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Microbial Forensics: Forensic Relevance of the Individual Person's Microbial Signature

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ABSTRACT

The forensic investigation involves the collecting, assembling, and analysis of all crime-related evidence with the aim of getting to a conclusion about a suspect. Humans have microorganisms present in the gut, mouth, and skin, unique to each individual. The individual microbiome can be distinguished based on the bacterial 16S rRNA to tell the bacterial species diversity between and among persons. Sterilized swab-sticks were used to sample fifteen individuals' fingertips, their personal items, office doorknob and a college photocopier. Skin-associated bacteria were readily recovered from surfaces and the structure of these bacterial communities can be used to link individuals to the objects they had touched. We compared the bacterial communities on objects and skin to match the objects to the individual. The 16S rRNA gene PCR polymorphism was used to analyze the bacterial community for each person and object. The higher similarity of bacterial community between individuals' and personal laptop keyboards, office chairs and office member's fingertips was more evident than between the doorknob and the photocopier. Highest bacterial species diversity was observed in doorknob followed by the photocopier. Hence, an individual's bacterial profile can be used as a human identification tool alongside other tools in forensic fields, especially in cases where there is evidence of deficiency.

Key-words: Fingerprint, Forensics, Individual person, Microbial signature, 16S rRNA, Skin bacteria

INTRODUCTION

To get sufficient human DNA from available biological evidence gotten from crime scenes for forensic identification is difficult most of the times. However, bacterial cells on the skin surface and on shed epidermal cells are often abundant and can be used to recover bacterial DNA rather than human DNA from touched or contact surfaces. This approach can also be useful for identifying objects from which clear fingerprints was not possible [1,2].

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The dynamic relationship between the human skin bacteria surfaces microbiome and objects that individual come in contact with demonstrates the degree to which the human microbiome can shape the microbial profile of our different ecology [2]. The bacterial cells in and on the body are said to be multiple times the total human cells in each individual man [3]. The accumulation of the individual microbiome has been attributed to the change in the gene counts and is said to be part of the individual normal development and existence [3].

Studies on the microbial exchange between an individual person and built environments, and between individual differences based on bacterial diversity have shown the forensic potential of the microbiome. Linkage study of the microbial signature of individuals with objects such as phones, dresses, shoes, computer keyboards, door knobs have been reported [4,1]. Microbial profile of different surfaces in different homes revealed that the

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microbial signature of families can be used to differentiate individual members within a home and in predicting of the family's home microbiome [2,5]. Also, microbes shared between and among persons who inhabit a given inhabitant or space may play a significant role in the persons' health and disease transmission [2]. This is so because a majority of human microbes are autonomous, self-replicating, transmissible, unavoidable, and, in general, ubiquitous and vary substantially (not less than 13%) from human to human [3-5]. Furthermore, post-mortem studies has also revealed that microbiome of animal hosts changes in a way that can be predicted which enables the use of microbial communities to help explore where an individual had been in recent time and the present current location [2,6].

Microbial forensics can employ the microbial profile of an individual together with their DNA and RNA which are often shed, deposited, and exchanged routinely in almost the same pattern to human DNA which is used for identifying individuals. These human microbiomes are complex and variable and may provide forensic signatures that could serve as a marker like the human molecular markers, such as short-tandem-repeat used for identification of individuals. The human microbiome may be another source of evidence that could be used to match or exclude individuals from crimes [3]. This study explored the potential of the use of individuals' microbial fingerprint to link them to items they have been in contact with.

MATERIALS AND METHODS

We hypothesized that bacterial DNA analyses could discriminate the different bacterial profiles between individuals in a way that has forensic value. To do this, we analyzed the bacterial signatures left by different individuals on surfaces including fingertips, personal laptop keyboard, personal office chair, photocopier and a doorknob using PCR based on the 16S rRNA gene.

Collection of samples

This study was carried out between December 2016 and November 2017 and all analyses were done in the Department of Biological Sciences, Redeemer's University and ACEGID laboratories Nigeria.

Autoclaved cotton-tipped swabs pre-moistened with normal saline were used to collect samples from fifteen recruited participants who had not taken antibiotics by rubbing their fingertips, personal laptop keyboards, personal office chairs, an office doorknob and a photocopier. Two of the participants (P12 and P14) were members of the office, which doorknob was used in this study; however, the office was accessible to all participants so was also the photocopier used in this study, all in Redeemer's University.

Bacterial Isolation and characterization- The samples were serially diluted in distilled water blanks up to 10^{-6} dilution. The 1 ml from dilutions 10^{-1}, 10^{-3} 10^{4} and 10^{-6} were spread over the surface of petri-plate containing nutrient agar medium using a sterile glass spreader. The plates were then incubated at 37^{0}C for 24 hours. After incubation colonies of different bacteria appearing on plates were streaked with the help of a sterilized inoculating loop separately on different plates of nutrient agar medium to get the pure culture of the isolates. The pure isolates were identified using morphological, biochemical and gram staining characteristics.

Bacterial DNA extraction- Bacterial DNA was extracted using a ZR Fungal/Bacterial DNA MiniPrep according to the manufacturer's instructions. The quality of the DNA was evaluated by measurement of the nanodrop.

16S rRNA PCR amplification- PCR amplification of the 16S rRNA was performed using the following primers: Forward primer 5'AGAGTTTGATCCTGGCTCAG-3' and Reverse primer 5'-ACGGGCGGTGTGTTC-3'. The amplification was carried out using an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec, extension at 72°C for 30 sec with a final elongation at 72°C for 5 min. PCR products were confirmed using 2% agarose gel electrophoresis with TAE buffer and the resolved species were visualized under UV light. These analyses were carried out in the Institution, Department of Biological Sciences and ACEGID laboratories.

Statistical Analysis- The basic analysis was conducted using SPSS 23.0 to generate the dendrogram and Microsoft Office Excel 2007 to construct the pie chart. Obtained bands of each PCR product from the different samples were scored visually.

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RESULTS

Twelve different bacterial strains were isolated in the study as shown in Fig. 1. *Staphylococcus aureus* was a most reoccurring strain (25%) and associated with all the samples collected from the participants together with the objects, while *Streptococcus mitis* and *Bacillus thuringiensis* were the least frequent shown in Fig. 1.

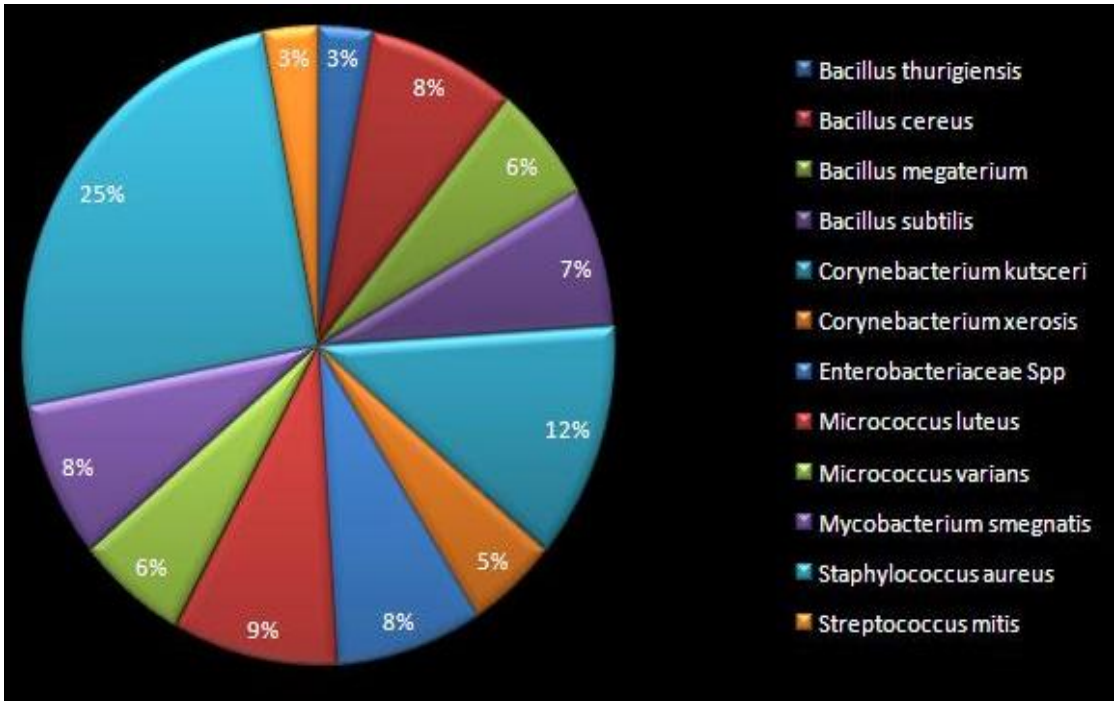

Fig. 1: Bacterial species diversity of all the samples

Doorknob sample had *B. subtilis, S. mitis, Enterobacteriaceae* sp., *B. cereus, M. luteus, S. aureus, M. varians, Corynebacterium kutsceri, B. megaterium,* and *Mycobacterium smegnatis.* From the photocopier *Enterobacteriaceae* sp., *B. cereus, B. megaterium, S. aureus, B. subtilis, S. mitis, M. luteus, M. varians,* and *C. kutsceri* were recovered, while the keyboards collectively had *M. varians, C. kutsceri, B. megaterium, S. aureus, M. smegnatis, M.luteus, Enterobacteriaceae* sp., and *B.cereus.*

Rich and diverse bacterial species were displayed by the doorknob followed by the photocopier than the other sample sources. This must have resulted from the frequency and diverse persons that have contact with the doorknob and the photocopier.

Bacterial communities (profile) between individuals and their personal items (laptop keyboard and or chair) clustered together in most of the cases than with other bacterial communities from others, such as the photocopier and doorknob. Furthermore, all participants were linked to the photocopier and doorknob, though at different levels participants 12, 14, and 15 made sub-clusters with the doorknob which is a likely reflection of the more contact time with the doorknob and photocopier shown in Fig. 2.

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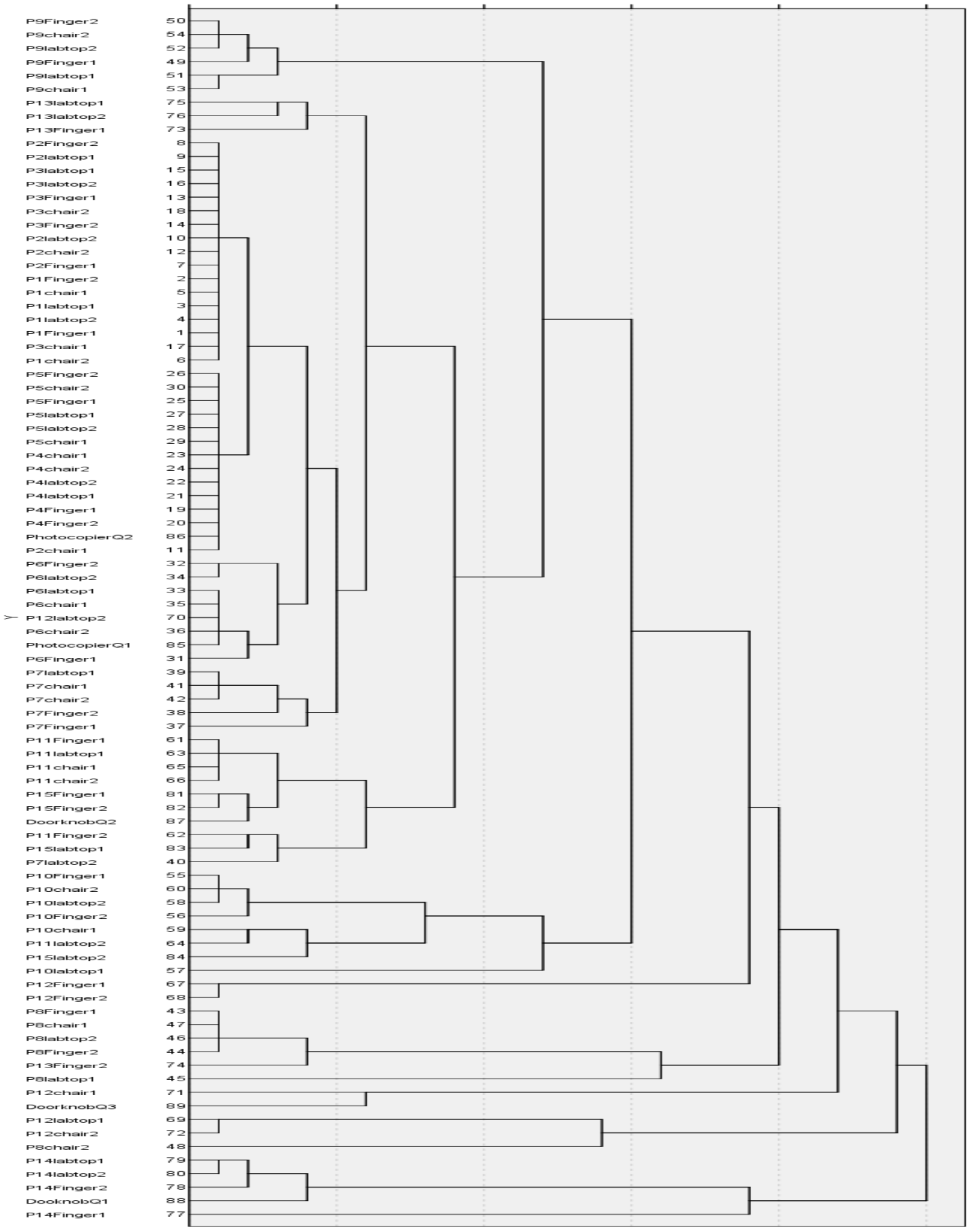

Fig. 2: Association cluster of bacterial community samples from the individual and items based on the 16srDNA PCR amplicon

Legend: P1 to P15= Individual one to individual fifteenth

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DISCUSSION

The use of genomic DNA based profiling in human identification has been employed in many fields including crime detection and paternity identification. The DNA fingerprinting technology is often used to generate evidence to establish a correlation between the crime scene, the suspect, and the victim conclusively beyond any doubt in the court of law. A considerable number of exhibits (genomic DNA samples) does not provide high-quality result or provide a partial DNA profile due to degradation, thus, a need of characterizing microflora over the case exhibits and or crime scene. This can be helpful in understanding the type of microbial population over the same variety of samples [7]. Forensic implications may be established, when the microbial profile of the microflora extracted from the case evidence or crime scene was studied because of the microbial interactions that exist between human-associated objects and the environments [2,8].

In the present study, which focused on the forensic application of skin microbiome, similar bacterial species were constantly observed in samples isolated from individual participants and their personal objects. Hence the limited diversity of bacterial species recovered from the samples, via the culture method used in this study. This observation re-verberates that the ability of different bacterial species to grow in the nutrient medium is limited [9,10]. We matched the bacterial profile generated from individual fingertips with those generated from their personal objects and other objects they had contact with. The recovered bacterial samples from the objects were successfully characterized, compared and linked. The rich diverse taxonomic bacterial species displayed by the doorknob and the photocopier was a likely reflection of the larger contact with the doorknob and the photocopier. This may be due to the greater contact diverse persons had made with the doorknob and photocopier.

Most samples from individual person's fingertips, laptop, and chair formed sub-clusters, although there were few case isolates but formed part of a larger linked cluster. A specific individual participant was linked to the objects the person had touched. Most participants' fingertips were linked to their personal laptops. Samples from participants P7, P13, P9, P10, and P14 fingertips were closely linked to their personal objects. However, P1, P3, P5, P4, P2, P11, P10, and P8 had closer links with their personal laptops and chairs. These are indications of the frequent contact between the individual participants' fingertips, their personal laptop keyboards, and chairs. This showed that each person had touched the object in each case. The samples from participants P6 and P12 fingertips did not make sub-clusters with those isolated from their personal objects as seen among the others, hence no direct linking with the objects, although all the samples were however linked in the major clusters. Lax *et al*. [2] had reported that different surface types may influence bacterial community structure. This may be the factor behind the variation in samples from P6 and P12. P12 and P14, who were members of the office, which doorknob was used for the study formed-sub clusters with the doorknob so also P15. This indicated that P12, P14, and P15 made regular or more contact with the doorknob and had deposited a greater number of bacterial samples than the other participants, hence their closer close link with the doorknob samples. However, other participants were also linked to the doorknob in the larger clusters. This showed that all the participants had touched the doorknob at one time or the other. This agreed with Fierer *et al.* [11] who reported that skin bacteria can be used to link touched surfaces to specific individuals. All participants in this study were also linked to the photocopier, though at different clusters levels, which was also an indication that each person had touched the photocopier. P6, P12, P2, P4 and P5 formed closer sub-clusters with the photocopier. This may be a reflection of the frequent contacts the individuals made with the object. Lax *et al.* [2] also reported a strong relationship between the microbial profiles from individuals, the objects they had touched and their environment. All samples from the doorknob nor the photocopier did not cluster together. This may be due to differences in the bacterial community present in the different part of the doorknob sampled, deposited by different persons who had made contact with the doorknob or the photocopier. These results showed the potential of identifying an individual based on the bacterial species composition of the analyzed surface.

The correlation between the persons and the individuals' personal objects and the objects they had interacted with support and strengthen the disposition of bacterial DNA analysis in forensic science. This study provides further encouragement, with the finding that individuals can be discriminated based on their bacterial DNA

fingerprints which can be recovered from objects they have made contact with Lee *et al.* [10] had reported that greater similarity exists in the bacterial profile between an individual and the personal objects than the relationship which exists between the persons and public objects. Jain and Shrivastava [12] had also reported that individual microbial profile can be an important tool in the identification of the individual.

CONCLUSIONS

Our findings on the use of bacterial profile to match individual persons to the objects, they have been in contact with reveals that the application of microbial forensics as an alternative to overcome limitations of current forensic science and as a complementary and not replacement for the standard DNA identification is realizable.

We plan to increase the number of samples and to employ the meta-genomic approach to enhance the results, which could be used to better discriminate between individuals and or link the individual to objects they have had contact with to establish the applicability of the microbial signature as forensic evidence. This was because culturing on common media recovers not more than 1% of the total available bacteria, which excluded the gene profile of the uncultured bacteria which is a disadvantage for a forensic potential of the approach. Microbial forensics studies have demonstrated that we can use skin bacteria to link touched surfaces to specific individuals. We hope standard practices are described and developed to promote global acceptance of the field of microbial forensics for scientific analyzes of microbial evidence in criminal and or civil cases for investigative purposes.

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CONTRIBUTION OF AUTHORS

Research concept- Abazuh Uchenna Desmond

Research design- Abazuh Uchenna Desmond,

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Final approval- Abazuh Uchenna Desmond, Oyejide Nicholas

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