A Review on Microbial Forensics: An Application of Microbiology in Forensic Science

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Abstract:

Few years back, the concept of living matter was undetectable to the naked eye but with improvement in technical skills, nowadays it is very easy to catch someone with all scientific evidence. This review is about the role of microbiology in forensic sciences. Microbiology has a role in every place whether it's medical sciences or any kind of postmortem. Forensic science plays the role with the life and after the life also. If the death of a person is not naturally occurred then forensic people will investigate the reason behind the death of that particular person. So, here the role of microbiologist and pathologist will take place with the expertise of every field they will cover all the facts and relate to the death and see which facts are suitable and what was the main reason behind the crime of criminal activity. Today the leading the way in both human health and anti-terrorism is microbiology. In a never-ending effort to protect people and find those responsible for their harm, forensic microbiologists research genes, infections, and contagions. To determine the post-mortem period for criminal prosecution or to identify who is responsible for biological attack, microbial forensics has been closely linked to these processes. This review's objective is to present basic microbiological ideas with a focus on forensic uses.

Keywords: *Forensic microbiology, microorganisms, microbes, bioterrorism, sexually transmitted infections (STIs).*

Introduction:

There are various definitions of "forensic science", but the simplest one is that it is the application of science to legal issues. In order to achieve their primary objectives, forensic scientists heavily depend on comparative analysis, where they compare evidence from a crime scene with evidence obtained from other sources, in order to identify the culprit. To illustrate, if DNA extracted from the victim matches the perpetrator's DNA, it can be said with a high confidence that the DNA belongs to assailant. The process of making such comparisons is based on Locard's exchange principle, which suggests that "every contact leaves a trace" means whenever two objects come into contact with each other, some material is transferred between them.

As a part of forensic science, forensic microbiology plays an important role in investigation and scientific analyses. Forensic microbiologists examine bioterrorism incidents, utilize small amounts of evidence to link individuals to locations of criminal activity and ascertain the cause and timing of death. The chain of evidence should be maintained following legal documentation of collection, transportation, and storage to make it admissible under the court of law. Every piece of physical evidence must be accompanied by a chain of custody form that provides information about the evidence's collection, testing, and handling, including the identities of all individuals who came into contact with it at the crime scene. In legal proceedings, it is crucial to demonstrate that the evidence was accessible only to authorized individuals, remained unchanged, and was appropriately stored to avoid any deterioration of the sample [1].

Forensic science comprises various fields, including biological analysis, fingerprint comparison, toxicology, and ballistics, among others, that are well-known. One of the newer areas is Forensic Microbiology, which gained significant attention after the Bacillus anthracis bioterrorism incident in 2001. However, forensic microbiology was not limited to bioterrorism and had been in practice before the anthrax event. This field of science deals with more than just bio-terrorism. "Microbes" holds great importance in field of forensic science, as microbial communities are ubiquitous and highly distinct and is present almost everywhere on Earth leads to various other advantages and their role and composition in humans, in the environment and in animals has been broadly studied and described in many types of research such as in molecular ecology and medical health [2,3]. NGS technology can be employed to sequence complete DNA extracts from any sample, enabling the identification of various bacterial taxa and strains to generate an overview of the existing microbial population. Prior techniques such as AFLP, DGGE and t-RFLP also produced profiles of population, but NGS surpasses them by overcoming many of their limitations, as it directly generates sequence information and requires no additional fragment analysis. A large number of samples can be analysed with a rapid decrease in costs which is initiating the growth in knowledge about microbial consortia which came as a new application in the field.

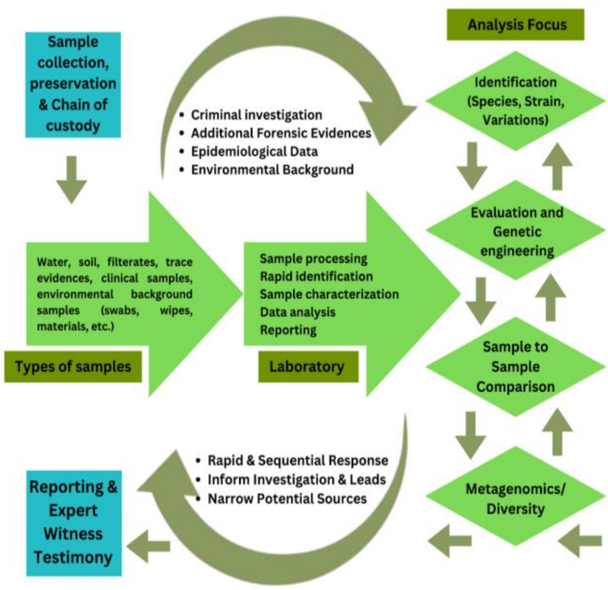

Figure 1. Process of Microbial Forensics

The field of microbial forensics entails examining a specific microbial isolate to identify dependable molecular differences between microbial strains and utilizing them to deduce the source, connections, or transmission route [4]. The examination focuses on an outbreak of a disease and aims to identify the responsible pathogen and trace it back to the source of the outbreak. These pathogens may establish new enzootic foci and can affect both human and animal by contaminating environment around them from decades. Here, the investigation involves the determination of the etiology which is, defining the origin of specific organisms, agents responsible, and the identification of causal agents. Discussions of different aspects of investigations under different cases have been well presented and explained in this review.

Development:

The historical use of bio-weapons, bioterrorism, and bio-warfare has been extensively recorded [5], far back as the 6th Century B.C., when rye ergot was used as poison by the Assyrians on their enemies' wells. Forensic Microbiology gained recognition after the 2001 anthrax threat [6], which was the most significant bioterrorism attack in recent times. This attack involved anthrax spores that emerged in New York in October 2001 [7], but Forensic Microbiology also addresses issues such as the outbreaks of food-borne diseases or hospital-acquired diseases, as well as the intentional spread of HIV by carriers [8]. In response to the anthrax letters, the FBI founded the Scientific Working Group on Microbial Genetics and Forensics (SWGMGF) to establish the basis of microbial forensics as a discipline.

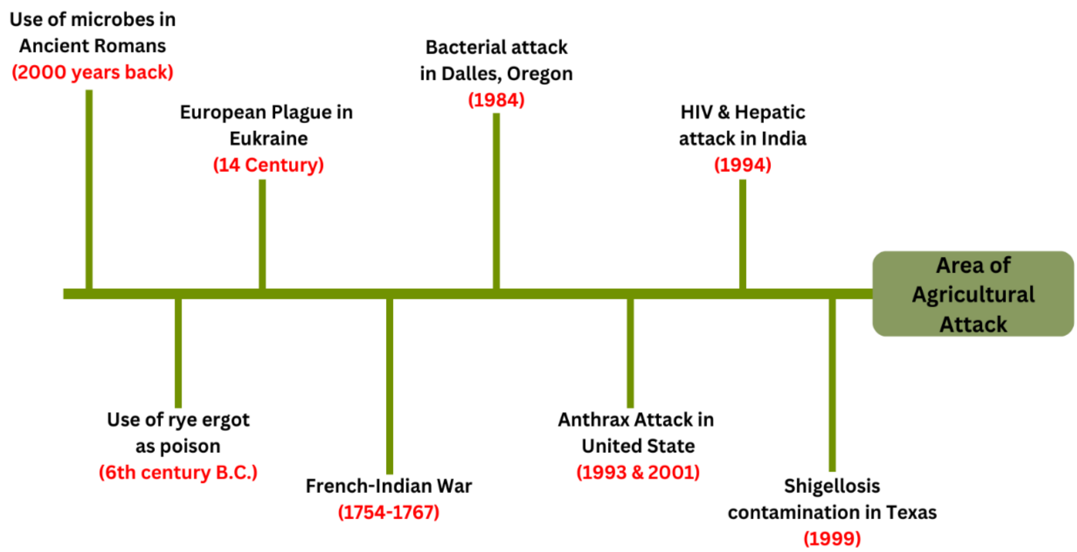

Figure 2. Timeline of Microbial Attacks

Applications:

Bioterrorism and bio-crimes:

The act of intending to cause harm to a person or group is referred to as a bio-crime or bio-threat. In these types of terrors, disease-causing agents or toxins are used as weapons. Here, actions can either be covert or overt.

In covert situations, a medical laboratory examiner or a physician is usually the first to detect a hazard, whereas in overt incidents, rescue personnel or emergency responders are the first to arrive at the place of the incident. Identifying the root cause of the infection causing agent is crucial in cases involving bio-threats, which can be accomplished by comparing isolates that were detected in victims to those associated with the offender. The molecular study of the genome is critical in this procedure. The methods used in analysing microbes are similar to those employed in studying human DNA, such as microsatellite and minisatellite loci typing, as well as single- nucleotide polymorphism analysis. Additionally, multi-locus variable-number tandem-repeat analysis, which is similar to the multiplex short-tandem-repeat typing used in humans, has also been utilized. As there is a variety of species of viruses and bacteria, it is challenging to do DNA fingerprinting in their case whereas it is easy to do so in the case of humans. In addition, bacteria are distinct from other organisms in that they are haploid, reproduce more frequently and mainly through asexual methods, and undergo horizontal gene transfer and recombination.

Forensic microbiologists have multiple techniques available to them, and the selection of methodology often be dependent on the required turn-around time. In cases where there is a possibility of bioterrorist attacks, prompt and precise recognition of the agents involved is crucial for reducing morbidity and mortality and avoiding panic. The rapid and accurate identification of numerous agents can be done by using microarrays or microchips as nowadays they are popular choices in these types of cases. Microarrays consist of a vast number of DNA sequences on their surfaces, which have the ability to attach to matching sequences present in test samples. The specificity of the microarrays is evaluated by the DNA sequences that are distinctive to assumed bio-threat elements. In such cases, environmental and bodily fluid samples are regarded as physical evidence, and multiple bacterial and viral phylogenetic studies are conducted and presented as evidence in court with approval from a judge. The United States has witnessed numerous incidents of bio-crimes. One of the earliest and well-documented cases of bio-crime was that of a dentist in Florida who had intentionally infected the individuals with the HIV virus [9]. The dentist faced accusations of infecting six patients during oral surgery without their knowledge, and he was charged accordingly. During the trial, the evidence provided included surgical records and phylogenetic analysis of HIV strains discovered in both the dentist and some of his patients. Genetic sequencing revealed that the viruses discovered in five patients were similar to the one detected in the dentist, However, they differed from test strains acquired from individuals who lived in the same region.

The same case involved Oklahoma, a dentist in Tulsa, who was charged for using non-sterilized and unhygienic methods while performing oral surgeries [10]. The accused dentist had a patient pool of about 7000 individuals. Out of the 4000 individuals who underwent testing, over 70 tested positives for HCV, five for HBV, and four for HIV. Consequently, the dentist relinquished his medical license voluntarily, and he is presently being sued by affected patients in multiple lawsuits.

A scientist at laboratory was charged with intentional infection of patients with HCV, leading to their arrest in 2012 on federal charges [11]. A laboratory scientist was taken into federal custody in 2012 for intentionally infecting hospital equipment and syringes with HCV using his blood.

The suspect's actions put an unknown number of patients at risk, as he worked in 18 different hospitals. Phylogenetic analysis indicated that the HCV strain detected in the suspect was identical to those found in 32 patients treated at a hospital in New Hampshire, where he worked.

Numerous individuals who have been diagnosed with HIV have faced charges of aggravated assault or have been labelled as sexual predators for intentionally infecting their sexual partners without disclosing their HIV status. In one case in Dallas, Texas, a homeless individual who was HIV-positive spat in the face of a police officer [12]. He was later found guilty of using a dangerous weapon (i.e., saliva) to assault a public servant and was given a 35-year prison sentence. The verdict was controversial because there is no scientific evidence to support the notion that saliva poses a significant risk of HIV transmission. Nonetheless, the judge contended that the attacker had the intention to cause harm to the officer.

There have been instances where people have been accused of murder when their partners infected with HIV died due to the infection. In 2009, a man in Canada was found accused of murder after intentionally infecting two women with HIV, who later passed away from HIV-related illnesses. This incident is thought to be the first time someone has been convicted of murder due to HIV transmission [13].

Governments and society are facing a significant challenge with the increasing risk and danger of bio-terrorism and bio-crime, which has prompted law enforcement agencies to investigate and evaluate activities related to such events. Boosting awareness and timely identification of threats are crucial factors in establishing an effective bio-security program, which can be achieved through a collaborative effort involving government, academic, private, and public resources with shared responsibilities and capabilities. Microbial forensic sciences are used in investigations to examine and identify forensic evidence, attribute it to a source, or reconstruct the crime scene. Molecular biology techniques, such as real-time polymerase chain reaction (PCR) and repetitive element PCR, are two examples of tools used to support the investigative process. These assays showcase the use of molecular biology techniques in assisting investigations.

Microbial forensic scientists ensure that the technologies they use are carefully examined and validated to ensure that the methods utilized are well-understood and that the interpretation of results can be conducted within the confines of the tests. Three types of validations are performed, which are preliminary, internal, and developmental. These validations are essential for ensuring the reliability and accuracy of the results obtained through the microbial forensic techniques used. The first validation is conducted to enable a swift response in the event of an imminent threat or a recent attack. The latter two validations are utilized for the regular implementation of established procedures.

A variety of microorganisms, including bacteria, viruses, fungi, and their toxins, collectively referred to as biological agents, can impact human health in several ways, ranging from minor allergic responses to severe illnesses, and potentially fatal outcomes. These microorganisms are prevalent in nature and can be found in animals, plants, soil, and water. Since many microbes have rapid reproduction rates and require minimal resources to survive, they pose a potential hazard in a broad range of work environments [14].

Microarray analysis and DNA fingerprinting, which are advanced molecular techniques, are utilized for various molecular identification purposes.

These techniques are used to measure the differences between related microorganism strains to link the causative agent's source which is associated with a specific person or group. In clinical microbiology, nucleic amplification and molecular-epidemiological techniques are vital tools for pathogen identification and for investigating outbreaks.

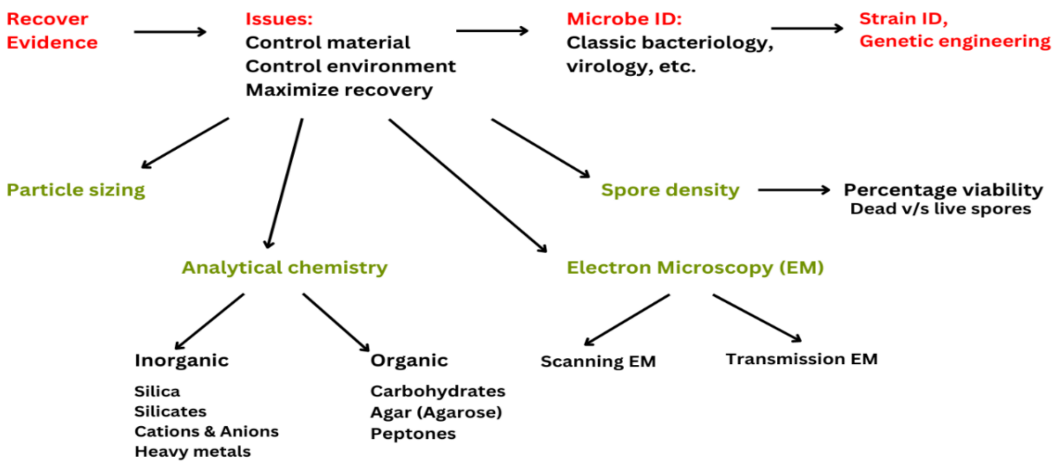

Figure 3. Flowchart for microbial forensic analysis for evidence from bioterrorism

Determining cause of death:

Investigating a suspicious death requires determining the cause of death, which is crucial. One question that frequently arises is whether the deceased passed away due to natural causes or if there was involvement of any foul play. Forensic microbiology performs an important role in situations like the determination of the cause of "sudden infant death syndrome" (SIDS). SIDS is a loosely defined condition that refers to the unexpected passing of an infant less than a year old. It is a frequent cause of death among neonates in developed nations [15]. SIDS is associated with various risk factors, such as genetics, immunologic disorders, and sleeping position. Several types of disease-causing microorganisms, such as cytomegalovirus, human herpes virus 6 and Epstein Barr virus [16], and bacterial pathogens like Haemophilus influenza, Neisseria meningitides, and Streptococcus pneumoniae have been linked to unexpected deaths [17]. In addition, there has been a suggestion that examinations of sudden infant death syndrome (SIDS) cases should encompass respiratory illnesses caused by viruses such as human metapneumovirus, Bordetella pertussis, respiratory syncytial virus, and adenovirus [15]. According to a study [18], 10% of sudden, unexpected deaths in children under the age of one were caused by infectious diseases, whereas 32% of sudden, unexpected deaths in children between 1 and 14 years of age were linked to infectious diseases. It is suggested that some infants may have passed away due to undiagnosed infections caused by the rapid progression of some infectious diseases. As SIDS is a complex condition with a variety of contributing factors, and its underlying causes are not always clear, there are instances when parents may be under suspicion for the loss of their child. Identifying an infectious disease as the cause of death can alleviate any suspicions the parents may have had and provide them with some reassurance.

It can be challenging to establish that an infectious agent is responsible for a SIDS death because simply identifying a pathogen in post-mortem tissue does not necessarily prove that an infection caused the death. For instance, the presence of bacteria in a sterile area like the bloodstream or cerebrospinal fluid could be a result of post-mortem translocation or transmigration, which is a natural part of the decay process that occurs after death. Certain possible disease-causing microorganisms, such as H. influenza and S. pneumoniae, are present in the normal microbial population of the mouth but can result in severe invasive infections, particularly in babies. Earlier assumptions were that post-mortem translocation would lead to the proliferation of multiple bacterial types, referred to as polymicrobial growth, rather than just one species. Nevertheless, a research study involving over 500 SIDS-related autopsies revealed no increase in the occurrence of positive cultures or polymicrobial cultures, indicating that post-mortem translocation did not play a role in the proliferation of these microorganisms [19]. Refrigeration of the deceased does not increase the probability of post-mortem translocation. On the contrary, the number of bacterial isolates obtained from samples were taken for culture a few days after death was lower compared to those collected immediately after death. This could be due to the sensitive nature of some microorganisms.

Forensic microbiology can assist in cases of drowning where determining the cause of death is challenging. Typically, drowning is diagnosed when all other possible causes have been ruled out. Forensic pathologists can identify external and internal indications of injury, but these indications may only indicate a violent form of asphyxia. Electrolyte changes, as well as haemodilution for freshwater drowning and haemoconcentration for saltwater drowning, along with the concentration of strontium and iron in blood and tissue, have been somewhat effective in determining the cause of death for victims found shortly after their death. While controversial, some forensic pathologists recommend testing for diatoms as a means of identifying drowning victims [20]. Diatoms are a type of algae that are found everywhere, including freshwater, salt water, brackish water, soil, air, and food. If diatoms are discovered in the liver, bone marrow, and kidneys after death, it indicates that they were disseminated through the bloodstream from the lungs while the heart was still beating during the drowning event. It is also crucial to detect diatoms in the drowning medium, which is often water.

The detection of diatoms has traditionally been done by extracting them from tissue and then examining them under a microscope, which is a time-consuming process. Certain researchers have proposed utilizing polymerase chain reaction assays as an alternate way of detecting pathogens. However, there are concerns about its accuracy in detecting pathogens due to its sensitivity and its proneness to post-mortem contamination, which could lead to false positive outcomes. Because of these limitations, forensic scientists have studied the use of assays that can detect faecal coliform and streptococcus bacteria instead of diatoms in suspected drowning cases. The use of these assays has demonstrated their sensitivity in identifying drowning as the underlying cause of death [21]. To determine the cause of death in suspected drowning cases, forensic scientists have investigated the presence of bacterioplankton in the blood and tissue of victims. Bacterioplankton is bacteria that are suspended in bodies of water, with different species predominating depending on the salinity of the water. Halophilic organisms are found primarily in ocean water, while non-halophilic organisms live in freshwater. According to studies [22, 23], the detection of certain bacteria in a drowning victim's blood can indicate the salinity of the water in which they drowned.

However, there is a possibility of contamination after death if the body is dumped in water containing faecal bacteria or bacterioplankton. Studies [21, 22] have shown that in non-drowning victims retrieved from water that contains faecal organisms or bacterioplankton, there is no evidence of passive diffusion of the immersion medium in the lungs or bacterial invasion.

Mushroom poisoning is a potential cause of death that falls under the purview of forensic mycology, and it can occur either accidentally or intentionally. This type of poisoning can be fatal and often results from the ingestion of mushrooms that have been misidentified by untrained collectors, who are often related to the victims. While the severity of the symptoms varies according to the number of mushrooms consumed and the individual's tolerance, most cases are not fatal. The symptoms of mushroom poisoning can emerge quickly in some instances, while in others, such as those affecting the kidneys, they may not surface for several days. Gyromitrin (in *Gyromitra esculenta*), amanitins (found in species such as *Amanita virosa*), orellanine (in *Cortinarius orellanus*), and muscarine (in certain species of *Conocybe* and *Inocybe*) are some of the most poisonous fungi, with even tiny amounts potentially resulting in fatality [24]. True dangerous mushroom species are few, while more species have the potential to upset certain people's stomachs. Regrettably, some of the deadly species are quite widespread and, to a non-expert, can resemble some edible ones. Any leftover mushrooms should be stored safely, and stomach contents should be examined in the event of any suspected mushroom poisonings.

Certain molds are capable of producing toxins, either directly or through the reaction of their products with other compounds. *Scopulariopsis brevicaulis* is a notable example of such a fungus. This fungus has been linked to the generation of trimethyl arsine from compounds containing arsenic. When this fungus grows in damp conditions on wallpaper that contains "Paris Green," it can generate this gas, which can cause death if inhaled [25]. In the 1990s, a theory was suggested that *Scopulariopsis brevicaulis* reacted with arsenic-containing fire retardants found in cot mattresses, leading to the release of harmful gas and resulting in sudden infant death syndrome (SIDS). However, this hypothesis was not supported when it underwent a thorough examination [26].

Despite this, other fungi exist in bedding materials that can generate various toxins. When investigating suspicious deaths that occur in beds, officers who arrive at the scene should inspect for significant fungal growth.

Identification of individuals:

For the last 20 years, human identification has been a crucial aspect of forensic science, relying on distinctive genetic characteristics. Nevertheless, this approach may be restricted in certain circumstances, such as with twins. Recently, sequencing methods have become accessible, and they may provide extensive data that could be valuable in forensic investigations.

The use of human DNA is the mainstay of current forensic human identification methods. However, due to factors such as degradation and low quantity, there are limitations to its use. These limitations can make it difficult to identify offenders in cases such as sexual assault, where the victim's DNA typically masks the perpetrators.

To address this issue, the potential of alternative targets, such as bacteria, is being explored. Bacterial DNA is of interest in this context because it may be more abundant and less prone to degradation than human DNA, and it may be able to provide valuable information about the individual who deposited it. To begin with, bacterial DNA [1] is more resilient to degradation and better protected than human DNA, meaning that it can endure better once left on a surface. Additionally, bacterial DNA has the potential to differentiate between twins, which is not feasible with current human DNA-based techniques. Moreover, approximately 99% of bacteria present in the environment are uncultivable [2]. Next-generation sequencing (NGS) has revolutionized the analysis of composition of bacterial community, allowing for previously unattainable depths of analysis. As a result, there is now potential to utilize bacterial analysis for forensic purposes. In a study conducted by Fierer and colleagues [29], it was shown that the analysis of an individual's skin microbiome could establish a connection between the person and an object they had touched. Furthermore, the researchers discovered that the bacterial population present on the object was more akin to that present on the owner's hand rather than the communities present in the hands of 270 other people. This implies that this technique has the potential to be employed in forensic identification [3].

Table 1a. Different types of microbiomes found in skin, can be used for individual identification

SKIN MICROBIOME:	ACTINOBACTERIA *Propionibacterium* *Corynebacterium* *Other actinobacteria*
	FIRMICUTES *Staphylococcus* Other firmicutes
	PROTEOBACTERIA
	BACTERIODETES
	SOME OTHER MICROORGANISMS

Table 1b. Different types of microbiomes found in hair, can be used for individual identification

HAIR MICROBIOME:	BACTERIA *Propionibacterium spp.* *Staphylococcus spp.* *Corynebacterium spp.*
	FUNGI *Malassezia spp.*
	VIRUSES

Table 1c. Different types of microbiomes found in different types of body fluids, can be used for individual identification

BODY FLUID MICROBIOME:		
Body Fluid	**Microorganisms**	**References**
Blood	*Staphylococcus epidermis* or *Staphylococcus aureus*	[30]
Semen	*Lactobacillus* *Finegoldia* *Gardnerella* *Streptococcus* *Haemophilus* *Pseudomonas* *Prevotella* *Rhodanobacter*	[31]
Saliva	*Corynebacterium* *Haemophilus* *Campylobacter* *Porphyromonas* *Streptococcus* *Solobacterium*	[32]
Urine	*Enterococcus faecalis* *Klebsiella pneumoniae* *Staphylococcus saprophyticus* *Proteus vulgaris* *Streptococcus agalactiae* *Pseudomonas aeruginosa*	[33]
Vaginal fluid	*Lactobacillus gasseri* *Lactobacillus crispatus* *Escherichia coli* *Gardnerella vaginalis* *Staphylococcus spp.* *Streptococcus spp.*	[34]

Determining Time Since Death (Post-Interval Mortem):

For centuries, people have been studying whether there is life after death, but many questions remain unanswered. Forensic microbiologists have developed new methods for studying substitute in microbial activities that occur after a person's death. However, there are certain shortage of research on thanatomicrobiome (microbial communities on decomposing remains) and epi necrotic communities (microbial communities found on the surface of decomposing remains). Conducting further research can help in better understanding the function and structure of the post-mortem microbiomes. Studies on the human microbiome have revealed that 75-90% of cells present in the body before death are microbial.

After death, a complex process of autolysis (self-digestion) and chemical degradation of cells, as well as the removal of intestinal composition because of enzymes affected by both biotic and abiotic factors, leads to decomposition. These factors have predictable effects on post-mortem microbial communities which can be used in forensic studies to determine the cause and time since death [35].

Post-mortem microbiology is valuable in clinical and forensic autopsies, allowing for confirmation of suspected infections. It is regularly used in donor studies in clinical settings and sudden and unexpected death investigations in the forensic field. To minimize the risk of contamination, specific sampling techniques are employed during the autopsy. The use of molecular diagnosis and its integration with molecular biology and histopathology have established specific interpretation criteria for post-mortem cultures, making it a vital component of an autopsy. Collaboration among microbiologists, pathologists, and forensic physicians will enhance the potential of post-mortem microbiology, resulting in the prevention of infectious diseases and improving overall health outcomes for the population.

Accurately determining the time since death is crucial in death investigations, but currently used methods can be prone to errors and biases. Forensic entomology, for instance, can lead to a range of errors from days to months when assessing the post-mortem interval (PMI). Microbes offer a potential new technique for estimating PMI that overcomes many of these limitations. Researchers have demonstrated in a mouse model that changes in the post-mortem microbial community are significant, quantifiable, and consistent, enabling PMI estimation within 3 days over 48 days [36]. Microorganisms have been present at crime scenes for over a century and have been used as physical evidence. The introduction of DNA sequencing and computational methods has brought about significant progress in the utilization of microbiome techniques for forensic science. This has especially been useful in determining post-mortem intervals (PMIs), discovering hidden graves, and obtaining trace evidence from skin and soil. Because of inexpensive and high-throughput technologies and with the use of advanced machine-learning algorithms, it is now possible to gather molecular data fastly to develop predictive models that can be valuable to serve the criminal justice system. The combination of the microbiome and metabolomic data is a particularly promising arrange for advancing microbial forensics [37].

Determination of the post-mortem interval or time since death is crucial part for a homicide investigation. Forensic pathologists utilize different techniques to approximate as the time since death, also called as the post-mortem time. They may assess the body's core temperature and rigor mortis in the first two days after death, or they may rely on forensic entomology and decomposition for longer period of time. However, for the accuracy of these methods is not always reliable. Forensic microbiology has been used in some cases to help determine the post-mortem interval, but it is not a widely used technique. For example, in one case, Forensic microbiology played a role in the investigation of a man's body found in a closed apartment with multiple stab wounds, as the absence of flies helped confirm the post-mortem interval [38]. At the location where the crime occurred, it was found that the carpet and couch fabric contained traces of blood as well as fungal colonies. The investigators measured the measurements of the mold colonies. Their objective was to determine the time since death, and for that purpose, they replicated the crime scene by removing some fabrics and carpet that lacked bloodstains and adding bovine blood.

They then introduced fungi from the original crime scene into the carpet samples with bloodstains and kept them under controlled laboratory conditions similar to those of the apartment in terms of relative humidity and temperature. The primary fungal species identified were *Penicillium citrinum*, *Penicilium brevicompactum*, and *Mucorplumbeus*. Through comparing the size of the colonies grown in laboratory with the discovered at the scene of crime, they concluded that death occurred for about 5 days before the body was discovered. Subsequently, a suspect made a confession, and the estimated time since death was verified.

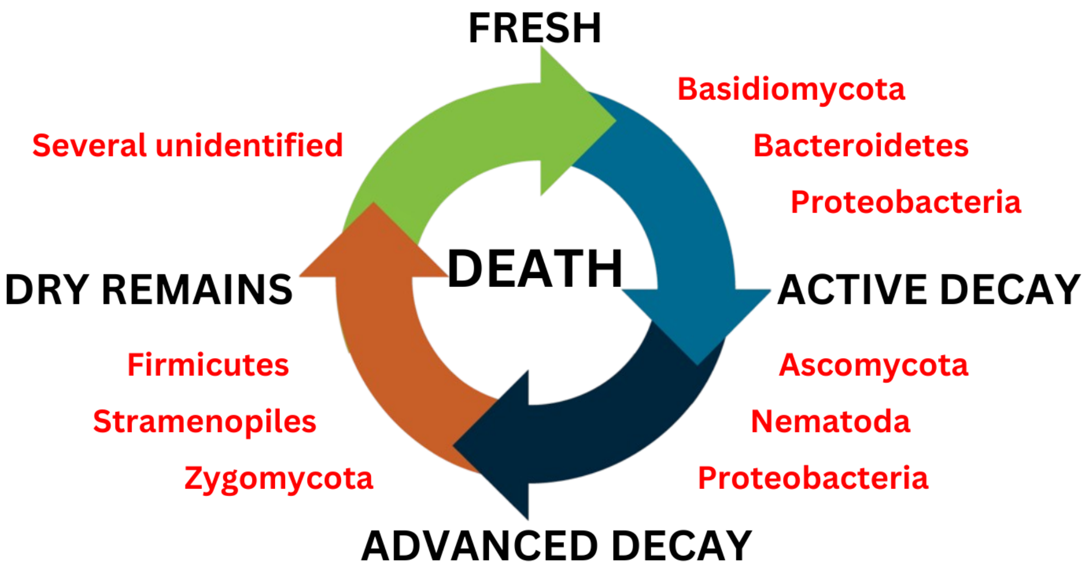

Figure 4. Some microbes which are found at different stages of decomposition

Other medico-legal aspects:

Various infectious microorganisms can be transmitted through sexual activity, including sexual assault. Some of the most common sexually transmitted infections (STIs) that can be contracted following sexual assault are *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and bacteria that cause bacterial vaginosis [39]. The occurrence of sexually transmitted infections (STIs) in a sexually active person cannot be automatically attributed to sexual assault, as they may have been acquired consensually before the assault. Although testing for STIs during an assault does not generally carry any legal weight, it remains a concern for survivors of sexual assault. Providing information about STIs and their symptoms, as well as offering subsequent therapy, is crucial. Whether or not to test a sexual assault survivor for STIs should depend on their particular situation. Certain survivors might be reluctant to take the test because they worry that having a STI could be used against them in court as proof of their sexual promiscuity. This is rule that prohibit using a survivor's past sexual history, including proof of prior STIs, in order to avoid undermining them [40]. To ensure the proper care and treatment of sexual assault victims, testing for STIs may be necessary for certain circumstances. This may involve testing individuals who are not sexually active as a baseline, to identify any subsequent development of an STD that can point to a sexual assault.

In cases where the transmission or threat of STI was part of the crime, such as the intentional transmission of HIV, testing is also important. To detect HIV, HCV, and HBV antibodies and/or antigens, blood serum should be collected. Additionally, NAATs and vaginal wet mounts can be used to screen for *N. gonorrhoea*, *C. trachomatis,* and *trichomoniasis*, respectively. It is essential to acknowledge that STIs contracted during a sexual assault may not be evident during the initial evaluation and may necessitate a subsequent examination. As with any physical evidence, evidence collected from sexual assault victims should be collected by trained personnel. Victims should also receive proper counselling and support, including information about STIs and potential symptoms to watch out for. Nurses who specialize in conducting medical assessments for victims of sexual assault are known as Sexual Assault Nurse Examiners (SANE), these nurses have completed specific education and practical training in order to do medical examinations for victims of sexual assault. Forensic nurse examiners have received additional training to collect forensic evidence related to various crimes. In cases where a child has an STI beyond the neonatal stage, it is usually indicative of sexual abuse [40]. However, certain cases differ from the typical pattern. For example, *C. Trachomatis* infections during the neonatal period can cause ocular and vaginal infections in young children, and the infection can last for up to 2-3 years. Furthermore, the presence of genital warts has been linked to childhood sexual abuse, but it has also been noted in children who do not show any other indications of abuse [41]. To ensure proper treatment, it is important to test children for STIs if they display symptoms, regardless of the cause. The majority of HBV infections in children occur due to exposure in the household from individuals with chronic HBV infections. The timing of the examination is crucial, as the infectious agents may not be detectable if the assault occurred recently. Therefore, a follow-up examination may be necessary after 2 weeks. It may take up to an additional 10 weeks to identify the infection through antibody scans. It is crucial to assess physical signs of genital abuse and symptoms of sexually transmitted infections (STIs) during both the initial and follow-up examinations. Vaginal, anal, and oral specimens must be cultured in the case of *N. gonorrhoea*; if urethral discharge is present, a swab culture of the urethral meatus is suitable [40]. To ensure accurate results, it is necessary to use standard culture methods, such as the inoculation of selective media, for identifying infectious agents. Simply conducting Direct Gram stains is not enough to confirm the presence of an infection. At least two distinct techniques, such as biochemical, enzyme substrate, or serologic testing, should be used to conclusively identify the isolates. Isolates must be kept in storage in case further testing is necessary in future. Culture is preferable for urethral or urine specimens from boys and for extra-genital specimens acquired from both male and female patients, even if NAATs can be used to examine vaginal specimens or urine from girls. Samples from the anus in boys and girls, as well as the vagina in females, are acceptable for detecting *C. trachomatis* [40]. To isolate *C. trachomatis* from samples taken from the vagina in girls and the anus in boys, standard cell culture techniques should be applied. Under a microscope, suspected isolates should be verified using a monoclonal antibody conjugated with fluorescein that targets *C. trachomatis* exclusively.

Preserving isolates for future testing is crucial, and confirmation of isolates through methods like enzyme immunoassays (EIAs) are not considered adequate.

It is not advised to employ direct fluorescent antibodies, non-amplified probes, or EIAs in child abuse situations due to their lack of specificity [17]. Girls' vaginal and urine samples can be tested for STIs using NAATs. However, there is not enough information available to recommend using NAATs for boys or for testing extra-genital samples. Cultures are still considered the preferred method for testing extra-genital specimens.

Analysis of microorganisms can be included in the investigation of medical malpractice, which refers to substandard medical care or disagreements between a patient and their physician about the quality of care provided.

To establish a case of malpractice, it is necessary to demonstrate negligence, meaning that

(i) It was the accused person's (the medical professional's) duty to tend to the patient,

(ii) The offender did not carry out duty, and

(iii) As a result, the victim (patient) suffered injury.

This indicates that in order for the patient to suffer injury, the doctor had to have either done something that was not regarded as conventional medical practice or neglected to do something that was.

Conclusions:

Forensic microbiology is a developing field that is still evolving. It is becoming increasingly important due to the ongoing risks of bio-terrorism and bio-crime. The most crucial aspects of forensic microbiology are the subtyping of infectious agents and rapid identification. In recent years, the cost of sequencing microbial genomes has significantly decreased, making this technique more accessible to laboratories working in this field.

The paper explores the involvement of microbiology in different investigative and evaluation tasks. Additionally, it presents a summary of how microbial forensics can assist investigations through the examination of forensic evidence to ascertain the source of the offense or recreate the crime scene. Biological agents or toxins produced by organisms can cause severe illness or death. Biological agents comprise a diverse range of microorganisms, including parasites, bacteria, fungi, and viruses. To improve confidence in scientific analyses and interpretations of results, microbial forensics has been employed.

Microbial forensics is presently pursuing progress in numerous domains, such as developing efficient techniques for gathering and conserving samples, exploring novel ways of detecting non-viable and minute materials, validating methodologies to assess the constraints of analyses, identifying genetic markers and matching criteria to establish benchmarks for contrasting reference and evidence samples, and creating unambiguous interpretation standards.

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