Clinical Microbiology
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Forensic Microbiology

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Abstract

The field of forensic science has obtained notoriety due, in part, to several sensational court cases, such as the murder trial of O. J. Simpson and the involuntary manslaughter conviction trial of Michael Jackson's physician, Conrad Murray, which captured national attention. In addition, although their roles are not always adequately portrayed, several modern television programs have brought to life the roles forensic scientists play in the criminal justice system. Forensic science encompasses many different areas. Most people are aware of DNA analysis, toxicology, fingerprint comparison, and ballistics, to mention a few. A newer area of forensic science is forensic microbiology. A landmark event in U.S. history, the *Bacillus anthracis* bioterrorism event in 2001, brought this field of science to the forefront. Forensic microbiology had been practiced before the anthrax event, and forensic microbiology involves more than just bioterror. This article discusses some of the roles that microbiology plays in forensic science.

Introduction

There are a number of definitions of forensic science, but simply put, it is the application of science to matters of law. One common goal of the forensic scientist, or criminalist, is attribution—determining who committed a crime. To meet this goal, forensic scientists rely extensively on comparison studies, comparing evidence from a crime scene to evidence found elsewhere. For example, DNA from an assailant found on a victim can be matched with almost certainty to DNA taken from the perpetrator. These comparisons are based on Locard's exchange principle, which states that when two objects come in contact, material is transferred from one to the other. Among other functions, forensic microbiologists may also use trace evidence to place people at crime scenes, investigate bioterrorism events, and determine the cause of and time of death. Discussions of bioterror investigations have been well presented elsewhere, so this review focuses on other areas that are less familiar.

For any evidence to be admitted into a court of law, it must have been legally obtained with collection, transport, and storage methods documented. A chain of custody form must be attached to all pieces of physical evidence. This form must account for the evidence from the time of collection to the time of testing and must include the names of all individuals who handled the evidence. During courtroom proceedings, the form must show that only appropriate individuals had access to the evidence, that the evidence was not tampered with, and that the evidence was stored properly, preventing degradation of the specimen.

Biocrime

A biocrime or biothreat is a situation in which a single person or a group targets a specific individual or group for harm. During a biocrime, a disease-causing agent or toxin is used as a weapon. The action can be either covert or overt. In the case of a covert event, it is likely that a physician or medical laboratory scientist will be the first to recognize the threat. In an overt action, emergency responders will be the first on the scene.

In biocrime cases, it is important to establish the source of the infectious agent by comparing the isolate found in victims to those associated with the assailant. Molecular analysis of the genome

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plays a critical role. Similar methods used in human DNA studies have been applied to microbial analysis, including microsatellite and minisatellite locus typing and single-nucleotide polymorphism analysis. In addition, multilocus variable-number tandem-repeat analysis, which is analogous to multiplex short-tandem-repeat typing in humans, has been used. DNA fingerprinting of bacteria and viruses is more difficult than that of humans because there are many species to consider. In addition, bacteria are haploid, reproduce more rapidly and primarily asexually, and undergo recombination and horizontal gene transfer.

Forensic microbiologists have several methods from which to choose. The choice of methodology depends partly on the turnaround time. In potential bioterrorist attacks, rapid and accurate identification of the agent involved is necessary to minimize morbidity and mortality and to prevent panic. In this case, DNA microarrays, or biochips, are popular choices because they can accurately and rapidly identify numerous agents at once. Microarrays contain many different DNA sequences on their surfaces that can bind complementary sequences in test samples. Specificity is determined by the DNA sequences unique to suspected agents of bioterrorism or biocrimes. In such cases, body fluids and environmental samples are treated as physical evidence, and phylogenetic studies of bacteria and viruses are performed and admitted into evidence by a judge.

There have been numerous instances of biocrimes in the United States. One of the first well-documented biocrimes involved a dentist practicing in Florida who was infected with the human immunodeficiency virus (HIV) (1). The dentist was charged with infecting at least six of his patients while performing oral surgery and failing to notify them of any risk. Besides records of the surgeries, evidence presented at the trial included phylogenetic analysis of HIV strains isolated from the dentist and some of his patients. Gene sequencing showed that viruses from five of the patients were similar to the strain from the dentist and that the strains were distinct from control strains taken from other individuals in the area.

In a similar case that occurred recently, a dentist in Tulsa, OK, was accused of using unsanitary and non-sterile procedures during oral surgeries (2). The dentist had approximately 7,000 patients, and over 4,000 were tested. More than 70 patients tested positive for hepatitis C virus (HCV), 5 for hepatitis B virus (HBV), and 4 for HIV. The dentist voluntarily surrendered his medical license and is facing several lawsuits filed by his patients.

In 2012, a laboratory scientist was arrested on federal charges for deliberately infecting patients with HCV (3). The suspect, who was HCV positive, used his blood to contaminate hospital equipment and syringes that were subsequently used on patients. It is unknown exactly how many patients were at risk from this event, since the defendant worked at over 18 hospitals. The strain of HCV from the suspect matched HCV strains detected in 32 patients treated at a New Hampshire hospital where he was employed.

Several HIV-positive individuals have been charged with aggravated assault or deemed sexual predators for deliberately infecting sexual partners by having unprotected sex and not notifying their partners of their HIV status. In Dallas, TX, a homeless man infected with HIV spit into the face of a police officer (4). He was convicted for harassing a public servant with a deadly weapon, namely saliva, and sentenced to 35 years in prison. The outcome was controversial, because contact with saliva is not a recognized risk factor for HIV infection. The judge argued that the assailant intended to harm the police officer. Murder charges have been filed in some instances where the victim died as a result of HIV infection. In 2009 in Canada, a man was convicted of murder for knowingly infecting two women who subsequently died of HIV infection (5). This is possibly the first case of a murder conviction following HIV transmission.

Trace Evidence

Trace evidence is defined simply as minute amounts of material that might be valuable in a court of law. By following Locard's exchange principle, trace evidence can place people at crime scenes. Fungi and fungal spores have been useful for this purpose. A young woman's body was found dumped on a patch of plants called stinging nettles (6). It is known that nettles support the growth of several species of fungi, about 15 of which are only found associated with nettles. Police identified a suspect in the death and dumping of the woman. Forensic scientists found two species of fungi in the suspect's car that were also found on the nettles where the body was found. While the suspect was dumping the body, he inadvertently picked up fungal spores on his clothing from the nettles and transferred them to his car. The evidence placed the suspect at the crime scene.

In a similar case, a man had made arrangements to meet an associate in a wooded area under the guise of making a drug deal (6). The plan, however, was to commit murder. The gunman hid in a forested area and shot his associate when he arrived. While waiting for the victim to arrive, the assailant had contaminated his clothing with a fungal plant pathogen that was found on cypress trees and ground litter where he had stood. Again, the fungal spores were found in the gunman's car, effectively placing him at the crime scene. Placing suspects at a crime scene does not conclusively implicate them, but it is an important part of the investigation.

Determining Cause of Death

Determining the cause of death is critical in investigating a suspicious death. A common question is, did the decedent die of natural causes, or was foul play involved? One example that relies heavily on forensic microbiology is the definition of "sudden infant death syndrome" (SIDS). This condition is loosely defined as an unexpected death of an infant less than 1 year of age. SIDS is a common cause of death in neonates in developed countries (7). Several risk factors are associated with SIDS, including sleeping position, genetics, and immunologic disorders. Numerous infectious agents are linked to sudden deaths, including human herpes virus 6, Epstein Barr virus, cytomegalovirus (8), and bacteria, such as *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* (9). In addition, it has been suggested that respiratory pathogens, such as respiratory syncytial virus, adenovirus, human metapneumovirus, and *Bordetella pertussis*, be included in SIDS investigations