



Forensic Microbiology, an Important Tool in Crime Investigation

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/SAJRM/2020/v6i230147

Editor(s):

(1) Dr. Chamari Hettiarachchi, University of Colombo, Sri Lanka.

Reviewers:

(1) Michael Adigun, Crawford University, Nigeria.

(2) Okpo, Ngozi O., Federal College of Agricultural Produce Technology, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/56905>

Review Article

Received 15 March 2020

Accepted 21 May 2020

Published 29 May 2020

ABSTRACT

Forensic science is the application of science to civil and criminal laws, also called as "criminalistics". Its branches are rooted in every branch of science and many other aspects of modern society. Forensic scientists are responsible to analyze scientific evidence during the course of investigation either by travelling to the scene of the crime or by performing analysis on objects in the laboratory. Forensic microbiology is the science by which microorganisms' behaviors are used to determine the origin of a particular microbial strain, the path of an outbreak or the identity of a criminal. Recently there has been a significant development in forensic microbiology as a result of the significant development in molecular biology, microbiology and biochemistry sciences. It was found that the rapid identification and classification of infectious microorganisms are of great importance in the case of biological and microbial threat, and the analysis of the microbial genome sequence, whose cost has decreased significantly in recent years, greatly helps in this area.

Keywords: *Microbial forensic; crime scene investigation; bioterrorism; bio-threat; pathogens; techniques.*

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1. INTRODUCTION

Microbial forensics is defined as the branch of science of applying scientific methods to the analysis of evidence related to the accidental release of a biological agent or toxin in any country in the world or the crimes related to bioterrorism. Recently, the development of technology in biochemistry, molecular biology and bioinformatics allow the identification and characterization of microorganisms for a variety of human forensic applications especially those concerned with biocrimes involving tracking of infectious agents [1]. Attribution of microbial evidence involves determining an associated source and/or perpetrator or group of individuals to the highest degree possible. Epidemiologists focus on the outbreak, the population(s) at risk, and spread of disease and characterization of the etiologic agent [2]. In parallel, traditional forensic methods, such as human DNA analysis, trace materials, fingerprinting and handwriting analysis, are exploited in a microbial forensic investigation, as ultimate attribution is the identification of the perpetrators of the crime. Taylor et al., [3] reported that there are more than thousands of microbial species or strains that are potential health threats to humans such as *Bacillus anthracis*. Technical advancements in recent years, through parallel sequencing allow analysis of microbes with speed that were not thought possible a short time ago. In addition, massively parallel sequencing (MPS) provides a methodology for human microbiome studies, which provide inference into different health and disease states, this tool have been used to characterize the complex community of the human microbiome and have been demonstrated for use in human forensic applications such as time-since-death decomposition analysis [4-6]. The aim of this review is to provide some information and concepts of microbiology with an emphasis on forensic applications.

2. ROLE OF MICROBIAL FORENSICS

Recently microbial forensic is a branch of science used mainly to analyze evidence for a bioterrorism act, bio-crime or spreading of microorganisms and their toxins in the environment [1]. This branch is still in the early stages of development and faces great challenges to provide new technologies about the detection of microbial and biological threat agents. Dealing with biological hazard agents without governmental agreement poses a great

danger to public health and environment which reflect directly to the economics of nations and global peace. International collaborations between scientists from all world countries before, during and after microbial threat (or bio-threat) should be strengthening to increase microbial forensics capabilities. Carus, [7] reported some cases from 1900 to 2000 of the use of biological agents by criminals and terrorists and he concluded the definition of bioterrorism as follows "assumed to involve the threat or use of biological agents by individuals or groups motivated by political, religious, ecological, or other ideological objectives" and he added that it is essential to separate the clearly criminal perpetrators from those with political agendas. In case of bio-crimes and bioterrorism public health protection requires that the microorganism must be first identified and its source located in order to stop further cases of exposure. The methods used in microbial forensics go deeper into identification of organisms and require standardization and validation, to meet legal standards for evidence. For example Brotke et al. [8] identified the facultative intracellular bacterium *Francisella tularensis* causes the zoonotic disease tularemia and reported that this bacterium resides within host macrophages in vivo, and this ability is essential for pathogenesis. The transcription factor MglA is required for the expression of several *Francisella* genes that are necessary for replication in macrophages and for virulence in mice. In another study, Hoffmaster et al. [9] reported that most isolates of *Bacillus cereus* appear to be harmless some are associated with food-borne illnesses, periodontal diseases, and other more serious infections. In one such infection, *B. cereus* G9241 was identified as the causative agent of a severe pneumonia and this isolate was found to harbor most of the *B. anthracis* virulence plasmid pXO1. These authors demonstrated that some *B. cereus* strains can cause severe and fatal infections in patients who appear to be otherwise healthy. In spite of science may not offer definite answers in all investigating cases in forensic science, it often plays a special role in the investigation of legal and policy matters. In order to protect public health quality to serve law enforcement, microbial forensics seeks to produce reliable conclusions quickly and different disciplines of science including microbiology, genetics, public health, agriculture, should participate to identify and characterize pathogens (or their toxins), implicated in biological events. Although microbial forensics includes basic science

research to develop the methods and techniques of investigation, the questions asked, expectations for, and outcomes sought may be different or more demanding than for basic research. Two conditions should fulfill in microbial forensics science: 1) obtained results of investigation must be validated and accepted by scientific and policy making stakeholders, 2) the obtained information can answer the legal and investigative questions. Another examples of microbial forensics investigations included tracing transmission of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) in criminal health-related matters [10,11], which fall under the biocrime category, therefore, microbial forensics focused on investigations where the microbe or its products (e.g., toxins) were used as weapons or bio-threats.

Murch [12] defined the scientific attribution as the assignment of a sample of questioned origin to a source (or sources), of known origin, to the highest possible degree of scientific certainty, while excluding origination from other sources. Examples are DNA analyses and fingerprint, which can provide a high degree of scientific certainty that evidence, came from one source to the exclusion of all others but Murch indicated that microbial forensics cannot yet claim that degree of certainty. The process of forensic investigation begins with gathering intelligence and information to justify an investigation followed by a time-driven, multidisciplinary, multisource investigation to pursue rule-out process. Crime scene investigation includes evidence identification, collection, preservation, and transport, as well as presumptive testing, which seeks to prove that it is the expected substance or not?. During the process investigators must place the interpretations of analyses and the conclusions drawn and transferring them to meet the needs of real-time and end users. Finally, all the information and evidence generated and other alternative explanations are provided to legal and policy decision maker.

3. DISCIPLINES OF MICROBIAL FORENSIC

Microbial forensic scientists indicating that the building of a microbial forensics discipline require a broad range of disciplines (e.g., epidemiology, genomic, biostatistics, biochemistry, analytical chemistry, bacteriology, mycology, virology, veterinary medicine, plant pathology, food science, ecology, materials science, physical

sciences, process engineering, computational science and bioinformatics. The most asking questions concerning microbial forensics seek to answer by specialists are the following:- a) What is the type of threat agent? B) Is the threat probative or relevant? C) Can it be linked to a source? D) What are the meaning of the conclusions?. Microbial forensics also investigates whether an agent has been genetically manipulated or chemically treated to make it more virulent or dispersible or to mask its characteristics. The source of a biological material are usually determined through physical and chemical analyses using mass spectrometry technique. There are many event scenarios to investigate using microbial forensics. These include introducing a highly aggressive new strain of influenza virus during flu season, various scenarios introducing biological threats into animal populations or agricultural crops and attacks employing pathogens that have been engineered. Until recently few culturable microorganisms were studied in laboratories using different techniques such as Gram stain, serology and growth characteristics. Today, however, the ability to sequence the genomes of microbes has provided a great deal of knowledge about nutrient cycling, gene regulation, and reproduction for some bacteria and viruses [13]. In addition, there have been few systematic efforts to collect and describe the microbes living in soil, seawater, freshwater lakes and streams, on plants. The presence of little information about microbial diversity, ecology, population genetics, evolution and phylogeny, represents a major scientific knowledge gap for microbial forensics purposes. Determination of a microorganism's source in the event of a bio-threat will be greatly hampered by the absence of baseline information on the natural abundance and distribution of pathogens. For this reason, understanding the endemic microbial background is necessary to provide proper contextfor microbial forensics analyses, interpretations, communication, and resulting decision making.

4. WORLD INVASION OF CORONA VIRUS COVID-19

Recently, the world was invaded by a dangerous and rapidly spreading corona virus called Covid-19, which started in Wuhan, China at the end of December 2019 and then spread to all countries of the world successively claiming many deaths and injuries, which entered the world and the inability to distinguish right from wrong in a way

dealing with this virus, and several speculations were made that the virus started spreading in Wuhan, China. Speculation has begun with forms of assumptions that began with talk about bats and even talk about the accidental leakage of a virus from a laboratory in this city. Since the start of the pandemic, and until now, some have seen it to be a terrorist attack, although many describe the behavior of countries and governments as conduct and as if they are facing war. The reference to terrorism may be in the letter from the Secretary-General of the United Nations to the Security Council, in which he warned that biological weapons could be used by various terrorist and non-terrorist groups. And some may see that terrorism has a hand in the manufacture of the Corona pandemic as a result of the defeat inflicted on terrorist bases while using conventional weapons, so it resorted to other methods that are surprising. Intelligence authorities from the major powers seek to search for the cause of the outbreak of this epidemic, and the place from which it specifically began using microbial forensic techniques and modern science.

5. METHODS AND TECHNIQUES USED IN MICROBIAL FORENSICS

These methods include both molecular genetics and non-genetic technologies. PCR, sequencing of 16S ribosomal RNA, sequence typing [14] and Advances in whole genome sequencing (WGS) have resulted in a reduction in the full economic cost of sequencing a typical bacterial genome [15]. WGS are molecular genetics technologies used to characterize microbial agents. Other physical science techniques, such as electron beam-based methods and mass spectrometry [16], can be used to analyze the physical properties of microbial forensic evidence [17]. The technique real-time PCR (qPCR) is a rapid and sensitive detection technology that has proven effective in both clinical and microbial forensic assay applications. Many other methods were able to detect hundreds of pathogens and hospital-acquired infections to the species and sometimes the strain level and a number of assays are FDA approved. MassTag PCR combines PCR with mass-spectrometry to provide high resolution, sensitivity, and specificity. This technology already has revolutionized the ability to identify respiratory pathogens and hemorrhagic fever viruses. Microarray platforms accomplish a similar goal of organism identification via detecting nucleic acid or protein signatures in a higher-multiplexed

format. Next-generation sequencing (NGS) technologies are allowing rapid sequencing of thousands of microbial isolates at far less cost and effort [18-21].

6. CONCLUSIONS

The main conclusions of this review are the following:

- International cooperation between all the interdisciplinary branches of forensic science to improve the capabilities to identify the agent(s) in a short time and to reach the source in order to provide this information as evidence in court.
- The awareness of forensic scientists and their institutions of the methodological and quality assurance requirements should be expanded, with greater support.
- It is of importance for forensic purposes to use the epidemiological tools to clarify the chain of infection and trace targeted strains.
- Improving the classical microbiological techniques and develop very rapid whole genome sequencing complement the polyphasic approach needed for diagnostics and typing.
- Microbial forensic investigations need to cooperate between gene banks (or gene libraries) from all over the world to characterize the microbes under investigation in a short period.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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