Bio Research Journal
ISSN: 2997-4232|
Volume 13 Issue 3, July-September, 2025
Journal Homepage: https://ethanpublication.com/articles/index.php/E20
Official Journal of Ethan Publication

BIOLOGICAL WEAPONS AND MICROBIAL TERRORISM: IMPLICATIONS FOR SOCIETY AND SECURITY

Raghavendra Suresh Sharma
School of Sciences, Noida International University, Greater Noida, Uttar Pradesh, India
DOI: https://doi.org/10.5281/zenodo.17425215

Abstract
Microbial terrorism as the name itself signifies is the use of microbes among the certain group of people or among animals which are close to the humans or by any other means which proves to be fatal and thus causes an epidemic to occur in the areas where these microbes have been released. As soon as the microbes are released they start showing there adverse effects like it can lead to mutation and these changes can lead to the genetic alterations which leads to death of the person once he gets effected by these microbial spores. The most well-known attack of bioterrorism in the present century is that by the anthrax spores in New York in October 2001. It is understood that even a small bioterrorist attack can lead to the destruction in the complete economy of the country and this can prove to be the most deteriorating situation. Microorganisms act as good weapons since their mass production from a single cell is easy without much skill and infrastructure in comparison to the devastation and disease they cause.

Keywords: microbial spores, genetic alterations, anthrax, devastation, disease, bioterrorism

Introduction

There are thousands of microorganisms which directly or indirectly affect the stability and economy of the place which has been hit by microbial terrorism. Many different medical and governmental organizations have created lists of the pathogenic microorganisms relevant to their missions; however, the nomenclature for biological agents on these lists and pathogens described in the literature is inexact. The emerging infectious disease is the unwanted situation in public health. However, there are several new emerging infectious diseases within the past decade [9]. The consideration on the worldwide outbreak of the new emerging infectious disease is set. An interesting point is the trend of bioterrorism using the new emerging infectious pathogen. Microorganisms make good weapons against bioterrorism which has been known to exist since centuries. Although such attacks of bioterrorism are few, forensic evidence to criminally prosecute the perpetrator is necessary. To strengthen defense against bio crimes, a comprehensive technological network involving various fields needs to be developed.

The impact of the attack has been seen multiple times in history. Microbial forensics is an elegant tool to tackle bio-crimes. This new branch is essential for the modern world where the technology possessed by the criminal or a terrorist could be disastrous. The impact of the attack has been seen multiple times in history. Bioterrorism is the intentional or threatened use of viruses, bacteria, fungi, or toxins from living organisms to produce death or disease in humans, animals or plants to accomplish political or social objective [1]. To strengthen defense against bio crimes, a comprehensive technological network involving various fields needs to be developed. There are important differences between the analyses of microbial genomes and those used in human DNA forensics. Because of the sheer number of potential pathogens that could be employed as a weapon, identifying genetic markers for microbes is a more daunting task than identifying human DNA.

Agents of bioterrorism can be altered or mutated in such a way so as to increase their virulence and ability to cause disease. They can be engineered to resist current medications. They can be spread through air, food, water, fomites, or through infected hosts (including humans, animals, insects, and other reservoirs). Microorganisms are indispensable components of our ecosystem. They make possible the cycles of carbon, oxygen, nitrogen, and sulphur that take place in terrestrial and aquatic systems. They also are a source of nutrients at the base of all ecological food chains and webs [1,4].

Microorganisms have indeed harmed humans and disrupted society over the millennia. Microbial diseases undoubtedly played a major role in historical events such as the decline of the Roman Empire and the conquest of the New World. In 1347 plague or Black Death struck Europe with brutal force. By 1351, only four years later, the plague had killed 1/3 of the population (about 25 million people). Over the next 80 years, the disease struck again and again, eventually wiping out 75% of the European population. Some historians believe that this disaster changed European culture and prepared the way for the Renaissance. Today the struggle by microbiologists and others against killers like AIDS and malaria continues [4].

The presence of viruses is obvious in host organisms showing signs of disease. Many healthy organisms, however, are hosts of non-pathogenic virus infections, some of which are active, while some are quiescent. Furthermore, the genomes of many organisms contain remnants of ancient virus genomes that integrated into their host genomes long ago. As well being present within their hosts, viruses are also found in soil, air and water [14].

Many aqueous environments contain very high concentrations of viruses that infect the organisms that live in those environments. There is therefore a requirement to understand the nature of viruses, how they replicate and how they cause disease. This knowledge permits the development of effective means for prevention, diagnosis and treatment of virus diseases through the production of vaccines, diagnostic reagents and techniques, and antiviral drugs. These medical applications therefore constitute major aspects of the bioterrorism [3].

Forensic scientists continue to evaluate the effectiveness of sample collection methods, the integrity of DNA samples, and storage of samples to ensure accurate and reliable collecting of DNA for further analysis. Before the sample collection process is started, it is valuable to know if even the DNA is intact. One approach for determining if the biological origin of an unknown sample could be blood, saliva, semen, vaginal fluid, faeces or urine is the use of mass spectrometry [9].

Examples of Biocrimes

- Intentional Salmonella typhi food contamination in France from 1910 to1918
- A Yersinia pestis attack by injection in 1933 in India
- Deliberate use of HIV-infected blood and secretions to inflict harm Source.

On the other hand, Microorganisms act as good weapons since their mass production from a single cell is easy without much skill and infrastructure in comparison to the devastation and disease they cause and this feature of microbes has led to the emergence of Microbial Forensics[29].

Microbial Forensics is an up and coming field which utilizes the basic concepts of forensic biology in the application of pathogens. It uses the concepts of forensic biology, DNA sequencing, and other sciences to determine the source of biological threats. Microbial forensics utilizes the identification and individualization techniques used in forensic biology to solve human criminal cases, to the application of microbial substances. It combines principles of public health, epidemiology and law enforcement to identify patterns in disease outbreak, determine which pathogen may be involved, and trace the organism to its source.

Microbial forensics may be defined as a scientific discipline bridging microbiology and forensic science dedicated to track and analyse bio crime. Microbial forensics includes a vast scope of forensic science clubbed with microbiology, which includes but not limited to analysis of microbes or their toxins and materials used to prepare, store and deliver the toxin or pathogen.

Bio terrorism in both crude and refined forms has been seen throughout the ages even from Biblical times. Terrorist or criminal use of pathogenic organisms and their toxins have been of great concern ever since the **Anthrax attacks in 2001 in the United States.** Microbial Forensics should play a lead role in sample handling, tackling, configuring, and prioritizing and validating bio crimes. The objectives of the research on Microbial forensics is to look back into the subtle events of the past, assess the shocking incidents of our present times and propose prospects to systematically deal with this emerging menace in the future and secure the nations with a special reference to India from the disastrous consequences of bio weapons [12,13].

At times an infectious organism can enter a latent state in which there is no shedding of the organism and no symptoms present within the host. This latency can be either intermittent or quiescent. Intermittent latency is exemplified by the herpes virus that causes cold sores (fever blisters). After an initial infection, the symptoms subside. However, the virus remains in nerve tissue and can be activated weeks or years later by factors such as stress or sunlight. In a quiescent latency the organism persists but remains inactive for long periods of time, usually for years.

A major thrust of microbial forensics will, therefore, be the analysis of nucleic acids that can relate the genome of the pathogen to specific sources. This analysis is analogous to human DNA forensic analysis, which is being widely used to prosecute criminals and to exonerate the innocent. But there are important differences between the analyses of microbial genomes and those used in human DNA forensics. Because of the sheer number of potential pathogens that could be employed as a weapon, identifying genetic markers for microbes is a more daunting task than identifying human DNA.

In the case of human identification, only one species is involved, and it is often possible to identify an individual person. Viruses and most bacteria are haploid. Microbes primarily reproduce asexually, but can also evolve by recombination, horizontal gene transfer, and gene duplication. Therefore, statistical methodologies and interpretation will require different tools than are currently used for comparing and estimating the rarity of (diploid) human DNA profiles. Nevertheless, obstacles due to genetic complexity can be reduced by obtaining samples as early as possible [10, 11].

If physicians suspect a bio crime, they should take steps to ensure the preservation of the diagnosticsamples so that they are not prematurely destroyed. Physicians may also advise the patient to preserve additional material that may prove useful for a criminal investigation. Just as in sexual assaults, in a suspected bio crime, the patient's personal articles may carry traditional forensic evidence that is of equal value to the information revealed by the microbe itself. Unlike sexual assault evidence, in a suspected bio crime, procedures used to preserve one particular microbe may be deleterious for other microbes and for physical evidence (such as fingerprints, culture media, isotopes, hair, and environmental material [30].

Although finding the perpetrator of a crime is a law enforcement function, the actions of attending physicians can help with microbial forensics—the scientific discipline dedicated to analysing evidence from a bio crime or an act of bioterrorism, and that seeks to authenticate a piece of the puzzle for attribution. Implicit in the term attribution is the identification of the responsible party or the exclusion of the innocent [15].

Microbial forensics includes the full scope of forensic evidence, such as analyses of microbes, materials used to prepare, stabilize, and deliver the toxin or pathogen, and fingerprints, hair, fibre, and pollen. The laboratory analyses used for microbial forensics may include molecular sequencing, microbiological cultures, biochemistry, electron microscopy, crystallography, and mass spectrometry. These analyses go well beyond those used for medical diagnoses and epidemiologic investigations [14, 15].

They require, however, the same substances used by the physician for diagnostics, for example, body fluid samples and microbial cultures. In this regard, the physician and the clinical laboratory have critical roles in the collection and initial analyses of samples for microbial forensics.

The founders of this new field have a unique opportunity to state clearly what the field is and what it is not. The definition of forensic science provided earlier in this article may aid in this matter. Additionally, the "boundary conditions" should be firmly established as to how it will be practiced and explored and what "rules of engagement" and expertise will be expected and required to do so. The new discipline of microbial forensics is in the process of being founded from an array of established and emerging fields, such as classical microbiology, microbial genomics, phylogenetics, and informatics, and it should embrace lessons learned from human DNA forensics and forensic informatics. The large body of knowledge and experience in forensic science itself should be accessed and applied as well [11].

Genetically modified agents; with the advent of recombinant DNA technology, researchers have developed standard methodologies for altering an organism's genetic makeup. Application of this technology to enhance traditional biological warfare agents has led to the classification of genetically modified BW agents as a separate category of BW agents. Examples of potential modifications include antibiotic resistance, increased aerosol stability, or heightened pathogenesis [10,11].

Technologies Involved In Microbial Forensic Analysis

The diagnostic methodologies used in the analysis are Classical microbiology techniques, serology and nucleic acid based techniques. Currently, more emphasis is on the molecular signatures or molecular markers, which are reliable and quantifiable. Microbiological evidences could include viable samples of the microbial agent, protein toxins, nucleic acids, clinical specimens from victims, laboratory equipment, dissemination devices and their contents, environmental samples, contaminated clothing, or trace evidence specific to the process that produced and weaponized the biological agent [1,5].

The first steps would be to follow routine microbiological methods keeping the fact in mind that the organism may be totally unknown or unidentifiable by traditional microbiology practices. Once the possible organism is found, all possible analysis such as genetic fingerprint, unique DNA sequences protein signatures may be required to establish and strengthen the data statistically[21,22].

The characterization, analysis and validation of evidence that concludes the description of an intentional act of bio crime or bioterrorism represent the main target of microbial forensics. But, even nowadays with the most recent technical advances, it remains extremely difficult, labour-demanding and time consuming to investigate and confirm the origin of microbial strains and to identify the person or motif behind the crimes. The true extent of microbial diversity in indoor or outdoor environments may still be underestimated, but its origin and maintenance is starting now to be better understood [21].

Other researchers sustain that microbes are simply so abundant that dispersion and migration capacities support a probable global distribution. Additionally, they possess a remarkable tolerance and adaptation abilities to the most unfavourable environmental conditions. Human body represents other sort of microbial community different from soil and water communities that may be ruled by other variables, particularly the individual genetic background.

Nowadays, there is no doubt that molecular markers are important for microbe discriminating and, thus, genotyping methodologies are increasing interest for forensic sciences. The advent of large-scale genotyping studies on microbial populations may provide a unique opportunity to compare genetic diversity within and among populations. The present mini-review focus the most recent developments regarding microbial genetics and population diversity, both community richness and genotype diversity, and its application for criminal cases and forensic evidence [11].

The characterization, analysis and validation of evidence that concludes the description of an intentional act of bio-crime or bioterrorism represent the main target of microbial forensics. Numerous cases of use and release of microbial agents as bioweapons have been employed for centuries in order to commit murder or injury against other people [16].

Microbial community profiling and characterization is an important issue for microbial forensics. Biogeographic patterns had been occasionally described for bacterial communities, as well as for fungal populations. Microbial genomes have been widely studied and new terms, like met genome or pan-genome, became increasingly common in the literature [6]. In fact, genomic technologies brought considerable advances to microbiology, allowing the detailed analysis of populations and complex microbial environments, as well as the comparison of multiple genomes [19, 21].

Airborne metagenome studies performed in indoor environments showed an extensive number of new bacterial and fungal phenotypes, but the overall microbial diversity of airborne samples may even be far below the estimated diversity for soil and water samples [7].

Thus, microbial diversity origin and maintenance is starting now to be better understood. Microbial biogeography is still in its infancy and great prospects are expected during the coming years. The advent of large-scale genotyping studies on microbial populations may provide a unique opportunity to compare genetic diversity within and among populations. The present mini-review focus the most recent developments regarding microbial genetics and population diversity, both community richness and genotype diversity, and its application for criminal cases and forensic evidence.

Another future topic of interest for microbial forensic researchers and technicians is the value of the data coming from the human microbiome project. This international consortium expects to verify whether humans share a core microbiome, to correlate changes in the human microbiome with changes in human health, and to develop technological and bioinformatics tools necessary for this microbial genetic research Microbial communities are influenced by and modulate human development, immunity, and nutrition. Future results may considerably explain and change the way we interact with the environment around. Meanwhile, the first results show a high diversity in the salivary microbiome within and between individuals from different continents, but small correlation with geographic distance was found [14].

Speciation and microevolution can also be a useful tool for forensic sciences. Mutations occur constantly and may link samples to specific places. Mutation rates in coding or non-coding genomic regions become the driving force for microbial diversity and might be used for forensic determinations. ―Close-related strains‖ and ―near matches‖ are highly problematic in a court, particularly when scientists try to explain relatedness to a people from other subject areas than life sciences [14, 16].

Special techniques and methods are needed to gather and preserve microbial forensic evidence — and to protect the health and safety of the first responders who gather this evidence at the crime scene. Developing and validating sample collection, preservation, handling, storage, packaging and transportation methods and procedures requires a much higher priority. Existing processes need to be standardized, compiled, and shared worldwide, while new, more efficient ones should be sought.

Bio Research Journal
ISSN: 2997-4232|
Volume 13 Issue 3, July-September, 2025
Journal Homepage: https://ethanpublication.com/articles/index.php/E20
Official Journal of Ethan Publication

In microbial forensics the same rules apply, but more detailed investigations may be necessary to obtain relevant information about the history of a specimen, environmental conditions, chemical and physical constitution of the matrix, presence of pollen etc. Microbial Forensics is the most effective method of the analysis of the samples if there is presence of proper scientific information among the analyst and under the presence of proper scientific analysing conditions which can provide to be proper optimum conditions for the pathogens or the given microbial samples.

Similar to a crime investigation, microbial forensic investigation involve collecting evidence, which in this case is microbial, and documenting, analysing and interpreting. Nevertheless, DNA sequence analysis remains the most reliable source of microbial information, when compared with proteins and other macromolecules. Even though the fingerprinting of the microbe, such as bacteria is possible the fact remains that different strains of the microbe can be similar at the different sequence level.

Similarly in case of a bio-crime, HIV infected individuals can carry more than one HIV strain, making it very difficult to prove a crime beyond any reasonable doubt in a court setting. It all began in a week after SEP 11 when a bio-crime attacked America when various news and media offices received the letters containing the spores of the anthrax. This is the best example of the bio-crime although there were only a few individuals affected there were some fatalities, and it turned the spotlight on MICROBIAL FORENSICS [20].

Cases and Evidences

Culture methods present considerable limitations in order to get the complete description of microbial communities living in specific ecotypes, particularly when comparing to genetic techniques that revealed an extensive microbial diversity previously unknown. However, a considerable number of pathogens can still be grown from a single cell and multiplication through culturing and dispersion can be carried out easily without sophisticated technology [7]. Few organisms, such as **B. anthracis**, are stable in the environment through the production of spores and persist for longer periods even after spread and under unfavourable conditions.

Compared with other weapons, microbial dissemination is inexpensive and difficult to detect because residual quantities can be prepared [1].New genetic assays have been developed in order to verify the origin of microbial strains and general transmission routes for a particular microorganism. Detection of pathogens with increased pathogenicity is crucial and several genomic tools may offer clues for detection of the most dangerous pathogens that sometimes are remarkably similar to the non- pathogenic relatives [12].

Microbiological investigations in forensic sciences are basically similar to other forensic investigations, following the same steps and involving crime scene investigation, evidence collection and storing, analysis of evidence, interpretation of results, and court management. Excellent descriptions of the procedures used for evidence treatment and analysis can be consulted in few previous reviews [1, 12]. In any case, the first step of such an investigation is the classification of the attack as natural or resulting from an intentional act.

Bio Research Journal
ISSN: 2997-4232|
Volume 13 Issue 3, July-September, 2025
Journal Homepage: https://ethanpublication.com/articles/index.php/E20
Official Journal of Ethan Publication

A planned attack might be identified by the unlikely occurrence of a disease (atypical season or geography) or unusual illness in people, as well as evidence of genetic engineering or other strain genetic indicator [9]. Microbial forensic was employed in a few reported criminal cases described below for clarification of bio-crimes, nevertheless it is expected that greater number of forensic investigations will be conducted in near future using genetic microbial data.

In 1993, the **Aum Shinrikyo** cult (that was also responsible for a **sarin** nerve gas attack in a Tokyo subway) attempted to spread anthrax in Tokyo causing no victims. The MLVA profile of the isolate confirmed that **B. anthracis** strain was the non-virulent veterinary vaccine strain **Sterne [1**]. In this case, information regarding strain pathogenicity was sufficient to alert health services and limit the public panic.

By exploring the case of the **State of Louisiana vs. Richard J. Schmidt, Budowle & Harmon** [19] reported few limiting, encouraging and challenging topics regarding the close relationship between genetic microbiology and forensic evidence. The criminal case occurred in 1995 and **Richard J. Schmidt** was accused of second degree attempted murder with a contaminated HIV injection against Janice Trahan [2].

The clinician used blood samples from patients infected with HIV and hepatitis C. Sequence data were generated from two genes from HIV isolates and phylogenetic data supported the hypothesis that the HIV variants from the victim (Janice Trahan) and the Dr. Schmidt's patient were closely related [5]. The Court of Appeal of Louisiana believed that was clear evidence that Richard Schmidt had the opportunity to acquire the contaminated HIV blood sample.

1959 Sweden Murder case: Soil washed from the clothing on a murdered woman, liquid sent to a palynologist at the University of Sweden to examine Surface dirt (control samples) was collected at the crime scene from areas near the body, pollen from the control samples did notmatch pollen from the soil on the clothing of the victim, That told the police the victim was killed somewhere else[21].

1959 AustriaMurder case:A passenger was missing when a sightseeing boat on the Danube River arrived in Vienna, Austria. His friend and business partner was on board but he claimed he did not know what happened to his friend. He becomes a suspect so the police search his cabin. They found a pair of hiking boots with mud on them; send boots to the crime lab. Mud on one boot contain a Miocene-age pollen grain of Carya Control samples taken along boat's route confirm only one spot where a Miocene outcrop is exposed with the same type of Caryapollen grains. Suspect is taken to the crime scene location, he was so shocked that he admitted the murder, showed where the body was buried in a shallow grave [46].

Mid 1970s USA Honey case: During mid-1970s, the USDA begins its search for illegal, non-domestic honey purchased under the US Farm Subsidy Purchase Program. Under the Federal Subsidy Program, the USDA agrees to purchase USA-produced honey at the subsidy price. Recession and falling honey prices make the USDA subsidy price higher than the price of honey selling on the world market. During the 1970s and 1980s several hundred honey samples were collected and sent from Beltsville, MD to Texas A&M for testing (to determine honey origins). Government samples consisted of a very small amount of the total honey purchased under the subsidy program. Pollen studies confirmed the nectar source and the geographical location (by pollen prints) of the honey samples. Results revealed ~6% of samples were from non-domestic honey [17,21].

Methodology And Tools

The diagnostic methodologies used in the analysis are Classical microbiology techniques, serology and nucleic acid based techniques. Currently, more emphasis is on the molecular signatures or molecular markers, which are reliable and quantifiable.

In forensic research, the sample collection is the first and the most important step. The method of collection should be sensitive, reliable and robust to clinch the presence of possible organism or the toxin.The samples to be collected include every material found in the scene which is labelled with time and site of collection. Also, Timely environmental sampling is important as it may be rapidly destroyed and the evidence of intentional spread may be lost. Each sample should be considered potentially hazardous and processed only in a well-equipped laboratory, or ideally sent to a reference laboratory equipped with stringent bio safety levels [33].

Microbiological evidences could include viable samples of the microbial agent, protein toxins, nucleic acids, clinical specimens from victims, laboratory equipment, dissemination devices and their contents, environmental samples, contaminated clothing, or trace evidence specific to the process that produced and weaponized the biological agent [29,32].

The first steps would be to follow routine microbiological methods keeping the fact in mind that the organism may be totally unknown or unidentifiable by traditional microbiology practices. Once the possible organism is found, all possible analysis such as genetic fingerprint, unique DNA sequences protein signatures may be required to establish and strengthen the data statistically [27].

Various New methodologies used for the analysis and identification in microbial forensic and pollen analysis are-

4.1Matrix Assisted Laser Desorption Ionization- Time of Flight (MALDI-TOF)

MALDI-TOF uses the time for a target particle to traverse a specific distance after being dislodged
From a surface by a precise amount of energy. This allows a determination of the molecular weight of the target.

4.2Matrix Assisted Laser Desorption Ionization- Time of Flight- Mass Spectrometer (MALDI-TOFMS) Potential use of MALDI-TOF-MS includes rapid microbial identification, rapid sub-typing and direct detection of bio-markers from samples. One of the advantages of this instrument is the speed of analysis. MALDI-TOF-MS could be applied directly to crude cellular fractions or cellular suspensions to produce chemotaxonomic signature profiles. It can establish the identity, purity, or other aspects of protein samples, genetic materials, and microorganisms. It has been successfully used for analysis of bacterial RNA and DNA, detection of unknown protein and their characterization, bacterial proteomics, rapid characterization of bacteria at the genus, species, and strain level [11, 13].

Bio Research Journal
ISSN: 2997-4232|
Volume 13 Issue 3, July-September, 2025
Journal Homepage: https://ethanpublication.com/articles/index.php/E20
Official Journal of Ethan Publication

4.3Gas Chromatography- Mass Spectroscopy (GC-MS)

It's an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-MS has been widely heralded as a gold standard for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample.

4.4Liquid Chromatography-Mass Spectroscopy (LC-MS)

The gas chromatography-mass spectrometry (GCMS) and liquid chromatography-mass spectrometry (LCMS) are widely used methodologies in identification and quantification of cocaine and its metabolites in forensic samples.

4.5Microarray

They have the ability to detect both nucleic acid and protein signatures. Microarray panels are available for microbial detection, SNP detection, and genome wide association studies, gene expression, and protein presence and abundance.

4.6Restriction Fragment Length Polymorphism (RFLP) Help in identifying genetic signatures and polymorphisms.

All the above methods help in resolving minor difference in proteins or small molecules. These may help in identifying the unique protein signatures [33].

Objectives of Study

The research will help us to have a robust and rational understanding of the application of microbial Forensics and palynology and will be able to:

5.1 **identify and prioritize biological threats.**

Create an information database and develop protocols for identification which includes determining unique genetic signatures, protein signatures, develop programs for ensuring the validity of results and constantly update based on existing literature.

5.2 Generate palynomorph data for forensic studies.

5.3 Assist in the investigation to bring perpetrators to justice and to deter future attacks causing bioterrorism.

5.4 Focus on characterizing evidence and include collection, analysis and interpretation of evidence so that it can eventually be used in court.

5.5 Identify and develop effective procedures for microbial sample collections at the attack site, educate first responders about self-protection, deciding the most reliable and appropriate methods of specimen analysis, and develop quality control standards.

Microbial Forensic Science in association with other Sciences

Microbial forensics is an emerging discipline that blends elements of various disciplines including microbiology, forensic science, molecular biology, epidemiology, phylogeny and evolutionary science, public health and medicine, agricultural sciences.

6.1 The application of microbiology to environmental forensic investigations involves the application of range of sub-disciplines including microbial physiology, molecular microbial ecology and microbial biochemistry.

6.2 Other sub-disciplines include Microbial genetics, genomics and bioinformatics that are crucial to microbial species identification, virulence determination, pathogenicity, characterization and source investigation.

6.3 The discipline of plant pathology offers much knowledge as well as technologies and resources, developed for peaceful purposes that can be adapted and applied to the development of the new sub discipline of plant pathogen forensics.

6.4 Forensic botany is other sub-disciplines which is the application of plant sciences to criminal investigations. Forensic botany incorporates several sub-disciplines: palynology (the study of pollens), dendrochronology (the study of tree rings), limnology (the study of aquatic environments), systematics (the classification of plants), ecology (the study of ecosystems), and molecular biology.

6.5 Palynology is the study of modern and fossil pollen and spores, with particular reference to their identification. The sub disciplines include plant pathology that deals with the diseases of plants, economic botany that deals with plants of practical use to man and ethno botany that covers the use of plants by aboriginal peoples, now and in the distant past.

6.6 In addition to the above major sub-disciplines, several specialized branches of botany have developed. Among them are bacteriology, the study of bacteria; mycology, the study of fungi; algology or phycology, the study of algae; bryology, the study of mosses and liverworts; pteridology, the study of ferns and their relatives and paleo botany, the study of fossil plants.

Forensic sciences also focus their actions on several materials, particularly cadaveric materials, and microbial genetics can also be useful to study these materials. A rapid colonization by local microbial communities happen in cadavers and the communities are replaced over time (or space if the cadaver is moved from one to another location).

Microorganisms can also release repellent toxins that sometimes could also be used for a better identification of where a certain crime took place [6]. More research is still necessary about these materials, but there is a large background to be explored by microbial metagenomics in this field.

Presently, there is a growing recognition that management of point and diffuse sources of microbial pollution is indispensable. Microbial agents may represent the strategic tool that environmental technicians and researchers needed. Microbial tracer studies, using for example **Bacillus globigii**, can be employed to follow a limited number of inputs at any time [3].When typical (cultivable) methods are used to trace pollutant movements in surface waters, it is very difficult to attribute the source of pollution to an individual source [9].

Even more when multiple sources are present and different pollutants should be detected. In these cases, it is necessary to define the relative importance for each pollutant and, finally, select appropriate methods of remediation [4]. Simple microbiology is not sufficient to track faecal pollution to individual sources and a long way is still needed to get the expected microbial source tracking (MST) results.

Relationship to Other Sciences

Microbial Forensic contributes a broad spectrum of other sciences to the human society. At one end of the spectrum are those sciences that contribute their methods and philosophical concepts to serve the needs of forensic expert, either in research or in the application of microbial forensic to human affairs. At the other end of the spectrum are those sciences to which are partially or completely related to the contributions of forensic science [15].

In the first group chemistry, biochemistry, pathology, physiology, epidemiology, immunology, ecology, and biomathematics has played an important role while molecular biology has, in the last two or three decades, contributed vast advancements in the field of forensic science.

In the group of sciences to which microbial forensic contributes significantly are such aspects of medicine as forensic medicine, clinical toxicology, pharmacy and pharmacology, public health, and industrial hygiene [45]. Microbial forensic also contributes in an important way to veterinary medicine, and to such aspects of agriculture as the development and safe use of agricultural chemicals. The contributions of microbial forensic to environmental studies have become increasingly important in recent years [28].

Clearly, microbial forensic is pre-eminently an applied science, dedicated to the enhancement of the quality of life and the protection of the environment. It is also much more. Frequently the perturbation of normal life processes by toxic chemicals enables us to learn more about the life processes themselves [44].

The field of microbial forensics has expanded enormously in recent decades, both in numbers of forensic expert and in accumulated knowledge. This expansion has brought a change from a primarily descriptive science to one which utilizes an extensive range of methodology to study the mechanisms involved in toxic events [22].

Much of the early history of forensic science has been lost and in much that has survived of almost incidental importance in manuscripts dealing primarily with medicine [33]. Some, however, deal more specifically with toxic action or with the use of poisons for judicial execution, suicide or political assassination. Regardless of the paucity of the early record, and given the need for people to avoid toxic animals and plants, toxicology must rank as one of the oldest practical sciences [25].

Biological warfare agents to be used against humans and animals were developed and weaponized in the fifties of the last century in several countries including the USA, the former Soviet Union and the United Kingdom [42, 44]. An international arms control and disarmament treaty, the Biological Weapons Convention (BWC), banned the use of biological weapons in 1972. Today only few states are under

Bio Research Journal
ISSN: 2997-4232|
Volume 13 Issue 3, July-September, 2025
Journal Homepage: https://ethanpublication.com/articles/index.php/E20
Official Journal of Ethan Publication

suspicion of having biological warfare programs. Politically-binding confidence building measures provide a permanent transparency tool for building confidence in compliance with the BWC [23, 25].

Centralized reporting and surveillance systems on the national and international level are essential as single cases may be regarded as sporadic although they are part of a larger transboundary outbreak. Surveillance systems have already been established that store and provide DNA fingerprints of microbes being major causes of hospital-acquired or food borne infections [47, 49].

Microbial forensic data must hold up not only to the scrutiny of scientists in the health care community, but also to the scrutiny of judges and juries and national policy and decision makers. It poses a great challenge to develop newer techniques as the present techniques like gene sequencing, hybridization, microarray, spectrophotometers, PCR etc. are inadequate. A national microbial forensics plan needs to be developed [50].

Future Aspects

In context to the Indian scenario, the potential of microbial forensics is incredibly vast. As a country which is commonly threatened by terror attacks, there is no doubt that biological weapons will be made use of by illegal organizations. Establishment of a national organization which integrates specialties from various fields of science will prove to be beneficial despite the cost that will be incurred in creating and maintaining such a team.

Three components will be absolutely essential to establish a fully functional National Microbial Forensic Laboratory. The first would be a knowledge center composed of databases on genomics, microbiology, forensics methods, SOPs, evidence assays such as fingerprinting, bioinformatics and standardized tools. The second component will be maintenance of strong partnerships between the existing government, the laboratory in charges, scientists and investigating agents.

The third component will be quality control and validation of newer assays. To the best of the author’s knowledge, there is no documented case of biological materials used by individuals in India, to intentionally cause harm to others.

This may be interpreted as no case occurring or as lack of forensic microbiology work up. The case of Anthrax spores (Amerithrax) was under study for many years. This is partially because United States was not prepared to face it, though a strong similarity between human forensic and microbial forensic DNA analysis exist, such as use of population databases, qualitative conclusions of test results, and the application of quality assurance / quality control practices. The differences would include the database size, contents and the protocol for analysis. This information can direct law enforcement officials (like a central investigating agency of that country) to expertise on specific threat agents. To achieve this goal, a national database (s) of pathogens, pathogen profiles and individuals authorized to have access to these pathogens and their data must be established [2].

Unlike an epidemiological survey, here the samples should be maintained till the clearance of the report, that it is a natural outbreak by a designated authority based on scientific observations, or hold it as an evidence for judiciary purpose. The strain may be required to be maintained by the judicial system or may be destroyed as required by the system [46].

Conclusion

In a nutshell, Microbial forensics is a naïve branch that involves multi-disciplinary approach to detection, tracing and evidencing the bio crime, with a predominant microbiological approach. This field is emerging as a requirement for civil security rather than luxury (Traverse, 1988).A national and international collaborative approach against the menace of bioterrorism can be done by setting up a national and international reference laboratory, transparency of analysis and strict action against all bio crime perpetrators. Considering all the scientific facts already discussed, ―Microbial Forensics‖ should be an ideal requirement in India.

References

Pattnaik P, Sekhar K. Forensics for tracing microbial signatures: Biodefence perspective and preparedness for the unforeseen. Indian J of Biotechnology, 2008; 7: 23-31.

Chakrakodi NV, Kuruvilla TS, Furtado Z. Microbial Forensic-Past, Present and Future. Int J Biol Med Res, 2012; 3(2): 1546-48.

Barns SM, Grow CC, Okinaka RT, Keim P and Kuske CR. Detection of diverse new Francisella like bacteria in environmental samples. Appl. Environ. Microbiol. 2005; 71: 5494 -500.

Ussery DW, Binnewiesm TT, Oliveira G, Jarmer R, and Hallin PF. Genome update: DNA repeats in bacterial genomes. Microbiology, 2004; 150: 3519–521.

Chakrakodi NV, Kuruvilla TS, FurtadoZ. Microbial Forensic-Past, Present and Future. Int J Biol Med Res, 2012; 3(2): 1546-550.

Budowle B, Murch R, Chakraborty R. Microbial forensics: the next forensic challenge. Int J Legal Med, 2005; 119(6): 317-25.

Budowle B, Johnson MD, Fraser CM, Leighton TJ, Murch RS, Chakraborty R et al. Genetic Analysis and Attribution of Microbial Forensic Evidence, 2005; (450-80).

Budowle, B.; Johnson, M.D.; Fraser, C.M.; Leighton, T.J.; Murch, R.S.; Chakraborty, R.Genetic analysis and attribution of microbial forensics evidence. Crit. Rev. Microbiol., 2005, 31, 233-54.

Whitaker, R.J.; Grogan, D.W.; Taylor, J.W. Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science, 2003, 301, 976-78.

Whitaker, R.J.; Grogan, D.W.; Taylor, J.W. Geographic barriers isolate endemic populations of hyperthermophilic archaea. Science, 2003, 301, 234-40.

P.Aggarwal, A.K. Chopra, S .Gupte, S.S. Sandhu. Microbial Forensics – An Upcoming Investigative Discipline. J Indian Acad Forensic Med. April - June 2011; 33 (2): 115. Microbial forensics: A new forensic discipline, Sharad Jain, Ashish Kumar, Pratima Gupta, Ramjee Prasad, Dept. of Microbiology, Himalayan Institute of Medical Sciences, Dehradun, JIAFM, 2005; 27 (2).

JW Drake, B Charlesworth, D. Charlesworth, J.F. Crow. Rates of spontaneous mutation, Genetics 1998: 1667- 686.

ML Kotewicz. EW Brown. JE LeClerc. TA Cebula. Genomic variability among enteric pathogens: the case of the mutS-rpoS intergenic region, Trends Microbiol. 2003: 2-6.

JE LeClerc. BG Li. WL Payne. TA Cebula. High mutation frequencies among Escherichia coli and Salmonella pathogens, Science 274 1996; 1208-211.

Craig A.Cummings and David A.Relman. Microbial Forensics "Cross-Examining Pathogens. Science. 2002; 296.

James B, Theodore R and Jack A. Biotechnology Impact on Biological Warfare and Biosecurity and Bioterrorism. Biodefense Strategy, Practice, and Science. 2003; 3 (1).

Budowle B. Quality assurance guidelines for laboratories performing microbial forensic work. Science supporting Online Material.: 2003.

Pattnaik P., Sekhar K. Forensics for tracing microbial signatures: Biodefence perspective and preparedness for the unforeseen. Indian J of Biotechnology, Vol 7, Jan 2008, pg 23-31.

Budowle B, Schutzer S E, Einseln A et al. Building microbial forensics as a response to bioterrorism, Science, 301(2003) 1852 - 853.

Centers for Disease Control and Prevention's Pulse Net site (the U.S. National Molecular Sub typing Network for Forborne Disease Surveillance)

Wiltshire, P.E. 2006. Hair as a source of forensic evidence in murder investigations, Forensic Science International, 163: 241-48.

Biodefense Perspective and Preparedness for Unseen, Priyabratta Pattnaik. . 29. Salyers, Abigail A. —Microbes in Court: the