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Review article

Estimating the postmortem interval using microbes: Knowledge gaps and a path to technology adoption

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ABSTRACT

Microbes have potential to be used as physical evidence for forensic science because they are ubiquitous and have predictable ecologies. With the advent of next generation sequencing technology and the subsequent boost to microbiome science (study of the genes and molecules of microbial communities), it has become possible to develop new microbial-based tools for forensic science. One promising approach is the use of microbial succession during the ecological process of decomposition to estimate the time since death, or postmortem interval (PMI). This microbial clock of death is developed by building a regression model using microbiome data collected from postmortem samples (e.g. swab of skin) with known PMIs. In a death investigation, a similar sample type (e.g. swab of skin) would be collected, the microbes profiled using DNA sequencing, and the microbes would be matched to a point on the clock (i.e. the regression model). Recent research by several independent scientific teams has provided a proof of concept for this new microbiome forensic tool. However, developing and transitioning new forensic science technologies into the justice system requires overcoming scientific, investigative, and legal hurdles. In this article, I address the apparent knowledge gaps in the science of microbiome technology to estimate PMI, and discuss a path for bringing this technology into the justice system.

1. Introduction

For unattended death scenes, determining the time since death, also known as the postmortem interval (PMI), is important because it can help with reconstructing death scenes, issuing of death certificates, and the distribution of assets defined in wills. It is also one of the most commonly requested pieces of information by next of kin (C. Carter, personal communication, [1]). PMI can be estimated via a range of methods, including those associated with last communications (cell phone activity, electronic or hard copy communications, visual sightings, etc) as well as biological clues associated with the human body and surrounding environment (rigor mortis, lividity, insect activity, etc). Each method can potentially contribute important information to an investigation. However, each method is limited in its applicability and accuracy depending on the conditions and timeframe of PMI (e.g. days, weeks, months) of a given case.

Biological indicators of PMI are generally associated with the cadaver decomposition process, which can be classified into several major, and sometimes co-occurring, stages progressing from a wet to dry environment [2,3]. Payne [4] describes cadaver decomposition in six stages, which include fresh, bloated, active decay, advanced decay, dry, and remains. Megyesi et al. [2] defined 4 stages of decomposition, which include fresh, early decomposition, advanced decomposition, and skeletonization. Decomposition is a continuous process, so these stages are arbitrary, but provide a useful way to organize significant taphonomic landmarks during decomposition. During the fresh stage, major landmarks include rigor mortis (stiffening of muscles), livor mortis (blood pooling), algor mortis (body temperature equilibrating with environment), and appearance of blow fly eggs [5]. During the stages of bloated/early decomposition/active decay, fermentation and proteolysis [6] lead to changes in skin color, gas builds up from microbial fermentation, fluids purge/leak due to bloat pressure, and blow fly larvae hatch and form maggot masses [2,3,5,7]. At advanced decay/decomposition, the post-purge cadaver is characterized by sunken flesh [2,4]. In the final stages of dry, remains, and skeletonization, soft tissues are dry or no longer present, and insect and scavenging activity is low [2,4]. A comprehensive review of human decomposition taphonomic landmarks can be found in Deel et al. [5].

Some of these landmarks, such as rigor mortis and blowfly larvae development, can be used to estimate PMI. Rigor mortis, the stiffening of muscles, usually begins hours after death and can persists until ~1-3 days postmortem, although temperature can influence these timeframes and introduce error [6]. If a body is accessible to insects, blow flies will lay eggs on the cadaver, which will develop into maggots and eventually adult blowflies. A growth curve based on temperature can be developed and used to estimate PMI based on the lifestage of the oldest

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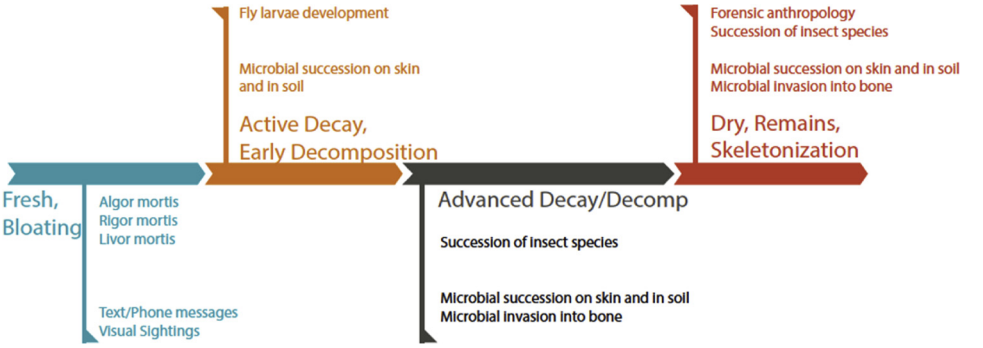

Fig. 1. Overview of the forensic tools available throughout the timeline of human decomposition. A variety of sources of physical evidence are available at fresh (algor, rigor, or livor mortis of the body, as well as last communications and visual sightings), early decomposition and active decay through advanced decay/decomposition (fly larvae development, succession of insect species, microbial succession associated with skin, bone, and soil), and dry, remains/skeletonization (forensic anthropology, microbial succession associated with skin, bone, and soil).

maggots [8]. This approach is generally useful over the initial 2 weeks of decomposition (depending on temperature), up until the first maggots become adult blowflies and leave the body. In cases in which robust growth curves for larvae development exist, accuracy can be within hours of the PMI [9]. However, this approach can be limited in indoor environments that exclude insects and seasons with low insect activity, and errors can be introduced due to misinterpretation of maggots as the oldest cohort when older cohorts have completed their lifecycle [5,10]. For a full review of biological approaches used to predict PMI, see Deel et al. [5]. Because each approach is generally most accurate for a particular time frame (Fig. 1), and is vulnerable to uncertainties, the field of forensic science will benefit from new techniques that complement existing ones.

A relatively new approach for estimating PMI that has been explored by several independent research groups is the use of cadaver-associated microbial communities to estimate PMI. The ecological hypothesis that underpins this method is that diverse suites of microorganisms driving mammalian decomposition are similar and repeatable across individual hosts and environments, at least to some extent. Because mammalian cadavers are an important, concentrated source of nutrients in an ecosystem, it is likely that communities of microbes have evolved over hundreds of millions of years to efficiently recycle these nutrients. Therefore, mammalian decomposition may include a predictable succession of microbes that reflects the different stages of metabolic function and pathways in decomposition. Indeed, several studies have utilized microbiome data (genomic content of microbial communities) to demonstrate repeatable shifts in microbial community composition during terrestrial mammalian decomposition associated with cadaver skin [11–14], gastrointestinal/rectal locations [11–13,15–17], oral sites [13,14,17], nasal, eyes, and ear cavities [17,18], internal organs [19], bone [20], and cadaver-associated soils [11,12,21,22]. Several studies with sufficient power have employed machine learning methods to create regression models that can be used to estimate the PMI of samples with unknown PMIs [12,17,18]. These microbiome-based models have provided accurate PMI estimates (as low as +/- 2–3 days) during some PMI ranges, and quantifiable error rates [12,18].

Machine learning is a powerful tool for discovering patterns in complex data, and it is commonly applied to microbiome datasets because they are large (hundreds of thousands of DNA sequences) and complex (DNA sequences represent thousands of different types of microbes). Amplicon sequencing data sets of taxonomically informative, universal genes (16S rRNA, 18S rRNA, ITS gene region) provide a snapshot of the microbial taxa in a sample, and are useful for creating predictive models using machine learning approaches. In order to build a robust model to estimate PMI, a time series of samples (hundreds to thousands of samples) with known PMIs are required. Additionally, environmental data such as temperature can be included in the model in a similar way to the relative abundance of microbial taxa (called “features”), and subsequently tested to assess whether these additional data improve the model.

Though research has provided a proof of concept for using microbiome data to estimate PMI in terrestrial ecosystems, there are a number of important knowledge gaps remaining. We do not know for which time frame(s) the microbial clock will be the most accurate or useful to forensic investigators. We do not know which environmental variables will improve the accuracy of the models. We do not know which sample types or body locations will host the most accurate succession of microbes. Finally, we do not know which machine learning approaches and parameters will result in robust models. Furthermore, it is important to consider the process of technology adoption in the justice system for this new method.

In this perspectives article, I outline scientific knowledge gaps that should be addressed by additional research and highlight the process for adoption of this new technology by the forensic science community and the justice system.

2. Knowledge gaps

2.1. For which time frames are microbes informative for predicting the postmortem interval?

Decomposer microbial communities should provide the most accurate estimates of PMI during timeframes in which microbial succession is rapid (measurable community shifts) and non-stochastic (predictable). This is likely during the early/active decay stage of decomposition, when the bulk of the cadaver nutrient pulse is processed by microbes [12]. Testing this hypothesis would require a fairly large data set with frequent (e.g. daily) sampling for a long period of time (depending on temperature, weeks to months). However, it is important not only to consider when this technique is the most accurate, but also when it might best complement or fill in gaps of existing forensic tools (Fig. 1). For example, microbial-based estimates of PMI may be most useful in death investigations during timeframes in which other forensic tools are limited or less accurate. In the sections below, I summarize the current state and gaps in knowledge for fresh, early decomposition/active decay, advanced decay/decomposition, dry, and remains/skeletonization stages of decomposition.

2.1.1. Fresh

Human skin microbiomes are personalized, or unique to each person, to the extent that they can be used to link people to the objects they have touched or spaces that they have occupied [23–26]. After death, these personalized skin microbial communities are overwritten by a succession of microbes that thrive on nutrient-rich cadaver resources. The timeframe in which the succession of decomposer microbes begins to erase the personalized signatures of the human skin microbiome is unknown, although mounting evidence suggests that it is after the first 48 h after death, at least in most indoor death scenarios [17,27]. However, most studies that investigate postmortem skin microbial succession for estimating PMI use donor bodies placed outdoors, which likely results in a different community of decomposer microbes

and more rapid decomposition [28]. If personalized skin microbiomes disappear more quickly in outdoor environments, a microbial clock of decomposition may be useful earlier than 48 h for this environment. Furthermore, although a number of forensic tools exist in the initial 48 h after death (Fig. 1), most death scenes are discovered in this timeframe so additional robust, complementary tools could be useful.

2.1.2. Early decomposition/active decay through advanced decay

Most research investigating the use of microbiome tools for estimating PMI has included experiments that span active and advanced decay stages of decomposition with sampling frequency ranging from daily to every 3 days over ~14-48 days [11,12,18]. This time frame is useful for leveraging the ecology of microbial succession because community turnover is rapid and appears to be a fairly generalizable process - similar groups of microbes becoming abundant in a successive pattern that is related to time, temperature, and possibly other variables [12]. Accuracies (or error rates) of models predicting PMI using microbiome data have ranged between +/- 2–5 days over 25–48 days of decomposition [12,18]. The frequency of sampling likely limits our knowledge of the true accuracy since the model accuracy cannot exceed the frequency of sampling used to develop it. Therefore, accuracy for this timeframe remains a knowledge gap.

Because active and advanced decay are driven by biological and biochemical processes, forensic scientists have developed a number of tools for PMI estimation during these time frames. One well established tool is the use of insects as an indicator of PMI, as discussed above. Forensic entomology has established itself as a reliable means to estimate the minimum postmortem interval in a range of temperatures [29,30]. However, as with any approach, forensic entomology has its limitations. For example, in the case of blow fly larvae development, there is uncertainty in the arrival time of the first flies laying eggs [8]. Therefore, complementary approaches such as the use of microbes as temporal evidence is valuable because microbes may remain active at temperatures too low for insects.

2.1.3. Dry, skeletonization/remains

Although most unattended death scenes are discovered in the first several days, some of the most challenging cases to solve are those in which the body is in extended decomposition [20]. Therefore, this time frame is rich with opportunity to discover new tools for forensic science. Microbial community succession associated with cadavers in late stages of decomposition has only been studied in a few cases. In Metcalf et al. [12], the skin and soil associated with human cadavers was investigated by sequencing the 16S rRNA (bacterial community), 18S rRNA (microbial eukaryotic community), and ITS (fungal community) gene or gene spacer regions. It was discovered that even in the extended postmortem period, PMI could be predicted with errors as little as +/- 5–7 days over the first 50 days of decomposition [31]. Two additional studies investigated microbial succession in soils over several months, and distinct microbial community compositions were characterized for each stage of decomposition [21,22]. In addition to skin and soil, the invasion of microbes into bone may provide a slower and extended microbial clock of death, a tool initially investigated with microbiome methods by Damann et al. [20]. A proof of concept for the use of microbes during late stages of decomposition are promising, but a more focused study of this time frame with more frequent sampling and larger sample sizes is needed before machine learning techniques can be robustly applied to assess error rates.

2.1.4. Which environmental variables need to be included in microbial models predicting PMI?

Environmental variables such as temperature [12,14,15,18,32] and the presence of soil [28] can influence the rate of decomposition, and thus microbial succession. Other variables such as mass of cadaver [33] and soil type [12] have been shown to have an insignificant or lesser effect on microbial decomposer communities, although these results should be confirmed with additional studies. If environmental parameters that influence microbial succession can be measured and recorded, they can be included as a feature in the PMI regression model, which should make the model more generalizable across environments. Currently, the only environmental feature that has been incorporated into a microbial decomposition regression model is temperature [12,18,31]. In Metcalf et al. [12], including temperature in the model allowed for a level of prediction accuracy that was better than random when generalizing the PMI model across two seasons in the same environment. Therefore, other environmental variables such as oxygen, humidity, precipitation, and the presence or absence of insects should be tested as important features in future studies. A study is underway to compare regression models developed using donor human cadavers placed at three anthropological research facilities in Tennessee, Texas, and Colorado. These models will test the importance of environmental parameters such as temperature, humidity, and precipitation and thereby help test whether a single, generalizable model can be developed, or whether models need to be trained within each geographic region to achieve high accuracy.

Another major knowledge gap is how different environments such as terrestrial, aquatic, and indoor/built environments affect the succession of microbes during mammalian decomposition. In a study of swine decomposition in freshwater, Benbow et al. [34] discovered a similar succession of microbes across individuals in two seasons. Similar to vertebrate decomposition in terrestrial systems (e.g [12].), early decomposer communities were dominated by bacteria in the phylum Proteobacteria and later decomposer communities were dominated by bacteria in the phylum Firmicutes [34]. Postmortem taxa appeared to be a mix of mammal-associated and water-associated organisms [34]. Furthermore, a study of swine cadavers in marine systems over two seasons revealed succession-like trends of postmortem microbes with many taxa being distinctly marine [35]. Therefore, it appears that estimating PMI using microbes in aquatic systems may be possible, but may include a different community of microbes than in terrestrial systems. It is unknown whether microbial succession during decomposition is predictable in built environments. A better fundamental understanding of the ecology of microbial succession across different environments will address these knowledge gaps.

Variables associated with the condition of the body itself, such as clothing, cause of death, and medication/drug history have not been tested. Thus far, proof of concept research on using microbiome methods to estimate PMI in mammals has generally included intact (non-autopsied, no puncture wounds or broken skin), unclothed cadavers. In studies using non-human mammalian cadavers, body condition has been highly controlled (e.g. age, diet, BMI, sex, living conditions, host genetics). This approach was critical for establishing whether the fundamental processes of mammalian decomposition included a predictable succession of microbes. In research using human donor bodies, the condition of donor bodies varies greatly (e.g. age, sex, BMI) with some of the variation unknown (e.g. medical and medication history). Despite the uncontrolled variation in human decomposition studies in outdoor scenarios, accurate microbially-based estimates of PMI have been demonstrated [12,18,31]. These studies have been critical to establish that the predictable succession of microbes after death is generalizable enough to overcome differences among body condition and personalized microbiomes during life. However, studies targeting particular body conditions (e.g. drug/medication, clothing) would potentially help improve model accuracies.

2.2. Which sample types and locations are the most informative for microbial clocks?

As a body is decomposing, microbial processes associated with decomposition are ubiquitous, but there is differentiation based on precise sampling location. Research has investigated microbial community change associated with mammalian cadaver skin [11–14],

gastrointestinal/rectal locations [11–13,15–17], oral sites [13,14,17], nasal, eyes, and ear cavities [17,18], internal organs [19], bone [20], and cadaver-associated soils [11,12,21,22]. Each of these locations has demonstrated promise, and one study comparing skin and soil did not find that one sample type was clearly superior to the other [31]. It may be that some sites will be more accurate for particular time frames of PMI (e.g. organs for shorter time frames, and bone for longer time frames). One consideration may be whether particular sample types are more accessible by death scene investigators, unlikely to be affected by the cause of death itself, or less invasive to the investigation. For example, skin is the largest organ of the human body and generally accessible without invasive sampling (e.g. swab of the skin) while gastrointestinal samples may be too invasive for some investigations. The most useful sample site(s) for estimating PMI using microbes remains a knowledge gap.

2.3. What are the most robust modeling methods and parameters?

There have been several data analysis approaches attempted for using microbiome data to estimate PMI. These include indicator species model-based analyses [14] and an exponential decay model based on declines in relative abundance of specific bacteria [15]. PMI estimates with the highest accuracies have used machine learning approaches [11,12,18,31], which utilize changes in relative abundance of all microorganisms in the entire community. The use of machine learning to build predictive regression models is promising, although due to the small number of data sets with sufficient sample size to power a robust model, knowledge gaps remain. Most importantly, we do not have well-established, general error rates for regression models estimating PMI using microbiome data sets, although we have several estimates from individual studies [11,12,18,31]. In a recent meta-analysis of several existing studies, Belk et al. (2018) found that skin and soil sample types provided similar estimates of PMI and that using the 16S rRNA genetic marker (compared to 18S rRNA and ITS gene region) with data summarized at the phylum level provided the most overall accurate estimates of PMI. For example, PMI error (in Accumulated Degree Day) was 48 ADD at the phylum level, 57 ADD at the class level, and 88.7 ADD at the sequence variant level. As more datasets are published, it will become clear if these results are generalizable. Furthermore, within the framework of machine learning approaches, there are a number of different regressors, including Random Forests [36], K-nearest neighbor [37,38], and elastic net [39], to name a few. A systematic comparison of machine learning approaches and parameters will be important for creating a robust model for estimating PMI, and establishing error rates.

The existing knowledge gaps for using microbes for estimating PMI are addressable and highlight the need for large-scale, coordinated projects that can include sufficient sample sizes to create models using a range of decomposition time frames, environmental variables, sampling locations, and regression models.

3. Technology adoption

The path to adopting new technologies into the justice system requires overcoming several hurdles, including identifying need, basic research, prototype development, validation and acceptance, and adoption (Fig. 2).

3.1. Need

Both research and forensic communities are important for identifying needs that new technologies may address (Fig. 2). When next-generation sequencing revolutionized the ability for microbial ecologists to rapidly and inexpensively characterize highly diverse microbial communities, new opportunities arose across scientific disciplines. Independent research groups in the fields of microbiome science, forensic microbiology, and forensic entomology recognized the potential for applying these new approaches to better understand decomposition ecology and its potential to develop a new tool for estimating PMI. For example, forensic microbiologists developed a workshop [40] to improve communication and understanding of needs within the research community. This effort has coincided with, and contributed to, the publication of two edited volumes [41,42]. A working group is currently in development to improve the communication and understanding of needs between the researcher and forensic communities, which will involve relevant personnel from microbiology, entomology, pathology, education, and jurisprudence. Furthermore, the medicolegal death investigation community has consistently expressed a need for reliable technology to estimate PMI across different scenes. Communication between research and medicolegal communities is critical to identify factors that will allow microbiome technologies to be adopted. For example, critical questions currently being addressed through this engagement are, “at what stage of the investigation should microbiome samples be collected? do microbiome samples need to be collected at the scene or can they be collected at autopsy?” [27]. The identification of these practical needs is critical for new science to be incorporated into the forensic workflow.

3.2. Research

Research, communication of findings, and the connection of researchers with the forensic community are critical for developing new technologies. Research generally begins with pilot studies that demonstrate feasibility and proof-of-concept of a new tool. This initial research and subsequent use of pilot study data to obtain grant funding for larger, more comprehensive research is essential to ensure that robust science is supporting the new technology. The use of microbiome data to estimate PMI was initially tested by several independent research groups that obtained funding (e.g. NIJ awards 2010-DN-BX-K243, 2011-DN-BX-K533, 2012-DN-BX-K023, 2014-DN-BX-K008, 2015-DN-BX-K016, 2016-DN-BX-4194) and published in peer-reviewed journals (e.g. [11,43,44]). To date, there are more than 10 peer-reviewed articles that support the science behind the use of microbiome data sets for estimating PMI [11,12,14–16,18,21,22,28,33,43,45], including several that provide preliminary error rates [11,12,18,31]. Furthermore, the use of microbiome data to estimate PMI has been published in two textbooks [41,42], and is being added to a third edition of *Microbial Forensics* [5] that is underway. Additionally, researchers have connected with the forensic community through numerous domestic and international presentations, workshops, and webinars [40,46–50]. By providing training for justice practitioners and law enforcement, future users of the technology will become informed about its utility and limitations. Additionally, research on PMI methods is being conducted in collaboration with the forensic community by working with forensic anthropology facilities and medical examiners to build robust datasets from which the justice system can benefit.

3.3. Prototype

The transition of a new technology into a cost-effective and robust protype that is useful to justice practitioners can be challenging. With genomic data types, technical and financial hurdles related to new sequencing technologies and bioinformatics requirements can result in new methods initially being restricted to particular laboratories or academic settings. Generating and analyzing microbiome data has become much more accessible, standardized, and less expensive over the past decade due to coordinated efforts such as the American Gut Project (http://humanfoodproject.com/americangut/; [51]), the Earth Microbiome Project (earthmicrobiome.org [52];) and the QIIME2 bioinformatics microbiome platform (https://qiime2.org/; [53], coupled with a rapid drop in DNA sequencing costs (https://www.genome.gov/27541954/dna-sequencing-costs-data/). The American Gut Project is a large-scale, citizen science project that required the development of a

Fig. 2. Transitioning new tools and processes from research into casework. An overview of the path to adoption of new technology into the United States justice system, including potential stakeholders and the role of researchers. The use of microbiome methods for estimating PMI is in the Research and Prototype development phases. *This graphic was developed by RTI International in operating the Forensic Technology Center of Excellence* http://www.forensiccoe.org/, *under Cooperative Agreement Award 2016-MU-BX-K110 from the U.S. Department of Justice, National Institute of Justice.*

deployable sampling kit and an automated, reproducible data analysis pipeline. The Earth Microbiome Project is a consortium of microbiome scientists that has worked to test and standardize data generation by developing, testing, and sharing protocols (http://www.earthmicrobiome.org/protocols-and-standards/), which are updated as improved methods are developed. The QIIME2 bioinformatics microbiome platform provides well-documented and supported analysis pipelines with features such as provenance tracking (all commands for a result are recorded), collaborative results sharing via a browser application (installation of QIIME2 not required), and frequent, inexpensive

training opportunities. Importantly, this analysis program has been developing machine learning analysis options relevant to estimating PMI. Additionally, academic and commercial microbiome sequencing service centers have become more common, removing the need to purchase expensive DNA sequencing equipment, with costs of amplicon sequencing of a DNA sample as low as $20 CDN or $15 USD (e.g. http://cgeb-imr.ca/pricing.html).

Even with these democratizing advances in the field of microbiome science, transitioning from academic lab research to a prototype useful for practitioners can be challenging. One significant hurdle is that the microbiome field evolves quickly with improved methods for both data generation and analysis published frequently, although this is true for genomic approaches in general [54]. Secondly, careful consideration will need to be taken on how to manage quality control. Microbiome data are notorious for being susceptible to batch effects and variation between laboratories, which has been studied extensively as part of the Microbiome Quality Control (MBQC) project [55]. It may be useful to include similar testing strategies as used in the MBQC project, such as standard blinded sample sets. Finally, it is important to consider the logistics of integrating microbiome sampling, data generation, and data analysis into the investigative community and crime laboratory infrastructure. Sample collection is generally done with a sterile cotton-tipped swab, which should be familiar to investigators. However, the importance of freezing the sample at -20C or colder within a short time frame could be a hurdle. This is important because microbial communities can continue to change after collection [56]. For DNA extraction, crime laboratories are likely already equipped to extract DNA from human samples or bacterial cultures. However, considerations of mixing potentially high biomass swabs of decomposing cadavers with low biomass trace human samples or bacterial culture-based work would need to be considered. Therefore, it is possible that a new section or dedicated DNA extraction equipment may be recommended for microbiome lab work. Finally, computational resources and bioinformatics expertise may need to be expanded, although resources for analyzing a microbiome amplicon data set (e.g. 16S rRNA gene data) sequenced on a Miseq Illumina or similar platform is not expensive computationally (e.g. 2 CPU days). Furthermore, the price of both DNA sequencing and computation are likely to continue to decrease (https://www.genome.gov/sequencingcosts/).

3.4. Validate & accept

Before new technology is accepted by the forensic science and the justice system, the science is assessed through an initial validation phase, which includes end-users such as crime laboratories and medical examiner offices (Fig. 2). Although medical examiner offices are not yet validating microbiome tools for forensics, they are directly involved in research [17,27], which should facilitate the transition to validation. A connection between researchers and crime laboratories is a critical future step for the validation of this new technology.

Acceptance of new technology by the legal communities requires a judge to establish precedent under the Frye and Daubert standards. The Frye ruling (Frye v United States 1923) states that science must be accepted by its relevant scientific community to be admissible [57]. The Daubert ruling (Daubert v Merrell Dow Pharmaceutical 1993) expanded on the Frye ruling by allowing a judge to ask if the science and technology has been subjected to peer-review publication, has been tested, has an established/acceptable error rate, and whether research was conducted independent of a particular case [57]. For new technologies to clear these hurdles, a lawyer must present the method to a judge, who will determine whether it can be admissible as evidence. This has not yet been attempted for introducing microbiome data into the justice system.

Once a robust prototype is developed that has been validated by end-users, the forensic microbiome technology will be ready for validation and acceptance. It may be useful for researchers to connect with end-users and lawyers in geographic areas with forensic anthropological research facilities where research is being conducted. Because microbial clock models were generated on data from particular geographic areas (e.g. Knoxville, Tennessee; Huntsville, Texas; Grand Junction, CO), end-users and the legal community may be more likely to validate and accept this new tool than in other areas of the United States.

3.5. Adopt

The final important hurdle is to integrate the technology into the investigative and legal workflows. This includes adoption of the technology by law enforcement, crime laboratories, and the legal practitioners (Fig. 2). These groups will ultimately decide if the technology is useful, and if so, share findings via conferences, trainings, and lab accreditation.

4. Conclusion

In this perspectives article, I provide an overview of scientific knowledge gaps that should be addressed for a better understanding of limitations and best use of microbiome data for estimating PMI. Major knowledge gaps include the timeframe during which this tool is most accurate or useful to forensic investigators, which environmental variables will improve the accuracy of the PMI model, which body locations or sample types will provide the most accurate reconstruction of PMI, and which modeling approaches and parameters will build the most robust models. Because a number of independent research laboratories are simultaneously working to improve the science underlying microbial decomposition succession, these knowledge gaps are addressable in the near future.

I also provide an overview of a path for technology adoption for using microbiome data to predict PMI as a new investigative tool in forensic science. The path requires overcoming a number of hurdles including identifying need, basic research, prototype development, validation and acceptance, and adoption. The use of microbiome data to estimate PMI is in the research and prototype development stages. Once a robust prototype is developed, it will need to be validated by end-users and accepted by the legal community via a Frye and Daubert hearing. To facilitate this process, it is important for researchers to connect with members of the forensic science, investigative, and legal communities.

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Other links

www.jessicalmetcalf.com.

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