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A Review of Forensic Microbes in Crime Investigations

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Abstract:- Advances in genetic sequencing technology, enhanced microbiological sample methods, and rapidly developing approaches in bioinformatics have all contributed to the meteoric emergence of microbiomics and metagenomics. Humans harbour vast microbial communities, both internally and outside, that are constantly exchanging information with and shaping their environs. These interactions may be crucial to forensics because information about them can be gleaned from human and environmental microbial profiles. Over the reports of hundred studies, as metagenome markers in forensic science is an emerging field of forensic science, microbiomes has a greater chance of becoming a specific tool kit for forensic science to provide answers for personal identification, cause and manner of death, interpretation of PMI and geolocation of a suspect or accused. Although the application of microbiomics to forensics has been extensively studied, most of its potential remains untapped because of insufficient sample numbers, inaccurate models, and unrepresentative experimental conditions. Law enforcement agencies are unlikely to benefit from the limited microbiomics data that is currently available. However, efforts are being made to find solutions to these problems, and evidence gleaned from the microbiome may one day aid in forensic investigations. Until more is learned about microbiomics, it is unlikely that this information will be useful to law enforcement. However, efforts are being made to find solutions to these problems, and it is possible that evidence gleaned from the microbiome can one day aid in forensic investigations.

Keywords:- *Microbiomics, Forensic Science, Law enforcement, Microbiology.*

I. INTRODUCTION

Over the past century and a half, microbiology's contribution to forensic science has been minimal at best (1). Sequencing of virally amplified DNA was implemented to lend credence to allegations that a dentist at Florida in 1990, United States had infected a number of his patients with the Human Immunodeficiency Virus (2). These allegations were made in support of a lawsuit that was filed against the dentist. PCR-based bacterial genotyping was projected to be a helpful tool in future forensics due to the rapid growth of technology; for instance, it was speculated during the time, forensic science will be a emerge are for application of PCR-based genotyping (3). Substrate items might be linked to specific sites after the development of fungal and pollen spore examinations in the mid-1990s (4,5). However, the "scientific discipline dedicated to analysing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes" of microbial forensics did not emerge until the early 2000s in reaction to the recently emerging bioterrorism threat (6,7).

Historically, a lack of accessible and affordable sequencing technology has hampered microbiological forensics (8,9) limiting many forensic applications to single-taxonomic investigations. This strategy has shifted significantly over the past decade because to developments in genomic sequencing technology and new approaches for analysing complicated community datasets (and sometimes low biomass samples). The advancement of the microbial forensic toolbox (10,11) has been aided by the study of the microbiome (12, 13) and metagenomics.

The homicide conviction rate in England and Wales [United Kingdom] was 79% as of March 2019 (14), which is just slightly higher than the homicide conviction rate in the United States (70%) as of March 2019 (15). Conviction of criminals is often difficult due to insufficient evidences (16, 17, 18, 19), and false convictions are common worldwide. So far, 375 people in the United States have been exonerated by DNA testing, including 21 who spent time on death row, according to the Innocence Project, falsely convicted individuals were exonerated by national legal and public policy organization (20). As a result, there is a considerable push from public, Judicial systems, and lawmakers to incorporate molecular techniques into the forensic arsenal. There are many types of microorganisms in and around the human body, in the environment, and on objects linked to a crime (21, 22, 23). It's expected that the number of microbial cells to be more than or equal to the overall number of human somatic cells (22,24). Conventional investigation techniques is been suggested which include the potential use of forensically relevant microbial profiles. (25, 26, 27, 28) Computational tools, processing tools, and improved methodologies are now being developed in the field of bioinformatics. It's unclear how useful microbiomic techniques could be in forensics at this time due to the field's early stage of development (29,30) and its lack of funding in the past (31). In this article, we discuss the use of microbiomics in forensics thus far and its possible future uses.

Over thorough research, the topics of interest include personal identification, determination of biological sex, analysis of trace evidence, investigation of the method and cause of death, PMI and exploration of alternative applications such as localization using animal microbiomes are just some of the areas that could benefit from geolocation.

II. CONCEPTS FROM FORENSIC BIOLOGY

➢ *Geolocation*

In recent years, researchers have worked tirelessly to better define the microbiome of different ecosystems, with a focus on urban areas and public transport. These studies have shown that there may be different types of communities in different parts of a city, as well as "molecular echoes" of environmental events and even a forensic capacity for geospatial microbiomic data (32, 33, 34). Two primary facets of geolocalization will be discussed here.

➢ *Analysing the Substrate*

The forensic microbiology community is beginning to see the value of analyzing soil microbial profiles. Microbiomes in the rhizosphere and the bulk soil vary greatly from one location to the next. Thus, with better techniques, samples of soil microbes could yield valuable bio-geographic information to identify source of the soil samples. As another possible use, this may be implemented to learn more about the history of a criminal case's evidence.

Distance-decay effects were shown by (35) [25-1000 m] local scale connections between soil microbial samples [N = 5 sites, N = 2-4 soil types and 5 replications]. Findings demonstrated that the larger distance between samples, larger the variation in microbial community composition was, indicating that the key determinants in shaping microbial communities are soil type and geographic location. Indeed, it has been shown that patches can be discriminated using the soil microbiome (36, 37) successfully attributed 95.4% of soil bacterial profiles were able to be traced back to their respective geographic origins by utilizing analysis of similarity, non-metric multidimensional, abundance charts, and k-nearest neighbor. Yet, because this was only credibility study-the total number of participants in the sample was rather small (n = 19). Additional research is needed with larger sample sizes and additional replications in order to get a complete understanding of the possibilities of this strategy. Analyzing the microbial communities found in a variety of soil samples [including those taken at crime scene, an pretext site and any intermediary locations] may be beneficial to a forensics inquiry. Using Ribosomal Intergenic Spacer Analysis and 16S rRNA gene sequencing, researchers were able to accurately classify samples from a variety of soil substrates (38). There is new data to suggest that at fine scales, 18S rRNA gene sequencing can be even more selective than conventional Mid-Infrared spectroscopy for eukaryotic organisms (39). In addition, the soil samples retrieved from the shoes can help to determine the site of origin by comparing the denaturing gradient gel electrophoresis-created soil bacterial 16S rDNA profiles (40).

Despite the promising future of this sector, there are still obstacles to be overcome. For instance, the results of microbiomic investigations should be interpreted with caution due to a number of potential limiting factors described by Pasternak (41). Microbiomes display a high level of biological, physical and chemical variability in space and time, and soil samples are heterogenous and immensely complex, presenting a huge challenge to employing these in a forensic setting. Actinobacterial fingerprints differ greatly between summer and winter at the same locations, which suggests that there may be concerns that are time-related. This is proved by the fact that there is a correlation between time and actinobacterial fingerprints (42). According to Keet (43), the fact that the composition of the soil microbiome is susceptible to brought change on by abiotic soil circumstances and the patterns of plant communities offers a substantial obstacle to the dependability of the results.

Gravesoil and soil collected near the human and animal cadavers can be used for the examination of metagenomics in forensic investigations. Studied seasonal changes in microbial community composition in soils near swine carcasses (44). They showed that microbial communities altered in predictable ways after death, but that the impact of decomposition on soil microbial communities varied greatly according on the time of year. Forensic investigations may benefit from the authors' proposed use of the ecological succession of microbial communities, although more study of the impact of seasonality on decomposition is needed. The ordination graphs suggest a small sample size (n 10 per treatment), however this was not indicated in the paper. As a result, the findings should be taken with a grain of salt.

Variations in the thanatomicrobiome that occur daily in the soil were investigated as a potential tool for determining PMI (45). In order to demonstrate the everyday successional changes that occur in the soil around the cadavers, one male and two female cadavers were used. Fast development of firmicutes was experienced between the bloat stage to the advanced decay stage of corpus. These were used by scientists to predict the PMI in Tennessee's summer condition; they advocate for future research to validate their findings with a larger sample size and in a variety of climates.

While these findings don't prove anything new, they do support the conclusions drawn (46) after they assessed the microbial communities linked to Human cadavers buried in graves (n = 18). Researchers left the remains to decay for varying amounts of time (three days to three years), discovering that the relative abundance of Firmicutes in surface bodies increased over time (from tenths of a percent at the beginning of decomposition to forty percent at the end).

The effects of human cadaver decomposition on the composition of soil microorganisms were studied (47). Soil samples were collected from each geographical buffer [N = 14 for the 0 m, N = 17 for both the 1 & 5 m] and they found predictable reaction of evidence to dead body decay that differed depending on the distance from the buffer. There were no statistically significant variations between the compositions of the bacterial communities at 1 and 5 meters, but there were substantial differences between the communities at 0 meters and 5 meters (measured as beta diversity).

Bacterial alpha diversity was also found to be much lower in the 0 m samples, which the researchers attribute to the increased nutritional supply from the corpses. This research adds to the expanding corpus of spatial compositional expertise in forensic microbiome applications.

However, more study is needed to establish the sensitivity and reproducibility of results (48) before soil microbiome analysis may be employed in forensics. Spatial and Temporal dynamics of the microbiome can be learned by more microbiome surveys in order to create methods to avoid these mistakes in the future.

- *The Potential of Machine Learning Across Various Spatial Dimensions*

There has been an explosion in the number of techniques that have been created to sample and predict environmental microbiome profiles over a wide range of spatial dimensions and orientations (for example, between houses, cities, states, and elevations) in order to trace the origins of people and things.

In spite of the fact that this study was conducted with a sample size [n = 3 for each office], the results of the analysis of office microbiome samples utilising sampling plates indicate that there is potential for further improvement (49). The inhabitants of households (n = 1625 from 18 participants in 10 residences) as well as the built environment that they lived in were investigated (50). While the authors' 82.9% success rate in matching foot microbiome samples to homes is low from an evidentiary standpoint, it does show the promise of these technologies for pinpoint bio-localization. Microbiota samples from 12 cities in 7 countries were whole-genome sequenced as part of 2018 CAMDA Meta SUB Forensic Challenge, and analysed the results (51). There were 30 total samples in the CAMDA dataset, and 3 of them were hidden. The authors used machine learning methods to determine where the microbiome samples had been collected. Despite the promising results of applying machine learning to biogeography (up to 90% accuracy), more proof is needed before these methods can be used in a legally binding setting. Ryan (2019) utilised a random forest classifier constructed from 311 city microbiome samples in a similar investigation (52). They were able to correctly categorize 83.3% of the mystery samples using their method. Using a novel approach that is based on deep neural network classifiers (53), researchers in the United States were able to improve their ability to predict the geo-site samples of fungus taken from dust (N = 1300). American researchers forecast geographical distribution of fungal samples extracted from dust (N = 1300) was enhanced. According to the authors, their algorithms can produce "good point predictions" on a worldwide dataset comprising samples from 28 countries; in a study covering the entire United States, over 50% of the geographic information errors fell within a hundred kilometers, and over 90% of the samples were correctly classified based on their origin.

The combination of machine learning and microbiomics is still in its early stages; for applying these methods in a forensic context, more research is needed to increase the sample size and improve classification accuracy.

The fact that microbiome compositions vary vertically as well as horizontally is a further relevant geographical consideration. Humans at high and low elevations have been found to have distinctive skin microbiomes. Human (n = 99) and swine (n = 82) skin microbiome samples were collected in Tibet (54). Several bacterial taxa (including Carnobacterium sp., Arthrobacter sp., and Paenibacillus sp.) were found to be enriched in samples taken at higher elevations. The alpha diversity of the people samples from skin who are living at higher elevations was similarly much lower. This hints at the possibility of using microbial samples for the analysis from the skin as a future method for determining geolocation based on altitudinal parameters; however, further methodological improvement will be required in this area, as well. It will also be important to think about how different skin microbiomes may change at different stages of life.

In general, classification is emphasized above prediction in all of the models. To allow simultaneous estimation of geographic coordinates from sampling data larger geographical and temporal sample sizes are needed, as are more rigorous approaches.

➢ *Individual Authentication*

There is mounting evidence that people can be distinguished from one another using only the microbes that are autochthonous, or naturally present in their surroundings. This has the potential to significantly affect forensic science, for instance in cases where the investigator is unable to collect enough human DNA. However, it is still unclear if the diversity of microbes present in the human body is high enough or stable enough to identify individuals within vast populations or to locate them through time.

Franzosa attempted to answer some of these problems by testing specific microbial profiles, matching with 25-105 microbial initial profiles and sampling site visits were repeated (55). According to scientists, profiles are let them distinguish individuals at the time of the initial sampling, and they were able to uniquely identify 30% of the individuals many months later. Eighty percent of participants (n = 120) were correctly located using gut microbiota samples. after a year, now. Although promising, especially on shorter time scales, the results are still plagued by considerable variation. As a result, more development is required, for example in methodology and sample effort, before such technologies may be beneficial in a forensic context.

Despite the limited sample size (n = 5), high-determination melting examination of 16S rRNA gene samples of oral swabs was used to show its possibility of discriminating among individuals (56).

Individuals' accurate identification (n = 12) using skin samples of swab from various body regions (n = 14) given by Schmedes et al. (2018). Using nearest-1 neighbor classification on the bacterial genome based on nucleotide diversity, they were able to achieve 96% accuracy by sampling palms and 97% accuracy by sampling shirts. In another recent study, the same method was used to track down participants (n = 51). Examined microbiome samples taken from the manubrium (the uppermost section of the sternum), the ball of the foot, and the palmar surface of the hand (57). When using a maximum nearest neighbor distance for diversity the researchers reached perfect classification accuracy, indicating promising future work if the results can be replicated in investigations with bigger sample sizes.

Minor taxa were proposed as a crucial component in differentiating individuals (58). Microbiome samples (n = 66) from 11 participants were analysed during a 2-year period, and 85% of the participants could be correctly identified. They also applied the same analysis techniques to the 89 individual skin microbiome samples that were made accessible to the public, correctly identifying 78 percent of them.

However, for forensic uses, this precision is probably not adequate. The scientists hypothesized that individual identification is feasible, but that the estimation accuracy drops off significantly for bigger cohorts as the prevalence of individuals with strikingly similar microbiome patterns increases. Microbiomics has the potential and technological viability to be used as a forensic tool for determining personal identification in cases the investigator is unable to extract significant quantity of DNA from human. However, there is not enough evidence to support the conclusions. The sensitivity and specificity of model's must be enhanced and a strategy must be developed to deal with the possibility of contamination. For the results to be useful in forensics, one must also get a deeper comprehension of the temporal and spatial dynamics of microbes.

➢ *The Role of Biology in Defining Gender*

In cases where large amounts of human DNA cannot be recovered, microbiomics may be able to help with personal identification by allowing for the differentiation of biological sex. There has been prior work characterizing the populations of airborne bacteria in indoor settings (59). The "aerobiome" was studied (60), who collected samples of airborne bacteria and fungi from 91 student dorm rooms across campus. Using machine learning methods, they were able to determine, with 79% accuracy, whose gender the room's inhabitants belonged to based on the ratio of their microbiota. Strangely, the microbial quantity was greatest in settings where males dominated. The authors postulated that this could be because men utilise less barriers against the elements, both natural and artificial, and because they shed more biological particles.

Microbial communities of the human thanatomicrobiome after the death they colonise the organs [thanatos, Greek for "death"](61), has been shown to have sex-related biological variations (62).

Using the V1-2 and V4 sections of the 16S rRNA gene, the authors analysed amplicon signatures in cardiac tissue from 10 deceased individuals and found significant variations among males, N = 6 and females, N = 4. Streptococcus sp was detected only in cardiac tissues of male whereas Pseudomonas species was far more common in females. A more refined version of this method might be used to establish the biological sex of a deceased person's body and their place of origin for various body parts.

According to the investigation of their pubic hair microbiomes, "readily differentiated " male [n = 3] and female [n = 4]participants. They discovered that the female participants had their own strains of Lactobacillus spp (63).

They also mentioned that pubic hair may be beneficial in forensics since it is protected from the elements and colonized by niche-specific bacteria. Sample size being small in this study makes it critical to draw any conclusions. Although this study was tiny, it was validated by another that used microbiota from pubic hair to determine the sex of individuals [n = 9]with ratio error of 0.027 0.058 and 0.017, 0.052 respectively (64). All of these investigations have small sample sizes, therefore more extensive validation studies are required before any firm conclusions can be formed.

Interestingly, discovered that the lack of the bacterium species Alloiococcus in microbiome samples of skin from both male and female [n = 45] could be effective in differentiating female biological sex (65). Certain bacterial species were found to be associated with individual traits (such as biological sex) in the study. Using leave-one-out cross-validation analysis to find out the microbiota from fingermarks if present or absent which were left behind on surfaces, they obtained a comparatively low accuracy in sex prediction of 67%. Further investigation of this strategy's potential requires larger sample sets and more precise machine learning. It is unclear whether particular strains of bacteria and other microorganisms are unique to women or whether differences are due to environmental factors (such as the use of cosmetic items on the hands).

Fig 1 Possible Sources of Forensically Relevant Microbiomes

III. OVERVIEW OF POSSIBLE SOURCES OF FORENSICALLY RELEVANT MICROBIOMES

- *Scanty Proof*

The study of microbiological profiles that are significant to forensics and left behind on items and surfaces is gaining popularity. Example: a number of research (66, 67, 68, 69) have demonstrated the prevalence of germs on common personal items like cell phones. In addition, it has been proven that human-associated objects like shoes and mobile phones support unique microbiomes (70, 71). Mobile phones have been studied (72), who looked into their potential as "personal microbiome sensors." Seventeen people were chosen, and samples of the touch screen, index finger, and thumb were taken. They showed that a person's mobile phone included a bacterial community that was more homogeneous with its owner than with anybody else's. A total of 22% among the detected taxa on fingertips of the participants were also present on their phones, while only 17% were common to all participants' phones. About five percent more OTUs shared between a index finger of the persons and their specific smart phones compared to all the other phones in the study combined. And between the index finger and the phone, 82% of OTUs were exchanged. While these findings are encouraging, more research is needed to ensure their validity.

It was discovered that postmortem skin microbiomes were strongly correlated with specific belongings. Medical equipment, eyeglasses, bottles, and coxswaining with 100% accuracy, while other objects like computers, there was an association between skin microbiota samples and cell phones and remote controls with an accuracy of over 67%, which suggests that, with some additional work, samples of microbiome of skin might be linked consistently to things near the scene. Up to 60 hours after death, the skin's microbiome has been shown to remain stable and mostly unchanged from premortem levels (73).

The microbial composition of various physiological fluids (n = 22) was studied. Different body fluids were tested, and source-specific microbiological signatures were discovered. The phylum Proteobacteria, for instance, has been linked to skin and semen sources, while the phylum Firmicutes has been found to be more common among saliva and vaginal secretions (74). Dobay and his associates demonstrate that the samples harbour specific body site microbial fingerprints even after exposure to indoor environments for a period of 30 days (75). Hanssen et al. showed excellent findings for the microbially mediated categorization of bodily fluids albeit having a limited sample size [n = 6]. They were successful in classifying 94 percent of saliva samples after performing recognition of the pattern with Principal Component Scores using a linear discriminant analysis model (76).

Recent research looked into the possibility of allochthonous microbiota (skin microbiomes from different people) being transferred between people and surfaces (77). They discovered that physical contact, such as when people shake hands, reliably transfers skin microbes. Microbiota can also spread through indirect touch, as seen when one person rubs a substrate and then exchanges it with another.

The authors claimed that this type of study could be helpful in providing further evidence of sexual assault and other crimes involving physical touch. In addition, they recommended that researchers investigate the effects of contact pressure, friction, and time.

➢ *Cause and Method of Mortality*

A death's "manner" is determined by an investigator. Natural, accidental, suicide, homicide, and unexplained are the five most common causes of death classified by medical science (78). Recently, microbiome samples from 265 decedents in the United States, Finland, and Italy (79). The causes of death among the examined bodies ranged from accidental [N = 88], to natural [N = 106], to homicidal [N = 23], and to suicidal [N = 45]. According to findings by them, several types of death were linked to the bacterial families belonging to Lactobacillus, Enterobacteriaceae, Sediminibacterium, and Rhizobiales.

Upon further research, these associations have the potential to be transformed into predictive markers that contribute to the process of determining the cause of death. The authors emphasize that Rhizobiales bacteria and Sedimini bacterium may signify pollution of the environment which has to be regulated. An additional validation approach towards improving the reliability through controlled experiments is required to establish the mode of death.

A recent study discovered distinct biomarkers that were connected with the mode of death. Hospital-related deaths were more likely to involve Xanthomonadaceae, while suicides were more likely to involve Actinomyces sp. In most cases, model accuracy improved as sample sizes grew larger (80).

The scientists noted that the number of anatomical sites analyzed and the machine learning algorithms employed both contribute to the prediction accuracy. According to the authors, this research provides essential groundwork for future efforts to include the machine learning process to create databases for reference that enable forecasts of deaths caused by microbes.

Kaszubinski et al. for postmortem cases used the data of microbes [n = 188 five body sites per case] to predict beta-dispersion and test for the association between method of death and cause of death. The study's authors proved that beta-dispersion and demographic information may differentiate between causes of mortality (81). In example, they discovered that 79% of deaths caused by cardiovascular illness or drugs were properly categorized. They concluded that models based on binary logistic regression were the most useful for boosting model performance.Manner and cause of death assessment was confirmed at the time by 88% which is a significant increase above the 62% accuracy achieved by multinomial logistic regression models.

This study demonstrates the potential of postmortem microbiomes as indicators of cause of death. Researchers stress the importance of larger samples. To train models with high success rates before they can be utilized in practical forensic scenarios, it is anticipated that enormous databases will need to be developed.

Researchers have looked into the significance of microbiological testing in defining the reason for death i.e., sickness or physiological disruption due to injury in the body leading to death. According to the author's study of autopsy data (n = 42), microbiological analysis was successful in determining the cause of death in 42% of the instances (82).

Increased CRP levels, for example, were singled out as a possible indicator of death due to microbiological causes in the study. Both sudden infant death syndrome (SIDS) and astronauts returning from space have been linked to increased CRP levels (83, 84, 85). In addition, newborn circumcision has been linked to potentially fatal bacterial illnesses such as infection and sepsis (86, 87).

An example of a forensic microbiome application that can be used to determine the reason for death is the analysis of ‘death using drowning’ which is ranked consistently among chief causes of fatalities which are unusual around the world (88, 89). The study of diatoms, which are single-celled algae and considered as the ‘gold standard’ for the era, despite the fact that their accuracy has been questioned (90, 91).

Several studies have used real-time polymerase chain reaction (PCR) as a primer test to distinguish bacterial species related with aquatic habitats i.e., Aeromonas spp. (92, 93, 94, 95). These assays have been designed, and they have been used, to strengthen the diagnosis of deaths caused by drowning. These investigations provided support for the finding of the cause of death on the basis of rather rates of microbiota with high detection, such as 84% [N = 32] , 75% [N = 20] and [N = 43], despite fact that levels of accuracy and sizes of the samples may again be considerably increased. Its suggested that bioluminescent microorganisms could serve as potential indicators for mortality caused by drowning at sea. An easy and straightforward assay that concentrates on the 16S rRNA gene was developed for the purpose of bioluminescent colonies detection such as Vibrio fischeri and Vibrio harveyi. Upon recent examination the expression of pulmonary surfactant protein (SPA) and the microbiota to establish a marker for the identification of drowning as the cause of death (96). They compared the microbiota and histological appearance of experimental rats treated with freshwater vs saltwater water, both during and after death. The authors counted 5513 marine OTUs and 5480 freshwater OTUs that were distinct from each other. In addition, they discovered that SP-A expression was higher in the lungs of drowning victims than in those of those who had been submerged postmortem. These results show promising possibilities for future applications and may have significant forensic relevance (e.g., identifying the environment type and the death time). Other research, as noted by Marella et al., have looked into coliforms, faecal and streptococcal bacteria presence to aid cause of death due to drowning. The femoral artery, vein, and ventricles are all sampled for these microorganisms (97).

Drowning victims are more likely to have faecal bacteria present than those with other causes of death (98). For instance, in their study of 22 cases of freshwater drowning, found that 100% had faecal streptococcal presence and 90% had coliforms. Thirty healthy volunteers served as controls, and they all tested negative for faecal microorganisms.

Subsequent research investigated if these bacteria might be detected in the drowning medium after death. Scientists obtained tissue samples from people who drowned (n = 5 in freshwater and n = 5 in marine) or were submerged (n = 3). All victims who drowned had coliforms and streptococci present, while those who were submerged after death did not. These results point to the possibility of using faecal coliforms and streptococci as indicators of drowning. However, the small sample sizes necessitate extreme caution when interpreting the results and calling for massively expanded sample sizes in subsequent research.

IV. POSTMORTEM LAG TIME

➢ *The Subatomic Structure of the Thanatobiome*

In many cases, it is crucial to a criminal inquiry to establish the PMI (the amount of time that has passed after a person's death). Many studies (99, 100) have looked at the thanatomicrobiome to better predict PMI. In the postmortem period, these communities proliferate and eventually colonise the host (101).

Microbial communities may go through significant changes in organs that help determine PMI, according to preliminary investigations. Preliminary research using model organisms suggests this is doable (102).

In order to develop a "microbial clock" that could forecast PMI over a period of 48 days of decomposition, Metcalf et al. sequenced 16S rRNA gene for archaeal and bacterial groups as well as 18S rRNA gene for micro eukaryotic communities (103). Their model yielded accurate PMI predictions (3 days; n = 223). Nonetheless, care is warranted when applying the data to 'real-world' scenarios given that study was done under conditions which are controlled utilizing mice models. The decay of pig corpses was also the subject of another investigation. With additional methodological for experimentation improvement, their model predicted the PMI within 2 to 3 hours of the time of death with 94.4% accuracy (104). Postmortem microbiomes were shown to not to have any changes, reflecting antemortem microbiomes of 24-48 hrs after death, according to a wide-scale investigation of body microbiome samples conducted (n = 188). Certain bacterial taxonomic groups were also discovered to be significant predictors of health status by the researchers (105).

The species of Hemophilus and Fusobacterium was found to be twofold as common in people who are healthy, but Rothia shown to be 0.09 times more common in those with heart disease. Clinical investigations of all types of deaths, from those that are slow and natural to those that are sudden and violent, could benefit from this in the future. It's worth noting that the approach of bioinformatics used to analyze OTUs and to give practical predictions is now deemed to be antiquated, although having been adequate at the time. Additional taxonomic detail may be available through Amplicon Sequence Variants (ASV) (106).

In order to estimate PMI researchers developed a algorithm based on data collected from skin microbiome samples taken from decomposing human cadavers. low error rates for skin samples, an accuracy for 2 days estimation of PMI [n = 144 from 21 cadavers], which was a significant change over past attempts (for example, using entomological inspection). By sequencing 16S rRNA amplicons, researchers were able to discover that models created at the phylum taxonomic class levels produced accurate and dependable predictions of PMI. This evidence supported research and showed its potential forensic applications (107, 108).

An additional study reported PMIs between 29.5 and 240 hours when utilising 454 pyrosequencing to analyse the postmortem microbiome of numerous important organs. These included organs like brain, heart, liver, and spleen. Postmortem microbial communities were shown to be dominated by anaerobic and spore-forming Firmicute bacteria, specifically Clostridium sp. Additional evidence for the future application of microbiomics in PMI estimation was provided and demonstrated that species of the Clostridium dominated at lengthy PMIs (109).

- *Animal-Based Microbiome-Based Localization*

Several studies have demonstrated that the microbiome of animals from different taxonomic groupings and settings is distinct. Genetics, geography, and altitude all plays a role in shaping gut microbiomes of numerous animals. all have distinct gut microbiomes (110, 111). Skin microbiomes of many species have been shown to be distinct (112, 113, 114, 115, 116, 117). This includes the skin microbiome of dogs Canis lupus familiaris. Humans and their pets have similar microbial ecosystems, according to research (118).

Such skills hint at the possible viability of tying a human based on shared microbes with animals, pending more research and methodological improvement. Although further research is required, forensic pathways may be able to link specific environments or occupations such as animal industries with profiles of trace microbes collected from other species. Detecting animal-specific microbiota on a suspect's or victim's skin or clothing could be helpful if there isn't required DNA of animals [i.e., from somatic and germ cells] evidence. As a supplement to more conventional forensic evidence, this profile might potentially be tracked to the interaction site with animal or animal-based contexts such as equine stables, Animal shops, or safari park. However, at the present time this strategy is largely theoretical, thus more investigation into its viability is required.

V. DISCUSSION

Almost no current criminal investigations or legal proceedings make use of forensics based on the microbiome. This can be better understood by classifying the various applications of microbial forensics can be categories for better understanding into either issue in reconstruction, such as the PMI and cause of the death, which focuses on "what happened?" and help shed light on the crime's content, or comparison issues are geo-sites and personal identification it includes similarity for these 2 DNA profiles? It may connect or disconnect a suspect from an entity or a place (for example, crime evidence). The applications of Forensic Reconstruction are simpler and easier to develop because they focus on issues including PMI and the cause of death. If sufficient time and effort are spent identifying characteristics of microbes related with each possible groups of temporal, spatial conditions and environmental, then reconstruction can be made relatively straightforward. If PMI is extrapolated, precision of high degree and inevitability from the discovery of a body buried deep around one meter in a desert in the summertime, shows that the gut microbial population has been specified for the environmental conditions at temporal succession. This is because PMI is based on the microbial population that was present in the gut at the time of discovery. The reconstructed microbiome's can pass easily the Daubert standard, which was set by the United States Supreme Court (119) it has been recognized as scientific evidence towards the bearing foundation. This includes widespread recognition among scientific community, acceptable rate of error, and a variety of other criteria and so on.

However, microbiome comparison techniques may be more useful for criminal investigations, but they are more challenging to build to a standard that would meet the Daubert threshold. In the same way the human DNA analysis in forensics compares the trace evidence DNA profile (from known samples and known locations) in a database, the productive means to use such tools would be in "one-to-many" configuration, the frequency of a profile in the relevant population is calculated in order to elaborate to encounter the probability chances of profile [i.e., initiating from a location or person unreal].

However, there are currently very few microbiome databases that may be used for forensic purposes and comparison to minute evidence in forensic settings, their absence necessitates a "one-to-one" arrangement. Samples from the scene of the crime, the alibi location, and other relevant areas are matched to samples from the suspect's clothing and other items.

In comparison to other methods, this one is less helpful because it can only provide either exclusionary or comparative results, such as "sample A and sample B show more similarities than compared to sample C". The European Network of Forensic Science Institutes recommends the Likelihood Ratio framework (120), and we are able to utilize this to statistically evaluate the evidence even for "one-to-one" research (121).

Forensic technologies based on comparative microbiomes still need to overcome three other significant obstacles. In the first case, a sample may contain substrates or DNA from multiple individuals or geographic areas. Mixtures of DNA analysis is challenging with relatively straightforward profiles of DNA, of humans much less with microbiome DNA profiles. The DNA profiles of the various microbiomes in a mixture are generally difficult to deduce, and it is often hard to know for sure how many different microbiomes are present. 2016's illustrious Independent analysis of complex DNA of human mixes by forensic professionals is not a credible methodology, according to a report from the US President's Council of Advisors on Science and Technology (122). As a result, a number of computer programs were created to objectively evaluate complicated human DNA mixes, and these are gradually gaining validation and acceptance for regular forensic use. Years from now, this foundation may be used to develop software for the impartial examination of microbial mixtures of DNA. Temporal fluctuation is a further substantial challenge. While human DNA tends to remain stable over time, microbial populations can vary, typically in response to variations in environmental factors like moisture and pH

(123). When comparing two samples collected at separate times under very diverse environmental conditions, it is necessary to first account for the temporal changes in community structure. Experimental research on this area is scarce; what little there is has mostly focused on using different carbon sources to "converge" (124) the diverse microbial communities.

The tertiary and potentially insurmountable obstacle is difficulty in transfer of DNA and Its identification from trace evidence are belonging to a exact person is now rarely borderline, because to the broad legitimacy and acceptance of human DNA evidence in the courts during the past decade.

Instead, it is increasingly common for the defense to question how the DNA was initially deposited, arguing that it could have been brought to the scene through lawful means or through DNA transfer (such as when the suspect who is innocent shook hands with the real perpetrator). Usage of probability ratio approach with activity level propositions can help get around this problem in some cases (125), but it's still an open question whether or not this will work reliably, and more work needs to be done before microbiomes can be used and accepted in the legal system.

VI. CONCLUSION

The field of microbiomics has blossomed over the past decade thanks to developments in genome sequencing and bioinformatics, resulting in an expanding suite of instruments designed to investigate the vast diversity of microorganisms that inhabit human bodies and the surrounding environment. Based on the evidence analysed in this study, microbiomics has the potential to become a useful and exciting forensic science with many potential applications, including but not limited to the following: identifying individuals, estimating PMI, detecting substrate provenance, and gathering trace evidence. These developments could eventually allow forensic investigators to employ different types of microbiomic data, such as that produced by thanatomicrobiome analysis, to answer problems connected to criminal investigations, or at least to use these data in conjunction with other forensic procedures.

Nutrient intake and the subsequent generation of breakdown products like metabolites are only two examples of the intricate interactions and co-adaptation processes that occur between humans and their microbiomes over the course of a lifetime. The microbiome's make-up and dynamics shift drastically after death as a result of these alterations in interaction. Conceptually, methodologically, and computationally, understanding these colonisations and fluctuations, as well as antemortem microbial dynamics, is extremely difficult. There is potential for further development of forensic resources through studies of related microbiomes in forensics setting, as well as through a deeper investigation of fungal and viral communities.

There are still a lot of challenges to be conquered, such as small study samples, contamination issues, model over-specification and misspecification, the accuracy of predictions made by machine learning techniques, the complexity of temporal and spatial variations in the dynamics of the environmental microbiome, along risks and ethical concerns (126). However, there is still a lot of potential for success in overcoming these challenges. The case of the Phantom of Heilbronn indicates that evidence from human DNA based which is somewhat known, is not completely correct (127). The majority of the previously published work relied on 16S or targeted sequencing procedures, both which are known to have limitations for taxonomic resolution. Metagenomics techniques (128, 129) and/or techniques that use longer reads (130, 131) have the potential to be an improvement upon this body of work. Furthermore, strict, titrated requirements for assuring the accuracy of these sequencing approaches throughout field-based testing and deployment would be beneficial (132). Given this, it's important to keep in mind that many of the literature applications are more like ideas of proof rather than actual forensic uses.

" It is abundantly evident that we are still in a moment of discovery and revelation, as Ogilvie and Jones write, "for an observation of the inherent of microbial ecosystem, newer methods and technologies are being provided."

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