

## A Microbial Approach to Forensic Science

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**Abstract:** The uniqueness of the microflora and microfauna increases the potential of microbes to reach from crime scene to courtroom as evidence. The microbes being very stable and unchangeable species and grows within the favourable and specific environment. They are present in abundance in and out of the body and millions are being shed on the objects that we touch. And they can survive there for a longer time than any other trace evidence like fingerprints or fiber. This will help in future to do the personal identification of the culprit if the microbial profiling is carried out for the suspects.

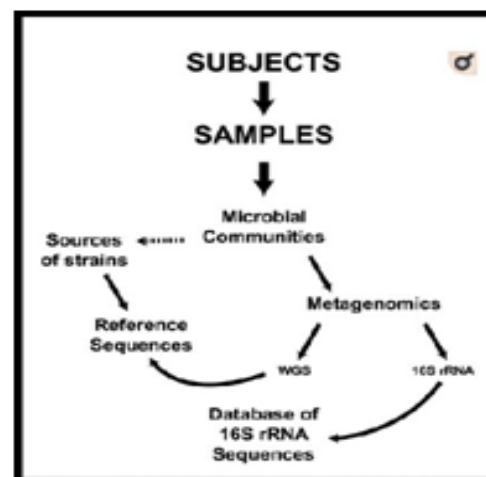
**Keywords:** Microbial Forensics, Locard Exchange Principle, Crime scene, Human microbiome sequence, Next Generation Sequencing, Personal Identification, Biocrimes

### 1. INTRODUCTION

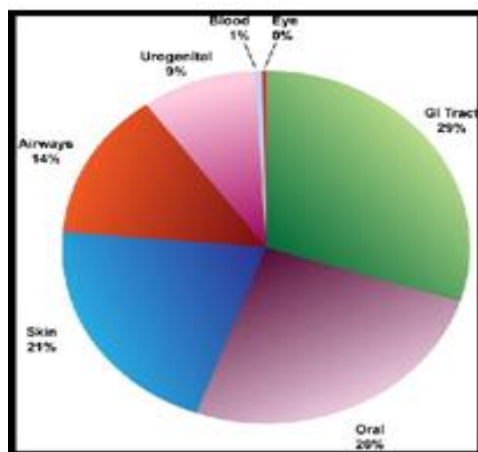
Bacteria's are the small single cellular primitive organisms on the earth (Rosenberg, E.2017). They are present ubiquitously, including exogenous and endogenous environment of almost all the living organisms existing on the earth. Many of these microbes are known to carry out various functions in the biosystems of the mammals for eg. Maintaining Skin pH (Linares, 2015), endogenous bacteria helps in digestion, metabolism processes, and in *Melanogaster drosophila* they help in choosing a partner for reproduction (Blum JE, 2013). Apart from the above mentioned functions they also help in degradation of complex organic substances. They are also very useful in the food industry to prepare cakes and breads to wine and alcohol (Breeze, 2018). Infact to be on more interesting part of these organisms that they are even present in extreme climatic conditions, these types of bacteria are known as extremophiles. The bacteria named *Prologues fumarii* grows in the temp. Of 80-113 deg C (Hafenbradl, 1997). Whereas, the bacteria named Psychrophiles can grow in very cold climatic conditions. Few of them are even noted to grow between the glacier layers (D'Amico et al, 2006).

In today's era various scientific communities are trying to map these species present Human skin and in internal organs to study its extent of

pathogenicity and their symbiotic relationship with the host organism (Chow, J, 2010). The human microbiome project started after few years of human genome project (By, National Institute of Justice, as the scientists could trace only 20000-23000 genes in the body but the rest of few functions were still unknown so they started Human microbiome project in which scientists are trying to find out the functions of each microbe present in and out of the body [Fi1 nd2] (Peterson j, 2009).



**Fig1:** Tells the pathway of analyzing human microbe's sequence, which can be analyzed by using met genomic sequencing. 16S rRNA from the collected samples are compared with database sequencing of 16SrRNA. Whereas, whole genomic shotgun is compared with reference sequences

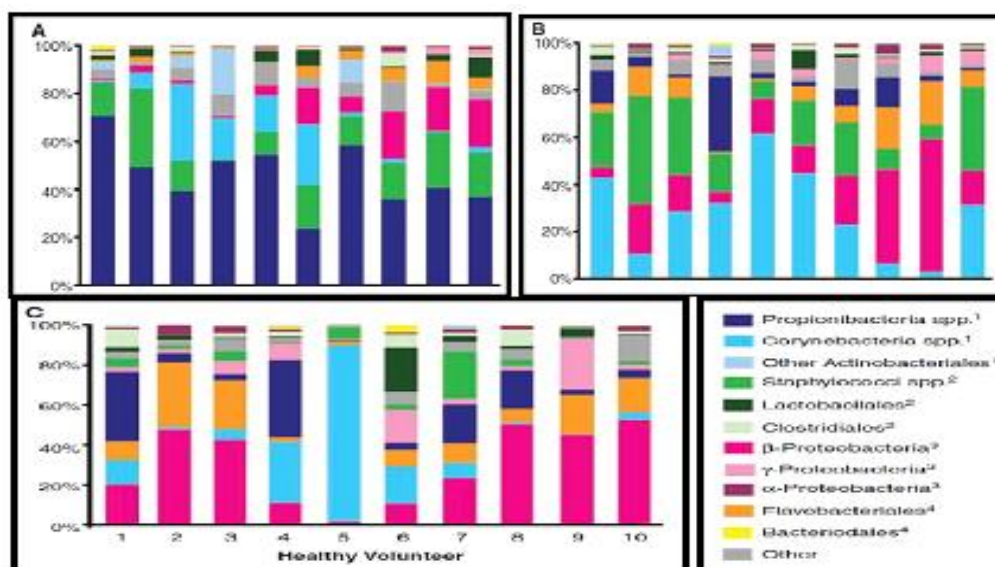


**Fig2:** Shows distribution of bacterial samples sites sequenced by HMP.

Next Generation Sequencing allows the scientific group to understand the genomic sequence of pathogenic bacteria. NGS came into the limelight when *S.aureus* outbreak happened in NICU in UK. It was not only revealed but traced back to the source as well (Behjati, S., & Tarpey, P. S. 2013, Chiu RW et al, 2008).

## 2. EVIDENCE OF HOW MICROBES CAN GIVE BASE FOR FORENSIC IDENTIFICATION

Apart from the above mentioned techniques and fact the attempt was also made to identify the difference between Amerindians and US citizen specimens. US showed dominated Propionibacteria. But in Amerindians the two diversities were observed amongst which one had similar diversity of bacteria as in the US samples, but instead of Propionibacteria, staphylococcus was dominating. And in remaining groups the proteobacteria diversity was observed. The Multiplexed V2targeted 16sRNA gene pyrosequencing was used for the analysis Purpose (B laser et al, 2012). In another study from 10 healthy humans the skin swabs were collected from 20 sites. These were analyzed again by 16sRNA technique. The results showed 19 bacteria phylas out of which most of the sequences were from 4 phylas named Actinobacteria(51.8%), Firmicutes (24.4%), proteobacteria (17%) and bacteroidetes (6%)(Grice et al, 2009).



**Fig3:** The above fig. Shows skin sites and associated microbiota, they represent three different microenvironments A) Sebaceous B) Moist and (C) Dry.

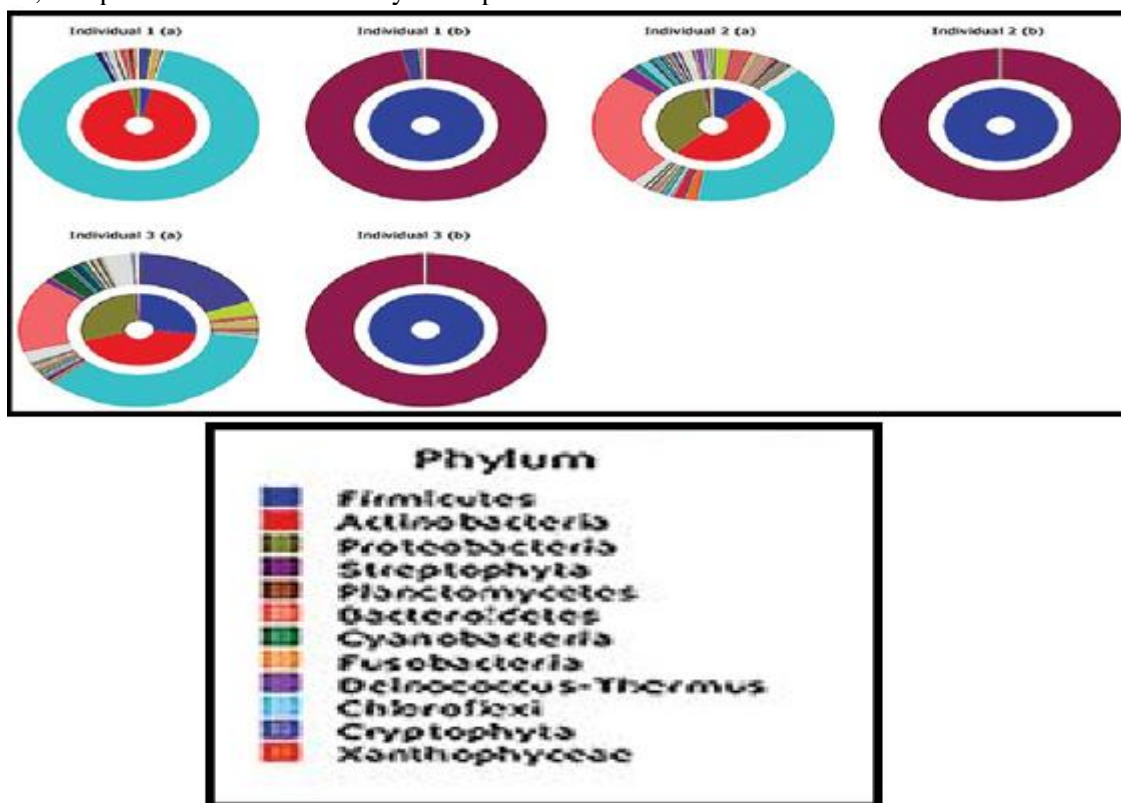
Pyrosequencing method was also used to survey the hand microbes based on various factors like the sex of a person, which handed the person is and how frequently the person washes the hands (Fierer et al, 2008). It's not only bacteria but even fungus can also show the uniqueness in its presence according to its geolocation, whether the skin has any infection or not. A study was conducted in South Valley University, Quena, Egypt. They found that *Aphanoasus* was the most common genus from the students of both sexes (Gherbawy et al 2006).

On the basis of above all observations we can microbial communities are very specific to their geolocation and day to day habitat of the host human. The microbial signatures usually do not change unless any drastic change occurs in the environment or host body. This ability of the microbes can be proved as the trace evidence where there is the crime scene which does not have efficient finger imprints or the fingerprints are damaged or they are not complete. As per the locard's exchange principle, whenever two bodies come in contact with each other they leave traces on each other's body (Roux et al

2015). The adequate characterization can be made from objects which people have touched (Fierer .N et al, 2010).

In another study conducted by the College of Medical Science, Soonchinyang University, Asan, Republic of Korea. They compared

culturing and non culturing methods to compare the bacterial variations in the palm. They got various phylas and species in non culturing methods whereas they did not get many variations in phylas in culturing methods (Fig 4) (Lee et al, 2016)



**Fig4:** The inner circle represents the cultured method in which not much variation is seen in the variations of microbial community. The outer circle represents Non- cultured method in which the variations of bacteria are observed.

The actual application of microbiology in forensic science pulled more attention after 2001 US Anthrax attack. On 18<sup>th</sup> Sept. 2001, Letters contained anthrax, mailed in US news agencies. Two suspects (Government scientists) freed as no exact evidence of attack is found. Suspect 1, Dr. Steven Hatfill eventually

exonerated. Second suspect, Dr. Bruce Edward Ivins committed suicide by acetaminophen overdose, on 29<sup>th</sup> July 2008. 6<sup>th</sup> August 2008, Dr.Ivins declared sole culprit. As the spores were tracked and found that they belong to Dr. Ivins Lab (Fig.5) (*ScienceDaily*,2011 and Rasko et al 2011).



**Fig5:** (Pic source- FBI)



Another incidence shows *S.aureus* strains from paraphernalia users to track drug network (B Quagliarello, 2002). The investigative cholera outbreak had two theories in which one states the climatic outbreak of cholera and other states the human transmission by water (Artibonite River). The strain difference in two ruled out the climatic outbreak of cholera (Orata, et al, 2014). *E.coli* outbreak in Germany to mention more cases of tracking the source of origin.(Grad, Y. H,2012). It's not only bacteria, but viruses study has also helped in solving the criminal and accidental cases. In the 1990's the dentist from florida was suspected to transmit the virus to his patients in his care. Later the patients were studied and observed that no other risk factor was assessed to get HIV infection. So, each patient's blood samples were collected and Virus genome was compared with Doctor's HIV genomic sequences. They found that the two sequences are closely related to each other.

As the fact is HIV's evolutionary rate is very high and it's difficult to get the same identical genomes (Bernard, E etal 2007). In another case of deliberate HIV transmission occurred in 1995 when the physician from Louisiana, US injected HIV infected blood in his girlfriend (Metzker etal, 2002). Ebola outbreak in 2014 can also be studied here. The fact that certain bacterias are specifically associated with vaginal fluids for eg. *Lactobacillus crispatus*, *Lactobacillus jensenii* and *Atopobium vaginae* while *Lactobacillus iners*, *Lactobacillus gasseri* and *Gardnerella vaginalis* are found in other body fluids as well. This can help in solving rape cases (Akutsu etal 2012). *Streptococcus salivarius* and *Streptococcus mutant* were detected in the mock forensic samples of Cigar buds and cotton gauge wiped licked skin. The streptococcus species were detected even in 6 years old salivary stains (Nakanishi etal 2009).

The PCR results for *S. salivarius* and *S. mutans* identification from various body fluids and skin swabs.

| Sample        | n  | <i>S. salivarius</i> |              | <i>S. mutans</i> |              |
|---------------|----|----------------------|--------------|------------------|--------------|
|               |    | Detected             | Not detected | Detected         | Not detected |
| Saliva        | 20 | 20                   | 0            | 18               | 2            |
| Semen         | 20 | 0                    | 20           | 0                | 20           |
| Urine         | 20 | 0                    | 20           | 0                | 20           |
| Vaginal fluid | 9  | 0                    | 9            | 0                | 9            |
| Skin          | 20 | 0                    | 20           | 0                | 20           |

Fig6

### 3. CONCLUSION

The above mentioned explanations and evidences enlightens the scope of microbiome signature for identification of the perpetrator as well as of the victim where the victim has not come forward or if the victim is dead and body is hidden by the criminal.

A similar approach was made by Jack Gilbert et al (NCJRS, National Criminal Justice Reference Service, US) to characterize the microbes from trace evidence and link it to the suspect. We can also link the study of microbes in forensic documentation examination for age of the ink estimation, as the microbial communities were identified from the tattoo inks many of them were pathogenic. By implying the similar method we can find out the concentration of microbes in aged inks.

This in future can also be able to tell the exact perpetrator by utilization of the techniques of HMP.

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