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Research paper Integrating the microbiome as a resource in the forensics toolkit



Thomas H. Clarke, Andres Gomez, Harinder Singh, Karen E. Nelson, Lauren M. Brinkac*

J, Craig Venter Institute, Rockville, MD 28050, USA

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ABSTRACT

The introduction of DNA fingerprinting to forensic science rapidly expanded the available evidence that could be garnered from a crime scene and used in court cases. Next generation sequencing technologies increased available genetic data that could be used as evidence by orders of magnitude, and as such, significant additional genetic information is now available for use in forensic science. This includes DNA from the bacteria that live in and on humans, known as the human microbiome. Next generation sequencing of the human microbiome demonstrates that its bacterial DNA can be used to uniquely identify an individual, provide information about their life and behavioral patterns, determine the body site where a sample came from, and estimate postmortem intervals. Bacterial samples from the environment and objects can also be leveraged to address similar questions about the individual(s) who interacted with them. However, the applications of this new field in forensic sciences raises concerns on current methods used in sample processing, including sample collection, storage, and the statistical power of published studies. These areas of human microbiome research need to be fully addressed before microbiome data can become a regularly incorporated evidence type and routine procedure of the forensic toolkit. Here, we summarize information on the current status of microbiome research as applies to the forensic field, the mathematical models used to make predictions, and the possible legal and practical difficulties that can limit the application of microbiomes in forensic science.

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1. Introduction

Advances in DNA analysis in the 1970s, such as Southern blotting [1] and restriction enzyme digests, gave forensic investigators a new tool for criminal investigations, with DNA-based evidence first introduced in a court in 1985 [2]. DNA forensics rapidly matured and the classic techniques, involving the use of minisatellites [3] or a variable number of tandem repeats (VNTRs) [4], became widely used by the 1990s. These inherently polymorphic DNA fragments provide information on an individual's specific DNA 'fingerprint', and can be used to identify suspects and then assist in their conviction through matching the fragment patterns. However, as with all restriction fragment length polymorphism (RFLP)-based methods, the requirement of high molecular weight DNA limited the type of sample that could be processed for forensic purposes [5]. Depending on the forensic question and quality and quantity of DNA available, current standard methods of DNA profiling use one or more of autosomal microsatellites or short tandem repeats (STRs), or lineage markers

* Corresponding author. *E-mail address:* lbrinkac@jcvi.org (L.M. Brinkac). including Y chromosome STRs or mitochondrial DNA (mtDNA) [6]. STRs are more precise in determining human identity, and thus were found to be ideally suited for forensic applications when sufficient DNA is available. In contrast, mtDNA is found in much higher copy number than nuclear DNA and can be used in the analysis of heavily degraded DNA when there is insufficient DNA to examine STRs. It is also possible to use mtDNA to determine maternal lineage.

Next generation sequencing (NGS) technologies have exponentially increased the amount of sequencing data that is available for forensic analysis while in parallel significantly reducing the costs associated with the generation of sequencing data [7]. This increase in available sequencing data has expanded the body of useful DNA-based evidence usable for forensic investigation [8]. The human genome, given the enormous amount of discriminatory information contained within, has the potential to be more completely utilized by the forensic community, and integrated approaches analyzing both nuclear and mtDNA can be pursued. Data that is generated from other fields of human genome science such as epigenetics, gene expression data and miRNAs, can now be used to address numerous forensics questions [9].

One area of human biology that holds significant promise for forensic applications is the study of the microbiome (including

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both human and environmental). This exciting new area of research describes the microbial DNA from the microbiome, the collection of microorganisms (predominantly bacteria, with fungi, viruses and microeukarya) that colonize surfaces, including both externally and internally on the human body. This field initially focused on small subunit (SSU) ribosomal (r) sequences, including cloning of the 16S rRNA and fingerprinting methods (DGGE, T-RFLP, ARISA), to uncover the vast richness and diversity of the human and environmental microbiome [10,11]. However, with the reduced costs and increased capabilities of NGS, this field has expanded significantly over the last 10 years enabling researchers to profile hundreds to thousands of DNA samples simultaneously with increased diversity coverage and phylogenetic resolution. NGS has offered new avenues to address critical issues of potential interest to the forensic community. For instance, studies have shown that the human microbiome does not consist of a single species spread over multiple body sites. Instead, distinct bacterial populations exist at different locations in and on the body [12]. We also know that a person's lifestyle can be a major driver of the composition of the microbiome communities across many body sites, including what they eat [13], with whom they live [14], whether they have pets [15], where they are from [16], if they smoke [17,18], their health status [19] and whom they kiss [20].

Humans are not the only potential reservoirs of human-derived microbial species of significance for forensic science. Environmental samples contain human microbial signatures. Research has shown that humans can change the bacterial composition in the environment through direct interaction, including changing the components of the air [15,21,22]. Human microbial signatures can be recovered in several enclosed classrooms [23,24], houses [14]. dorm rooms [25], bathrooms [26], and offices [23,27], and even used to predict information about a person interacting with the environment, such as the sex of the inhabitant of dorm rooms [25]. While the signature is strongest immediately following the interactions, it remains detectible for up to several months later. Microbiomes in enclosed built environments are more likely to reflect human microbial signatures compared to natural and open environments [28]. The exchange between environmental and personal microbiomes is also seen on a micro-level, especially for the terms that they are in close contact with their users. Microbiomes harvested from fabrics [29], shoes [30], keyboards [31], and cell phones [30] have all shown that they partially share their user's microbial signatures. The changes are not entirely unidirectional, as the environmental bacteria can also alter the microbiomes of its inhabitants, as was seen between individuals that live together with cohabitating spouses, children and pets all sharing microbial signatures [14], though bacterial viruses also can lead to increased taxa similarity across families [32].

The ability to capture and leverage these differences in the human and environmental microbiome presents exciting new possibilities for forensic science, including the possibility of linking specific human subjects to a crime scene [8]. However, several questions remain to be addressed before these microbial biosignatures can become routine and highly effective in forensic science. The differences between bacterial communities on the body and in the environment require consideration as to what these communities can reliably address and what statistical power is needed to rely on the microbiome-derived data. Additionally, NGS can generate enough personal information to alter the current legal framework of data collection and application. This review summarizes the current state of human microbiome research in the forensic field, and addresses the possibilities and the limitations, while proposing approaches that can be applied to overcoming some of the potential issues, as visualized in Fig. 1.

2. Human microbiome sequencing techniques

The human microbiome is potentially a very powerful forensics tool given the variety of surfaces that can be used to obtain a sample and the diversity of information available to researchers from these samples. The first large-scale comprehensive survey of the human microbiome, the Human Microbiome Project (HMP), recruited approximately 300 people at two locations in the United States, and sampled 15-18 body sites that represented skin, urogenital tract, oral cavity and gut. The results from this study. which included analysis of both 16S rRNA gene and metagenomic data, indicated that the microbial diversity on an individual body site was more similar to the same body site on a different person than to other body sites on the same person [12]. Later studies also demonstrated that microbiome composition (taxonomic arrangements) vary across time as well [33], with gut and saliva being more consistent in their bacterial compositions than skin. Microbiomes from other body sites, including those of interest to the forensic community like scalp and pubic hair, have begun to be examined for use in forensics [34], but they remain understudied compared to the body sites sampled in the HMP.

Human microbiome diversity can be characterized in several ways. The most common technique is to examine the taxonomic distribution of the species in the sample via targeted sequencing of the variable regions of the 16S rRNA gene. Several bioinformatic



Fig. 1. Current Microbiome and Challenges. A simplified schema summarizing the microbiome analysis pathway including: collecting samples from various reservoirs of microbiomes (Sampling); the different sequencing technologies available to quantify the microbiomes (Sequencing); and possible question that developing analysis can address (Forensic Uses). The current limitations and challenges of these uses are also briefly listed (Challenges). Each topics is addressed in more detail in the text.

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