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Microbial Forensics: A New Breakthrough in Forensic Investigations

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Abstract:

Microbial Forensics is a promising branch of Forensic Science that fills the knowledge gaps and empowers the conventional and traditional forensic investigative procedures. Microbial fingerprinting can assist in the field of forensics in the areas like source tracking, geolocation, circumstances of death and trace evidences. The studies performed for the forensic investigations mostly focus on the internal organ samples and the soil samples, whereas the microbiome present in mouth, skin and vaginal samples are routinely collected in the cases like femicide and sexual assault. Microorganisms are ubiquitous in nature and hence, are involved in the natural metabolic activities, and therefore they are considered as physical evidence in the forensic investigations. The microbial species attacks the cadaver after the death of an organism and starts the decomposition of the body. The whole process of decomposition is an amalgamation of microbial and entomological attack which is dependent on various factors like soil, surrounding environment, temperature, humidity etc. The study of the microbial species can be an important aspect of the forensic investigations as it helps in the estimation of Post Mortem Interval (PMI). From the last few years, microbial forensics is gaining limelight as microbes can prove to be better evidence than entomological species for estimating Post Mortem Interval.

Keywords: *Microbial Forensics, Post Mortem Interval, Microorganisms, Fingerprinting*

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Introduction

The field of science is a collaboration of multiple disciplines like physics, chemistry, geology, biology, and many more. The application of these basic sciences in the field of law is what we call as "Forensic Science". Forensic science itself consist of fields like examination of Questioned Documents, Cyber Forensic, Toxicology, Forensic Physics, Forensic Chemistry, Forensic Biology and Serology etc which are based on the application of general laws of sciences and hence helps in the courtroom for solving different types of criminal and civil cases. One of these fields of forensic science is "Microbial Forensics". This field is concerned with the viruses, bacteria, algae, and other microbes which are present in and around us, living or dead. The focus is on the type of microbes they are, their structure, classification, function, life span, life cycle, presence and are the microbes good or not so good for the humans. All these aspects related to microbes were studied and applied in the field of forensic science where the information that is obtained is very crucial for solving the case. The information like time of death, place, origin, body type, etc can be extracted **(Janaway *et al.*, 2009).**

The microbes play an important role in the decomposition process as they recycle the organic matter present in the living bodies and the nutrients which we extract from the food we consume, and hence the food webs are formed in the environment. Also, 99% of nutrients and organic matter is cycled by microbes from plants organic matter and nutrients **(Carter et al., 2008).**

The presence of microbes during the process of decomposition provides the major chunk of the information about the cadaver. As decomposition is a process in which multiple factors like temperature, climate, humidity, the condition of cadaver, insects etc. The decompositions process acts as an intimate association of these factors which in turn act an important factor for the ecological setup. Studying these physiologic and metabolic changes that occurs in the cadaver due to the microbial action provides the information about the manner of death, time since death, location of death etc. and these microbes which are associated with the cadaver are termed as "Thanatomicrobiome" **(Vass, 2001).**

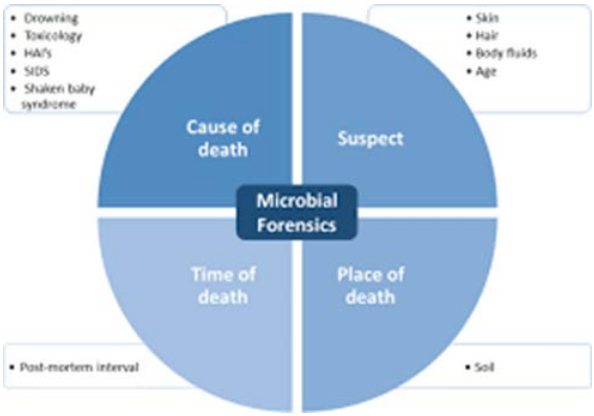

Figure No. 1: Different Aspects of Microbial Forensics

This community of microbes which are termed as "Thanatomicrobiome" includes the microbes which are present on the external surface of the body as well as inside our bodies also. The term "Epinecrotic Microbial Community" is given to the microbes which attacks the external body surface. During decomposition process, both the microbiome acts as an important factor for decaying the body and providing the details which in turn are forensically important factors. The internal microbiome, starts proliferating in the organs after 24 hours of death as there is a bacterial community which is present in intestine, oral opening and rectum. If the microbial action stops in the cadaver, then the chemical decomposition will take place at a very slow rate and due to this the formation of biochemical waste will increase at a very high rate. The carrion community of microbes basically produce the volatile molecules which in turn is related to the physiology and behaviour of the necrophagous insects **(Hopkins *et al.*, 2000).**

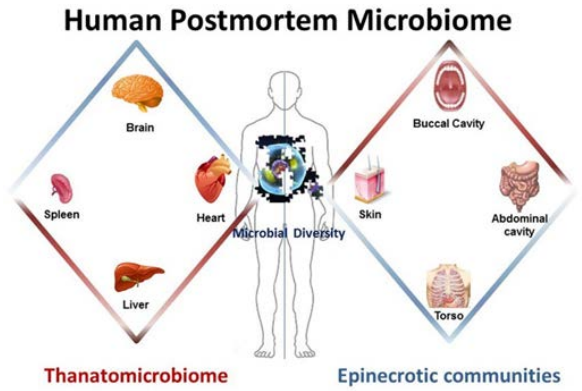

Figure No. 2: Human Postmortem Microbiome

When a person dies the human cells become hypoxic as the heart stops pumping the blood and it gets

collected inside the cells which in turn increases the rate of discharge of intracellular elements. These elements degrade the organelles which are mobile in movement by using the autolytic enzymes. The enzymatic action happens to be on the cell membrane and initiate its lyses, which liberates the cells including lipids, minerals, amino acids, carbohydrates and water to the tissues. These components are metabolised by the microbes and hence their population increases drastically. During the process, there is a formation and leakage of the gases like methane, hydrogen, hydrogen sulphide, sulfur dioxide and ammonia due to the unavailability of oxygen which transfers the whole process from aerobic to anaerobic fermentation **(Hewadikaram and Goff, 1991).**

As we know that the microbes are not present in the organs like coronary heart, brain, spleen and liver as the immune system keep these organs under check but when a person dies, the microorganisms increase at a very high rate in ileocecal area and in turn spreads in liver as well as in spleen because of the fact that immune system becomes weak and dies off with person only. The spread of microorganisms is then continued towards the heart and brain also. There is an interconnected communities of prokaryotic and eukaryotic species which are related to the decomposition of human corpses and animal dead bodies. Their bodies present at the crime scenes were observed to be in different decomposition stages. The cadavers which are in early decomposition stage, the cadaveric changes are so quick that they are very difficult to observe and make an analysis. After the studies on the cadavers, it has been found that the microbe “Lactobacillus” and few anaerobic species of microbes were present on the cadavers who have shorter post mortem interval. “Clostridium” was also found on multiple dead bodies having different intervals of decomposition. And by these experimental data, it was suggested that Post Mortem Interval (PMI) can be calculated by studying the presence of microbes on dead bodies **(Howard *et al.*, 2010).**

The microbiota even gets altered by the human genotype, diet, antibiotic use and disease states. The intestinal microbiota is around 1013 to 1014 microorganisms whose size of the genome is 100 times as that of humans. Because of the amalgamation of human and microbial attributes, humans are considered as superorganisms.

There is an extensive intestinal microorganism which plays an important role in providing nourishment to the human body, regulating the innate immunity. The health and disease are the vital platforms for the endogenous gastrointestinal microbial flora. The microbial species present in human consist of the eukaryotic, archaeal and bacterial microorganisms. These microbes help in food digestion, helps in maintaining the development of immunity, drug metabolism and fight with the pathogenic organisms. The intestinal illness is even concerned with the gut microbes and can provide ample number of clues about it. The ecosystem present in the intestinal gut is dominated by the anaerobic microorganisms like *Clostridium, Eubacterium, Bacteroides, Bifidobacterium, Peptostreptococcus, Ruminococcus* and *Propionibacterium* and the sub dominant bacterial species like Enterobacteriaceae family, majorly *E. coli, Lactobacillus, Fusobacterium, Enterococcus, Streptococcus, Methanobrevibacter* and *Desulfovibrio*. The information about the range of bacteria in the gastrointestinal tract has been acquired by using the 16S rRNA genes. The techniques used were the fingerprinting procedure, quantitative PCR, microarrays, fluorescent in situ hybridization and 16S rRNA gene amplicon sequencing **(Parkinson *et al.*, 2009).**

The microbial sampling was also done using fecal sample of humans. The information about the bacterial species is mostly gained by the cultivation of fecal samples. The fecal microorganisms might be the combination of poorly adhered microbes which gets shed away. The microbial species changes in the fecal sample depending upon the time as it depends upon the oxygen concentration and nutrient availability.

The distal gut of the humans is comprised of the microbial species. Gut microbiota is the most complicated network of bacteria present inside the humans. The microbiota which are present in GI tract have facultative. Also, it consists of the genome which in turn codes for the starch and sucrose metabolism. The metabolism of mannose, xylose, arabinose, fructose, galactose is also from the gene coding mechanism of these microbes. The microbiome in the human gut metabolizes xenobiotics, amino acids, glycans. And the biosynthesis of vitamins and isoprenoids is a 2-methyl-o-erythritol mediated process **(Chen *et al.*, 2011).**

Out of 70 species of bacteria and 13 species of archaea, Firmicutes and Bacteroidetes are used for surveying the gut and fecal microbiota. These species contribute for more than 99% of phylotypes, *Methanobrevibacter smithii* and methanogenic archaeon. The microbiota present in the mucosal region also plays an important role in regulating the immunity as it is capable of destroying antigenic proteins and activates the production of IgA **(Durbán *et al.*, 2011).**

Previously the experimentation regarding the bacterial samples includes the cultivation techniques but nowadays, culture independent studies are in use as these techniques are specific and more reliable in case of results and data. Using these data and analysis, the therapeutic and preventive interventions can also be targeted.

Knowledge Gaps in Microbiome Studies

The most accurate estimation of Post mortem interval by the decomposer microbial communities can be done in case of rapid and non- stochastic timeframes. This process is likely to be possible during the early/ active stage of decomposition, specially when the maximum cadaver nutrient pulse is processed by microorganisms **(Metcalf *et al.*, 2016).** The experiment required for the establishing considerable results should be in large data sets with frequent/ daily sampling for a long period of time. This technique will also fill the current gaps which exists in forensic investigation field. Microbial based estimation of post mortem interval will be useful in the death investigation in which other tools are less accurate or are limited.

The following sections include the knowledge gaps present in fresh stage, early/ active decay stage, advanced decay stage, dry stage and skeletonization stages of decomposition.

Fresh Stage

The microorganisms are personalized and unique to the specific organ, which will also be different in each person, which can be used as a link between the people and the objects they have touched or their occupied spaces **(Lax *et al.*, 2015).** After the death of a person, the specific skin microbiome is overwritten by the microbial succession that thrives on the cadaver which will serve as the nutrient rich source for the microorganisms. The successional decomposer microbiome will begin to erase the personalized signatures from the skin microbiome is still unknown but the mounted evidences suggests that the changes occur in the first 48th hour after death of the person, especially in indoor cases **(Pechal *et al.*, 2018).** Although the skin microbial succession for estimating post mortem interval can also be used for the bodies placed in outdoor conditions where the decomposition will be comparatively more rapid **(Lauber *et al.*, 2016).** These microbial species can show changes in the first 24 hours also in outdoor cases which will be helpful in the investigative procedures as most of the death scenes are discovered in the early timeframes, hence this can prove as an additional complementary and robust tool.

Early Decomposition/ Active Decay Stage

The researches performed using microbiome tools for Post mortem interval estimation has been done on the active and advanced decay stages of decomposition in which the frequency of sampling ranges from 1-3 days **(Metcalf *et al.*, 2013).** This time range becomes useful for the ecology of microbial succession as the community turnover is quick and is a generalizable process. The microbial succession depends on different factors like temperature, time and possibly other factors which will also be the variable points in the studies **(Johnson *et al.*, 2016).** The accuracy rate in case of microbial succession ranges from 2-5 days during early decomposition stages **(Amendt *et al.*, 2007).** As the advanced decay of the decomposition process is driven by the biochemical and biological processes, there are many different tools used to calculate PMI. The field of forensic entomology have also established as a field on which forensic investigators can rely for estimating post mortem interval for various temperature ranges **(Amendt *et al.*, 2004).** But due to some of the uncertainties in this field like the arrival of the first fly which lays egg, therefore new approaches are required for the better estimation of post mortem interval and microbial forensics can become a valuable tool as microbes can remain active even at low or high temperatures which becomes unsuitable for the entomological species.

Dry/ skeletonization remain Stage

There are incidents where the crime scene gets discovered during the later stages of decomposition which becomes challenging as compared to the cases which gets discovered during the early stages of decomposition. This phase is also known for the extended decomposition of the body **(Damann *et al.*, 2015)**; hence, this phase has abundant opportunities for discovering new tools for the forensic investigation. In the study carried out by Metcalf et al., investigated the soil and skin samples of the human cadavers by sequencing the ITS (fungal species), 18S rRNA (eukaryotic microbial community) and 16S rRNA (bacterial community) gene. It has been discovered that during the period of extended post mortem, the post mortem interval has been predicted for the period of 50 days with error rate of about 5-7 days **(Belk *et al.*, 2018).** There were studies which investigated microbial succession for several months in soil samples and it was analysed to have various microbial species which were characterized for each stage of decomposition. Other studies were also carried out in which the microbial invasion into bones was analysed. This study was carried out by Damann et al. The studies based on the later stages of decomposition can be promising but needs a greater

number of experimental data based on frequent sampling and larger sample sizes.

Figure No. 3: Changes during different stages of Decomposition

Conclusion

In this respective article, the knowledge gaps that should be addressed for better understanding of the best use of microorganisms that can be used in the forensic investigations. The data collected from the microbiome study for the estimation of post mortem interval can be helpful to understand the decomposition process. Major knowledge gaps include the concepts related to the environmental variables, temperature ranges, body location etc. Also, microbial forensics can become the most useful field of forensic science that can provide the most accurate estimation of Post mortem interval.

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