Redefining Microbial Forensics: A Broader Scope of Applications

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May 1st, 2021
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Introduction

Microbial forensics is currently defined as the study and analysis of evidence from a bioterrorism attack, biocrime, or any other release of biological agents, with the primary purpose of attribution to criminal activity (Budowle et al., 2003). Working alongside law enforcement and epidemiologists, microbial forensic scientists focus on identifying the agent or toxin and determining how it was produced and spread. Since its inception, the field of microbial forensics has been focused primarily on bioterrorism, concentrating on investigations of microbes being used or threatened to be used as a weapon. Recent advancements with technology like massively parallel sequencing (MPS) have created new possibilities of identifying practically any microbe, regardless of the quantity or quality of the sample, in a time- and cost-efficient manner (Schmedes et al., 2016). Such advancements give rise to the potential of studying microbes for other areas of forensics, such as human identity testing (Fierer et al., 2010). For example, short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) can be studied to rule out individuals who may have been falsely identified through DNA or other forms of biological testing (Schmedes et al., 2016). Thus, microbial genetic markers can be used to expand upon the preexisting DNA-based identity testing to create more comprehensive and accurate methods of human identification. The various applications of microbial testing in forensic human identity testing demonstrate that the scope of microbial forensics is in fact much broader than its definition suggests.

The human body consists of ten times more bacterial cells than human cells, all of which make up the human microbiome. The human microbiome is highly complex and is able to
provide forensic signatures that are distinct and variable between individuals (Schmedes et al., 2016). Similar to human DNA, human microbes are constantly shedding and can be traced to identify people involved in various types of criminal activity. Microbes from different areas of the body can be investigated based on the type of crime and the type of evidence present at the crime scene.

**Collection & Analysis of Microbial Evidence Samples**

Microbial evidence is typically collected from the crime scene by one of three general approaches. One approach is to collect the whole contaminated item and transport it to a biocontainment facility for further sampling and analysis. However, an item may sometimes be too large or too bulky to be removed from the crime scene or to study in a laboratory. Alternatively, there may be a high possibility of losing trace evidence during transport. In these cases, an investigator can isolate a portion of the item using techniques like vacuuming, filtration, and water sample collection. The third and notably effective approach for trace material collection is swabbing or wiping materials or surfaces with sample collection devices, including dry or pre-moistened swabs, wipes, high-efficiency particulate air vacuums and filters, and aspirating needles. Depending on the type of evidence being isolated and the surface that it is present on, it may sometimes be beneficial to collect multiple samples and use multiple preservation methods so that several different analysis techniques can be used to study the evidence (Budowle et al., 2006).

**Microbial Analysis of Touched Objects**

When it comes to objects of evidence that may have been touched, fingerprints typically do not leave much DNA behind, and most of the current technologies cannot accurately make
conclusions with such small samples (Schmedes et al., 2016). Additionally, these methods are highly unreliable; false positive rates of latent fingerprint DNA analysis have been found to be as high as 1 in 18 (Holdren & Lander, 2016). However, since there are many more bacterial cells than human cells, more of the former will likely be deposited on touched objects. One study showed that 10,000 bacteria could be collected by swabbing one square centimeter of skin, and five times this amount could be obtained by scraping the skin (Grice et al., 2009). These larger quantities of markers can then be sequenced and typed. Isolating bacteria found in shoes and subsequently matching it to plantar skin of tested individuals has been found to be possible and relatively accurate (Goga, 2012). Additionally, bacterial DNA has been found to be more resistant to changes in environmental conditions than human DNA (Tozzo et al., 2020). However, despite this, microbe analysis from hands has not been found to be very suitable, as bacterial samples tend to vary before and after hand washing (Fierer et al., 2008). Thus, while further studies are certainly necessary to establish best practices and methods for optimal matching with bacterial samples, there is sufficient evidence to indicate that microbial testing is an effective tool in human identification from touched objects. Particularly in cases where only smudged fingerprints or partial prints are retrieved, microbial analysis has the potential to be a useful supplement.

**Microbial Analysis of Bodily Excretions**

Another important use of microbial forensics in human identity testing is to study samples of bodily excretions. Fecal material or vomit may be found in crime scenes, particularly in cases of homicide or rape. In one specific case involving a robbery, fecal material found on the suspect’s clothes was matched with a fecal sample from the bathroom at the location of the robbery (Norris & Bock, 2000). Feces and vomit are primary sources of gut bacteria sampling.
The microbiome of fecal material is typically stable but can be affected by environmental changes (Spor et al., 2011). The stable microbiome may be used for identity matching, and the fluctuating microbiome can be used to determine recent dietary consumption or geolocation (Schmedes et al., 2016). In addition to human identification, this information can help trace a suspect’s tracks before or after the crime occurred or may help identify behaviors that connect the suspect to the crime.

Certain bacteria have unique genetic signatures that may help identify the source of the sample. Specific bacteria (*Streptococcus salivarius* and *Streptococcus mutans*) can be used as markers for forensic identification of saliva samples (Nakanishi et al., 2009). Similarly, *Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Atopobium vaginae* have been found in and determined to be effective markers of vaginal fluid (Akutsu et al., 2012). Such information could be helpful in cases of rape. Sexually transmitted diseases (STDs) can also be tracked using microbial forensics. In one intriguing case, such microbial analysis was used for a medical malpractice investigation and was able to identify more than 270 patients that a physician infected with Hepatitis C (González-Candelas et al., 2013). This testing was so thorough that it was able to accurately identify the date and time of infection for each of these patients, even after about 25 years since the initial infection.

**Microbial Analysis in Identifying Behavior & Social Networks**

Bacteria found through microbial forensics can also be used to trace and identify criminally associated networks. Nose and throat commensal bacterial samples are prime candidates for determining recent contact information and geolocation because they are able to survive and remain viable for long periods of time (Smith et al., 1996). *Staphylococcus aureus*
has been found to be common among drug users (Scheidegger & Zimmerli, 1989). One study was able to track down fourteen social networks linked to a crack house by identifying similar strains of this bacteria in users and in drug paraphernalia (Quagliarello et al., 2002) through extensive screening of nasal swabs for *S. aureus*. These samples were then compared to the microbiota present on the drug paraphernalia samples. Meanwhile, only two of these networks were identified by social network analysis alone. Therefore, such bacterial sampling techniques can be beneficial in intelligence gathering and can also be used to verify suspects’ claims about their whereabouts.

**Next Steps in Microbial Forensics**

As microbial testing becomes more commonly used in forensic investigations, the development of more advanced technologies is a primary focus. Future goals should include filling some existing gaps in this science in order to refine the existing techniques to be more accurate and cost-efficient. It is also necessary to consider several steps in terms of setting standards and guidelines. For one, it is important to properly define operational protocols for sample collection, handling, and maintenance. If evidence is degraded, contaminated, or not collected, accurate analysis of these samples is at risk of being compromised. Thus, it is essential to set protocols that ensure that sufficient quantities of samples are obtained and maintained in order to maximize the characterization of the evidence. These protocols must be validated for a broad spectrum of bacterial species and other toxins. Additionally, law enforcement, first responders, and other related personnel should be trained in preserving potential microbial evidence while securing the crime scene and ensuring operational safety (Budowle et al., 2006).
Conclusion

Advances in MPS and other microbial testing techniques have the potential to revolutionize the field of forensics. Bacterial samples in bodily fluids and touched objects have been promising not only in the identification of suspected individuals, but also in studying behavioral, social, and geographical networks. With increasing potential and more studies being done to further explore and refine these applications, it might be worth revising the definition of microbial forensics. This study can be expanded beyond the scope of solely investigating bioterrorism and can be used to characterize human samples that may aid law enforcement in a broader range of criminal investigations. While this paper focused primarily on the use of microbial testing for human identification, there are many other applications of these techniques in the field of forensics, such as postmortem analysis. Thus, microbial forensics may be more accurately defined as the characterization of microbiological evidence to identify leads or evidence in criminal and civil cases.
References


doi:10.1038/nrmicro2540