Microbial Forensics: A New Boon to Legal Justice

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Abstract

Forensic microbiology is a thriving new discipline combining both microbiology and forensic sciences. The growing field deals with profound effect of microorganisms weapons which identify and prioritize bioterrorism. Bioterrorism uses microorganisms as weapons which has been known to exist since centuries. The major components of a successful microbial forensics investigation are detection, identification, characterization, attribution and interpretation of weapon pathogen. Advance technologies in this field no doubt develop better resources and treatment for the microbial diseases which affect humans.

Keywords

Bioterrorism · Pathogen · Attribution · Forensic

22.1 Introduction

A microorganism or microbe is a microscopic organism which may exist in single celled form or in colonies (Schmedes et al. 2016). There are different varieties of microorganisms in our environment. These microbes are both boon and bane for us and for our surroundings.

Because of their universal nature, they can act as a commendable forensic indicator for investigation. Microbial forensics has been defined as "a scientific

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discipline dedicated to analyzing evidence from a bioterrorism act, biocrime, or inadvertent microorganism/toxin release for attribution purposes"(Budowle et al. 2003).

Microbial forensic investigation includes characterisation and detection of both biological and non-biological evidence. Biological agents consist of protists, toxins, bacteria, fungi and viruses. Non-biological evidence, such as growth media, additives, and delivery devices, which can be useful in microbial forensics, potentially provides investigative leads and helps infer methods of manufacture and dissemination (Budowle et al. 2008).

In this study, proper classification of specific organism is considered beside its origin and impacts. Indeed our human body contains botches of bacteria inside it; consequently they can act as a proficient tool in forensic investigation. For instance, if the sample collected from the crime scene which could be a whitish liquid, the microbe will tell almost the specific fluid by the assistance of microbiome inside the human body.

The microorganisms are amassed inside the body on or inside various body fluids, tissues, skin or glands like lungs, placenta, ovarian follicles, seminal fluid, uterus and gastrointestinal tracts. This aggregate of microorganisms inside the human body is known as human microbiota. Microbes which reside on the human body are typically excluded from this elucidation. The human microbiome interprets precisely the collective genomes of resident microbes. Many microbes reside inside the human body. The conventional estimate is that the average human body is dwelled by 10 times as many non-human cells as human cells, but the recent estimate had dropped that value to 3:1 (Sender et al. 2016).

On the basis of their interaction with the host or human body, the microorganisms are classified into:

1. Commensal (they coexist without harming the host/human being)
2. Mutualistic (both host and parasite are benefitted)
3. Non-pathogenic (parasite can harm human hosts through their secretions or metabolites such as trimethylamine)

There are certain microbes which are favourable for the human body. These include:

22.2 Bifidobacteria

A diverse microbial community has evolved to adapt and survive in the human GIT and is commonly referred to as the gut microbiota (Thursby and Juge 2017). It helps in converting complex food into simpler form and helps in absorbing nutrients for providing energy to the body. The demerit of this bacteria is that it liberates gas. It helps the body to get rid of constipation and diarrhoea.

22.2.1 *Escherichia coli*

These are present inside the human intestine. This bacteria is useful in digestion and helps in providing nutrition to the body. Moreover, it fights with harmful bacteria inside our body and inhibit their growth.

Hence, their location can be easily determined from this human microbiota. We can easily analyse the type of sample by knowing the origin of the microbe. The most important thing is that these microbes are present universally. Therefore, they play a major role in contamination of the sample. In forensic scenario, we have no hold over the purity of the sample. If the sample is contaminated by bacteria, pathogens, fungi, etc., *Staphylococcus* and *Bacillus cereus* are the microorganisms that are most often recovered from contaminated blood. Forensic scientists can also analyse the time period, the progressive changes that occurred simultaneously by time since death decomposition analysis. The progression can typically be divided into a number of distinct stages:

1. Fresh
2. Bloated
3. Decay
4. Post-decay
5. Dry skeleton

Each stage is additionally associated with a rough time period during which it is likely to occur, subject to the components that can modify these time periods. Each of these stages are also associated with the arrival of distinctive species of microorganisms and insects. DNA sequencing is an everlasting technique used in the forensic scenario for the purpose of research. Moreover, this technique has various roles and applications which were not known earlier. Hence, DNA sequencing is the newer methodology in microbial analysis. A much better understanding of the environment, how it changes over time, and how it interacts and changes the ecology of its more extensive environment might have vital applications in forensic science. It could, for example, lead to new, more accurate ways of estimating time of death and of finding bodies that have been hidden (Pattnaik and Jana 2005).

In case of transmissions of diseases especially in sexual assault case, these microbes are utilitarian for forensic investigation as they tell about successive changes through their life cycle. The population of microbes increases when they act on any sample and their colonies may form. Variations are analysed in the sample and in the microbes and hence identification is done. Microbiology along with forensic science focuses on the reliability to describe the nature, origin and classification of these microorganisms along with their stimuli to different external and internal factors. If these microbes are present in a trace amount, forensic analysis can be achieved to yield desired results.

22.3 Bioterrorism

Bioterrorism is an illicit, menacing use of microbes or their toxins to cause death and destruction to humans, animals or plants (Murch 2001). Many of these microbes or bio-agents are isolated from the environment and are manipulated genetically to make them more hazardous and pathogenic so that they can cause destruction at a very high scale when used as a bioweapon.

The microbial agents used for this purpose include:

- *Bacillus anthracis*
- *Brucella abortus*
- *Brucella suis*
- *Clostridium*
- *Tularaemia*
- *Filoviruses and arena virus*

These are supplied/sprayed over large geographical areas and are transferred into the human body by inhaling, contact or via the gastrointestinal tract. In some cases these pathogens are mixed with the water bodies of geographical area which is to be destroyed or are mixed in the crops. The crop and water samples are collected by the investigators with great care and are sent to the laboratory for analysis. In the laboratory samples are analysed and interpreted by using certain tools and techniques, and hence the result is made. The techniques used most commonly are enlisted below.

These microorganisms are divided into three different categories:

Category A: Easily disseminated
Example: *Bacillus anthracis, Yersinia pestis, Variola major*
Category B: Moderately easy to disseminate
Example**:** *Brucella* spp., *Salmonella* sp., *Shigella* spp.
Category C: Engineered for mass dissemination

22.4 Bioterrorism and Forensic Science

Forensic science plays a major role in bioterrorism. Microbial forensics deals with study related to classical microbiology, microbial genomics, phylogenetics and informatics. The samples collected from the attack site are analysed by various scientific tools and techniques. This includes dealing with knowledge of development and preparation of microorganisms, the arrangement of toxins, the different approaches for weaponisation and dispersal of biothreat agents, and the use of synthetic biology (Tims et al. 2010).

The techniques equipped for the analysis include:

DNA typing, genomic polymorphism, genetic mapping, massively parallel sequencing.

Microbial forensic investigators focus mainly on three important steps:

Identification: Identification includes identifying the microorganism used in the attack. Depending upon the destruction caused, the pathogen can be analysed by manifesting the signs and symptoms on the particular population (human/animals/plants) in the affected geographical area. It is the main step because if the pathogen is identified the causes, origin and study become quick easy.

Characterization: It deals with the successive events that took place while destruction. This includes whether the attack was intentional or unintentional. It is a very useful step for determining the cause of death.

Attributions: In the third step – the source – tracing is done, i.e. the origin of the pathogen is identified. It helps in locating the perpetrator and weapon strain pathogen.

The tasks involved in identification and attribution include:

Identifying and collecting sample
Handling and preservation of the collected sample
Choosing appropriate analytical methods
Casework analysis
Interpretation of results
Quality assurance and validation

Forensic science works by initially collecting the samples from attack site. Further the recognition of the attack is done; it is based upon parameters like intentional, unintentional, covert, overt attacks. The next step is diagnosing the diseases caused to particular inhabitants of attack area. This is followed by analysis of the specimens and their identification to claim out the origin of the pathogen and culprit. Last but not the least, validation is done in which the quality assurance and quality control is checked. Because microbial forensics focuses on tracking and establishing the link between microorganisms and individuals along with the locations, different strategies are implemented, depending on the nature of the attack and the type(s) of evidence collected (Budowle et al. 2010). In an overt attack, for instance, the evidence collected such as the package, the weapon and associated materials (hairs, fibres, fingerprints) can be analysed. In a covert attack, the evidence gathered from the crime scene may be limited to medical histories, diagnoses and isolates taken from victims.

Forensic science is the science of identification and comparison. The comparison of a genetic profile from a reference sample with that of an evidentiary sample can have three possible general outcomes: “match” or “inclusion”, “exclusion”, or “inconclusive.” Because of lack of database, genetic testing and unknown diversity, there is a lot of inaccuracy, but on the flip side, the advancement in the forensic microbial tools triggers certain amount of accuracy which is very apprehensive (Budowle et al. 2005).

22.4.1 Techniques/Methods Used in Forensic Investigation: Microarray Analysis

This is useful in genomic study of pathogens and other microbes involved in bioweapon.

It involves complex and statistical tests.

Sample preparation →Hybrids→Washing→
Image acquisition→
Data analysis

Microarray is a pattern of SSDNA probes which are not allowed to move and are stuck on a chip or slide.

Hybridisation is used to detect specific DNA or RNA in a sample.

22.4.2 DNA Fingerprinting

In this technique, the DNA is extracted from the pathogen cell. Cutting of DNA is done by enzymes, then the DNA fragments are separated and transferred onto a paper. Radioactive probe is added an end X-ray film is set Electron Microscopy (Budowle et al. 2008).

22.4.3 Serological Assays

Serological assay technique is used for diagnosing infectious diseases. Some pathogens are highly infectious and are difficult to cultivate, so this technique is used to determine the diagnostic accuracy of specific tests.

22.4.4 PCR

PCR (polymerase chain reaction) is used as an effective tool in DNA fingerprinting. This technique is useful in individualization. For example, any minute DNA sample collected from the crime scene/attack site can be compared with other suspected samples (Hampton-Marcell et al. 2017). PCR-based DNA fingerprinting is also useful in solving maternal and paternal disputes. Quantitative polymerase chain reaction is used for detection of nucleic acids in many biological fields.

22.4.5 Subtractive Cloning

This technique is also termed as subtractive hybridization. This technology works in the way that it removes dsDNA formed by hybridization between standard and reference sample. It is used for identification of strain-specific DNA sequences in a variety of microbes like bacteria.

22.4.6 Advanced Techniques in Forensic Science

22.4.6.1 Next-generation sequencing/high-throughput sequencing/ massively parallel sequencing (MPS)

This technique has overcome the problem of identification of unknown pathogens and microbes. It helps in detecting less amount/minute microbes in complex mixture sample.

MPS is useful in the characterisation of microbe and checks its abundance and nature (whether degraded or intact). It rapidly diagnoses and oversees the infections using culture-independent methods and hence trails the disease outbreaks.

MPS provides tactics for human microbiome and is useful in human identification, body fluid characterisation and time-since death decomposition analysis (Budowle and Chakroborty 2004).

22.4.7 Microbiome Profiling

Microbiome profiling is useful in identifying a person or lifestyle characteristic according to the studies. Humans shed 30 million bacterial cells into their vicinity every hour, e.g. bacterial community found on our fingerprints which could be traced on keyboard in cases where computer has been used and, hence, a person can be identified according to bacterial residue with the help of microbiome profiling.

22.4.8 Metagenome Classification

This technique is used for the extraction of essential information of the organism through the traces left by it in a given environmental sample. It helps in depicting origin and generating composition profile along with diversity of the microbe. It leads to fast and accurate classification after sequencing entire samples and it allows a database to be built without restriction.

22.5 Case Studies in Support of Microbial Forensics

- ***Salmonella enterica***

Salmonella enterica serovar Typhimurium is a Gram-negative, rod-shaped, facultative anaerobic pathogen. This pathogen infects both humans and animals. Infection due to *Salmonella* occurs due to contaminated food and water, which leads it to intestinal epithelium and triggers gastrointestinal diseases. When this bacteria first enters the human body, it initially propagates inside the intestinal tract and spreads throughout the peripheral lymphatic system, such as the bone marrow and causes typhoid fever.

Case Study

The **Rajneeshee bioterror attack (1984)** was the food-poisoning attack of 751 individuals in Dallas, Oregon. Salad bars contaminated with *Salmonella* were used for the attack at 10 local restaurants by Rajneeshee followers (later known as Osho) led by Ma Anand Sheela. Among them, 45 individuals were hospitalised but none died. The incident is considered to be the biggest bioterrorism attack in the United States until now (Lightfoot et al. 2001).

- **Scrub Typhus**

It is a mite-borne disease and is also well known as bush typhus. It is mainly caused by bacteria *Orientia tsutsugamushi* (Rickettsia tsutsugamushi) and through infected larval mites or chiggers which belong to family Trombiculidae and genus and subgenus *Leptotrombidium.* Symptoms of scrub typhus will begin after 10 days of being bitten by the bacteria like body chills, muscle pain, rashes, fever, chills, headache, etc.

Case Study

Scrub typhus disease was diagnosed in the troops of Assam and West Bengal during World War II. During the Indo-Pakistan War of 1965, the same symptoms appeared in Jammu-Sialkot sector troops. A similar scrub typhus outbreak in north-eastern India came under suspicion and India's defense and typhus outfits were alerted to this outbreak of pneumonic plague (Singh 2004).

- **Diatoms**

Diatoms are unicellular algae found in freshwater or sea water. They exhibit great role in investigation of drowning cases in the field of forensic sciences. Through diatoms forensic scientists can establish ante-mortem and post-mortem drowning. When a person gets submerged into water, the diatom will enter the lungs through water and then carried to distinct parts of the body through the circulation process. But when the person is already dead, the water will enter the lungs but there will be no circulation. By examining the body tissue for diatom, we can establish the ante-mortem or post-mortem drowning.

Case Study

A dead body of a male aged 30–40 years was found in a highly decomposed condition near Shimla bypass, Himachal Pradesh, India, in the month of November. The person was identified as Lal Pani Nullah (canyon). The cause of death could not be ascertained in autopsy. The femur bone was sent to the laboratory with water sample of canyon for the detection of diatom. After examination, diatoms *Cymbella sp., Aulacoseira sp. and Gyrosigma sp.* were detected in the water sample. However,

diatoms could not be detected in femur bone, which signifies that it was post-mortem drowning (Kaushik et al. 2017).

22.6 Forensic Application

Microbial forensics elucidate the new and growing field of microbial forensic that is the science that will help in providing justice to victims and apprehending criminals and terrorists who use biological material to cause harm and destruction. Microbe forensic is a very vast field in forensic scenario. It is very helpful in identification and attribution of the pathogens used as a bioweapon. Forensic scientists use different tools and select appropriate analysis methods and tools according to the sample. Databases of these microbe genomes are burgeoning promptly and prove to be commendable for forensic analysis. Forensic microbiology works as a base for interpreting the result. It uses certain tools and techniques to explore the identity and origin and to initialize the investigation.

– **Helpful in Solving Crimes**

Microorganisms because of their universal nature are very helpful in solving crime cases. They are part of decay process; therefore, by analysing the extent of decomposition and type of microns, one can tell about the age of dead body and reason of the death. In case of drowning, the microbes inside the body will give detailed information regarding death (during post-mortem).

Using genomic analysis of the pathogen and identifying its origin in most cases can be solved i.e. bioterrorism attack by *Bacillus anthacris* in USA, 2001 was solved by genomic analysis. The 2001 anthrax attacks are also called Amerithrax. It took place in Washington, DC. This attack killed five people; it has become obvious that biocrimes can only be solved when genomic information is used to identify the source of an organism.

– **Identification of Source**

In forensic microbiology certain methodologies are there which can be used to detect and trace back the spread of microorganisms in the context to the crime. By forensic analysis of microbes, the perpetrator pathogen and type of events can be analysed. It can tell whether the cause was intentional or unintentional or whether it was covert or overt.

– **Scrutinizing the Human History by Studying Ancient Microbial DNA**

Study of ancient microbes and their DNA is known as paleomicrobiology. Paleomicrobiology is the study of microbes which are associated with primeval material. This field of science is hauled from various other branches like microbiology, anthropology, history, palaeontology and archaeology. It tells about the disease

and infections that our ancestors had suffered from their dietary habits were also analysed through this method. So, this study tells about the evolution and lifestyle of ancient pathogens.

– **Tracking Culprits in Public Health Crimes Along with Their Sources**

If pathogens are mixed with either food, crops, water bodies, by monitoring their quality and the interaction between the host body and pathogen is analysed. This analysis is done by microarray analysis (DNA) sequencing qPCR. qPCR is used to detect, characterise and quantify nucleic acids for numerous applications. It involves fluorescent labelling of the data which quantifies the amplified DNA molecule.

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