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REVIEW ARTICLE

Microbial Forensics

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ABSTRACT

Microbial forensics has been defined as the discipline of applying scientific methods to the analysis of evidence related to bioterrorism, biocrimes, hoaxes, or the accidental release of a biological agent or toxin for attribution purposes. Over the past 15 years, technology, particularly massively parallel sequencing, and bioinformatics advances now allow the characterization of microorganisms for a variety of human forensics applications, such as human identification, body fluid characterization, post-mortem interval estimation, and biocrimes involving tracking of infectious agents. Thus, microbial forensics should be more broadly described as the discipline of applying scientific methods to the analysis of microbial evidence in criminal and civil cases for investigative purposes.

Keywords: Anthrax, Bioterrorism, Forensics, Microbes.

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INTRODUCTION

Microbial forensics combines principles of public health epidemiology and law enforcement to identify patterns in a disease outbreak, determine which pathogen may be involved, and trace the organism to its source. Since investigators must consider potential prosecution and presentation of evidence in court, biocrime investigations demand careful controls and standards for validation and evaluation of technologies and the data they produce. Scientists can easily evaluate new methods of detecting organisms implicated in a bioterrorist attack, but taking the resulting evidence into a court is another matter. Any microbial evidence, such as anthrax spores, that links to a suspect has to meet stern standards.[1]

Microbial forensics is a rapidly evolving scientific discipline. In the last decade, and particularly due to the anthrax letter attacks in the United States, microbial forensics has become more formalized and played an increasingly greater role in crime investigations. This has brought renewed interest, development, and application of new technologies, and new rules of forensics and policy engagement. It has many applications ranging from biodefense, criminal investigations, providing intelligence information, making society more secure, and helping protect precious resources, particularly human life. A combination of diverse areas is investigated, including the major disciplines of biology, microbiology, medicine, chemistry, physics, statistics, population genetics, and computer science.[2]

Forensics and Epidemiological Investigations

Disease outbreaks naturally occur every year throughout the world, and investigations into these outbreaks often include both epidemiology and microbial forensics investigations. Epidemiology studies the occurrence, features, and determinants of disease in populations. The same general principles of epidemiology for disease investigations apply to a bioterrorist attack or crime. Therefore, microbial forensics investigations are based on the same well-established principles of epidemiological investigations. Microbial forensics and public health share common interests regarding the identification and genetic characterization of the biological agent and how it was disseminated in the population. However, public health officials tend to focus on

- Determining that an outbreak has occurred,
- Defining the population at risk,
- Determining the method of spread and reservoir, and
- Characterizing the agent.

A common thread of public health and microbial forensics is determining whether the outbreak is natural, accidental, or intentional. While microbial forensics and epidemiology are integrated disciplines, microbial forensics scientists and law enforcement concentrate on attempting to individualize the agent or toxin and how it

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was produced and disseminated for attributing the event to a person or group of persons, while maintaining a chain of custody for legal purposes or for decision makers and their responses.[3,4]

Procedure for Investigation

The first step in case of any suspected biocrime is the gathering of evidence at the scene of crime. The experts should understand the biology of the organisms and be aware of maintaining the chain of custody to preserve validity for subsequent criminal proceedings.[5] The next major step is the identification of the organism. Though phenotypic evidences offer initial clues about the type of microorganism involved, they cannot be used as forensics marker. Currently, more emphasis is being given to molecular signatures or molecular markers, i.e., finger prints and polymorphisms, which are reliable and quantifiable.[6] Microbial forensics is an extension of fingerprint analysis to microbial agents that are known as bioweapon agents and is primarily intended for identification at strain level for attribution purposes.[7] Nucleotide sequencing and comparative evaluation of the sequence polymorphism is the classical approach to detect variations in signature sequences. Even comparative evaluation of specific gene targets having more number of synonymous mutations has been explored for molecular typing and tracing the source of pathogens in disease outbreaks.[8] Though such information tells clearly about molecular phylogeny of a given isolate,[9,10] it is insufficient for criminal investigation.

Development in deoxyribonucleic acid (DNA) fingerprinting class has made it easier to screen expression libraries and patterns. The DNA microarray has been well established for its potential use in study of population structure, species evolution, and acquisition of virulence.[11,12] Hyphenated technologies like matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), gas chromatography–mass spectroscopy (MS), and liquid chromatography–MS are becoming popular for detection of differences in protein or small molecules. One of the major advantages of MALDI-TOF–MS instrument is the speed of analysis. This technique can be applied directly to crude cellular fractions or cellular suspensions to produce chemotaxonomic signature profiles, analysis of bacterial ribonucleic acid and DNA, and rapid characterization of bacteria at the genus, species, and strain level. Studies have shown that subtle differences between closely related strains can be clearly delineated using MALDI-TOF–MS.[13] Stable isotope ratio as a tool in microbial forensics has also been explored recently.[14] Kreuzer-Martin et al[15,16] demonstrated that the source of a microorganism carries specific signature of the environment where they were grown in. The range of variations of 13C, 15N, and 2H contents of bacteriological media can yield differences in microbe isotope ratios, which are readily measurable.[16]

Need for Microbial Forensics

For law enforcement agencies to investigate, attribute, deter, and prevent biological threats and for the Justice Department to prosecute criminals, the field of microbial forensics must be developed. In order to establish the field and the needs of law enforcement, the following short-and long-term goals must be met:

- Federal agencies must develop, maintain, and regularly update prioritized lists of biological agents that have the potential to cause severe harm to humans, animals, plants, or the environment and material resources.
- Currently, there is an insufficient number of validated analytical tools to identify many of the most important threat agents rapidly in the field at the site of an incident.
- With defined quality assurance and quality control guidelines, the users, the legal community, and the public can have confidence in the results obtained from a crime scene.
- Genetic analyses are not the only way to identify a microorganism or provide forensics attribution. Such topics as trace material analysis, immunology, culturing, and so forth also need to be addressed.[17,18]

CONCLUSION

Microbial forensics is an interdisciplinary field that involves scientists, public health, law enforcement, the intelligence community, and policy and decision makers. Together, they provide the interconnected system that helps protect us from naturally occurring disease outbreaks and acts of biological terrorism and biocrime. New advancements in molecular techniques, especially sequencing technologies, provide tools for the microbial forensics scientist to extract more information at dramatically reduced costs and faster turnaround times than previously possible.

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