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Article

Forensic Human Identification for Cutaneous Microbiome, a Brief Review

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ABSTRACT

Forensic Science compounds many study areas in context of solving crimes, one of which is the forensic microbiology. Combined with genomic approaches, microbiology has shown strong performance in studies regarding the relationship between microorganisms present on human skin and environment. The Human Microbiome Project (HMP) has contributed significantly to characterization of microbial complexity and their connection to human being. The purpose of this work consists of a historical overview of scientific articles, demonstrating the growth and possibility of using skin microbiome in forensic identification. Studies about use of cutaneous microbiome in human identification, as well its forensic approaches, were looked into for writing of this review. Comparisons among cutaneous microbial communities and manipulated objects have been tested using 16S rRNA, as well as a thorough sequencing of the bacterial genome. From use of ecological measures of distance to genetic markers with nucleotide variants and predictive algorithms, research has shown promising results for advances in field of forensic identification. The development of metagenomic microbial panel markers, named hidSkinPlax for targeted sequencing has been designed and tested with great results. Research results show satisfactory potential in human identification by cutaneous microbiome and the possibility for contributive use in elucidating crimes.

Keywords: microbiome; genome; cutaneous; forensic.

RESUMO

A ciência forense compõe várias áreas de estudo no trabalho de elucidação de crimes, uma dessas áreas de estudo é a microbiologia forense, que associada à abordagens genômicas tem mostrado forte desempenho em estudos da relação de microorganismos presentes na pele, microbioma, com o ambiente. O Projeto Microbioma Humano (HMP) tem contribuído significativamente na caracterização da complexidade microbiana e sua relação com o ser humano e também como banco de dados de resultados de características exploráveis nos estudos subsequentes. Comparações entre as comunidades microbianas cutâneas e objetos manipulados vem sendo testadas através do gene rRNA 16S e também de sequenciamento do genoma completo de bactérias, utilizando desde medidas ecológicas de distância a marcadores genéticos com variantes nucleotídicas e algorítimos de predição, apresentando resultados satisfatórios e promissores para os avanços no campo da identificação forense. O desenvolvimento de um painel de marcadores metagenômicos microbianos, chamado

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hidSkinPlax para o sequenciamento Targeted Sequencing foi desenhado e vem sendo testado, mas ainda requer estudos para uma possível validação. Este artigo aborda brevemente os avanços das últimas décadas no campo relativamente novo da comparação do microbioma humano em aplicações forenses.

Palavras-chave: microbioma; genoma; cutâneo; forense.

1. Introduction

Forensic Science is composed by many areas involved in elucidation of crimes, such as forensic biology and within it, the microbial area. Microbial forensic science rose in response to possible threats of biological weapons and bioterrorism. The lack of preparedness in response and investigation of biological weapon-related crimes brought up the importance of study in field, which led to creation by (FBI) Federal Bureau of Investigation of Scientific Working Group on Microbial Genetics and Forensics (SWGMGF). Besides promoting research, the group also developed quality control guidelines for other laboratories in United States (Budowle et al. 2003; Budowle, Eisenberg, and Van Daal 2009; Peterson et al. 2009; Roux, Crispino, and Ribaux 2012).

Studies of microorganisms has since then grown as well, and, in 2007, the Human Microbiome Project (HMP) was officially created as an extension of the Human Genome Project, given that humans are habitat to countless commensal, symbiotic or pathogenic microorganisms, commonly named Microbiome. The project aims at characterization of human microbiome and a greater understanding of its influence on human health (Turnbaugh et al. 2007).

The (HMP) leveraged growing studies in forensic microbial area, which extends to field of testing for human identification through the cutaneous microbiome (Schmedes, Sajantila, and Budowle 2016). Recent studies demonstrated this possibility of exploring human skin microbiome and its potential to be unique to each individual, while genetic and environmental factors influence its composition and specific microbial lineage signatures (Fierer et al. 2010; Franzosa et al. 2015; Oh et al. 2016).

Human skin is a physical barrier against countless invasive and mechanical factors, it protects the body from dehydration and pathogenic microorganisms (Proksch, Brandner, and Jensen 2008). However, at the same time, it is natural habitat for an infinity of other microorganisms that are mostly harmless, among which a wide variety of bacteria. This bacterial abundance varies topographically, due to differences in skin anatomy, and physiology, such as oiliness, thickness, salinity, moisture, and pH level (Grice and Segre 2011; Oh et al. 2014).

Further elements that contribute to microbial diversity compound environmental, temporal and personal factors such as location, occupation, frequency of hygiene, for instance: handwashing, age and gender. Gender and age based differences influence skin acidity, substances produced, sweat and sebaceous glands activity, and production of different hormones (Costello et al. 2009; Dao and Kazin 2007; Fierer et al. 2008; Somerville 1969; Ying et al. 2015).

Work occupation, clothing, cosmetics, hygiene products and even diseases and medication use have an influence on skin bacterial flora and therefore affect the conditions of epidermis and substances expelled by glands. These conditions may also modulate the skin colonizing microbiota, possibly contributing to greater bacterial variation (Dao and Kazin 2007; Flores et al. 2014; Grice and Segre 2011).

Microbial variety in skin is also related to incidence of ultraviolet (UV) light, since a wide range of microorganisms are sensitive to specific light wavelengths and present different recolonization patterns after its incidence (Burns et al. 2019). The microbiota variation is distinguished topographically due to characteristics of each area and in temporaly comparison it is more similar on diferent areas in own person than it same areas in diferent people. In strain level, the microbiota variation still remains stable in healthy individuals (Costello et al. 2009; Grice et al. 2009; Oh et al. 2016).

When it comes to couples, the individual microbial composition is altered due to cohabitation and direct contact. Intimacy increases the sharing of bacteria between two individuals, although it is still possible to find differences between both bacterial communities. There is less bacterial differentiation between couples than among other people, making it possible to infer greater intimacy between individuals (Ross, Doxey, and Neufeld 2017).

Although temporarily stable, individual bacterial composition appears to be transferable among individuals as well as the environment (Fierer et al. 2010; Flores et al. 2014; Oh et al. 2016). This transfer is not only restricted to physical contact, but also by

exhale. Individuals are able to emit their own individual microbial cloud that contributes, like direct contact, to surface bacteria, especially indoors (Meadow et al. 2015).

Considering the transfer of bacterial cells between individuals and objects, both generate potential for a forensic approach, analyzing similarity degree between bacterial communities (Fierer et al. 2010; Goga 2012; Meadow, Altrichter, and Green 2014). The present article aims to demonstrate by means of a brief review the evolution of studies in human identification through the cutaneous microbiome. It aims to assess whether this is a viable approach that can be applied into real forensic investigations, therefore enabling a new contributive alternative in elucidation of crimes.

2. Methology

Bibliographic research was performed on the online platforms of Google Scholar, PubMed, Science Direct and Oxford Academic Journals websites between April and September 2019, regarding the use of cutaneous bacterial genetic material in forensic approaches. Research involved terms such as *microbiome*, *skin microbiome*, *skin microorganisms*, *use of 16S rRNA*, and *microbiology*, some associated with topics such as *sequencing*, *forensic*, and *human identification*. Through citations of some articles researched, although some often coincide with others already studied, new articles have been found. The coincidence of articles in this case probably accounts to fact that there are still very few studies in human forensic identification with cutaneous microbiome.

The selection of articles was made based on topicality, complementarity in present work and an attempt of developing a historical view of the approach. Beyond the more recent articles, some older ones were sought for further clarification of original approach or for a more complete answering of questions proposed. To build a historical overview, we used pioneer articles as well as more up-to-date articles, demonstrating the progress in research comparing human skin microbiome with environment and human identification possibilities that arise from it. Thus, we selected 43 out of the 67 articles analysed to establish a connection and therefore build a discussion regarding a possible contribution for the identification by cutaneous microbiome in criminal cases.

3. Results and Discussion

Current forensic identification methods use autosomal profiles of Short Tandem Repeats (STRs) of human DNA to create profiles and compare them to biological evidence (Butler 2006; Hares 2015). Despite the efficiency of method, cases and sample variables such DNA mixing, degraded samples, and low DNA copy numbers may affect a satisfactory result, which entails the need of applying alternative methods such as mitochondrial DNA (Amorim, Fernandes, and Taveira 2019), increased concentration of collected DNA samples or even the number of PCR cycles, post-PCR purification (Budowle, Eisenberg, and Van Daal 2009; Smith and Ballantyne 2007) or High Resolution Melting (HRM) genotyping (Jiang, Zhang, and Pang 2020). The use of human cutaneous microbiome also proves to be a possible contributive alternative method to generation of (STR) profiles using microbial genetic markers employed in comparison of cutaneous microbiome with manipulated objects.

Countless studies have shown the comparison of an individual's microbial profile and objects related to them. In order to obtain a characterization and comparison between bacterial communities in individuals and objects, it is necessary to employ molecular techniques. Molecular analysis in characterization of cutaneous bacteria has been proven as being much more efficient than bacterial culture method, revealing a greater diversity of organisms (Griece and Segre 2011). The molecular approach enables characterizing microorganisms regardless of their source, eliminating the use of bacterial culture. As an example, we point to use of bacterial ribosomal RNA (rRNA) genes that are highly conserved, but also with variable regions, allowing the isolation of sequence of interest regarding the specific gene, without need of cultivating the organism (Theron and Cloete 2000).

3.1 Use of 16S rRNA

In bacterial genome study, rRNA sequences, especially of the 16S rRNA gene, have been an important target, including their universal distribution, present in all bacteria and archaebacteria, but not in eukaryotes. The 16S rRNA gene has specific variable regions making it an effective species taxonomic classification tool, and its highly conserved regions allow the development of Polymerase Chain

Reaction (PCR) primers or hybridization probes (Clarridge 2004; Hugenholtz and Pace 1996; Větrovský and Baldrian 2013; Woese, Kandler, and Wheelis 1990).

Most approaches commonly used over time in study of cutaneous microbiome have involved the sequencing of Variable 3 and 4 (V3 and V4) regions of 16S rRNA gene (Flores et al. 2014; Lax et al. 2015; Meadow, Altrichter, and Green 2014; Ross, Doxey, and Neufeld 2017; Williams and Gibson 2017). However, other areas of bacterial genome and methods independent of 16S RNA, also have been explored witn Whole Genome Sequencing (WGS). For instance, Shotgun Metagenomic Sequencing and Targeted Sequencing techniques that enable not only taxon-level analysis and phylogenetic classification, but also metagenomic analyses enabling strain-level classification by using entire bacterial genome (Franzosa et al. 2015; Schmedes, Woerner, and Budowle 2017).

3.2 Comparison Between Microbiome and Individuals

By means of 16S rRNA sequencing, Fierer and co-workers compared microbial communities found on computer keyboards and mouse devices with communities found on fingers and hands of different individuals, thus discovering a bacterial community that is more similar to objects of their respective owners (Fierer et al. 2010). Even between the two hands of one individual, bacterial communities differ. Dominant hand has a greater abundance of microbial composition, yet hands of different individuals show greater variation and share less bacterial phylotypes (Fierer et al. 2008; Grice and Segre 2011).

Bacterial similarity between feet and shoes has been subject of a further approach by using 16S rRNA, which demonstrated that the bacteria present in shoes come from feet of their owners and can be identified, even with use of socks. Thus, it is possible to establish a comparison between crime scene shoes and their owner's identification (Goga 2012). Nishi and co-workers created an amplified 16S rRNA gene profile and analyzed bacterial profile of individuals' hands and traces of touch and handprints, establishing similarities capable of identifying an individual (Nishi, Bergamaschi, and Campos 2015). Another approach has succeeded to generate microbial hair profiles that proved themselves unique to each individual and was kept stable even after six months, demonstrating viability in forensic identification, since hair is often found in crime scenes, but not always applicable under currently available methods (Nishi et al. 2017).

Variant regions of 16S rRNA gene were used in taxonomic approach for hand and cell microbiota comparison analysis (Meadow, Altrichter, and Green 2014). The microbiome analysis for a possible forensic scope comparing mobile phones and shoes was also proposed, using 16S rRNA gene, comparing community composition of smart phones with owners and yours shoes surfaces with floor. Results showed the possibility of identifying people from their microbial communities and objects manipulated by them but could hardly estimate the recent location of individuals due to high and rapid turnover of microbial comunites surface in environments (Lax et al. 2015).

Applying a metagenomic code creation algorithm to four different types of metagenomic sampling obtained from (HMP): Operational Taxonomic Units (OTUs) of 16S rRNA sequencing; (WGS) Species Abundance; (WGS) Kilobase Windows and Species Specific Markers also from (WGS), was possible identifying strain level metagenomic codes specific for individuals, enabling the identification in a fixed population over time (Franzosa et al. 2015). Schmedes and co-workers identified genetic markers with possibility of testing forensic samples by evaluating nucleotide diversity of stable markers specific for clade (group in phylogenetic tree that shares the same common ancestor) (Schmedes, Woerner, and Budowle 2017). Thus, showing less variation in nucleotide diversity of the same individual over time than between different individuals.

Results of several studies in human cutaneous microbiome identification and your genetic markers specific for individuals and stable over time led to creation of hidSkinPlex. This is a new marker panel for Targeted Sequencing that uses the most informative skin microbiome markers in human identification. The HidSkinPlex contains 286 markers of 22 bacterial and phage clades, from family, genus, species and subspecies levels, and also predicts the body site of origin of probing samples. Thus, being able to obtain high coverage of microbial profiles for forensic identification (Schmedes et al. 2018).

3.3 Promising Bacteria

In phylum and species level analyses, it was observed that hands' communities of different individuals, among all 102 sampled hands, shared only 5 phylotypes out of a total of 4,742 sampled phylotypes. Sequences by more than 25 phyla were detected, where

Actinobacteria, *Firmicutes* and *Proteobacteria* represented 94% of analyzed sequences. The most abundant genera of sequences were *Propionibacterium*, *Streptococcus*, *Staphylococcus*, *Corynebacterium* and *Lactobacillus* (Fierer et al. 2008).

In subsequent studies using strain level analyzes for *Propionibacterium acnes* and *Staphylococcus epidermidis*, it was observed that different individuals and different body sites harbor variations of bacteria, with *P. acnes* strains unique for individuals and *S. epidermides* strains unique to each different body site (Oh et al. 2014). Adding a Single Nucleotide Variants (SNVs) approach, *P. acnes* also showed a temporal stability in strains level, varying more individually than by new strains acquisition from the environment or other individuals (Oh et al. 2016).

In search of appropriate species for a forensic focus, Schmedes and co-workers performed a taxonomic classification to identify the main bacterial species of microbiome that is stable over time and shared by all sampled individuals, resulting in 10 species: *Corynebacterium aurimucosum, Corynebacterium jeikeium, Corynebacterium pseudogenitalium, Corynebacterium tuberculostearicum, Micrococcus luteus, Propionibacterium acnes, Propionibacterium granulosum, Pseudomonas sp.* unclassified, *Rothia mucilaginosa* and *Staphylococcus epidermidis*. *P. acnes* was the only species that was present in all body sites sampled (Schmedes, Woerner, and Budowle 2017).

Most hidSkinPlex markers are from *P. acnes*, and most *P. acnes* strains are more closely related to individuals by personal and specific body locations, thus evidencing that *Propionibacterium acnes* presents strong potential as a target species for a forensic approach (Oh et al. 2016; Schmedes et al. 2018).

3.4 Privacy

A concern with human genomic advances is the privacy of results and individuals, not only by identification, but also by possibility of providing data that characterizes the individual. Hereditary conditions, risk of disease, possibility of undiagnosed psychiatric conditions, physical and ethnic characteristics are some examples (Akgün et al. 2015; Greenbaum et al. 2011).

The genotyping of human microbiome, although discarding human contaminants, also raises this question once it is influenced by environment (Oh et al. 2016; Ying et al. 2015), sex (Dao and Kazin 2007; Fierer et al. 2008), age (Somerville 1969), lifestyle (Grice and Segre 2011) and even diseases and medications (Flores et al. 2014). However, as with the Human Genome Project, methods that get around these possibilities are also being studied for Human Microbiome Project.

The building of metagenomic codes to identify individuals by strain level microbiome helps maintain this privacy. Since they use "insights from computing theory and microbial ecology" in construction of metagenomic codes, they restrict the direct identification by bacterial genes associated with personal and environmental variations that contribute to their coding (Franzosa et al. 2015). However, despite the use of codes a fraction of samples could still be traced back and it is expected that advances in forensic microbial identification will also promote the privacy for individuals.

4.Conclusion

The use of human microbiome presents itself as a possibility of complementary alternative method in forensic identification. The analisis demonstrates the potential for a possible contributive consideration in elucidating crimes by presenting ability to predict origin of crime sites. For practical use, studies have yet to be performed, such as new comparisons between cutaneous microbiome and environment, and with other individuals, using the latest methodologies and in different time frames.

Deeper studies of more promising bacteria is also needed, like bacteria *S. epidermides* that shows potential, mainly coupled with hidSkinPlex, to predict the body site of origin of samples, once each body site has unique strains. So is necessary the development of more markers for enrichment of hidSkinPlex panel. Thus, it is possible to make more robust the human identification through the skin microbiome approach, contributing to elucidate real criminal cases in the future.

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