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Using the postmortem epinecrotic microbiome as a tool for time since death estimations

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Abstract

The estimated time since death, or postmortem interval (PMI), is a crucial piece of information in forensic death investigations. Current scientific methods used to estimate this timeframe do not always provide the most accurate predictions and often rely on subjective interpretations. The microbiome has recently been recognized as a large impactor of human decomposition and current research shows its potential to provide additional accuracy to PMI estimations. As bacteria are ubiquitous, persistent, and due to recent advancements in technology genetically identifiable, microbial analysis effectively complements other forensic science approaches. However, this new field of forensic research requires standardization, foundational validity, and research collaboration if it is to be considered reliable for use as evidence in the court of law. This review discusses the potential for forensic microbiology to be used as an additional estimator for the PMI, the advantages of epinecrotic microbiome sampling, and outlines further steps needed for the integration of this discipline into forensic practice.

Impact Statement

This review covers and discusses the potential of using the time-dependent changes in the postmortem microbiome as a tool for estimating the postmortem interval (PMI) of deceased victims. We discuss the recent advances in forensic microbiology, give an overview of where the field is at present as well as future directions needed in order to elevate forensic microbiology to be used as a reliable source of evidence in criminal cases.

Keywords: postmortem interval estimation; time since death; 16S rRNA sequencing; external necrobiome; epinecrotic microbiome; thanatomicrobiome; decomposition

Introduction

Death investigations are performed when a person's death is considered suspicious, unexpected, violent, or unexplained. This includes homicides, suicides, and deaths by misadventure (Hanzlick 2003). A primary focus of these investigations is determining when the death occurred, inferred from the postmortem interval (PMI), or the time since death (Maile et al. 2017, Pittner et al. 2020, Franceschetti et al. 2023). Knowing this time window is crucial to establish a timeline of events, corroborate witness statements, or exclude or include potential persons of interest. However, accurate estimation of the PMI has proven difficult (Maile et al. 2017, Tozzo et al. 2022, Franceschetti et al. 2023). This is due to knowledge gaps regarding how variables impact decomposition, intrinsic differences between human bodies, and even the manner of death itself (Zapico and Adserias-Garriga 2022, Dawson et al. 2023). Extrinsic or environmental factors also affect decomposition, decreasing or increasing the rate of decay due to temperature, humidity, exposure, and scavenging (Kõrgesaar et al. 2022). Further fundamental research into postmortem events is needed to fully understand the biological, chemical, and physical changes that occur during human decomposition to ultimately increase the accuracy of PMI estimation methods (Finley et al. 2015, McIntyre et al. 2024).

Stages of decomposition

After death, the human body undergoes five major stages of decomposition: fresh, bloat, active decay, advanced decay, and skeletonization (Goff 2009, Shrestha et al. 2023, Almulhim and Menezes 2024). These stages can vary in timeframe due to intrinsic and environmental variables, and it is possible for adjacent stages to overlap where some parts of the body decay faster than others—referred to as differential decomposition (Wescott 2018). How the stages are classified and currently employed in PMI estimation within forensic sciences are outlined below, noting that while these tools are discussed in literature, not all are routinely applied in operational forensic casework:

Fresh (0–48 hours)

Changes in the body occur immediately following death. Within hours, the chemical composition of the vitreous humour in the eyes changes and can be examined using forensic chemistry techniques such as liquid chromatography and

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mass spectrometry (Cordeir et al. 2019). This technique is only applicable if the eyes are not compromised, limiting its application to the early stages of decomposition. The body will also undergo holistic changes: livor mortis (dark purple discolouration of skin) as the blood pools from gravity, algor mortis (cooling cadaveric temperature) as the body undergoes thermodynamic equilibrium with the environment, and rigour mortis (stiffness of the muscles) as adenosine triphosphate is depleted and lactic acid builds up in muscles (Goff 2009, Cieśla et al. 2023). These approaches are strong predictors, but only for the first few days postmortem (Webber and Connor 2015, Wilk et al. 2021). The temperature of the cadaver is susceptible to the environment, particularly the ambient temperature and depends on intrinsic factors of the deceased such as illness, fever, or stress, and cannot be used to estimate the PMI after reaching equilibrium. Livor mortis will change according to the positioning of the body and, as such, is not reliable if the cadaver has been moved by external forces, and rigour mortis subsides within 36 hours after death (Singh et al. 2025). Histopathology can be performed on the blood cells of the cadaver, observing their depletion over time, but is only reliable for the first few days postmortem (Babapulle and Jayasundera 1993). The same method can be used to observe degradation of the DNA from certain tissues over time, which research shows has a negative linear relationship, but again is only able to be used in the early postmortem stage (Tozzo et al. 2020). Insects will also begin to colonize fresh remains, laying eggs in natural orifices such as within the face, as well as wounds (Matuszewski 2021). Any physical barrier such as underwater submersion, extreme weather, burial, body encasement, or indoor location will delay insect activity (Brownlow et al. 2023, Sharma et al. 2025). The biomolecular changes occurring within tissues can be analysed via lipidomics, metabolomics, and proteomics (the 'multi-omics' approach) (Ueland et al. 2021, Bonicelli et al. 2022). Analysis within this approach may focus on transcription, proteins, lipids, metabolism, and genome present in the deceased's cells (Woodland et al. 2025). The degradation of proteins especially is of interest, as they are biomarkers of decomposition (Chhikara et al. 2025). The complex nature and relative novelty of these analytical techniques cause varying reliability of results and little overlap in examined biomolecules (Secco et al. 2025). This approach is still in development in forensic research, but is highly complementary to microbiology (Pérez-Llarena and Bou 2016, Xian et al. 2025).

Bloat (2–7 days)

Major internal events occur during the bloat stage of decomposition. Anaerobic bacteria within the internal organs produce gases as they start digesting tissue from inside out (Javan et al. 2017). This, in combination with the gases produced by cellular putrefaction and autolysis, causes the characteristic swelling of body parts, primarily distension of the abdomen (Brooks 2016, Wilson et al. 2020). The gastrointestinal bacteria also invade blood vessels and create a dark colour within the veins and arteries, appearing as 'marbling' on the skin (Goff 2009). Sulphur, a compound produced by decay, binds to haemoglobin in the blood to form a sulfhemoglobin complex (Almulhim and Menezes 2024). This molecule creates the distinct green colour in skin associated with the bloat stage. Hydrolytic enzymes from cells enter the space between the dermis and epidermis, causing skin to 'slip' off the body (Goff 2009, Shrestha et al. 2023). These visual changes—tissue swelling, skin marbling, green skin discolouration, and slippage—can be visually measured and scored (Wilson et al. 2020). However, these features can vary between humans and environments and require extensive expertise in human decomposition to accurately estimate the PMI, making their analysis subjective (Hu et al. 2024). Entomologically, larvae will begin hatching from laid eggs (Matuszewski 2021). Massive larval masses may be seen as the juvenile insects accumulate and feed (Carter et al. 2023). The age of these larvae can be used to estimate when oviposition (egg laying) occurred, but is again subject to external factors. Significant odours will come from the cadaver as tissue starts to breakdown and bacteria ferment, creating gaseous compounds referred to as volatile organic compounds (VOCs), which can be recognized and analysed with gas chromatography coupled with mass spectrometry (Cieśla et al. 2023, Thurn et al. 2024). VOC-based postmortem estimation methods can be used continuously during decomposition, as even bones have a unique smell (Paczkowski and Schütz 2011). This newly developing scientific field remains susceptible to the heterogeneity of humans and requires further robust studies (Cieśla et al. 2023). Chemical and biological shifts occur in the grave soil (soil underneath and surrounding the cadaver) during the bloat stage (Dent et al. 2004). Forensic geology can use analytical techniques to assess the composition of the soil sampled from clandestine graves to estimate the PMI (Tibbett and Carter 2009). Such a method is again, reliant on the chemical profile of the soil prior to disturbance, and the circumstances of the cadaver's placement (i.e. encased, buried, surface deposition, concealed). As with the multi-omics methods and the VOC sampling, soil analysis can be done continuously throughout the subsequent decomposition stages.

Active decay (1–2 weeks)

The shift between bloat and active decay is marked by the collapse of the cadaver's abdomen (Goff 2009). The liquefaction of internal organs and tissue is 'purged' from the body via the mouth, nose, and distal gastrointestinal orifice (Shedge et al. 2023, Shrestha et al. 2023). This expulsion of fluid can also be noted in the late bloat stages, showing overlap between adjacent decomposition stages. In moist environments, saponification of tissue may occur, where human fat tissue is hydrolyzed into adipocere (Goff 2009, Ubelaker and Zarenko 2011). Adipocere, a waxy by-product of decay, can hinder decomposition as it acts as a preservative in certain conditions, making PMI estimation more difficult (Alfsdotter and Petaros 2021). Significant insect activity occurs at this stage: previously laid larvae continue the insect life cycle by aggressively feeding and growing, and more adult flies are drawn to the cadaver by smell (Matuszewski 2021). Sections of soft tissue will be eaten away by the growing maggots. The eggs and larvae of other insects, such as beetles, are present. This successional colonization of carrion (decomposition) insects is what is measured by forensic entomologists to estimate the PMI. The now enormous maggot masses will generate considerable heat, getting up to dozens of degrees Celsius above ambient temperature, and can differ based on fly species (Johnson and Wallman 2014, Johnson et al. 2014). All of these are subject to environmental variables. The use of multi-omics, VOC, and soil analysis continues to be applicable to this stage.

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Advanced decay (2–4 weeks)

As mentioned previously, different parts of the body will decay faster than others, a process known as differential decomposition (Dautartas et al. 2018). This means that some sections of the cadaver will be in advanced decay before others finish active decay. Typically, this is true for the head and limbs of the cadaver. Signs of advanced decay include hardening of adipocere, desiccation of tissue as fluids evaporate, concavity of the eyes, and reduction of biomass as the cadaver starts to mummify (Goff 2009, Shedge et al. 2023). The advanced decay stage shows a reduction in holistic changes as the body approaches the end of the decomposition process. Taphonomically, the skin will appear hardened, yellow or brown, shrunken, and leathery due to drying, but may still be greasy (Goff 2009, Shrestha et al. 2023). Mould may bloom on the external surfaces of the cadaver, an observation specific to late stages of decomposition (Richard Beaulieu et al. 2025). Insect larvae will have pupated and grown into adult insects and a transition from flies to beetle scavenging occurs (Matuszewski 2021). The production of butyric acid associated with this stage attracts certain insects not typically present in earlier decomposition stages (Martín-Vega et al. 2025). Forensic anthropology starts being most applicable in the advanced stage of decomposition, as bones will become visible during this time (Wilson et al. 2020, Franceschetti et al. 2023). Multiple techniques are used during anthropological testing to date the bones, such as luminol chemiluminescence, radiocarbon (C14) and other radioisotope dating, citrate extracellular matrix component analysis, and molecular studies like protein analysis and DNA degradation (Franceschetti et al. 2023, Arora et al. 2024, Ermida et al. 2024). Visual analysis of bone condition can also be utilized to estimate the time since death but requires extensive expertise and is subjective (Marhoff-Beard et al. 2018). Volatile organic compounds specific to this stage can be recognized using the analytical chemistry techniques mentioned previously. Specific chemicals increase in the soil profile during advanced decay (Taylor et al. 2023). This stage can last for weeks depending on the environment.

Skeletonization (1 month+)

The final stage of the complex decomposition process is skeletonization. During this time, only the bones and some hair and teeth will remain (Shrestha et al. 2023). As the connective tissues between bones decay, the ribcage and other upheld structures will collapse. Exposure to the elements will bleach the bones, and eventually the bones erode (Wilson et al. 2020). This process can take months or years depending on the environment and fossilization may occur under very specific circumstances (Joannes-Boyau et al. 2020). Forensic anthropology is highly applicable during this stage, and visual comparison, protein analysis, DNA degradation, and VOC analysis can be used to estimate the time since death. There is a noticeable decline in insect activity during this timeframe due to a lack of soft tissue as a food source (Matuszewski 2021). Some insects that consume very dry tissue and bone may be present and are indicative of this stage (Rai et al. 2020). A clear issue with late PMI estimations is that as more time goes on, the harder it is to accurately estimate the time since death and the greater the uncertainty (Garcés-Parra et al. 2024). This is likely due to a decrease in measurable biological, chemical, and physical changes happening to the cadaver in the very late PMI.

There is a significant amount of physical, chemical, and biological processes that occur during human decomposition. A summary of the timeline, visual changes, and commonly used methods of PMI estimation for different stages of decomposition are outlined in Table 1.

Even with the numerous methods available, accurate estimation of the PMI remains a difficult task in death investigations (Franceschetti et al. 2023). Understanding all variables relating to decomposition and their relationships with one another is the key to improving accuracy (Dawson et al. 2023, McIntyre et al. 2024). One major decomposition variable that only has recently begun to be explored in depth is microbiology; a factor that is undeniably linked with all components of decomposition and changes as decomposition progresses (Pechal et al. 2018, Dash and Das 2020, Tarone et al. 2022, Dawson et al. 2023). Bacteria are present in nearly all contexts that would be relevant to human decomposition: abundantly present in living human's microbiome, both internal and external, found in plants, water, soil, indoors, and dust, and can be specific to species of animals and insects (Finlay and Clarke 1999, McFall-Ngai et al. 2013, Compant et al. 2019, Dekaboruah et al. 2020, Shan et al. 2020, Some et al. 2021, Coolen et al. 2022, Kim et al. 2022, Chen et al. 2024). The near-constant presence of bacteria is coupled with fast growth. Some bacteria's doubling times is only hours long, and in the right conditions, some bacteria can double in less than half an hour (Gibson et al. 2018). These dynamic changes occur at a fraction of the time other postmortem processes take. This means that samples taken at fine time increments will show measurable changes occurring at that temporal scale. Such small shifts in the microbiome could provide greater accuracy when predicting the PMI, especially in conjunction with other time-dependant data.

Forensic microbiology as a tool for time since death estimations is becoming more prominent in recent research. This review will discuss the necrobiome and its components, outline the rationale for prioritizing the epinecrotic microbiome as the preferred sampling location, technology used in forensic microbiology studies, recent publications observing the epinecrotic microbiome over time, and the standardization required for future necrobiome work.

Forensic microbiology as a PMI estimator

The postmortem microbiome impacts—and is in turn impacted by—each of the other variables regarding decomposition (Tarone et al. 2022, Dawson et al. 2023, McIntyre et al. 2024). Reciprocal relationships have been shown between postmortem microbes and extrinsic factors, such as insects (Tomberlin et al. 2011, Junkins et al. 2019, Iancu et al. 2020, Tarone et al. 2022), water (Ashbolt 2004, Dickson et al. 2011, Dmitrijs et al. 2022, Bone et al. 2024), and soil (Adserias-Garriga et al. 2017a, Lauber et al. 2014, Cobaugh et al. 2015, Finley et al. 2016, Weiss et al. 2016, Samaddar et al. 2017, Mason et al. 2023). Significant intrinsic properties of the deceased can also affect the postmortem microbiome even before death (Alan and Sarah 2012, Javan et al. 2017, Zhou and Bian 2018, García et al. 2020, Nodari et al. 2024)—such as overall health of the person, diet, biometrics, prescription or recreational drugs, therapy, and diseases (Bull and Plummer 2014, Hills et al. 2019, Rajasekaran et al. 2024). Social interactions,

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Table 1. Summary of the five stages of decomposition (fresh, bloat, active decay, advanced decay, and skeletonization), the associated approximate timeframe, observable changes that occur to indicate the stage, and applicable methods used to estimate the time since death within the timeframe.

	Fresh	Bloat	Active decay	Advanced decay	Skeletonization
Approximate timeframe	0–48 hours	2–7 days	1–2 weeks	2–4 weeks	1 month +
Changes in cadaver	Dark purple discolouration (livor mortis), muscle stiffening (rigour mortis), and cooling of body (algor mortis) (Goff 2009, Cieśla et al. 2023)	Swelling of abdomen and limbs (Brooks 2016, Wilson et al. 2020) Dark marbling on skin (Goff 2009) Green discolouration of skin (Almulhim and Menezes 2024) Skin slippage (Goff 2009, Shrestha et al. 2023)	Collapse of abdomen (Goff 2009) Purge of liquefied organs and tissue (Shedge et al. 2023, Shrestha et al. 2023) Saponification of fat (Goff 2009, Ubelaker and Zarenko 2011)	Differential decomposition (Dautartas et al. 2018) Eyes concave (Goff 2009, Shedge et al. 2023) Skin appears yellow/brown, hardened, shrunken, or leathery (Goff 2009, Shrestha et al. 2023) Mould on outside surface (Richard Beaulieu et al. 2025)	Presence of only hard cartilage, bones, and hair (Shrestha et al. 2023) Eventual erosion of bones (Wilson et al. 2020)
Estimation methods	Visual taphonomy (Megyesi et al. 2005) Cadaveric temperature (Nokes et al. 1992) Liquid chromatography, mass spectrometry of tissue (Cordeir et al. 2019) Tissue histopathology (Babapulle and Jayasundera 1993) DNA degradation (Tozzo et al. 2020) Insect colonization (Matuszewski 2021) Multi-omics (AKÇAN et al. 2020, Ueland et al. 2021, Bonicelli et al. 2022)	Visual taphonomy (Megyesi et al. 2005) Presence of insect larvae and larval masses (Matuszewski 2021, Carter et al. 2023) Multi-omics (AKÇAN et al. 2020a; Ueland et al. 2021, Bonicelli et al. 2022) VOC analysis via GCxGC-MS (Cieśla et al. 2023, Thurn et al. 2024) Grave soil analysis (Dent et al. 2004, Tibbett and Carter 2009) DNA degradation (Tozzo et al. 2020)	Visual taphonomy (Megyesi et al. 2005) Presence of insect pupae (Matuszewski 2021) Heat signatures from insect activity (Johnson and Wallman 2014, Johnson et al. 2014) Multi-omics (AKÇAN et al. 2020a, Ueland et al. 2021, Bonicelli et al. 2022) VOC analysis via GCxGC-MS (Cieśla et al. 2023, Thurn et al. 2024) Grave soil analysis (Dent et al. 2004, Tibbett and Carter 2009) DNA degradation (Tozzo et al. 2020)	Visual taphonomy (Megyesi et al. 2005) Visual anthropology (Marhoff-Beard et al. 2018) Presence of late-stage insects (Matuszewski 2021) Dating of exposed bone (Arora et al. 2024, Ermida et al. 2024) Multi-omics (AKÇAN et al. 2020a, Ueland et al. 2021, Bonicelli et al. 2022) VOC analysis via GCxGC-MS (Cieśla et al. 2023, Thurn et al. 2024) Grave soil analysis (Dent et al. 2004, Tibbett and Carter 2009) DNA degradation (Tozzo et al. 2020)	Visual taphonomy (Megyesi et al. 2005) Visual anthropology (Marhoff-Beard et al. 2018) Presence of very late-stage insects (Matuszewski 2021) Dating of bone (Arora et al. 2024, Ermida, Cunha and Ferreira 2024) Multi-omics (AKÇAN et al. 2020a, Ueland et al. 2021, Bonicelli et al. 2022) VOC analysis via GCxGC-MS (Cieśla et al. 2023, Thurn et al. 2024) Grave soil analysis (Dent et al. 2004, Tibbett and Carter 2009) DNA degradation (Tozzo et al. 2020)

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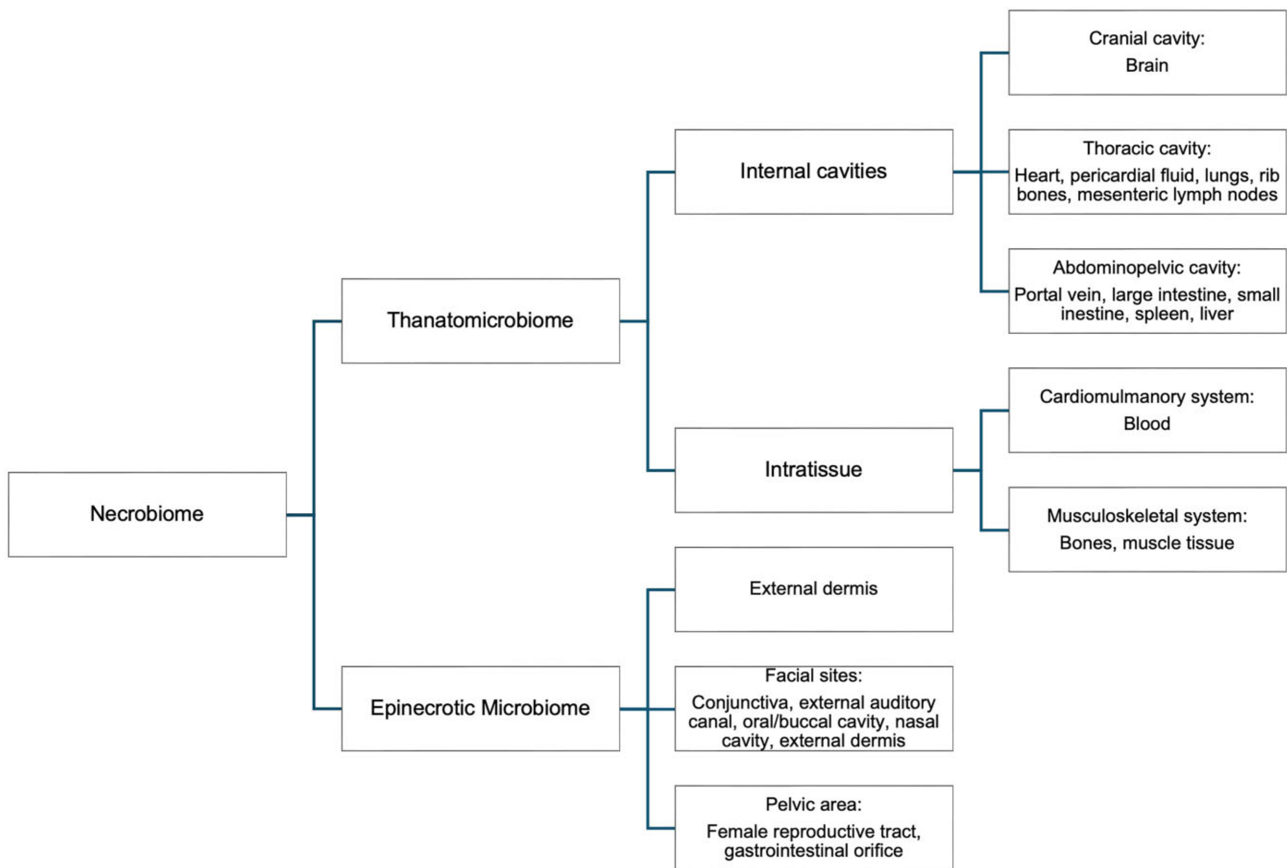

Figure 1. A hierarchical graph showing the categories that compose the postmortem microbiome. The necrobiome has two major facets: the thanatomicrobiome, which encompasses internal microbes, and the epinecrotic microbiome, which is made of external sites. Within the thanatomicrobiome, samples can come from internal cavities (cranial, thoracic, and abdominopelvic) or intratissue systems (cardiopulmonary and musculoskeletal). Epinecrotic samples come from exterior sampling sites, such as the overall external dermis, facial sites (eyes, ears, mouth, nose, skin), or the pelvic orifices (female reproductive tract, gastrointestinal orifice).

location, and hygiene also impact a person's microbiome, affecting the overall microbiome that they will die with (Tarone et al. 2022, Beghini et al. 2025).

The necrobiome itself is defined as the microbes that compose the microbiota found in and on deceased human individuals, measured by the presence and relative abundance changes in these microbes over time (Cláudia-Ferreira et al. 2023). Within the necrobiome, samples are categorized into two regions, the epinecrotic microbiome and the thanatomicrobiome (Javan et al. 2016a, Oliveira and Amorim 2018, Zhou and Bian 2018, Cláudia-Ferreira et al. 2023). Etymology wise, the Greek '*epi*' means upon, or above, and '*necrotic*' refers to dead tissue. Greek '*Thanatos*' derives '*thanato*', meaning death. The difference between these two regions is their locality within the body (Fig. 1). The epinecrotic microbiome includes samples taken from the outside of the body non-invasively. Inversely, the thanatomicrobiome specifically includes samples taken invasively from the internal portions of the body.

The thanatomicrobiome is a major focus of current forensic microbiology research (Tuomisto et al. 2013, Can et al. 2014, Hauther et al. 2015, DeBruyn and Hauther 2017, Javan et al. 2017, Bell et al. 2018, Lutz et al. 2020, Deel et al. 2021). Internal organs within human cadavers are microbially dense after death (Can et al. 2014, Dash and Das 2020, Abdoun et al. 2023), and these microbes migrate within the body during decomposition in a predictable manner (Javan et al. 2016a, Tuomisto et al. 2013, Ventura Spagnolo et al. 2019), allowing them to be used as microbiological predictors for the PMI (Hauther et al. 2015, DeBruyn and Hauther 2017, Javan et al. 2019, Dash and Das 2022). As organs within the human body degrade at different rates and harbour signature bacteria, sampling and comparison of these locations can provide strong predictive power for PMI (Pechal et al. 2018, Chen et al. 2024). The microbes of internal organs are also less vulnerable to external microbial variables, such as insects and soil, in the early postmortem period (Javan et al. 2016a). However, thanatomicrobiome samples are subject to the microbial variability seen between humans (Can et al. 2014, Bell et al. 2018). A logistical factor to consider is the requirement of an autopsy to allow for thanatomicrobiome sampling. As this process involves highly trained forensic pathologists, part of an already strained field of medicine, any factor that prevents or delays the completion of an autopsy will hinder the sampling of internal organs (Weedn and Menendez 2020, Chisari et al. 2025). In the extreme case of mass disasters, as per the INTERPOL Disaster Victim Identification (DVI) recommendations, cadavers may be stored for days at a time before being seen by a medical examiner (INTERPOL 2023).

In contrast, the epinecrotic microbiome offers a more favourable sampling approach as it focuses on non-invasive procedures targeting areas of interest that are easily accessed

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by first responders and forensics teams (Metcalf 2019, Roy et al. 2021, Cláudia-Ferreira et al. 2023). Epinecrotic microbiome sampling sites include external surfaces or orifices that do not require invasive sampling. The mouth and gastrointestinal orifices of cadavers, parts of the epinecrotic microbiome, naturally harbour large amounts of bacteria (Adserias-Garriga et al. 2017a, Zhang et al. 2023a). Therefore, large quantities of bacteria will be present during early stages of decomposition, before extrinsic factors impact the body. This advantage is important in a forensic context because measurable microbial data is present immediately following death (the deceased's intrinsic microbiome), which over time will be influenced by the environment's extrinsic microbes (Zapico and Adserias-Garriga 2022). Epinecrotic surfaces undergo predictable microbiome changes that can serve as indicators of the PMI (Dash and Das 2020). These quantifiable changes—the shift between internal to external bacteria in the microbiome and the relative abundance changes for each microbe over time—are what would be measured to estimate the time since death using a forensic microbiology approach. These advantages are also instrumental in times of medicolegal system stress, such as during autopsy staff shortages, overcrowding of mortuaries, incidents with mass casualties, or DVI scenarios (Ascolese et al. 2024). The latter two circumstances are highly relevant to the current world climate due to an increase in natural and man-made disasters (Yamamura 2015, Topluoglu et al. 2023).

One major component that influences the epinecrotic necrobiome is the interaction between carrion insects and cadavers over time. Certain species of fly are well known 'decomposers', in that they are globally ubiquitous, prevalent, and well involved in the postmortem process (Ren et al. 2018, Matuszewski 2021, Hodecek et al. 2024). They accelerate decay as they introduce extrinsic bacteria, provide larval offspring that consume necrotic tissue using proteolytic enzymes, and increase the heat signature of the cadaver (Campobasso et al. 2001, Valachova et al. 2014, Griffiths et al. 2020, Syed Mohd Daud et al. 2024, Johnston et al. 2025). As a result, host-specific microbes interact with bacteria introduced by these insects (Johnston et al. 2025). In addition to the predictive PMI possibilities offered by successional carrion insect colonization during decomposition (Griffiths et al. 2020, Matuszewski 2021), the bacteria associated with these flies and beetles have the potential to be used to estimate the PMI (Iancu et al. 2018, Junkins et al. 2019, Iancu et al. 2020). Specific examples of entomological bacteria that could be used for PMI estimation are genera *Wohlfahrtiimonas* and *Ignatzschineria*, both almost exclusively associated with carrion flies and myiases infections, which is the live larval colonization of flesh on living mammals, including in humans (Tóth et al. 2001, 2008). The link between these two bacterial genera and flies has been demonstrated worldwide in both medical documentation and forensic cases (Almuzara et al. 2011, Campisi et al. 2015, Kõljalg et al. 2015, Le Brun et al. 2015, Heddema et al. 2016, Hoffman et al. 2016, Cipolla et al. 2018, Katanami et al. 2018, Lysaght et al. 2018, Connelly et al. 2019, Al-Qahtni et al. 2020, Deslandes et al. 2020, Fear et al. 2020, Dovjak et al. 2021, Hladík et al. 2021, Karaca et al. 2022, Demurtas et al. 2023, De Smet et al. 2023). While this link is well established, the predictive power of these extrinsic bacteria regarding estimating the PMI is not fully explored.

How decomposition variables influence microbes is yet to be fully understood. What is known, is that bacteria in the necrobiome behave in measurable and typically predictable patterns: the 'microbial clock', popularized by Metcalf et al. 2013 (Metcalf et al. 2013, Metcalf 2019). The theory is that due to the successional nature of human decomposition and external factors such as invertebrate colonization, microbes within the necrobiome will behave in a predictable time-dependant manner (Guo et al. 2016). This timeline of bacterial events has been the focus of many proof-of-concept studies involving animal analogues in place of human cadavers, including rodents (mouse or rats) (Metcalf et al. 2013: 20 252 025; Iancu et al. 2018, Dong et al. 2019, Martínez Aragonés et al. 2022, Li et al. 2023, Liu et al. 2023, Wang et al. 2024, Su et al. 2025) and swine (pigs) (Pechal et al. 2013, Dibner et al. 2019, Wang et al. 2021, Yang et al. 2023, Green et al. 2024, Iancu et al. 2024). Such animal models are typically used in forensic research when human donors are not available. While these studies do suggest that the postmortem microbiome changes in ways that can assist in time since death estimations, the compatibility between animal data and human studies is highly contested, considering that other studies show that humans decompose differently from animal models (Belk et al. 2018, Knobel et al. 2018, Dawson et al. 2020, DeBruyn et al. 2020, Collins et al. 2022). Using the necrobiome timeline as a method of PMI estimation has also been successfully shown in human studies, with varying levels of predictive accuracy (Javan et al. 2016a, Adserias-Garriga et al. 2017b, Hyde et al. 2013, 2014, Damann et al. 2015, Johnson et al. 2016, 2016b, DeBruyn and Hauther 2017, Pechal et al. 2018, Ashe et al. 2021, Tarone et al. 2022, Burcham et al. 2024, Chen et al. 2025). These studies vary in geographical location, sampling methods, the part of the necrobiome being focused on, and level of bioinformatics used in analysis (Zhou and Bian 2018, Ventura Spagnolo et al. 2019, Dash and Das 2020, García et al. 2020, Cláudia-Ferreira et al. 2023, Mason et al. 2023, Nodari et al. 2024, Singh et al. 2025).

Techniques involved in forensic microbiology

In forensic microbiology sampling, there are four primary components that impact the quality and format of resulting microbial data: the sample collection method, which DNA extraction kit is used to extract the bacterial DNA, the sequencing system is used, and what bioinformatics platform is used to analyse the resulting data (Galloway-Peña and Hanson 2020, Villette et al. 2021, Fierer et al. 2025).

Sample collection for microbial data can be done in many ways, typically involving swabs, scrapers, and tape-stripping, each providing different microbial biomass amount and diversity collected. Tape-stripping and scraping both provide similar amounts of microbial data as swabs but are less utilized in studies (Ogai et al. 2018, Smith et al. 2024). Between swabs, comparison studies show flocked swabs are more efficient at microbial sampling than cotton swabs (Wise et al. 2021).

The efficacy of commercially available DNA extraction kits is often compared for specialized microbial samples (Claassen et al. 2013, Elie et al. 2023, Galla et al. 2024). Microbial DNA extraction kits that target low-biomass samples are especially important for forensic microbiome research, as some areas of the body targeted for sampling may have low amounts of bacteria, such as the external epidermis (Gall-David et al. 2023, Kurokawa et al. 2023). The choice of extraction kit used for a forensic microbiology project may depend on availability of certain brands, the experience of use within

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the laboratory team, or compatibility with downstream processes such as sequencing. Commonly used bacterial DNA extraction kits that have shown promising results for forensic microbiology applications include ones from MoBio (e.g. PowerLyzer/PowerSoil Pro DNA Isolation, Bacteremia DNA Isolation) (Adserias-Garriga et al. 2017b, Hyde et al. 2013, Metcalf et al. 2013, 2014, Johnson et al. 2016, Junkins et al. 2019, Burcham et al. 2024), ThermoFisher (e.g. PureLink Genomic DNA Mini) (Pechal et al. 2018), QIAGEN (e.g. RNeasy PowerMicrobiome, DNeasy PowerSoil, QIAamp DNA stool mini) (Lawrence et al. 2019, Ashe et al. 2021, Chen et al. 2025), Omega Bio-Tek (e.g. OMEGA Soil DNA) (Liu et al. 2023), MP Biomedical (e.g. FastDNA SPIN Kit for Soil) (Tarone et al. 2022) and Illumina Nextera XT (Truong et al. 2021, Zeden and Gründling 2023).

Some studies do not use commercially available kits but instead perform extractions using standard laboratory methods, such as simply heating the samples or placing them in a solution to lyse the cells (Javan et al. 2016b, Pechal et al. 2013, Can et al. 2014, Dong et al. 2019, Iancu et al. 2023).

Advancements in technology have revolutionized bacterial DNA sequencing for forensic microbiology, allowing microbiome sequencing to recognize higher taxonomic resolutions at a reduced cost (Tozzo et al. 2022, Franceschetti et al. 2024, Oliveira et al. 2024). With next-generation sequencing (NGS), or high-throughput sequencing, it is possible to generate significantly more data compared to using traditional Sanger sequencing methods, and thus increasing the efficacy of results while decreasing time and expense (Satam et al. 2023, Yuan et al. 2023). Two of the most widely used NGS methods are shotgun metagenome sequencing and 16S rRNA gene sequencing (Johnson et al. 2019). Both methods have the capability of proving species, or even subspecies-level taxonomy identification (Johnson et al. 2019, Jeong et al. 2021). Shotgun sequencing looks at the entire bacterial genome and is therefore more powerful than 16S rRNA sequencing in that it provides a larger breadth of identified microbial gene results and recognizes them at smaller quantities (Johnson et al. 2019, Durazzi et al. 2021). However, this specificity comes at a cost, both financial, as samples are expensive to run, and analysis wise, as the resulting data are more complex (Sharpton 2014).

Due to its relative simplicity compared to whole genome sequencing, 16S rRNA gene sequencing has generally been the preferred method in forensic microbiology research thus far (Nodari et al. 2024, Yang et al. 2024). It provides taxonomic identification and relative abundance via the 16S ribosomal gene, a highly conserved region of coding DNA that is found in virtually all bacteria (Janda and Abbott 2007). The cost of running samples is more affordable than whole genome sequencing, and the resulting taxonomy and abundance data is accessible to non-experts in its analysis. These advantages are critical to forensic and law enforcement agencies, as the resulting data can be analysed by appropriately trained forensic biology personnel as opposed to specialist microbiologists or bioinformaticians, and is arguably more digestible to layperson juries in courtroom settings.

Analysis-wise, there have been significant developments that have made microbiome examination more accessible to forensic researchers. Bioinformatics software that is used for microbiome analysis includes Illumina BaseSpace (Tan et al. 2019), Galaxy (Afgan et al. 2018), mothur (Schloss et al. 2009), MicrobiomeAnalyst (Lu et al. 2023), and most notably QIIME (Quantitative Insights Into Microbial Ecology 2), or QIIME2 (Caporaso et al. 2010, Bolyen et al. 2019, Estaki et al. 2020). The preference for QIIME2 is evident in forensic microbiology research (Zhang et al. 2023a, Lutz et al. 2020, Burcham et al. 2024). The programme QIIME2 is an open-source professional microbiome analysis pipeline used to turn pure genetic data into easy-to-digest taxonomic and relative abundance visualizations (Bolyen et al. 2019, Estaki et al. 2020).

Recent studies on the dynamics of the epinecrotic microbiome

Recently published reviews mainly focus on forensic microbiology as a whole (Ventura Spagnolo et al. 2019, Speruda et al. 2022, Cláudia-Ferreira et al. 2023) and the thanatomicrobiome (Zhou and Bian 2018, Javan et al. 2019, Dash and Das 2020, 2022). There has been considerably less of a focus on the epinecrotic microbiome specifically. Out of dozens of recent necrobiome research studies, only a handful focus on the epinecrotic microbiome over time (Table 2). The majority of these were performed in the USA, due to the number of available taphonomic facilities (colloquially known as body farms) there. Facilities used in these studies include:

(i) The Southeast Texas Applied Forensic Science Facility (STAFS) in Texas (Hyde et al. 2013, 2014, Burcham et al. 2024),
(ii) mortuary complexes in Alabama (Javan et al. 2016b) and Michigan (Pechal et al. 2018),
(iii) the University of Tennessee Anthropological Research Facility (ARF) in Tennessee (Adserias-Garriga et al. 2017b, Johnson et al. 2016, Burcham et al. 2024),
(iv) the Forensic Osteology Research Station (FOREST) in North Carolina (Ashe et al. 2021),
(v) the Texas State University Forensic Anthropology Center in Texas (Tarone et al. 2022), and
(vi) the Colorado Mesa University Forensic Investigation Research Station (FIRS) in Colorado (Burcham et al. 2024).

Two studies sampled cadavers outside of the USA, but neither in body farms. Human cadavers were sampled in mortuaries in Romania (Iancu et al. 2023) and China (Chen et al. 2025). At this time, advanced decomposition studies remain an uncommon forensic research topic due to the limited number of facilities that can accommodate for such experiments. Eight forensic taphonomy research centres exist in the USA, one in Australia, Canada, and the Netherlands each, for a total of eleven facilities worldwide (Miles et al. 2020).

Table 2 shows the experimental design of papers that have sampled the epinecrotic microbiome of humans. Three of the studies did not follow cadavers over time (Javan et al. 2016b, Pechal et al. 2018, Chen et al. 2025). These studies did, however, have large sample sizes (for a forensic context) and therefore were able to report on observed bacteria at different stages of early decomposition. When comparing the methods used, there are clear reoccurring sampling consumables and techniques seen between the different papers. Each of the papers used swabs when sampling and used 16S rRNA sequencing with varying primers and target regions (Zhang et al. 2023b, Smith et al. 2024). The predominant software used for bioinformatics analysis was QIIME [or QIIME2 after its introduction in 2018 (Bolyen et al. 2019)]. Javan et al. (2016) did not list the bioinformatics platform used in their study, and

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Table 2. Recent published studies following epinecrotic samples over time.

Set up	Location	Sampling	Sample site	Collection method	Sequencing	Analysis software	Genera of interest	Source
Outdoor, terrestrial	STAFS, Texas, USA	2 cadavers, 1 sample at placement and 1 after bloat	Mouth, (rectum)	Cotton swab, plastic spatula scrape	16S rRNA sequencing, primers 357F and 926R, V3-V5 region	QIIME	*Staphylococcus, Streptococcus, Peptoniphilus, Clostridium, Ignatzschineria*	Hyde et al. 2013 (Hyde et al. 2013)
Outdoor, terrestrial	STAFS, Texas, USA	2 cadavers, every 1–2 days for ∼3 weeks	Mouth, cheek skin (rectum, bicep skin, torso skin)	Cotton swab	16S rRNA sequencing, primers 357F and 926R, V3-V5 region	QIIME	*Ignatzschineria, Wohlfahrtiimonas, Acinetobacter, Clostridium*	Hyde et al. 2014 (Hyde et al. 2014)
Indoor, mortuary	Alabama, USA	27 cadavers, 1 time point	Mouth (blood, brain, heart, liver, spleen)	Cotton swab	16S rRNA sequencing, primers 515F and 806R, V4 region	No software listed in methods	*Streptococcus, Veillonella, Prevotella, Staphylococcus, Rothia, Haemophilus*	Javan, et al. 2016 (Javan et al. 2016b)
Outdoor, terrestrial	ARF, Tennessee, USA	4 cadavers, every 2/3 days for ∼3 weeks	Nose, ear	Phosphate-buffer saline soaked cotton swab	16S rRNA sequencing, V3-V4 region	Illumina Base-Space	*Staphylococcus, Vagococcus, Symploca*	Johnson et al. 2016 (Johnson et al. 2016)
Outdoor, terrestrial	ARF, Tennessee, USA	3 cadavers, every day for 10–12 days	Mouth	Dual swab, one water-soaked	16S rRNA sequencing, primers 515F and 806R, V4 region	QIIME	*Streptococcus, Ignatzschineria,*	Adserias-Garriga et al. 2017 (Adserias-Garriga et al. 2017b)
Indoor, mortuary	Michigan, USA	188 cadavers, 1 timepoint	Eyes, ears, mouth, nose, (umbilicus, rectum)	Cotton swab	16S rRNA sequencing, primers 515F and 806R, V4 region	QIIME	*Alloiococcus, Corynebacterium, Dialister, Finegoldia, Peptoniphilus, Staphylococcus, Streptococcus, Prevotella, Bacteroides, Lactobacillus, Bifidobacterium, Clostridium, Haemophilus*	Pechal et al. 2018 (Pechal et al. 2018)
Outdoor, terrestrial	FOREST, North Carolina, USA	3 cadavers, every 3–4 days for 2–3 weeks	Mouth	Water-soaked swab	16S rRNA sequencing, primers B969F and BA1406R, V6-V8 region	QIIME2, Deblur	*Rothia, Lysinibacillus, Lactobacillus, Staphylococcus*	Ashe et al. 2021 (Ashe et al. 2021)

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Table 2. Continued

Set up	Location	Sampling	Sample site	Collection method	Sequencing	Analysis software	Genera of interest	Source
Outdoor, terrestrial	FAC, Texas, USA	20 cadavers, 4 timepoints each	Nose	Swab with MP lysing matrix	16S rRNA sequencing, primers 515F and 806R, V4 region	QIIME2	*Clostridium, Ignatzschineria, Corynebacterium, Enterococcus, Sporosarcina, Lactobacillus, Providencia, Gordonia, Bacteroides*	Tarone et al. 2022 (Tarone et al. 2022)
Indoor, mortuary	Iași, Romania	8 cadavers, 2 samples at 0 and 12 hours	Face skin, (hand skin)	Cotton swab	16S rRNA sequencing, primers 357F and 806R, V3-V4 region	QIIME2, FastTree	*Staphylococcus, Streptococcus, Lactobacillus*	Iancu et al. 2023 (Iancu et al. 2023)
Outdoor, terrestrial	ARF, Tennessee, FIRS, Colorado, STAF, Texas, USA	36 cadavers, every day for 21 days	Head skin, (hip skin)	Cotton and polyester swab	16S rRNA sequencing, primers 515F and 806R, V4 region	QIIME2	*Oblitimonas, Ignatzschineria, Wohlfahrtiimonas, Bacteroides, Vagococcus, Savagea, Acinetobacter, Peptoniphilus*	Burcham et al. 2024 (Burcham et al. 2024)
Indoor, mortuary	China	34 cadavers, 1 time point	Mouth, nose	Cotton swab	16S rRNA sequencing, primers 343F and 798R, V3-V4 regions	QIIME2	*Streptococcus, Haemophilus, Prevotella, Corynebacterium, Staphylococcus, Escherichia-Shigella, Acinetobacter, Klebsiella, Leptotrichia*	Chen et al. 2025 (Chen et al. 2025)

Papers that have observed the necrobiome by sampling external sites that are non-invasive and accessible, such as conjunctiva (eyes), the nasal cavity (nose), external auditory canal (ears), buccal/oral cavity (mouth), and external dermis (skin). Each study has either followed the microbial changes of cadavers over time or has sampled a large sample size at one timepoint to average the data in decomposition timeframes (Javan et al 2016a, Pechal et al. 2018, Chen et al. 2025). Details for each study include the experimental set up, geographical location, sampling location on the cadaver, sequencing details, analysis software, and bacterial genera of note. The studies are listed in publication date order.

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the methods in the Johnson et al. (2016) paper use Illumina BaseSpace.

Previous forensic microbiology studies report their findings either as a list of 'prominent' bacteria or following a timeline while sampling longitudinally. Published forensic microbiology reviews have compared key bacteria at different decomposition; however, these are typically reported at a phylum level or are not comprehensive (Dash and Das 2020, Moitas et al. 2023). Bacteria of interest that have been associated with different stages of decomposition based on the studies examined in the above Table 2 are outlined in Table 3. Genera *Acinetobacter*, *Clostridium*, and *Ignatzschineria* are of note within nearly all stages. During early decomposition, bacterial genera that are typically associated with the human microbiome, such as *Streptococcus* (Velsko and Warinner 2025) and *Staphylococcus* (Campos et al. 2023), are prominent. Although less bacteria are presented during the later stages of decomposition, it should be noted that this may not be a result of diversity truly decreasing over time but may be reflective of a reduction in studies that sample during late decomposition.

A clear trend emerges where most studies have followed a similar structure in sampling procedures (Table 2). Visualized in Fig. 2, the papers largely align in five core sections in the methodology: sampling details, the swabs and biological fixative used, DNA extraction protocol, sequencing procedures, and data analysis (Fig. 2). The compared methodology does not include geographical location or cadaver demographic information, as these are not as controllable in the experimental setup.

The overall advances in epinecrotic microbiome understanding calls for standardized sampling, extraction, sequencing, and bioinformatic analysis procedures. Developing unified protocols is key to provide comprehensive operational industry standards allowing forensic microbiology evidence to be considered reliable, reproducible, and comparable across cases and jurisdictions, meeting both scientific and legal expectations.

Rigorous requirements for forensic science

Studies observing the necrobiome over time show similarities in methods, suggesting potential for standardization. However, countries, states, and forensic agencies have differences in the standard operating procedures relating to the collection, testing, and analysis of evidence. Leading authorities such as INTERPOL, the International Organization of Standardization and the Federal Bureau of Investigation provide recommended procedures for evidence (Butler 2015, Ward 2017, Wilson-Wilde 2018, Uberoi et al. 2024), yet global standardization, and unification remain ongoing effort. As forensic microbiology remains in its infancy, there is a valuable opportunity to draw on best practices from more established and regulated forensic disciplines—helping ensure its effective integration into routine forensic workflows and legal standards.

For any science to be considered applicable to medicolegal inquiry, and subsequently, be used in a court of law, it must be proved reliable, standardized, and robust. As outlined by the National Academy of Science's 2009 critique on forensic science (National Research Council 2009), the Daubert principle's criteria (National Institute of Justice 2023) and 2020 Australia and New Zealand Policing Advisory Agency roadmap (ANZPAA 2020), the integration of a new forensic (sub)discipline, such as forensic microbiology, into routine analysis requires rigorous testing and uniformity in practice and analysis and demonstrated foundational validity. To apply forensic microbiology for PMI estimation in a judiciary setting, a reference database that catalogues observed necrobiome events and changes over time needs to be founded. Such a collection for PMI data must:

(i) Have uniformity amongst collected samples from different experiments, i.e. be treated the same both in the field and in the lab, with resulting data being in a compatible format.
(ii) Be robust enough to include and compare variables between experiments, such as location, weather, intrinsic human metrics, and all other effects that influence decomposition.
(iii) Be accessible to policing and forensic agencies so that the reference data may be used in real-world medicolegal cases with confidence.

There is currently no universal method for sampling and analysis in forensic microbiology in respect to neither epinecrotic microbiome sampling nor thanatomicrobiome sampling (Fernández-Rodríguez et al. 2015, Cláudia-Ferreira et al. 2023). Once sampling methods are standardized, the practice of forensic microbiology can meet the standards needed for medicolegal application in the future (Fernández-Rodríguez et al. 2019, Metcalf 2019).

Conclusions and directions for future research

To meet the requirements proposed by forensic authorities, the scientific methods involved in forensic casework must be standardized, foundationally valid, unbiased, and replicable. The current space of forensic microbiology is still evolving, particularly in establishing standardization in methods (Cláudia-Ferreira et al. 2023, Guo et al. 2023, Nilendu 2023, Franceschetti et al. 2024, Nodari et al. 2024, Singh et al. 2025). With additional sampling within this field, a key limitation of lacking diverse demographic data may be addressed. Building a database including varying ages, races, and sexes is needed in order to be applicable to more scenarios in the future. As with any field that researches massive datasets, such as the microbiome, the application of machine learning models is a critical tool for postmortem estimations in the future. The machine learning model primarily used in forensic microbial studies is the random forest regression model, which has been trialled in previous papers (Zhang et al. 2019, Emmons et al. 2020, Deel et al. 2021, Dmitrijs et al. 2022, Iancu et al. 2024, Chen et al. 2025, Su et al. 2025), but there is currently no centralized model pipeline. Random forest regression applies well to forensic microbiology, as large datasets can be used to train the model, which can then be validated with 'blind' testing (Hajipour et al. 2020). This should be a consideration for all future studies in the field. In addition, microbial data may be combined with complementary decomposition data, such as the multi-omics approach or VOCs, for further impact (Akçan et al. 2020, Procopio and Bonicelli 2024).

To strengthen the field of forensic microbiology to the point of use in judicial courts, the methodology and analysis involved in reference data collection needs to be uniform and validated. This will allow for forensic practitioners to accurately compare findings to previously published literature to

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Table 3. The five stages of decomposition (fresh, bloat, active decay, advanced decay, and skeletonization), the approximate timeframe of these stages, and associated microbes as reported by previous forensic microbiology studies.

	Fresh	Bloat	Active decay	Advanced decay	Skeletonization
Approximate timeframe	0–48 hours	2–7 days	1–2 weeks	2–4 weeks	1 month +
Microbial data	*Acinetobacter* (Chen et al. 2025) *Actinomyces* (Ashe et al. 2021) *Clostridium* (Hyde et al. 2013, Ashe et al. 2021) *Corynebacterium* (Adserias-Garriga et al. 2017b, Chen et al. 2025) *Escherichia* (Chen et al. 2025) *Haemophilus* (Javan et al. 2016b) *Ignatzschineria* (Adserias-Garriga et al. 2017b) *Lactobacillus* (Iancu et al. 2023) *Peptoniphilus* (Hyde et al. 2013) *Prevotella* (Hyde et al. 2013, Javan et al. 2016b, Chen et al. 2025) *Pseudomonas* (Hyde et al. 2014) *Rothia* (Adserias-Garriga et al. 2017b, Ashe et al. 2021) *Staphylococcus* (Hyde et al. 2013, Pechal et al. 2018, Iancu et al. 2023) *Streptococcus* (Javan et al. 2016b, Adserias-Garriga et al. 2017b, Pechal et al. 2018, Ashe et al. 2021, Iancu et al. 2023, Chen et al. 2025) *Veillonella* (Javan et al. 2016b)	*Acinetobacter* (Adserias-Garriga et al. 2017b, Ashe et al. 2021) *Capnocytophaga* (Chen et al. 2025) *Clostridium* (Hyde et al. 2013) *Corynebacterium* (Adserias-Garriga et al. 2017b, Chen et al. 2025) *Ignatzschineria* (Hyde et al. 2013, 2014, Adserias-Garriga et al. 2017b, Ashe et al. 2021) *Lysinibacillus* (Ashe et al. 2021) *Peptoniphilus* (Hyde et al. 2013) *Prevotella* (Chen et al. 2025) *Pseudomonas* (Hyde et al. 2014, Adserias-Garriga et al. 2017b, Chen et al. 2025) *Rothia* (Javan et al. 2016b) *Staphylococcus* (Javan et al. 2016b, Pechal et al. 2018) *Streptococcus* (Javan et al. 2016b, Pechal et al. 2018, Chen et al. 2025) Unknown *Clostridiaceae* (Hyde et al. 2013) Unknown *Planococcaceae* (Hyde et al. 2013) *Vagococcus* (Adserias-Garriga et al. 2017b, Ashe et al. 2021) *Veillonella* (Adserias-Garriga et al. 2017b) *Wohlfahrtiimonas* (Ashe et al. 2021)	*Acinetobacter* (Adserias-Garriga et al. 2017b) *Clostridium* (Hyde et al. 2014) *Ignatzschineria* (Hyde et al. 2014, Ashe et al. 2021) *Sporosarcina* (Ashe et al. 2021) *Tissierella* (Ashe et al. 2021) Unknown *Bacillaceae* (Adserias-Garriga et al. 2017b) Unknown *Clostridiaceae* (Hyde et al. 2014) Unknown Clostridiales (Adserias-Garriga et al. 2017b) Unknown *Planococcaceae* (Hyde et al. 2014, Adserias-Garriga et al. 2017b, Ashe et al. 2021)	*Clostridium* (Hyde et al. 2014) *Ignatzschineria* (Hyde et al. 2014, Ashe et al. 2021) *Pseudomonas* (Ashe et al. 2021) *Sporosarcina* (Ashe et al. 2021) Unknown Bacillales (Ashe et al. 2021) Unknown *Clostridiaceae* (Hyde et al. 2014) Unknown *Planococcaceae* (Hyde et al. 2014, Ashe et al. 2021)	*Acinetobacter* (Ashe et al. 2021) *Corynebacterium* (Ashe et al. 2021) Ignatzschineria (Ashe et al. 2021) *Pseudomonas* (Ashe et al. 2021)

Note that a number of studies shown previously in Table 2 have been omitted from the above table as they do not provide relative abundance data correlated with a sampling timeline (Johnson et al. 2016, Pechal et al. 2018, Tarone et al. 2022, Burcham et al. 2024).

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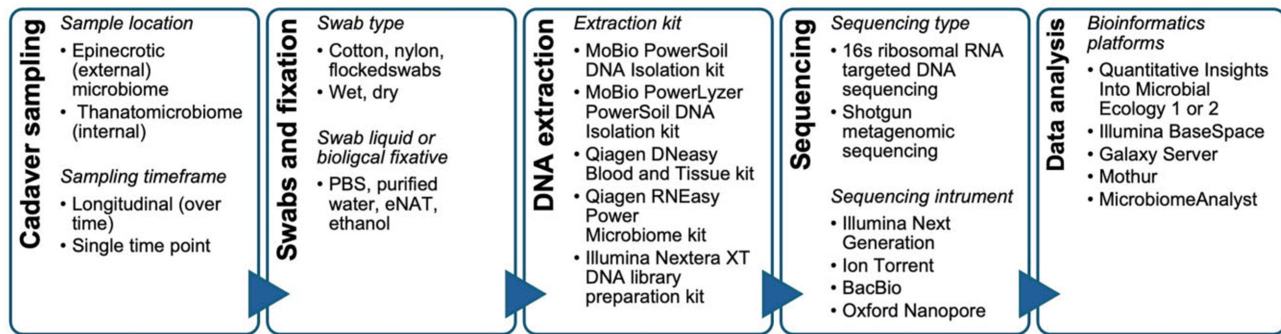

Figure 2. Summary of options for steps involved in forensic microbiology sampling. The five overarching methodological phases of necrobiome sampling are how the cadaver is sampled, the type of consumable swab and fixation methods used, bacterial DNA extraction, genetic sequencing, and the bioinformatics and data analysis.

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develop a reference database. It is only with reproducible protocols and comparable data that forensic microbiology can be considered for medicolegal applications.

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Author contributions

Diana Marshall (Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing), Maiken Ueland (Funding acquisition, Supervision, Writing – review & editing), Annemarie Nadort (Writing – review & editing),and Bill Söderström (Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing)

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Data availability

All datasets given and summarized in this study are included in the manuscript and is available from the corresponding author(s) upon request. Original and underlying raw data presented in this text is available in the source papers cited within this review.

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