampling site	Case-specific information and body storage/environmental conditions	Sampling time	Sampling mode	Microbiological technique applied	Statistical analysis	Results
									causes of death in 42 autopsies.	
(2015) [25]			microbiological procedures and findings at our forensic institute.	−Feces	institute from the period from 1/1 – 2009 to 31/12 – 2011, a total of 669, 42 cases where microbiological testing had been applied were Identified Of the 42 microbiologically tested cases, only 5 included the throat, nose, and feces among the sampling sites, and the microbiological analysis for these was negative. * Environmental conditions not reported		Feces: Collection method not specified.		No inferential statistical tests (e.g., t-tests, chi-square, regression) were performed. The analysis focused on summarizing and interpreting the data without assessing statistical significance.	Microbiological sampling remains an important part of the autopsy yielding the cause of death in 42.8% of the cases in which it was performed.

Article	Type of study	Place the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Microbiological technique applied	Statistical analysis	Results
J.J.C. Sidrim et al. (2009) [9]	Descriptive/ Cross-Sectional Study	Medical Mycology Center, Federal University of Ceara´, Fortaleza, Ceara´, BRAZIL	Ttracing out the profile of fungal communities of human cadavers for the first time in order to use it in forensic science	−mouth −rectum,	−Human bodies in 3 stages of decomposition, whose cause of death wasn't specified 60 cadaversa	N/A	~~Sterile swabs~~	Microscopic exam with 40% KOH using glucose Sabouraud agar, Sabouraud with vancomycin + polymyxin B (to inhibit bacteria), and Sabouraud with vancomycin + polymyxin B + cycloheximide (to inhibit environmental molds)	The study applied descriptive statistics to analyze fungal presence across decomposition stages: −Frequencies and percentages were used to report how often specific fungal species (Aspergillus spp., Penicillium spp., Candida spp., Mucor sp.) appeared during bloat,	Fungal Species by Decomposition Stage

(*continued on next page*)

Table 3 (*continued*)

Article	Type of study	Place the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Microbiological technique applied	Statistical analysis	Results
									putrefaction, and skeletonization.	
				−vagina,	−34 in bloating stage,		Sterile swabs were employed to obtain samples from the mucosal surfaces of the mouth and rectum.		−Cross-tabulations were employed to compare the distribution of fungal species across these stages, highlighting changes in fungal communities over time.	Bloated stage:
				−under the −foreskin, −lungs, skin, −scalp hair, −clothing −surrounding area	−6 in putre-faction stage −20 skeletonization stage * Environmental conditions not reported					−Aspergillus spp. −Penicillium spp. −Candida spp. Putrefaction stage: −Candida spp. (only) Skeletonization stage: −Aspergillus spp. −Penicillium spp. −Mucor sp.

Post-mortem preservation status is classified by decomposition stage: F = Fresh; E = Early; A = Active decay; AD = Advanced decay; DS = Dry remains / Skeletonization; M = Mummification; FZ = Frozen. ADD = Accumulated Degree Days (cumulative sum of temperature above a base threshold, typically 0–5 °C; calculated either from high–resolution measurements [e.g., every 30 min] or from daily mean temperatures using the formula (Tmax+Tmin)/2–Tbase(Tmax+Tmin)/2–Tbase, summed across the postmortem interval).

Table 4
Summarisation of the information of Microbiota obtained from the articles under analysis.

Article	Type of study	Place the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Microbiological technique applied	Statistical analysis	Results
C. Campobasso et al.	Descriptive/ Cross-Sectional Study	Department of Experimental Medicine, Legal Medicine Section, University of Study of Campania,	The aim of this study is to verify the application of the mass spectrometry technique, better known as MALDI-TOF MS (matrix-assisted laser des orption/ionization time-of-flight mass spectrometry), as a cheap	−**eyes,**	18 human bodies	−7 cases with a PMI of < 72 h;	Microbiological swabs were collected from external anatomical sites (eyes, ears, nose, mouth, and rectum). For each site, a separate swab was gently rubbed and rotated for a few seconds.	MALDI-TOF MS (matrix-assisted laser des	The study used descriptive statistics to report microbial frequencies across body sites and PMIs, highlighting dominant phyla (e.g., Firmicutes, Proteobacteria) and trends in decomposition.	Most common genera by site:
(2022) [26]		ITALY	and quick method for microbe taxonomic identification and for studying the PM microbiome.	−**mouth,**	−15 enclosed environments (10 cases in hospitals, 4 cases in apartments, one case in jail)	−6 cases with a PMI of 72–168 h,		orption/ionization time-of-flight mass spectrometry		**Eye**: Enterococcus spp., Staphylococcus spp., Candida spp.
				−**rectum**	−3 found outdoors (one suicide, and 2 natural	−5 cases with a PMI of > 168 h	Microbiological swabs (swab type not specified)		Cadavers were grouped by PMI (<72 h, 72–168 h, >168 h) to compare microbial succession over time.	**Rectum:** Enterococcus spp. (most prevalent), followed by Escherichia spp.
				(Ears, nose, brain, spleen, liver, and heart)	deaths).		Four different agarized plates			**Oral cavity**: Candida spp., then Lactobacillus spp. and Streptococcus spp.
							as follows:		Due to limited sample size, no advanced statistical models were applied. The authors emphasized the need for larger studies to improve PMI estimation using microbial data.	
					(2 cardiac deaths due to myocardial infarction and arrhythmogenic cardiomyopathy, and 3 respiratory deaths due to pneumonia) and 9		−Columbia CNA agar with 5 % Sheep Blood,			Phyletic shifts over time:
							−MacConkey agar,			**PMI < 72 h**:

(*continued on next page*)

Table 4 (*continued*)

Article	Type of study	Place the study was performed	Main goal	Sampling site	Case-specific information and body storage/ environmental conditions	Sampling time	Sampling mode	Microbiological technique applied	Statistical analysis	Results
					violent deaths (3 suicides, 3 car accidents, and 3 homicides by blunt and ballistic injuries).		−Chocolate agar,			Proteobacteria dominated the eye, ear, nose, and rectum
					17 males		−Sabouraud agar			Firmicutes dominated the oral cavity
					1 female,		Incubation: 37 ∘C 24 h (chocolate agar in anaerobiosis condition), Sabouraud agar at 30 ∘C for 48 h.			**PMI > 72 h**:
					(age range from 4 months to 82 years old, mean age of 43.27 years)					Firmicutes became dominant in eye, ear, oral cavity, and rectum, followed by Proteobacteria
					*					Temporal trends: In the oral cavity, Proteobacteria increased with time, replacing Firmicutes
					Cadavers stored in mortuary, then refrigerated at + 4 °C (24–36 h) in individual chambers. Autopsies within 72–96 h; storage time included in PMI calculation.					In the eye, the opposite occurred: Proteobacteria decreased and Firmicutes became dominant

Table 5
Qualitative assessment of the included studies according to the CASP Checklist for Cohort Studies and the CASP Checklist for Descriptive/Cross-Sectional Studies.

	CASP Checklist: For Cohort Studies														
	Section A: Are the results valid?									Section B: What are the results?			Section C: Will the results help locally?		
	Did the study address a clearly focused issue?	Was the cohort recruited in an acceptable way?	Was the exposure accurately measured to minimise bias?	Was the outcome accurately measured to minimise bias?	Have the authors identified all important confounding factors?	Have they taken account of the confounding factors in the design and/or analysis?	Was the follow up of subjects complete enough?	Was the follow up of subjects long enough?	What are the results of the study?	How precise are the results?	Do you believe the results?	Can the results be applied to the local population?	Do the results of this study fit with other available evidence?	What are the implications of this study for practice?	Score
C.Sguazzi et al (2022)	yes	yes	yes	yes	can't tell	can't tell	n/a	n/a	yes	yes	yes	can't tell	yes	yes	9
Xin Huang et al. (2024)	yes	yes	yes	yes	can't tell	can't tell	n/a	n/a	yes	yes	yes	can't tell	yes	yes	9
Hyde E.R. et al (2013)	yes	yes	can't tell	yes	no	no	n/a	n/a	yes	no	can't tell	no	yes	no	5
Hyde E.R. et al (2015)	yes	can't tell	yes	yes	no	no	n/a	n/a	yes	no	can't tell	no	yes	can't tell	5
Ogbanga et al (2023)	yes	can't tell	yes	yes	yes	can't tell	n/a	n/a	yes	can't tell	yes	can't tell	yes	yes	8
Pittner et al (2020)	yes	yes	yes	yes	yes	yes	n/a	n/a	yes	yes	yes	yes	yes	yes	12
Pechal, J. L., et al. (2017)	yes	can't tell	yes	yes	no	no	n/a	n/a	yes	yes	can't tell	no	yes	can't tell	6
Pechal, J. L., et al. (2018)	yes	yes	yes	yes	yes	yes	n/a	n/a	yes	yes	yes	yes	yes	yes	12
Gulnaz T. Javan et al. (2016)	yes	yes	yes	yes	yes	yes	n/a	n/a	yes	yes	yes	yes	yes	yes	12

CASP Checklist: For Descriptive/Cross-Sectional Studies												
Section A: Are the results valid?												
	Did the study address a clearly focused issue?	Did the authors use an appropriate method to answer their question?	Were the subjects recruited in an acceptable way?	Were the measures accurately measured to reduce bias?	Were the data collected in a way that addressed the research issue?	Did the study have enough participants to minimise the play of chance?	How are the results presented and what is the main result?	Was the data analysis sufficiently rigorous?	Is there a clear statement of findings?	Can the results be applied to the local population?	How valuable is the research?	Score
Emily C. Ashe et al. (2021)	yes	yes	yes	yes	yes	yes	no	yes	yes	can't tell	yes	9
J. Adserias-Garriga et al (2017)	yes	yes	yes	yes	yes	no	yes	yes	yes	can't tell	yes	9
S. Christoffersen et al (2015)	yes	can't tell	yes	yes	can't tell	yes	yes	yes	yes	can't tell	can't tell	7
J.J.C. Sidrim et al. (2009)	yes	yes	can't tell	yes	no	can't tell	yes	yes	yes	can't tell	no	6
Campobasso CP et al. (2022)	yes	no	yes	yes	yes	yes	yes	no	yes	can't tell	can't tell	7

preference for 16S rRNA gene sequencing techniques. While molecular approaches offer a broader and more sensitive characterization of microbial diversity, culture-based analyses—although less frequently employed—retain specific informational value, particularly regarding microbial viability and metabolic activity.

The findings indicate the presence of relatively consistent microbial succession patterns, especially in more protected anatomical sites such as the rectum. In these regions, the post-mortem increase in anaerobic taxa and concurrent decline in aerobes represent potentially valuable indicators for the development of PMI prediction models. However, significant heterogeneity among the studies—regarding experimental design, sampling strategies, preservation protocols, and analytical methods—remains a critical limitation, hindering both result comparability and translation into forensic practice.

The observed variability across anatomical regions, along with the sensitivity of microbial communities to environmental factors such as temperature, humidity, and preservation conditions, suggests that no single site can currently be considered universally reliable for PMI estimation. Nonetheless, a multisite approach that integrates data from both the microbiome and microbiota may enhance the robustness and accuracy of predictive models.

Looking ahead, the operational forensic application of these methods will require the establishment of internationally standardized protocols governing sample collection, preservation, and analysis. In parallel, large-scale, multicenter studies with representative case cohorts should be promoted to validate findings across diverse conditions. Only through such coordinated efforts can the post-mortem microbiome evolve from a promising research avenue into a reliable medico-legal tool for determining the time since death.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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