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Microbiome and Metagenome Signatures: The Potential Toolkit for Futuristic Forensic Investigations

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Abstract

Advancements in genome sequencing technologies, improvements in microbial sampling methods and recent approaches in bioinformatics have driven the rise in microbiomics and metagenomics. Diverse ranges of microbial communities are hosted by human bodies which interact with its environment and cause change in it. Human and environmental microbial profiles can be extracted and analyzed to get information of these interactions and they are likely to be applicable in forensics. In this review we explain microbiome and metagenomics with emphasis on its application in forensics and for identification purposes, and factors affecting the microbiome diversity in the light of previous studies. No doubt this field is still in its beginning but the use of microbiomics and metagenomics signature for this purpose has potential to increase the forensic toolkit. Most of time, studies have been affected by sample size limitation and model accuracies which leads to less exploration of the complete potential of microbiomics in forensics. Furthermore, currently the information obtained from microbial forensics is not considered in dubious by the law enforcement agencies. However, extensive research is needed to overcome these challenges to make evidences based on microbiome served to futuristic revolutionary forensic investigations.

Keywords: Microbiomics; Microbiome; Phylotypes; 16S Rrna; DNA

Abbreviations: GI: Gastrointestinal; MPS: Massive Parallel Sequencing; DGGE: Denaturing Gradient Gel Electrophoresis; RpoB: RNA Polymerase Beta Subunit; RFLP: Restriction Fragment Length Polymorphism; MLST: Multi Locus Sequence Typing; EBS: Expirated Blood Spatter; SP-A: Surfactant Protein; PMI: Postmortem Interval; FMD: Forensic Microbiome Database; HOMD: Human Oral Microbiome Database; HOT: Human Oral Taxon Number.

Introduction

Microbiology plays a diminutive role in forensic science for over 100 years [1]. It is considered as a valuable emerging tool in forensics due to the technological advancement's rapidity in PCR mediated genotyping. It is suggested that it opens up a major area for the application in forensic science [2]. Due to the lack of cost-effective sequencing technologies microbial forensics has been constrained to restriction of many forensic applications to individual taxa analysis [3,4]. In last few decades a new field of microbiomics has set after the advancements in genomic sequencing technologies and processing of complex community datasets by new methods. Microbial forensic kit Development is enhanced after the Combination of microbiome with Meta genomics [5-8]. It was suggested that for complement traditional investigation methods forensically relevant microbial profiles can be used

as evidence [9-12]. Different computational tools are being currently used for analysis of microbial profiles as evident but still this field is in its infancy [13,14]. In this review potential applications of microbiomics in forensics are discussed and also factors affecting the diversity of human microbiome are explained.

Oral Microbes

Leeuwen hook discovered bacteria in 16th century. Later on, culturing, isolation and characterization of many microbial species were studied [15]. A large number of microbial cells are hosted by human body [16]. The total genetic content of microbes present in human body is known as microbiome [17]. Microbiome varies in number and species composition in different body sites and location [16]. Five major sites are observed by microbial colonization in human body which is shown in Figure 1. Oral cavity contains diverse composition of microbiome. Almost 1000 species of bacteria are present in oral microbiome which represents different phyla [18]. Types of microbes present in mouth are ecologically different from other surfaces mouth characteristics do not allow all microorganisms to persist and colonize. Furthermore, due to the different ecological condition in mucosal surface (cheeks, tongue, lips, palate, teeth) mouth habitat supports the growth of only few microbial communities. During the life of an individual, microbial habitat will change. After birth only mucosal surface is present for colonization of microbes but teeth eruption supports the accumulation of larger mass of microbes. In an individual ecology of mouth vary due to any dental treatment or placements of orthodontics band .Oral microbiome of human is very complex in nature composed of fungi and bacteria. 74 cultivable and 11 non cultivable fungal genera are present in oral cavity [19,20]. There is need to identify al large no of oral microbiome which are still uncultivable [19]. Six major phyla that include *Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, Spirochaetes,* and *Fusobacteria*, contain 96% of oral bacteria while phyla *Euryarchaeota, Chlamydia, Chloroflexi*, SR1, *Synergistetes, Tenericutes* and TM7 contain only 4% of oral bacteria [21]. The major entrance to human body is oral cavity which contains viruses, fungi and many other species of bacteria. Numerous microbial habitats are offered by oral cavity with mucosa or solid surface. Specific ecological niche which selects distinct microbes present on different habitats such as the sulcus, dental surface, the tongue and different non keratinized and keratinized mucosal surfaces [22]. According to a hypothesis 100M microbial cells are present in one ml of human saliva from a healthy adult. In every 24hrs about 8×1010 bacteria shed from oral cavity in normal salivary flow rate which is 750ml per day [23]. Human oral microbiome constitutes almost 200 bacterial taxa and great number of opportunistic pathogens. As Second most diverse microbial community is represented by oral microbes which play very important role in determining human health, diseases and forensic investigation. Complexity of oral microbes is much understood with the advent of DNA sequencing advances [24]. Microbial community present in oral microbiome is different among different individuals. It is very unique in each individual and highly individualizing [25]. Therefore, traces of oral microbes are collected from crime scene which leads investigation in criminal and civil cases.

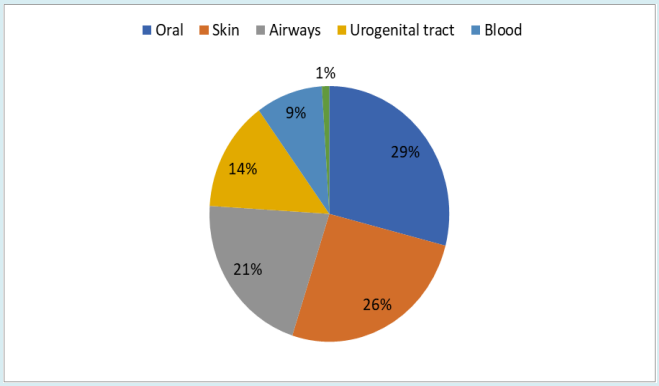

Figure 1: Percentage of microbial content in different parts of human body.

Skin Microbes

A large number of microbes are harbored by human skin which can be easily transferred and displaced to other surfaces after touching, Therefore proper hygiene is very important [26,27]. As some of the bacteria are resistant to moisture, temperature, UV radiations and environmental stresses so they can present on touched surface for extended period [28,29]. So, during our daily activities a large number of skin associated bacteria is deposited on those surfaces which we touched. Surprisingly a diverse range of microbial communities is present in skin microbes which have high degree of variation at different skin locations [30,31]. Between two individuals 13% of bacterial phylotypes are shared on the palm surfaces. There is much stability in skin microbial community with time even after washing hands with in few hours microbial community of palm surface is recovered [32]. Individual's skin microbes can be used as a fingerprint for forensic investigation due to the stability, uniqueness and transferable property of these bacterial communities. For linking of skin bacteria of specific individual to its touched surface following requirements are needed. Acceptable characterization and contrast of bacterial DNA must be done after recovery from touched surface, Surface whom are touched must be linked with individual who touched it and there must be long term persistence of skin microbial communities on that surface. So, currently different objects are linked with specific individuals by comparing with the

microbial community of that objects to the hand of owner. A data set is present which contains microbial community information for more than 250 hands [33].

Gastro Intestinal Tract Flora

One of the largest interfaces between environmental factors, host and antigen in the human body is represented by human gastrointestinal (GI) tract. A huge threat was imposed on gut integrity by the abundance of microorganisms which passes through GI tract with food [34]. Less diversity is existing in gut microbiota as compared to the microbial communities of other body sites [35]. Gastro intestinal tract flora is basically a collection of archaea, bacteria and eukaryotes which co evolved with host over thousands of years [36,37]. It is estimated that number of microorganisms in GI tract exceeds 10^{14} which is 100 times of microbiome genomic content of human genome and it incorporates bacterial cells which are 10 times of human cells [36]. But later on, it was estimated that human bacterial cells ratio is near to 1:1 [38]. Many benefits are offered by gut Microbiota which perform following physiological functions such as involvement in shaping of intestinal epithelium, strengthening the gut integrity, protection against pathogens, host immunity regulation and energy harvesting [39,40]. Culture independent approaches like High-throughput and low-cost sequencing methods improves the ability to survey the breadth of gut microbiota in which bacterial 16SrRNA gene targeting is popular approach because of its high variability and its existence in all archaea and bacteria [41,42]. Recently the focus of scientist from sequencing of 16SrRNA is shifted towards analysis in greater depth of shorter sub regions of gene [42]. But error can be induced by the use of shorter read lengths. Whole genome shotgun metagenomics is a technique which provided reliable estimation of microbiota composition and diversity because of its high sensitivity and resolution [41].

Applications of Human Microbiome

Massive parallel sequencing (MPS) have promote significant progress in microbial forensics such as microflora helps in criminal investigation as additional evidence, cause of death, for post mortem interval estimation and estimation of geographical diversity. For the purpose of forensic applications human micro biota sampling, sequencing techniques used and analysis has been shown in Figure 2:

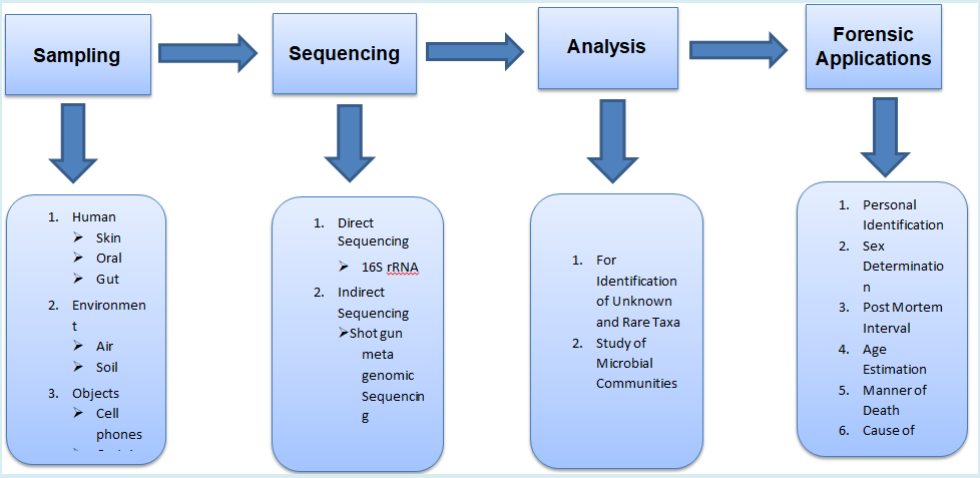

Figure 2: Scheme of Micro biome analysis pathway showing its sampling, sequencing, analysis and forensic applications.

Personal Identification for Forensic Purposes

Microbial composition must be same in collected samples or suspect's oral cavity for forensic purposes. If salivary microbiomes change over time and variations occur then there is a chance of failure in match due to which real preparatory is excluded from the investigation. In early 2000s characterization of salivary microbes firstly studied on the base of genetic finger printing technique including denaturing gradient gel electrophoresis (DGGE). For characterization and profiling of bacterial communities DGGE is widely used for qualitative and semi quantitative information of microbial population and change in community composition. After that taxonomic data is obtained from the gel by the excision of amplified band [43]. It was suggested that stable autochthonous microbial profiles can be used for unique identification of human individuals which have a considerable impact on forensic sciences in conditions when investigator does not have sufficient amount of human DNA for identification purposes. But it is still unknown whether microbial community variation between people is enough for

unique identification of individuals within large populations. For answering of these questions specific microbial profiles on different body sites were tested and attempts are made to match them with microbial profile of the person during his first and second visit to the sampling site. Authors found that at initial sampling time of these microbial profiles were able to distinguish among individuals. Even several months later 30% of the individuals were uniquely identified. Up to year later 80% of individuals were pinpointed by gut microbiome samples. No doubt these results still have relative high variability but can be used mostly in shorter time scale but before the use of such methods in forensic settings improvements are still required in its methods and sampling efforts [44].

High resolution melting analysis has potential for differentiation between individual's which target 16SrRNA gene from oral swab. Accurate identification of individuals on the base of skin swab samples from different site of body n = 14 is demonstrated by Schmedes and his coworkers and results was surprising. 97% accuracy is achieved by shirts sampling and 96% accuracy is achieved by palm samples based on diversity of bacterial genome. 100% classification accuracy was achieved by researcher when classification is based on maximum neighbor distance for diversity. For classification of publicly available skin microbiome samples from individuals was used and identification accuracy is 78% which is not sufficient for forensic applications [10]. However, for the determination of personal identification microbiomics use as a forensic tool which has a potential and technological feasibility and can be used in conditions when sufficient amount of DNA is not retrieved by the investigator and findings show shortage of proofs. But still there is requirement of improvements in model sensitivity and specificity and methodology to prevent contamination issues. Also, better u understanding of forensic value of microbial dynamics is required across time and space [45].

Biological Sex Determination

Microbiomics has another contribution in biological sex determination when sufficient amount of DNA is not retrieved by investigators for personal identification for examples in indoor environments air borne bacterial communities have been characterize [46]. Airborne fungal and bacterial diversity is investigated from different dormitory rooms of university by Luongo and his colleagues and Biological sex of occupants of rooms is predicted with 79% accuracy on the base of relative abundance of microbiota by using machine learning techniques [47]. It was found that high relative abundance of microbiota exhibited by males that can be due tothe use of less cosmetic barriers or may be males shed more biological particles. Bell and his coworkers also demonstrated the biological sex related differences in humans [48]. Amplicon signature of 10 individuals in corpse heart tissues is compared by using 16SrRNA gene in V1-2 and V4 region and an important difference is found in between males and females. In male heart tissues exclusively *Streptococcus* spp was found while in females *Pseudomonas* spp is in high occurrence. So, this approach can help us in determination of corpse biological sex and source of its body parts. Another study depicts the the sex on the base of pubic hair microbiomes analysis. In female participants they found that *Lactobacillus* spp were unique and they suggest that insulation of pubic hair is from the environment and their colonization is with niche specific microbiota which can be effectively used for forensic investigation [49]. Study by Willams and Gibson, et al. determine biological sex and identified individuals on the base of pubic hair microbiota with very less error ratio. However, for all these studies sample size is different so for any reliable conclusion it is necessary to further validate these studies with larger sample size [50].

Researcher analyzed skin microbes for determination of sex in both genders and found that female biological sex can be predict on the base of absence of genus *Alloiococcus* and it is showed that connection is present between personal characteristics and certain biological species. They use leave one out cross validation analysis for prediction of sex with 67% accuracy to discovered the microbiota presence and absence from fingermarks which are left on the surfaces [12]. For further exploring the potential of this approach sample size improvements and machine learning accuracy is required. It is also necessary to research that certain bacterium are only distinct to females or to check either they are related to external factors.

Trace Evidence (Touch Evi)

Interest in Forensic study of microbial profiles of different surfaces after touching is increasing day by day. It is showed by numerous studies; High level of microbiome is present on personal objects of any person [51-53]. So, interest is increasing to study these left behind microbial profiles. Additionally, it is showed that distinct microbiomes are supported by Personal associated items such as mobile phones and shoes [54,55]. Potential usefulness of Mobile phones is investigated as personal microbiome sensors, for this purpose 17 individuals were selected and samples were taken from three surfaces (Index finger, Thumb and cell phones touch surface). It was found that Microbiome samples of mobile phones is more associated with the owners than other person. 22% of participants finger taxa also found on their phones and 17% of participants taxa is shared with other person's mobile phones. A person index finger taxa share 82% with their phones and 55 more OTUs was shared by a person index finger to its mobile phone than other

mobile phones. But still there is need to increase sample size and accuracy of results in future [56]. Postmortem of skin microbiomes shows that it can be associated with high degree of accuracy to the personal objects. Bottles, Medical devices, eyeglasses and steering wheels can be associated with 100% accuracy while cell phones, remote control devices and computer devices can be associated with 67% accuracy. Skin microbiome is suggested as a reliable way to link objects to the scene. Moreover, it is suggested that postmortem microbes are stable and similarity exist between postmortem and antemortem skin microbes for up to 60h of postmortem [57]. Salzmann and his co-workers investigate Microbial profiles of different body fluids source specific microbial signatures were identified from these body fluids as in saliva and vaginal secretions higher occurrence of *Firmicutes* were showed while in semen and skin high prevalence of Phyla *Proteobacteria* was found [58]. It is suggested by Dobay, et al. that sample possesses site specific microbial signatures even after 30 days exposure of body fluids to the indoor conditions [59]. Recently it was investigated by Neckovic, et al. between individuals and substrates skin microbes are potentially transferred. Through direct contact skin microbiota is reliably transferred but microbiome is also transmitted through indirect contact. It is suggested by authors that these analyses can be helpful in investigation of sexual assault cases and other contact related crimes. It is suggested by authors that it must consider in further research on this which is pressure, friction, area of contact and the time of contact [60].

Bite Mark Analysis

The critical factor in forensic investigation is human identification. Correct identification of preparator and victim either dead or living is very important. Bite marks can be used as evidence for identification of preparator in sexual assault cases. Saliva sample of bite can be used for extraction of DNA But there is chances of degradation due to presence of enzymes [61]. According to Locards exchange principal bacteria is also transferred to the skin during bite infliction as saliva also contains bacteria. A new way for identification is found by analysis of bacterial DNA as bacteria can resist harsh conditions like putrefaction, degradation and drying [43]. Oral microbial community is totally different from skin microflora. Most isolated bacteria from bite mark are Alpha-hemolytic *streptococcus*. In dental plaque *streptococcus* group of bacteria is present. The major species found in saliva is *streptococcus salivarius*. 45-50% of bacteria are lost from site of infliction per hour. From dead individuals' microbes can be found for more duration then living individual [43]. The oral streptococcus from bite mark DNA samples of individuals shows significant results through PCR followed by DGGE denaturing gradient gel electrophoresis [62]. RNA polymerase beta subunit (RpoB) and 16s RNA shows that *streptococcus* as most common genus in saliva. RpoB2 gene analysis shows that Rothia as most abundant genes in saliva. All these experiment shows that oral microbial profile of two individuals is not same. All individuals' samples are different from each other [63]. For detection of polymorphism of 16s rRNA gene of bacteria and comparison of bite marks to the suspect teeth Restriction Fragment length Polymorphism (RFLP) is very important techniques [61]. For isolation and differentiation of microbial species Multi Locus Sequence Typing (MLST) has been developed for oral *streptococci* [64]. The most satisfactory results are given by rpoB gene while ITS and 16SrRNA has less discrimination power (Kennedy, 2011). Following species are present in infected human bite mark with abundance of *streptococcus, staphylococcus aureus, prevotell, veilonella, streptococcus, fuscobacterium* [65]. Hence for identification purpose microbial profile can be used for extraction of bacterial DNA because it resists the degradation and two individuals can be differentiated on the base of this. Identical twins can also be distinguished on the base of oral microbes because genetics do not have effect on this [43].

Oral Microbial DNA Analysis for Expirated Blood Spatter

Expirated blood spatter (EBS) is the blood which comes from nose, mouth and chest after any wound. The differentiation of EBS from impact spatter is a new front in forensic science. For this purpose, a new PCR method is developed for detection of DNA from *streptococcal* bacteria as biomarker which is specific to human mouth or saliva. The sensitivity of this method is too much that it can even detect 60fg of target DNA. Oral cavity bacteria can be used as marker for blood stain detection and can differentiate blood stains from expirated blood spatter [66]. 184cm distance can be travelled by saliva from the mouth of any person during laughing, talking or coughing [67]. So, the surface associated with human outside the mouth contains small number of oral microbes but for short time period 2-6 days [68]. *Streptococcal* DNA which is present on unstained material can be detected either it is in small amount but, the ratio of detection is five folds less than the stained sample detection. *Streptococcal* DNA can be used for distinguishing mouth expirated blood spatter from other types of EBS such as which comes from nose because it does not contain oral microbes [66]. *Streptococcal* DNA can be detected from denim even after washing [69].

Prediction of Body Fluids Saliva by Oral Microbes

Saliva is collected from crime scene and analysis of salivary microbial composition can helps to point out the preparator and can exclude or associate the individuals and

suspect with crime. Monozygotic twins in which genetic makeup is same can be distinguished by salivary microbial analysis [70]. Salivary microbiome profile can be helpful in forensic identification of those cases in which preparator DNA is mixed with victim DNA or preparator DNA is not detectable like in sexual assault cases. Illumina high throughput sequencing can be used for the identification of salivary sample from two different individuals. It was studied that salivary microbe's composition is different in different geographical and climatic areas which helps in narrow down the suspect [71]. Rasiah, et al. study reports that the bacterial composition of saliva is stable. Although some transient changes were observed or conclude that pressure imposed by environment can cause the change in oral bacterial communities [72]. Lazarevie, et al. compared the salivary sample of 5 different individuals and results showed that microbial community of saliva appear to be stable almost for 5 days [73]. Stahringer, et al. checked the oral microbe's variability on twins and siblings. He also examined the composition of microbes to check how it is varied by gender, age, weight, class and human genotype. In total he collected 264 saliva samples. Main bacterial phyla in saliva are *actinobacteria, proteobacteria, Bacteroidetes, Fusobacteria* and *fumicutes* [70]. Following genera were observed in almost 955 of samples *fusobacterium*, *Rothia, Preutella, Granulicatella, Neisseria, Gamella, Veillonella and strepto coccus*. In more than 505 populations additional 13 genera were also detected. Usually, body fluids are found at crime scene in trace amount as biological evidence. Different presumptive test is performed which shows result by change in color for identification of the type of body fluids to understand the nature of crime. Most commonly used presumptive test for saliva detection is Phadebas test. Sometimes these results are false positive because salivary enzyme may exist in other body fluids (sweat, semen, vaginal fluids, feces, breast milk) also. Another test which is used for detection is SALIGaE test which is more sensitive than Phadebas test but still false positive results can be observed [74]. So, for detection of saliva specific gene products have been recently introduced [75]**.** Microbial community is well established in mouth and nose or varies with individuals and with age or health condition. Detection of oral streptococcus species by amplifying ribosomal RNA gene and by amplification of streptococcus specific glucosyl transferase gene is successfully used for identification of these bacteria in detection of saliva [74]. Taxonomic profiling of microbes is an alternative method for body fluids (saliva, semen, feces, menstrual blood, and vaginal secretion) detection. For discrimination of saliva collected from human body or crime scene 16sRNA microbial gene were sequenced. Oral *streptococcus* such as *S. salivaeius*, *S. mutans* used as a new marker for saliva detection because it is only found in saliva or not in other body fluids [75].

Age Estimation by Microbial Flora

When current method of human DNA typing is not utilized then human identification can be done through the analysis of inter individual's variation of salivary microbiome. Narrowing of suspects can be done through age prediction. Microbial analysis of saliva could point out subject age within 5 years. Low bacterial count and diversity shows that suspect's age is more than 65 years [76]. Personal habits like smoking indicated by oral microbial analysis because it alters the microbial composition and increase pathogens in oral cavity [77].

Manner and Cause of Death

Manner of death is generally determined by the person who is involved in the investigation. Generally considered manner of deaths are five which are suicide, natural, accidental, undetermined and homicide [78]. 256 cadavers were collected from three different countries and found that these cadavers are different in manners of deaths. *Sediminibacterium, lactobacillus, Rhizobiales* and *Enterobacteriaceae* are associated with different manners of deaths but with further research these maybe developed as predictive markers for determination of manner of death and researcher also noted that *Rhizobialed* and *Sediminibacterium* can be contaminated by environment which is need to be control for its reliability in determination of manner of death [79]. It was found that different biomarkers are associated with manner of death in a cases related to hospital death. *Xanthomonadaceae* was more predominant and in suicidal cases. Researcher modeled beta dispersion test with association of microbiome data set of postmortem cases for testing of cause and manner of death. Researchers found that demographic data and beta dispersion can differentiate cause and manner of death. Specifically, they demonstrated that in 79% of cases cardio vascular diseases and drugs related deaths are found. After knowing the results of this study, it is showed that postmortem microbes have potential to indicate the manner of death. Furthermore, large database is required for training of models with high success rate before using it into in practical forensics [80]. In case of determining cause of death Due to any disease or injury researcher started to investigate microbiological testing importance. After studying the autopsy results it was found that in 42% cases cause of death can be determined by microbiological analysis [81]. Different factors were highlighted for indication of microbiological related cause of death for example, CRP measurements which can be implicated in case of SIDS as cause of death [82,83].

Another application of microbiome is determining death by drowning, because worldwide it is the major

leading cause of unnatural deaths [84]. No doubt for over a year diatoms analysis has been used as gold standard but its reliability is questionable [85,86]. Death diagnosis by drowning can be done through Real time PCR assays of bacterial species (*Aeromonas* spp) which are associated with aquatic environment [87,88]. It is recommended that in case of death by drowning in sea water bioluminescent bacteria may be worked as a biomarker. A simple assay was developed for identification of bioluminescent colonies (*Vibrio harveyi, vibrio fischeri*) by targeting the 16SrRNA gene [85]. Recently Lee, et al. developed a marker for death diagnosis of drowning by analyzing the microbial composition and surfactant protein (SP-A) expression. Microbiota and histological appearance of experimental rats were analyzed for both drowned and experimental groups, Fresh water and marine water treatments were compared and studies conclude that 5480 and 5513 OTUs were unique to fresh and marine water respectively [89]. Another study points out that current focus is on presence of *streptococcal* bacteria, coliforms and fecal bacteria which helps us in determination of death by drowning. Sampling of these bacteria can be done from right and left ventricles, femoral artery and veins. It was considered that in drowned subjects' fecal bacteria is always present as compared to other in which other cause of deaths is diagnosed [90].

Estimation of Postmortem Interval by Using Oral Microbes

Traditionally used methods for estimation of postmortem interval (PMI) are gross changes, temperature changes and entomology study of cadaver. Microbial succession can also be used for PMI estimation [91]. Cellular autolysis is started after death by hydrolytic enzymes. Putrefaction started by the activity of enzymes and bacteria present in and outside of body during the time of death. So, therefore, both microbes (gut and oral cavity) play role in decomposition of carcass. Variation in mouth microbial population is occurred due to decomposition so; oral microbial composition is used for calculation of time after death [18]. *Actinobacteria* and *fumicutes* are the major phyla observed in cadaver at starting stage. *Lactobacilli*; *staphylococcus aureus*, *veilonella,* and *streptococcus* are the main species which observed in early stage of cadaver. Accurate time of the death of dead victims can be estimated by carefully analyzing the oral micro flora. An important part for criminal investigation is determining the PMI [91,92]. It was suggested from initial studies successional changes occur in microbial composition which helps in determining the PMI [18]. Estimation of PMI can be provided by sequencing of 16S rRNA gene of achiral and bacterial communities and 18S rRNA gene of micro eukaryotes over the period of decomposition [9]. 94.4% accuracy is showed by decomposition of pig cadavers the PMI within 2-3 hr of the time of death [93]. A large-scale study was performed on microbiome samples which show stability in postmortem microbiomes. Anaerobic, spore forming *firmicute* bacteria and *clostridium* sp. is most abundant taxa found in post mortem microbial communities. Evaluation of post mortem human microbiome was performed to evaluate that the microbiome found in first hour of death is related with health state of individual before death. Samples were collected with post mortem intervals for different hours till 73 hours. And results shows that in different anatomical region strong differentiation occur in microbiome and it is also suggested that in first hour after death the state of human health can be indicated by ante mortem microbial communities which persists. There is limitation in value of past-postmortem microbiome at 48hr of death and reduction in time ranges occur with high temperature which enhance the proliferation of specific taxa of microbes [94]. A study was carried on two bodies which were found in freezer samples were analyzed at three different states frozen, partially frozen and thawed condition from eyes, ear canals, mouth, navel, nostrils and rectum. Increase in microbial diversity of nostrils, eyes and rectum was mostly observed during thawing process [95]. Another study was performed on 66 samples from liver, blood, oral cavity, heart, spleen and brain and it was observed that change in microbiome is dependent upon sex of corpse and PMI [92]. *Clostridial* and *pseudomonas* is dominant in female cadavers while *clostridial, streptococcus* and *clostridium* are present in high abundance in male cadavers. *Pseudomonas* is most abundant in Female cadaver's while *rother* is only identified in male.

Factors Affecting the Diversity of Oral Microbes

Multiple factors (spatial and temporal) have effect on human microbiomes as it is highly dynamic system. Variation in oral microbes occurred with respect to age,. After few hours of birth bacterial colonization is started in oral cavity. Throughout the life span of human micro flora of oral cavity is change due to physiological changes [96]. Extreme environment, diet, pH of oral cavity can affect the microflora of oral cavity. Microbial shift is occurred due to low pH changes of oral cavity. Immune system of the individual affects the growth of bacteria and prevent from diseases by keep in control. Various combinations of bacteria are led by all these conditions and due to this every individual is unique. Microbiome condition is more similar if there is a similarity in the genome of individuals. Oral microbe heritability is affected by enzymes (amylase, lysozyme), Immune molecules, biomolecules (histatins, cystatins, defensins), proline proteins and mucin which controls the microbiome composition [97].

Geolocation/ Geographical and Altitudinal Soil Etc

There is difference in composition and function of microbial community due to different environmental conditions (rainfall, altitude, climate and soil condition) across different geolocations and cities [98,99]. Geographic location can be specified by knowing microbial composition of host and its environment [100]. For example, different strains of bacteria are only linked to specific geographical settings such as *Helicobacter pylori* [95,101,102]. Now Human trafficking cases can be assisted by microbiome data of humanitarian programs which helps in linking the children to their families on the base of microbiome geographical signatures and intermediate locations by which that child is trafficked [103]. Even there is a different geographical bacterial signature are present in buildings and 16S rRNA bases taxa is varies between offices of different states of America [100]. According to different studies Harvesting of microbiome of different body sites of host can be linked to its country origin. It is confirmed that nonliving things can retain specific microbes of its geographic locations. For geographic information's most commonly examined microbiome is gut microbiome which can be collected from toilets [104,105]. Specific microbial signatures and 16SrRNA based taxa can be used for differentiation of individuals in different geographical locations [106,107]. Along with gut microbe's microbiome of saliva and skin can be successfully used for getting geolocation information [71]. For differentiation of geographic locations of random forest analysis, the most commonly used technique is machine learning. Any change occurs due to illness, diet, travel Can alter the microbial composition over only days [108]. But still the most robust geographical signature can be derived from gut microbiome [109].

Forensic Microbiome Databases/Database of Human Oral Microbe

A human microbiome analysis tool which compares the 16SrRNA datasets (obtained from different body sites) to metadata for forensics analysis is basically forensic microbiome database (FMD). The aims of FMD are:

1. Provision of evidence-based tool to the forensics scientist after documentation of provided microbial sample sequences.
2. Results, metadata and related analysis are maintained in the form of database.
3. Development of websites which allow users to evaluate, interpret, compare and quantification of the data.

The FMD 16SrRNA gene data is obtained from different body sites is used by forensic scientist as a human microbiome analysis resource.The most studied human microflora is human oral microbiome. Oral microbes which are found in human oral cavity is present in human oral microbiome database. Taxonomic and genomic information are the two main types of information provided by Human oral microbiome database (HOMD). The main aim of creating HOMD is to provide comprehensive database of microbial community present in oral cavity. Phenotypic, genotypic, phylogenetic, clinical and bibliographic information for each taxon is interlinked by human oral taxon number (HOT). For studying the function of human associated microbes in health and disease first characterized database of human region is human oral microbiome database (HOMD). Almost 700 species present in HOMD which provide information to the research community. In the last 20 years 600 16s RNA gene were sequenced and 35000 clone sequence were collected. Currently HOMD collection consists of almost 150 genera and 700 species and genomes of 400oral taxa and 1300 species of microorganisms. Information coming from cultural and non-cultural inhabitants are compiled in HOMD [43]. Abundant group is streptococcus which has 43 species in HOMD.30 oral taxa and 202 streptococcus species also found in HOMD [110]. Main focus of HOMD is cultivation of microorganisms but 20-60% of oral microbe data is uncultivated due to limited growth condition [43].

Conclusion

Human microbiome consists of bacteria, fungi and viruses which are found inside human body. 2-3Ibs of human body mass is counted by these microorganisms only. They have immense importance in forensics as it works as an evidence-based tool for developing relation and exclusion of people to any illegal activity. With the advancements in forensic investigation using micrbiota the forensic community will need education on the difficulties of utilizing microbiome data, as well as the proper use of databases and informatics tools, as microbiome research gains traction in the field of forensics. Further research is needed to explore the effectiveness of human microbe as general and particularly use of oral microbes in research and for forensic purposes. A variety of human-related metadata, including ethnicity, geographic origin, nutrition, and social information, must be included in reputable microbiome databases before they may be used more widely in forensics. The existing legal criteria for genetic privacy need far more samples to be gathered and processed in a manner that is acceptable to the community.

Conflict of Interest

All authors declare no conflict of interest.

Ethical Declarations

This article does not contain any studies with human

participants or animals performed by any of the authors.

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