

# Microbial Forensic – An Investigative Approach

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**Abstract:** The study of microbes' sources can aid in the investigation of bioterrorism, biocrimes, and cases of disease outbreaks. Microbial Forensics is a new discipline within the field of forensic science. As bacteria have been used as biological weapons for ages, anthrax letter attacks were very popular in 2001 and posed a serious threat to the general public. Even though there aren't many examples of this kind, it's still important to catch the offender. In this subject, forensic science and microbiology are combined to serve the criminal justice system. Microbial forensics is a new field that examines evidence from bioterrorism, biocrimes, and the intentional or accidental release of biological agents or poisons for attributional purposes. Microbe investigation is similar to forensic science, but in order to maintain the viability of the microorganisms, collection, preservation, and transportation must be different. To determine the source of the causative agent, it also employs cutting edge methods including molecular signatures, new DNA fingerprinting techniques, and other hyphenated methods. In addition to assisting the medical community's specialists, this field also aids judges and juries in their deliberations. Microbial forensics has to advance more in order to handle the issues posed by bioterrorism assaults.

**Keywords:** Microbial forensics, Bioterrorism, Biological weapons, Molecular signatures.

## 1. Introduction

Pathogens are being discovered and transmitted more frequently today than ever before [1], and since their unauthorized usage puts people, the public, the environment, and animals in grave danger, it is crucial to educate both the scientific and general public about microbial forensics.

For the purpose of establishing responsibility, "microbial forensic is an emerging field to investigate the evidences gained from bioterrorism, bio crimes, purposefully or unintentionally disseminating bio toxins or biological agents" [2]. The term "attribution" refers to the process of locating the individuals responsible for bioterrorism, bio crimes, including the purposeful or accidental release of microbes or toxins [1]. This branch of study, which incorporates numerous fields (microbiology, biochemistry, genomics, and molecular biology), is known as forensic science [3]. More frequently than ever before, pathogens are being identified and spread [1], and since their unauthorized.

## 2. Epidemiology and Forensics

The term "epidemiology" refers to the study of illnesses, their

risks, and methods for population control. The same rules apply to bioterrorist acts or crimes where disease-causing microorganisms or biological substances are purposefully disseminated or altered [4].

In order to fully understand the infectious disease epidemic, it is crucial to take the following into account:

1. To prove that an outbreak has occurred.
2. Calculate the population's risk.
3. To determine the distribution and manipulation techniques.
4. To determine the kind of agents.

Forensic epidemiology is the application of epidemiological techniques to examine health issues where there is a suspicion that criminal activity is a contributing element [6].

## 3. Deliberate Introduction of Biological Agents

Attacks classified as bioterrorism include the deliberate discharge of viruses, bacteria, and other germs with the goal to infect or kill humans, animals, plants, and to harm cattle and crops. Biological agents are divided into three groups according to their level of risk:

Primary healthcare practitioners and the US Public Health system are required to prepare a list of biological agents.

### A. Category A

This category includes easily transmitted and disseminated agents which result high mortality rates and have higher risk of public health impact.[7]

Examples:

- Botulism (Clostridium botulinum toxin)
- Anthrax (Bacillus anthracis)
- Plague (Yersinia pestis)
- Tularemia (Francisella tulnensis)

### B. Category B

This category includes moderately easy to disseminate agents which result in moderate morbidity rates and low mortality rates & require specialized diagnostic ability and disease surveillance. [7]

Examples:

- Brucellosis (Brucella species)
- Q fever (Coxiella burnetii)
- Ricin toxin (Ricin communis)

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### C. Category C

This category includes the agents that could be engineered for mass dissemination in future because of their availability, easy to produce which result high morbidity and mortality rates. [7]

Examples: Nipah virus and Hanta virus

Biological agents classified on the basis of characteristics:

- a) Infectivity: The ability of agent to penetrate and multiply in the host.
- b) Pathogenicity: The aptitude of the agents to cause a disease after penetrating into the body.
- c) Transmissibility: The aptitude of agent to transmit the disease from infected person to normal person.
- d) Ability to neutralize: It means to have a preventive tools and therapies to control & prevent the effects of agents.

### 4. Mode of Transmission

Biological Agents can be transmitted in various ways:

- a) Parenteral: Agents transmitted through body fluids.
- b) Airways: Agents which are inhaled in surrounding infected environment.
- c) Contact: Agents transmitted through when contact with the infected surfaces.
- d) Oral Faecal route: Through objects, food or other items infected by the faeces or sexual contact [8].

### 5. Field Investigation of Outbreak

Gathering evidence at the crime scene is the first stage in any bioterrorism strike. To protect the integrity of those biological evidences, forensic scientists should be knowledgeable about the causes, negative effects, and preventive measures of biological agents before collecting any evidence. [9].

### 6. Sample Collection of Microbes

A precise standard operating procedure (SOP) is needed for the collecting of microorganisms. However, there are often three main methods for gathering the evidence.

- 1) To prevent contamination in sampling and analysis, collect the entire item and transport it using a bio contaminant facility. Once the evidence has been transferred to a secure place, analysis and sampling are carried out under the necessary bio contaminant conditions in the proper setting.
- 2) An investigator will remove a portion of the item if it is too large and bulky for them to handle
- 3) Swabbing or wiping materials, vacuuming with a suitable filter, and aspirating needles with the bio contaminant facility are all ways to collect trace materials and prevent contamination during sampling and analysis [10].

### 7. Transportation and Preservation

Transport and storage circumstances may also have an

impact on results. Therefore, experts need to be aware of the nature and traits of various agents. For instance, aerobic microorganisms will perish if not exposed to enough oxygen [11]. Depending on the type and features of the evidence gathered, each piece of evidence should be transferred into a specific container. While certain specimens should be transported at room temperature, others should be stored in the refrigerator to protect the protein content and reduce contaminant overgrowth [12]. However, plant tissue, insects, rodents, soil, or water should be packed specifically in containers such plastic bags, paper bags, and ice bags to avoid distortion and protect the integrity of microorganisms [13]. Some microorganisms are kept and transported in glycerol to decrease distortion

### 8. Identification of Microorganism

Microorganism detection is a crucial next step. The type of microorganism involved can be determined using a variety of microbiological tests. However, molecular fingerprinting, or polymorphisms, is currently more popular [14].

DNA Knowing the libraries and patterns of screen expressions is made simpler by fingerprinting. The common method for identifying molecular signatures is nucleotide sequencing and comparative examination of sequence polymorphism [15]-[17].

Rapid characterization of microorganisms is possible thanks to advancements in PCR-based DNA fingerprinting techniques like Terminal Restriction Fragment Length Polymorphism (TRFLP), Amplified Fragment Length Polymorphism (AFLP), Single Stranded Conformation Polymorphism Analysis (SSCP), Thermal and Denaturing Gradient Gel Electrophoresis (TGGE, DGGE), Amplified Ribosomal DNA Restriction Analysis (RAPD) [18].

Other hyphenated methods are gaining popularity in the field of microbiological forensics, including MALDI-TOF (Matrix Assisted Laser Desorption Ionization- Time of Flight), GC-MS (Gas Chromatography- Mass Spectrometry), and LC- MS (Liquid Chromatography- Mass Spectrometry) [19].

### 9. The Value of Microbiological Forensics

The field that is just starting out is microbial forensics. This will enable law enforcement locate the targeted agents and create a database of the targeted viruses that are most likely to be harmful in the future. It aids in tracking disease outbreaks and deters criminals from obtaining these pathogens [20].

### 10. Conclusion

The review paper's goals are to pinpoint research gaps and offer suggestions for future directions in the field of microbial forensics. The collection, handling, storage, and transportation of microbiological evidences are essential components of the microbial forensic inquiry. The entire inquiry process is jeopardized if these procedures are not followed. The nation's biosecurity will improve as methods and techniques to track bioterrorism and bio crimes are developed.

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