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REVIEW

Thanatomicrobiome in forensic medicine

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SUMMARY

The circumstances of death and the estimation of the post-mortem interval (PMI) are often a great challenge for scientific and judicial investigators, especially when some time has elapsed since death. Several techniques are used; nevertheless, each presents its own limitations. In the quest for new techniques that are more reliable or at least complementary to those existing and sometimes less expensive, researchers have in recent years turned toward exploring the dynamics of the different microbial communities of a corpse according to their different stages of decomposition. This article summarizes the various works done in the field and shows the different sources of microorganisms in the different parts of the human corpse and their potential interest in the field of forensic medicine.

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INTRODUCTION

Microbes have long been recognized for their importance in various fields, such as medicine, ecology, food sciences, etc., but have been largely ignored by forensic science. In recent years, forensic scientists have focused their efforts on the cadaveric microbial flora and its most-mortem evolution. Indeed, bacteria colonize cadavers according to particular sequences, just as necrophagous insects do (Alan and Sarah, 2012).

Throughout life, the human microbial flora performs health-related functions, including nutrient acquisition, energetic functions, and immune defense. The microbiome is also implicated in metabolic and immune diseases, as well as in health disorders via the gut-brain axis (Gilbert *et al.*, 2016; Gilbert *et al.*, 2018). The microbiome knowledge that has been accumulated, which also extends to fields other than medicine, has so far found few forensic applications. Indeed, although it is well known that several transformative processes are driven by the diffusion and proliferation of microorganisms to the various tissues, few efforts have so far been conducted in the search for elements that could justify a microbiome role in determining the time and causes of death.

After death, the body becomes a home for microorganisms. During decomposition, microorganisms and necrophagous insects enter the corpse to promote bacterial proliferation. The body thus becomes a huge source of nutrients containing nitrogen, carbon, phosphorus, etc. (Metcalf *et al.* 2016 a, b). The postmortem microbiome plays an important functional role in the postmortem decomposition of the host (Can *et al.*, 2014).The thanatomicrobiome is therefore composed of certain external commensal microbes that begin to colonize body orifices after death, and of the internal microbial community that colonizes human organs, whereas the necrobiome exclusively defines the external bacterial communities related to a human cadaver (Javan *et al.*, 2016b). Changes in post-mortem microbial communities in a decomposing cadaver generally occur through horizontal migration from the host's digestive tract and through contact with the external environment (Javan *et al.*, 2019; Can *et al.*, 2014; Javan *et al.*, 2016b; Javan *et al.*, 2017). Bacteria that colonize the surface of the decaying cadaver undergo uncontrolled reproduction, leading to the dormancy of other microbes. This rapidly affects the surface epinecrotic communities as compared to the internal microbiome (Pechal *et al.*, 2013).

Bacterial associations vary in different internal organs of human cadavers. They can be exploited to predict essential forensic data, such as cause and time of death (Lutz *et al.*, 2020). Therefore, the than-

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atomicrobiome has important forensic applications for estimating the PMI (post-mortem interval) and assessing the mode and cause of death, as it reflects antemortem health status (Kaszubinski *et al.*, 2020 b). Information on fluctuations in the thanatomicrobiome and necrobiome communities of human cadavers as well as on associated microbial genes from visceral parts and natural body orifices is limited (Dash and Das, 2020). Genomic analyses of autopsy samples have emerged as a possible approach for estimating PMI and determining cause of death (Roy *et al.*, 2021). The study and knowledge of variations in thanatomicrobiome communities in internal tissues and on surfaces of cadavers, during decomposition, is very important to accurately interpret microbiological parameters tested during post-mortem examinations. This article summarizes the importance of variations in thanatomicrobiome communities and their applications in the forensic field.

HUMAN MICROBIOME

The human body harbors a large number of microorganisms in specific areas, such as skin, oral mucosa, nasal cavity, vagina and gastrointestinal tract, while most other internal organs are sterile, except in cases of major infection (Ribet and Cossart, 2015). The colon has the highest total bacterial load in the human body, quantified in approximately 10^{11} bacteria/ml; high microbial concentration is also found in saliva and in dental plaque (10^{9} and 10^{11} bacteria/ml, respectively), but their contribution to the total count of bacteria is negligible due to their small volume (Sender *et al.*, 2016). Each part of the human corps has a defined microbial taxa predominance, such as proteobacteria on the skin, lactobacilli in the vagina, bacteroidetes and firmicutes in the intestine, and fusobacteria in the mouth (Grice and Segre, 2012).The list of main commensal microbes specific to each part of the body is shown in (*Table 1*). The occupation of these anatomical sites by specific commensal microflora guarantees protection from pathogens, for example, by competing with pathogens for various metabolic substrates (Khan *et al.*, 2019). On the other hand, the microbiome balance is prone to modifications, as microbial composition can be influenced by factors related to the individual's health, diet, environmental factors, and drug use (Langdon *et al.*, 2016).

The digestive, respiratory, and urogenital mucosa are majors sites of contact with bacteria, as they represent a surface area of approximately 300 to 400 square meters, which is 200 times larger than that of the skin (Ribet and Cossart, 2015). The human gastrointestinal tract harbors the highest bacterial load in the body, with 10^{13}-10^{14} bacteria of 500-1000 species (Hao and Lee, 2004). The predominant bacterial groups are Cytophaga-Flavobacterium-Bacteroides (CFB) and Firmicutes belonging to three major groups, including Clostridium rRNA subcluster XIVa, Clostridiums under rRNA group IV and Bacteroides (Manson *et al.*, 2008; Wang *et al.*, 2018). The oral cavity contains the second largest and most diverse human microflora in the human body. The dominant species in the oral cavity are Fusobacterium nucleatum, and Streptococcus, Lactobacillus, Actinomyces, Neisseria, and Veillonella begin to colonize the oral mucosa as early as the first year of life (Deo and Deshmukh, 2019). The low microbial load found in the stomach and upper small intestine may derive from contaminants which enter from the outside through the mouth and nose, as these areas are considered sterile (Bornside *et al.*, 1966). However, the lower part of the small and large intestine harbors large and diverse populations of bacteria. The ileum of the small intestine harbors a high number of microflora (10^{7}-10^{8}) followed by the jejunum (10^{5}-10^{7}) and duodenum (10^{4} -10^{5}) (Booijink *et al.*, 2007), and the most frequent microbial species in the small intestine are Clostridium, Lactobacillus, Streptococcus, Staphylococcus, Bacteroides, and others (Hayashi *et al.*, 2005; Ahmed *et al.*, 2007). The human colon also harbors a large load of microbes, with 10^{12}-10^{14} bacteria per gram of colonic content. It contains more than 30 genera and 500 species belonging mainly to obligate anaerobes (Canny and McCormick, 2008). The dominant microbes in the human large intestine are Bacteroides and Gram-positive anaerobic cocci such as Peptostreptococcus, Eubacterium, Lactobacillus, and Clostridium (Swidsinski *et al.*, 2002).

The human skin provides a unique habitat for the

Table 1 - *List of commensal microorganisms at various body parts (Dash and Das, 2020)*

Buccal cavity	*Small intestine*	*Large intestine*	*Female reproductive tract*	*Skin*
Streptococci	*Bacteroides*	*Bacteroides*	*Lactobacillus Actinobacteria*	*Corynebacterium*
Lactobacillus	*Clostridia*	*Clostridia Prevotella*	*Bacteroidetes Firmicutes*	*Micrococcus*
Haemophilus	*Streptococci*	*sp. Eubacteria*	*Proteobacteria Gardnerella*	*Propionibacterium*
Actinomyces	*Lactobacilli*	*Ruminococci*		*Pseudomonas*
Prevotella sp.	*Enterococci*	*Streptococci*		*Rothia*
Gemella sp.	*γ-proteobacteria*	*Bifidobacteria*		*Staphylococcus*
Veillonella sp.	*E. coli High G+C*	*Enterococci*		*Malassezia*
Granulicatella sp.	*microbes*	*Lactobacilli*		
Fusobacterium sp.		*Fusobacteria*		

association and growth of specific sets of bacteria, including fungi, viruses, and mites (Cogen *et al.*, 2008). The skin microbiome comprises core microbial taxa consisting of 10 bacterial species, namely: Corynebacterium aurimucosum, Corynebacterium jeikeium, Corynebacterium pseudogenitalium, Corynebacterium tuberculostearicum, Micrococcus luteus, Propionibacterium acnes, Propionibacterium granulosum, Pseudomonas sp. unclassified, Rothiamucila ginosa, and Staphylococcus epidermidis, Malassezia globosa as an isolated fungal species, and Propionibacterium phage P101 A as an isolated virus (Schmedes *et al.*, 2017).

The female genital tract, in particular the human vagina, hosts a large number of microbes that constitute a complex ecosystem. The glycogen present in the vaginal environment facilitates the growth of Lactobacillus species, which guarantees a low vaginal pH (Amabebe and Anumba, 2018). A recent report prepared a repository of 581 microbial species belonging to 10 different taxa of the human vagina, with Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria as the main phyla discovered by a non-culturable method (Diop *et al.*, 2019). Similarly, in the male genital tract, Prevotella and Lactobacillus species are isolated in human semen; a similarity with the vaginal microbiota of female partners is also noted (Zozaya *et al.*, 2016). Circumcision modifies an individual's normal microbial flora, hence the male genital microbiome. Thus, Pseudomonadaceae are revealed to be the predominant microbial population regardless of circumcision status (Gray *et al.*, 2008; Price *et al.*, 2010). In addition, the human body undergoes infections that also affect the microbial population and the total microbiome load, such as skin infections, gastroduodenal diseases, inflammation of tonsil tissues, bacterial vaginosis, smoking habit, and others.

THANATOMICROBIOME COMMUNITIES CHANGES AND SUCCESSION

The human thanatomobiome includes microbial populations represented both inside and outside the cadaver, potentially helping to modulate cadaveric decomposition (Lutz *et al.*, 2020). After death, the corpse becomes an enormous source of nutrients rich in nitrogen, carbon, phosphorus, water, and others, thus becoming a hot spot for microbial action (Metcalf *et al.*, 2016 a, b). After death, the environment of the corpse becomes hypoxic and decomposition proceeds to the process of anaerobic fermentation. Gases such as HS2, CO2, methane, ammonia, sulfur dioxide, and hydrogen are then released (Vass *et al.*, 2002). The release of cell contents after lysis significantly alters the composition of human microbial communities. In addition, during this process, certain external microbes begin to colonize the surface and the orifices of the body (Can *et al.*, 2014). After death, failure of the host's immune system allows many germs to proliferate in internal organs, which are considered sterile, within 24 hours (Gevers, 1975; Morris *et al.*, 2006). These organs begin to degrade sequentially due to the bacterial concentration present (Can *et al.*, 2014).

The dominant microbial communities during human cadaveric decomposition at different stages are summarized in *Table 2*.

Throughout the post-mortem interval, thanatomicrobiome communities undergo changes in composition and turnover of taxa. Burcham *et al.* (2016) investigated the constitution and functional dynamics of Staphylococcus aureus and Clostridium perfringens in a murine animal model. The highest load of S. aureus occurred after 5-7 days of death, whereas these microbes were not present on the thirtieth day. A post-mortem investigation of the human gut microbiota demonstrated a shift in the gut bacterial community from Bacteroides and Parabacteroides to decaying communities such as Clostridium, Anaerosphaera, Ignatzschineria, and Wohlfahrtiimonas. It also showed that bacterial richness increases with decreasing diversity (DeBruyn and Hauther, 2017). A proportional increase in intestinal microbial DNA from Bifidobacteria, Bacteroides , Enterobacter, and Clostridia has already been noted in the gut with increased post-mortem time (Tuomisto *et al.*, 2013). Another study on post-mortem microflora in the large intestine found that the relative abundance of Bacteroides and Lactobacillus decreased exponentially, while Bifidobacterium abundance remained unchanged (Hauther *et al.*, 2015).

Post-mortem swabs from the oral cavity and the rectum revealed that γ-proteobacteria were most common in both samples as the decomposition process went on, while Firmicutes and Bacteroidetes de-

Table 2 - *Microbiome signature at different stages of degradation of a human cadaver (modified from Payne, 1965; Lee Goff, 2009)*

Fresh	*Bloat*	*Active Decay*	*Advanced Decay*	*Dry Remains*
Proteobacteria	*Proteobacteria*	*Proteobacteria*	*Firmicutes*	*Acidobacteria*
Pseudomonas	*Wohlfahrtiimonas*	*Pseudomonadaceae*	*Sporosarcina*	*Bacteroidetes*
Enterobacteriaceae	*Ignatzschineria*	*Chromatiaceae*	*Lactobacillales*	*Firmicutes*
		Proteus	*Acinetobacter*	*Soil microflora*
			Planococcaceae	

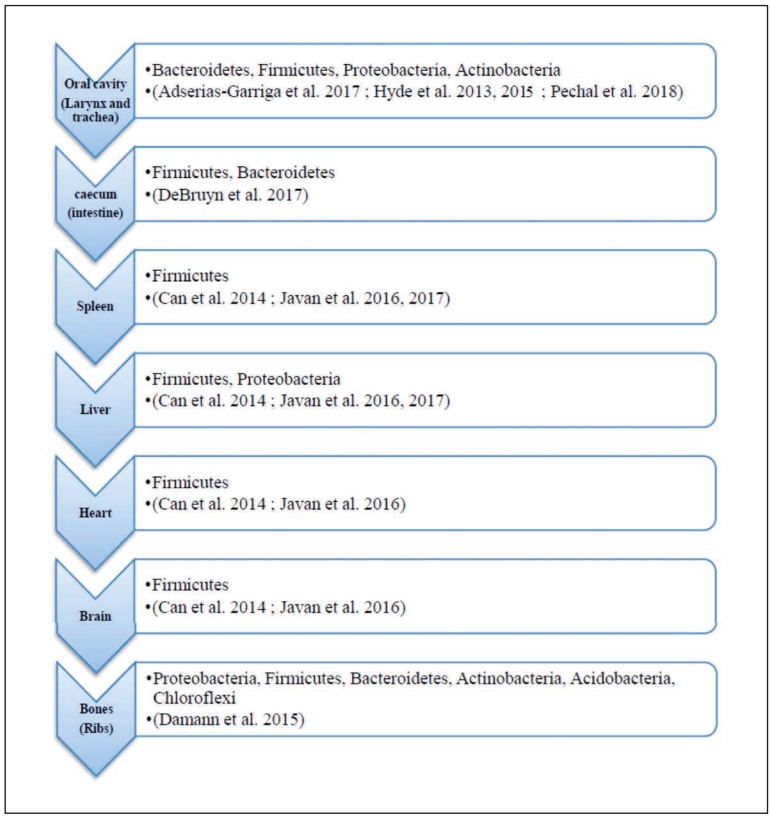

Figure 1 - *The dominant Thanatomicrobiome communities in specific parts of the body, with the progression of the natural order of decomposition of these organs.*

creased, respectively (Guo *et al.*, 2016). In another analysis of oral swabs from human cadavers, Firmicutes and Actinobacteria were the most dominant in the fresh stage and Tenericutes in the bloat stage (Adserias-Garriga *et al.*, 2017 a, b). Investigation of the necromicrobiome by analyzing the skin microflora, ear, and nasal cavity showed that the diversity of the ear microbiome was linked to the nasal cavity with the necrofloral genera Staphylococcus and Vagococcus (Johnson *et al.*, 2016). In samples from the cheek, biceps and torso during the first two days after death, the Proteobacteria group was revealed to be the most dominant, and the pattern of abundance shifted towards Firmicutes and Actinobacteria as decomposition progressed (Hyde *et al.*, 2014).

The bacterial colonization of normally sterile internal organs that occurs when an individual dies plays an important role in understanding the changes underlying cadaveric decomposition (Can *et al.*, 2014). Samples from autopsy cases postulated that pericardial fluid and the liver remain sterile up to 5 days after death (Tuomisto *et al.*, 2013). Similarly, careful bacterial cultures in blood and cerebrospinal fluid were positive for bacterial infection after death (Morris *et al.*, 2006). Weinstein (2003) compiled the first list of major microbes found in blood after death. The dominant post-mortem bacterial taxa in the visceral organs and the progression of their natural order of decomposition are summarized in *Figure 1*.

FACTORS INFLUENCING THANATOMICROBIOME COMMUNITIES

The human microbiome is highly diverse and differs among individuals depending on age and gender, ethnicity, dietary habits, geographical location, disease history, and other factors. Similarly, the thanatomicrobiome communities varies and changes depending on various biotic and abiotic factors, both intrinsic and extrinsic (David *et al.*, 2014; Panizzon *et al.*, 2015; Rehman *et al.*, 2016; Gupta *et al.*, 2017; Gaulke and Sharpton, 2018; De la Cuesta-Zuluaga *et al.*, 2019). Therefore, it is important to understand the interactions of various factors on cadaveric decomposition and the evolution of the post-mortem microbiome in order to design models that would be useful in forensic science.

Generally, abiotic factors affecting the thanatomicrobiome are weather, temperature, humidity, pH, and ante mortem living habits related to diet, and drug and antibiotic use. However, biotic factors include necrophagous insects, scavengers, commensal microorganisms, and microbes associated with ante mortem infections (Dash and Das, 2020).

Environmental conditions influence the thanatomicrobiome and its rate of succession in a cadaver. High temperature significantly affects cadaveric decomposition and significantly alters bacterial colonization of tissues (Benbow *et al.*, 2018; Iancu *et al.*, 2018). On

the other hand, cold blocks the process of decomposition as well as activities of microorganisms, whereas the storage of cadavers in the morgue, usually between -1°C and +4°C, does not prevent the decomposition process (Alan and Sarah. 2012). Thus, refrigeration does not prevent microbial proliferation over time, and may also lead to variations in the microbiome in some tissues 48 hours after death (Lawrence *et al.*, 2019). In addition, high humidity promotes bacterial growth and accelerates cadaver decomposition, but microbial growth decreases relatively in a cold environment with low humidity (Abad Santos, 2019; Jordan and Tomberlin, 2017). The activity of necrophagous insects can also influence the predictable succession of necrobial populations during decomposition. Microbial succession during cadaveric decomposition can be determined independent of the presence or absence of necrophagous insects (Guo *et al.*, 2016). The soil is also a factor influencing decomposition through interaction of its microorganisms with the corpse (Metcalf *et al.*, 2016b).

The composition and diversity of the gut microbiota is closely related to diet and different dietary habits. The association of microbial populations with a high-fat diet differs from that with a high-fiber diet (Wu *et al.*, 2011; Senghor *et al.*, 2018). This difference was demonstrated in a comparative study of animal and plant-based diets (David *et al.*, 2014). However, the high microbial diversity of populations from different countries cannot be explained by diet alone (Nishijima *et al.*, 2016). Host genetics, lifestyle, altitude, and subsistence strategies are also involved (Nam *et al.*, 2011; Schnorr *et al.*, 2014; Das *et al.*, 2018; Jha *et al.*, 2018). The prolonged use of antibiotics also alters the gut microflora, as it makes the host vulnerable to further infections (Norén, 2010) and promotes the proliferation of resistant bacteria in the intestines (Keeney *et al.*, 2014; Nogueira *et al.*, 2019). These factors should be considered when searching for thanatomicrobiome signatures. Further work on human subjects is therefore needed to better understand the interactions between these factors and post-mortem microbial communities in order to define the microbial clock (Metcalf *et al.*, 2016 a, b).

THANATOMICROBIOME AND POST- MORTEM INTERVAL (PMI) ESTIMATION

Post-mortem microbiome analysis suggests that the evolution of bacterial communities after death may reveal important biomarkers that are useful in determining the time of death (Bell *et al.*, 2018; Ventura Spagnolo *et al.*, 2019). A positive correlation between post-mortem microbial changes and time was observed (Pechal *et al.*, 2018; Deel *et al.*, 2020). Several recent studies have monitored changes in the thanatomicrobiome and necrobiome communities during cadaveric decomposition for the estimation of PMI (Metcalf *et al.*, 2013; Damann *et al.*, 2015; Javan *et al.*, 2016 a, b; Adserias *et al.*, 2017 a, b; Lutz *et al.*, 2020). The post-mortem microbiome is influenced by changes generated by decomposition products, resulting in variations in the bacterial communities of the cadaver, despite the fact that the body's internal organs are not directly exposed to external environmental conditions during the early stages of decomposition (Javan *et al.*, 2019). The decay of human organs takes place in a different order of increase. The stomach, intestine, liver, and pancreas undergo decomposition first, followed by tendons, bones, and the nulligravid uterus (Javan *et al.*, 2016 b). Therefore, the intestinal bacterial community could provide better indices for determination of PMI (Javan *et al.*, 2019).

To determine quantifiable time-dependent variations in the gut microflora post- mortem, a quantitative PCR (qPCR) study targeting human large intestine microflora found that the two genera of Bacteroides and Lactobacillus decreased exponentially with time. In contrast, the third genus Bifidobacterium showed no significant trend. Therefore, the abundances of these two bacterial genera may be useful as quantitative indicators when estimating PMI (Hauther *et al.*, 2015). Another experiment performed on decomposing cadavers under natural environmental conditions showed that the composition of the gut flora communities changes similarly over time toward a common decomposition community, in which Bacteroides and Parabacteroides decreased while Clostridium and Anaerosphaera, as well as Ignatzschineria and Wohlfahrtiimonas, increased significantly (DeBruyn and Hauther, 2017).

In the study by Tuomisto *et al.* (2013), carried out on blood, liver, portal vein and mesenteric lymph node samples from 33 cadavers using bacterial culture and quantitative reverse transcriptase PCR (RT- qPCR) techniques to profile post-mortem microbial populations, 21 genera of bacteria were identified. The most abundant species were Staphylococcus sp., Streptococcus sp., Clostridium sp., Enterococcus sp., and Escherichia sp. Since human internal organs such as heart, liver, spleen, brain, and blood are sterile in healthy adult individuals, Can *et al.* (2014) examined the microbial communities from these organs in human cadavers. They found similar microbial populations in these organs from the same corpse, but variations among corpses that. These variations may be related to differences in PMI and/or external factors. Thanatomic analyses of the organs of 11 corpses within an interval of 20h to 240h have identified facultative anaerobes, such as Lactobacillus, predominant when the PMI is short (29.5h), whereas at longer PMI (240h), obligate anaerobes (such as Clostridium) are predominant. Javan *et al.* (2016 b) observed substantial changes in the thanatomicrobiome communities of the brain, heart, liver, spleen, oral cavity, and

blood over time in 27 human cadavers with known PMI. These organ-specific bacterial signatures can potentially be useful in estimating PMI. In 2017, Javan *et al.* also demonstrated that the high numbers of putrefying microbes in the internal organs of rotting corpses are obligate anaerobes (Clostridium pp). These bacteria are considered to be the etiological agents responsible for fatal infections. Clostridium spp. were also found in abundance in post-mortem liver and spleen tissue from subjects who received drug overdoses (Brackett, 2018). The study of internal organ tissues from 40 Italian cadavers with PMIs ranging from 24 to 432 h established that during human decomposition, the uterus and prostate are the last internal organs to decompose. Both organs have significantly higher alpha diversity and significantly different microbial compositions compared to non-reproductive organs (Lutz *et al.*, 2020).

Because of its ongoing contact with the environment, the external microbiome of the skin and natural bodily orifices is frequently changing. In order to explore the postmortem microbiome involved in cadaveric decomposition, sampling was conducted at various body sites at the beginning and end of the bloat stage of two cadavers left to rot in natural conditions in the Southeast Texas Applied Forensic Science Facility. The pyrosequencing results of the 16S rRNA gene revealed a shift from aerobic to anaerobic bacteria in the same body sites sampled and a variation in community structure between the two bodies. These preliminary results demonstrated that oral cavity communities change during the pre-bloat and terminal bloat stages of decomposition (Hyde *et al.*, 2013). To assess the evolution of epinecrotic bacterial community succession over time, the same researchers conducted another study on two other human subjects. Samples were taken from the left internal oral region, left and right external cheeks, left and right external biceps, torso, and rectum. The analysis results showed that Acinetobacter of the class γ-Proteobacteria were abundant in both cadavers in the later stages of decomposition. Thus, for the skin, mouth, and rectum samples, microbial richness and composition were comparable with advancing decomposition (Hyde *et al.*, 2015). Another experiment conducted to combine the deep characterization of the epinecrotic community, carried out on two human cadavers, the one allowed to decompose in two different seasons (spring and winter), indicated a series of bacterial and fungal groupings that create a repeatable network on a predictable time scale, which can be used as a PMI estimator (Metcalf *et al.*, 2016b).

The analysis of resident surface communities in the nasal and auditory canals of cadavers led to the development of a k- nearest neighbor regression model, which could calculate true PMI at less than 55 ADD with precision. This makes skin microbiota a potential method in forensic death investigations (Johnson *et al.*, 2016). A study conducted on three cadavers, targeting the oral microbial populations, revealed different dynamics in microbial composition during cadaveric decomposition and showed similar overall successive changes.

During the early stages of decomposition, Firmicutes and Actinobacteria were predominant, while from the bloat stage Clostridiales and Bacillaceae were the main representatives of Firmicutes (Adserias-Garriga *et al.*, 2017b). Pechal *et al.* (2018), in their work on eye, ear, nose, mouth, and rectum samples from 188 individuals, demonstrated that predictive models for determining PMI in a time interval of less than or more than two days could be used from composition and functional profiles in the context of each sample location. Taken together, these results offer a better explanation of the dynamics of the human microbiota after death.

Necrobiome communities isolated from soil in contact with a rotting corpse are also recognized as potential tool to determinate time since death. Analyses of soil in contact with 18 rotting corpses revealed increased taxon richness in buried remains with decreased uniformity and consistent diversity, while surface bodies showed a decrease in all parameters. This indicates that burial depth is important and can influence composition of the community of necrobiomes, and therefore estimation of PMI (Finley *et al.*, 2016). Similar results were found by Adserias-Garriga *et al.* (2017 a) in their studies using soil samples from three cadavers to identify the soil microbial communities involved in human decomposition; proteobacteria and bacteroids were dominant within the first 6 days of decomposition. Beyond day 6, they found a sudden increase in firmicutes, in parallel with a decrease in proteobacteria. Therefore, the growth curve of firmicutes collected from human remains can be used to estimate PMI. The similarity of bacterial succession through the different stages of human decomposition is considered as an interesting finding, as it reflects the possibility of applying the post-mortem microbiome in estimating PMI.

THANATOMICROBIOME AND PREDICTION OF THE CAUSE OF DEATH

Post-mortem microbiological examinations are performed in forensic pathology to identify cause of death in unclear cases (Heimesaat *et al.*, 2012). Researchers have been able to find a link between isolated microbes and cause of death. Cultures from blood and internal organ taken during autopsies provide many answers in the forensic field, such as: confirmation of a diagnosis of ante-mortem infection; identification of the etiological agent of a previously undiagnosed infectious disease; determination of the efficacy of antimicrobial therapy (Ventura Spagnolo *et al.*, 2019). In general, the detection of a single mi-

crobial species in post-mortem body fluids suggests an infection during life, whereas mixed species profiles indicate post-mortem proliferation (Alan and Sarah, 2012). A specific microbial species may constitute evidence, helping to explain the circumstances of death and possibly determine the main cause of death (drowning; toxicology; nosocomial infections, and sudden infant death syndrome), acting as bio-indicators that could improve forensic analysis (Oliveira and Amorim, 2018).

Cases of drowning deaths can be problematic for investigators. Pathologists use diatom tests or blood strontium levels, but a level of uncertainty is recognized in these tests. Rutty *et al.* (2015) applied probes for the detection of bacterioplankton in 20 cadavers, where drowning was confirmed as the cause of death in 16 cases. Another study conducted on animals by Lee *et al.* (2017) found that, when compared to the closed organs of the post-mortem immersion group, the drowned rat group had a considerable aquatic microbial presence, with the predominance of seawater Proteobacteria, freshwater Bacteroidetes, Actinobacteria, and Bacillariophyta .

In patients with advanced liver disease, gut dysbiosis and bacterial translocation are frequent, and bacterial translocation across the epithelial barrier has been demonstrated to drive experimental liver disease progression. Increased intestinal permeability and bacterial translocation were detected in early stages of liver illness, independent of microbiome or endotoxin changes (Fouts *et al.*, 2012). A kinetic study of post-mortem microbiota changes revealed bacterial translocation of intestinal bacteria exiting to reach extra-intestinal compartments such as mesenteric lymph nodes, spleen, liver, kidneys, and cardiac blood as early as 5 min. after death. This explains that translocation of gut bacteria occurs within minutes of death, which allows differentiation between relevant bacteria and bacteria contaminants, and provides relevant information for routine forensic applications (Heimesaat *et al.*, 2012). In order to predict cause of death and trace origins associated with the geographical location of human remains in forensic investigations, Burcham *et al.* (2016) studied the post-mortem transmigration of Staphylococcus aureus and Clostridium perfringens during the decomposition of murines. Another study on 336 body fluid samples from 129 Korean autopsy cases, using bacterial culture and 16S rRNA identification with the matrix-assisted laser desorption/ionization-time-of-flight (MALDI- TOF) technique, showed a correlation between bacterial isolates and cause of death in six out of eight seawater drowning deaths. In another study, Enterococcus and Enterobacter were found in cardiac and peritoneal blood liquid from samples taken from a case of death from colonic perforation and cardiopulmonary arrest, indicating migration of enterobacterial communities from the colon (Na *et al.*, 2017). A large-scale investigation by Pechal *et al.* (2018) on death cases in urban and industrial populations demonstrated that these microbiomes reflected ante-mortem health conditions within 24-48 hours after death. Decreased phylogenetic diversity of microbial populations was one of the predictors of heart disease in the <24 h time interval. Investigations of qualitative and quantitative variations in thanatomicrobiome communities are very useful for the identification of existing pathological conditions of death.

Unexpected death of an apparently healthy newborn baby in the post-neonatal period, known as Sudden Infant Death Syndrome (SIDS), leaving the cause of death unexplained, became the subject of numerous judicial enquiries due to the possibility of crime in infants under one year of age (Duncan and Byard, 2018). D'Argenio *et al.* (2017) used a combination of laser microdissection and microbial typing based on a microbial signature to determine the cause of death of an 18th-century child. The cause of death was associated with osteomyelitis caused by Pseudomonas aeruginosa.

Many bacteria and viruses in autopsy samples can be identified using routine biochemical and molecular post-mortem techniques. However, comprehensive microbial profiling has not yet been applied in this field, and can provide significant disease diagnoses specific to etiology and autopsy findings (Roy *et al.*, 2021). Thus, in criminal investigations, ante mortem clinical information is particularly useful in determining the cause of death due to infection and in excluding other causes.

CONCLUSION

Post-death decay is a dynamic process, influenced by many intrinsic and extrinsic factors that can provide valuable information about the date and circumstances of death. New techniques based on the analysis of the thanatomicrobiome and the epinecrobiome make it possible, by understanding community variations, to estimate PMI and provide possible explanations for causes of death.

Conflicts of interest

None

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