(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805

REVIEW ARTICLE

MICROBIAL FORENSIC'S - MICROBES AS A PART OF FORENSIC INVESTIGATION

Nancy Charaya, Hershita Singh, Supreet Kaur Sidhu, Monika Poonia, Monika, Sandeep Singh Sihmar, Shalini[1]

Post graduate students, Department of Oral Pathology, [1]Department of Oral Medicine and Radiology, Surendra Dental College & Research Institute, Shri Ganganagar, Rajasthan 335001

ABSTRACT:
Researchers in the field of microbial forensics work to track down the source of a microbe, whether in a criminal investigation of bioterrorism attacks or a study of naturally occurring disease outbreak. 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. Microorganisms can therefore provide evidence in many different forensic scenarios but most of the work is still at the experimental stage and there are therefore many opportunities for further research. The forensic potential of microorganisms is becoming increasingly apparent as a consequence of advances in molecular sciences and genomics. This review highlights the role of microbes in the field of forensic sciences along with recent update.
Key words: Bioterrorism, Forensic, Microbial.

Corresponding author: Dr. Nancy Charaya, Department of Oral Pathology, Surendra Dental College & Research Institute, Shri Ganganagar, Rajasthan 335001

This article may be cited as: Charaya N, Singh H, Sidhu SK, Poonia M, Monika, Sihmar SS, Shalini. Microbial Forensic's - Microbes as a part of Forensic Investigation. J Adv Med Dent Scie Res 2016;4(4):32-37.

Access this article online	
Quick Response Code	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2016.4.4.8

INTRODUCTION

In 1995, the first idea originated about the use of forensic science in investigation and solution of bioterrorism events. It is advantageous over routine investigation because of the view that investigations with the help of forensic science could be readily perpetrated without the availability of eyewitnesses, concerned citizen reporting, records available for review and analysis, or serendipitous or purposeful intelligence or evidence. Such sources of information usually enforce law by conducting investigations, making arrests, and disrupting, preventing, or deterring crime. The prediction was also based on the expectation that someday one or a small number of skilled individuals would succeed in such an operation, by possessing knowledge and materials, employing tight operational security, and using subtle means during the preparation and dissemination of a biological agent.[1] Although microbial forensics employs genetic tools for strain identification, it is important to draw a distinction between this and the use of human DNA analysis in conventional forensics. Human DNA analyses are important tools in modern forensics. Their main use comes when DNA from specific individuals matches the DNA found in crime-scene samples and samples from suspects of the crime. Conversely, DNA analyses may be important in eliminating suspects when mismatches are found.[2]

DEFINITION

Forensic science refers to the use of science and technology to investigate and establish facts in criminal or civil court of law'. It is a multidisciplinary endeavour involving pathology, toxicology, computer investigation, fingerprint analysis, anthropology, DNA technology and other elements. It focuses on the person or persons who perpetrated the crime, and also on the victims and their related microbiology.[3] Microbial forensics refers to the scientific discipline dedicated to analyzing evidence from a bioterrorist attack, biocrime or inadvertent microorganism/toxin release for attribution purposes (who was responsible for the crime). [2] In another words, it refers to the detection of reliably measured molecular variations between related strains and their use to infer the origin, relationships, or transmission route of a particular isolate or forensic sample.[4] Additionally, the "boundary conditions" should be firmly established as to how it will be practised and explored and what "rules of engagement" and expertise will be expected and required to do so. Other emerging fields such as classical microbiology, microbial genomics, phylogenetics, and informatics are giving contribution to this new branch of microbial forensics.[5] Microbial forensics seeks to produce reliable conclusions quickly to protect public health and with sufficient validity and quality to serve law enforcement and policy purposes. In microbial forensics, law enforcement may partner with scientists from microbiology, genetics, public health, agriculture, and many other disciplines to identify and characterize pathogens, or their toxins, implicated in biological events.[6]

CLINICAL AND FORENSIC APPROACHES TO MICROBIAL IDENTIFICATION

The law enforcement and legal context of microbial forensics and the challenges imposed by this context are emphasized and contrasted with clinical diagnostics in medicine. Dr. Dana Kadavy, Senior Microbiologist at Signature Science LLC, reviewed the microbial forensic process from systems and technical perspectives, described challenges in microbial forensics compared with the needs of clinical diagnostics and public health, and reviewed technologies applicable to identification of organisms for microbial forensics. Technologies related to this field are roughly categorized into four classes depending on their targets for detection: protein (antibody and toxin) signatures, nucleic acid signatures other than sequencing (e.g., PCR), gene sequencing, and mass spectrometry (IOM/NRC, 2014).[7] Analysis of the microbial forensics are carried in the context of a criminal investigation, which shapes the differences between its technologies and/or best practices and those used for clinical diagnostics and public health. Analysis extends to strain, subtype, or isolate; type and/or abundance of organism present in simple to complex samples; the presence of antibiotic resistance and virulence genes; evidence of genetic engineering and/or isolate evolution (is it endemic wild type or a cultured strain repeatedly passed around labs?); and in-depth, sample-to-sample comparison that may be informed by SNPs.[8] When reporting to a court,1 investigators must have confidence in their results and understand the power and limitations of the procedures they have used, which can only be gained through accumulated experience in testing and validation. Investigators also must consider reasonable alternative explanations. Moreover, they must provide known error rates and detection limits when possible for a judicial audience that is unlikely to include scientists. These factors inform the choice of analytical technologies to be used. Traditional microbiology and culture are the current gold standard in clinical diagnostics, but it is not always possible or practical to culture microbes in a forensic investigation.[9] A capability needed in microbial forensics is sample processing to achieve desired sensitivity/accuracy in complex sample matrices, such as soil samples from forensic exhumations of graves linked to the Bosnian– Serbian war. Sensitive nucleic acid–based and antibody-based detection technologies also are needed. Although these diagnostic assays may not provide in-depth information, they potentially offer rapid, inexpensive, high-throughput screening to rule in or rule out various samples. Investigators and responders need to know quickly what kind of threat they face; additional in-depth analysis can be performed later. Traditional microbiology methods and features do have forensic value. These methods include culture, phage sensitivity, staining, and microscopy (all used in the U.S. anthrax letters case), as well as fatty acid analysis and serotyping. It is not always possible or practical, however, to use these on complex, degraded, and/or inactivated samples, or for isolates or mixtures that cannot be cultured.[10]

MICROBES AND POST-MORTEM TOXICOLOGY

Microbes degrade certain drugs and also generate metabolites during the decay process, which can be mistaken as indicators of pre-mortem drug consumption. E.g., bacterias quickly break down nitrobenzodiazepines such as Clonazepam and Nitrazepam into amino compounds and may be difficult to find in the blood even when the victim has died of an overdose. By contrast, morphine can be detected in buried bodies up to 8 years after death although during this time morphine-3-glucuronide is converted to free morphine by bacteria. In a notorious case in the UK, it was the resistance of morphine to autolytic and putrefactive processes that provided evidence that Dr Harold Shipman was responsible for intentionally killing up to 240 of his patients by administering overdoses of diamorphine. If he had used a more labile drug it is possible he may never have been convicted.[11] Results of bacterial and fungal fermentation are the production of a range of short chain alcohols, although not methanol, from a range of metabolites. This needs to be considered in cases in which alcohol consumption might have contributed to the cause of death. The amounts and rates of ethanol produced vary between species of microorganism and substrate but can be enhanced by the presence of high blood sugar levels at the time of death. E.g. Appenzeller *et al*. (2008) described a case in which exceptionally high levels of alcohol were noticed in the blood of a 14-month old child at autopsy. Suspicion arose from this case, that the child might have been a victim of abuse (given the alcohol) or neglect (allowed access to the alcohol). Later, it was demonstrated that the child had been given a glucose infusion shortly before her death and the alcohol detected was a consequence of fermentation by *Lactococcus garvieae*. This bacterium is rarely recovered from humans and is unlikely to have been the cause of death and it is possible that it gained access to the body iatrogenically.[12]

BIOTERRORISM

In 2001, Seth Carus compiled a series of cases from 1900 to 2000 of the illicit use of biological agents by criminals and terrorists. According to him, Bioterrorism is "assumed to involve the threat or use of biological agents by individuals or groups motivated by political, religious, ecological, or other ideological objectives." He clearly noted, however, that "most individuals and groups who have used biological agents had traditional criminal motives." He believes that it is, therefore, essential to separate the clearly criminal perpetrators from those with political agendas, whether the motive is sectarian, religious, or ecological. In the light of available evidences, it was suggested that the vast majority of cases involve criminal motives. Extortion, revenge, a desire to terrorize particular victims to make them worry about their health, and murder are included in the list of such motives.[13]

IMPACT OF BIOTECHNOLOGY ON BIOLOGICAL WARFARE

The impact of biotechnology on biological warfare is the threat which is faced by both military and civilian populations is graphically represented in the Figure. The threat presented by traditional agents has been increasing since the early 20th century but eventually will level off because of two major factors:

1. Development of targeted medical counted measures probably will reduce threats posed by current biological warfare agents, and
2. The number of natural pathogens and toxins that contain properties suitable for biological warfare is finite.[14]

Like traditional agents, the threat posed by genetically modified traditional agents eventually will plateau partly because, ultimately, only a finite number of properties and genetic modifications can be used to enhance a traditional agent without altering it beyond recognition of the parental strain or serotype. Importantly, the large countable number of potential genetic modifications may well represent a multiplicative threat relative to that posed by the traditional agents. Unlike threats posed by traditional and genetically modified traditional agents, the capability-based threat posed by Anti Biological warfare agents will continue to expand indefinitely in parallel with advances in biotechnology.[15]

MICROBES AS A CAUSE OF DEATH

Whenever body fluids are examined and single microbial species are recovered from autopsy, it is suggested that infection occurred during life, whilst mixed species profiles indicate post-mortem invasion. However, this is not invariably the case and it can be difficult to avoid contamination when taking the samples. Indeed, in a well publicised case in the UK, a pathologist giving evidence to an inquiry on the death of an 8-week-old child failed to report recovering *Staphylococcus aureus*, from the cerebrospinal fluid and other regions of the body because this is an extremely common component of normal skin flora and he was sure that it was a contaminant.[16] The sudden death of otherwise apparently healthy children during infancy invariably causes consternation and accusations. In some cases, death is ascribed to 'Sudden Infant Death Syndrome' (SIDS) but there is little agreement on its causes. It can be precipitated by a transient bacterial infection which, although cleared before death occurs, generates toxins that cause haemorrhagic shock and encephalopathy. Interestingly, this can result in pathology that is also associated with so-called 'shaken baby syndrome'. Therefore, for the evidence of response to bacterial toxins in samples, it is an important test even if it is not possible to isolate organisms in the blood or cerebrospinal fluid, this would provide evidence of a recent bacterial infection.[17]

SOIL MICROBES AS FORENSIC INDICATORS

For linking a person, animal, or plant to a locality, soil is a useful forensic indicator. Soil analyses are routinely usually performed for determining the mineral and chemical composition but their effectiveness is sometimes limited by the need for relatively large amounts of material and the lack of soil databases. Even though soils contain an extremely diverse microbial community but most species cannot be cultured in the laboratory.[18] With the development of molecular techniques, this is no longer a problem and there are an increasing number of studies investigating the potential of soil microorganisms as potential indicators. Most of these studies have been undertaken using 16-S ribosomal RNA gene sequencing using terminal restriction fragment length polymorphism analysis (T-RFLP) or amplicon length heterogeneity polymerase chain reaction (ALH-PCR). The abundance of soil microbes means that very small sample sizes are required; also these techniques are relatively cheap and can be 322 automated.[19]

RECENT DATA

In 2001, Anthrax attack, in which spores of the bacterium Bacillus anthracis, were disseminated via the mail. Microbial forensics has led to some high-pro-file discoveries. For example, sequencing of amplified viral fragments from the dentist and the infected patients supported the alleged transmission of HIV from a Florida dentist to several patients.[20] Recently, using multiple-locus variable number tandem repeat (VNTR) analysis, the Aum Shinrikyo B. Anthracis bioterror strain was identified as the veterinary accine strain, Sterne 34F26. Both criminal investigation of bioterrorism attacks and studies of naturally occurring disease outbreaks will continue to be important applications of this technology. In fact, in some cases, it is difficult at the outset to distinguish mother nature from man as the perpetrator: The investigation of the West Nile virus outbreak in the north Eastern United States in 1999 eventually revealed a single strain from birds and humans in New York with greatest similarity to a strain originally isolated from a dead goose in Israel, leading to the conclusion that the outbreak was of natural origin.[21, 22]

VALIDATION PROCESSES REQUIRED IN MICROBIAL FORENSICS

General validation criteria are more specific to the situation; it is useful to divide the processes for microbial forensics that may require validation into four underlying categories: [23]

1. **Collection and preservation.** The proper collection and preservation of microbial forensic evidence are crucial to a successful investigation leading toward attribution. Retrieval of sufficient quantities and maintaining the integrity of the evidence increase the chances of characterizing the material to obtain the highest possible level of attribution. Thus, methods involved in this aspect of microbial forensics should be validated, as well as is possible, given that each crime scene is unique and all scenarios are not predictable or completely definable. In many undefined situations, it may be more appropriate to develop and validate more-generic approaches.[24] The other application of the analysis is to determine that the microorganism or toxin is not present at the site (or at least no longer active) to evaluate whether it is safe to re-enter the site. Each contextual use requires different sampling strategies. The degrees of confidence in a result will differ between these applications.[25]
2. **Extraction of target for analysis.** The extraction of the target from a collected sample may not be necessary before direct analysis. Examples of this are the collection of bacteria from surfaces by using replicate organism direct agar contact plates for culture and the direct collection of visible

powder for microscopy. In contrast, an extraction process is often necessary in microbial forensics analysis as a sample-processing step between collection and analysis. It may be necessary to extract the target from the collection device (swab, wipe, or filter, etc.), purify the target from the environmental matrix or substrate before analysis, and/or remove inhibitors that may affect subsequent analyses. For the extraction of targets, a minimum checklist of validation criteria should consider several other factors also.[26]

3. **Analytical component of the process.** For some types of analyses, there are substantial descriptions of validation procedures in the literature. Notable examples of these are analytic procedures for the identification and quantification of chemicals and analytical procedures for the detection and quantification of nucleic acid targets.[27] However, sometimes validation of an analytical process of an assay lacks the rigor necessary for high confidence or effective interpretation. As stated above, it is likely that traditional forensic laboratories will engage in partnerships to carry out microbial forensic analyses and thus will be subcontracting bio threat agent analyses.[28]

CONCLUSION

Microbial forensics is a very potential upcoming field which is being utilized for highlighting role of microbes in legal medicine. Because of the technologies like molecular biological assay, NGS, it is becoming a potential component of forensic science. However, still a lot of exploration is required in this area like molecular characterization of bacteria from crime scene exhibits, for further promoting the use of this science in the forensic cases.

REFERENCES

1. Marshall JC; Gastrointestinal flora and its alterations in critical illness. Curr Opin Clin Nutr Metab Care, 1999; 2: 405–411.
2. Priestley CJ, Jones BM, Dhar J, Goodwin L; What is normal vaginal flora? Genitourin Med, 1997; 73: 23–28.
3. Grice EA, Segre JA; The skin microbiome. Nat. Rev. Microbiol, 2011; 9: 244–253.
4. Maurice CF, Turnbaugh PJ; Quantifying the metabolic activities of human-associate microbial communities across multiple ecological scales. FEMS Microbiol Rev., 2013; 37(5): 830–848.
5. Marples RR; Sex, constancy, and skin bacteria. Arch. Dermatol. Res., 1982; 272: 317–320.
6. Fierer N, Hamady M, Lauber CL, Knight R; The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc. Natl Acad. Sci. USA, 2008; 105: 17994– 17999.
7. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, Bouffard GG, Blakesley RW, Murray PR, Green ED, Turner ML, Segre JA; Topographical and temporal diversity of the human skin microbiome. Science, 2009; 324: 1190–1192.
8. Marples RR, Downing DT, Kligman AM; Control of free fatty acids in human surface lipids by Corynebacterium acnes. J. Invest. Dermatol, 1971; 56: 127–131.
9. Giacomoni PU, Mammone T, Teri M; Genderlinked differences in human skin. J. Dermatol. Sci., 2009; 55: 144–149.
10. Kong HH, Segre JA. Skin Microbiome; Looking Back to Move Forward, Journal of Investigative Dermatology, 2012; 132: 933– 939.
11. Cummings CA, Relman DA; Microbial Forensics- "Cross-Examining pathogens. Genom and Micro. Science, 2002; 296: 1976- 1979.
12. Roth RR, James WD; Microbial ecology of the skin. Annu. Rev. Microbiol, 1988; 42: 441– 464.
13. Fredricks DN. Microbial ecology of human skin in health and disease. J Invest. Dermatol. Symp. Proc, 2001;6:167-9.
14. Dethlefsen L, McFall-Ngai M, Relman DA; An ecological and evolutionary perspective on human-microbe mutualism and disease. Nature, 2007; 449: 811–8.
15. Turnbaugh PJ, Ley RE, Hamady M, Fraser- Liggett CM, Knight R, Gordon JI; The human microbiome project. Nature, 2007; 449: 804– 810.
16. Gao Z, Tseng CH, Pei Z, Blaser MJ; Molecular analysis of human forearm superficial skin bacterial biota. Proc Natl Acad Sci USA, 2007; 104: 2927–2932.
17. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R; Bacterial community variation in human body habitats across space and time. Science, 2009;326:1694–7.
18. Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R; Forensic identification using skin bacterial communities. Proc Natl Acad Sci USA, 2010;107:6477–6781
19. Aggarwal P, Chopra AK, Gupte S, Sandhu SS; Microbial Forensic- An upcoming Investigative discipline. J. Indian Acad Forensic Medicine, 2011; 33(2): 163-165.
20. Eitzen E. M; use of biological weapons, 1997; 437-450.
21. Enserink M, Ferber D; Microbial Forensic: reports spells out how to fight bio crimes, Science, 2003;299:1164-1165.
22. Anonymous, epidemiologic notes and reports update: Transmission of HIV infection during an invasive dental procedure – Florida, Morbid Mortal Wkly Rep, 1999; 40: 21-27.
23. Albert J, Wahlberg J, Leitner T, Escanilla D, UHlen M; Analysis of a rape case by direct sequencing of the Human immunodeficiency virus- 1 *pol and gag* genes, J Virol, 1994; 68: 5918-5924.
24. Blanchard A, Ferris S, Chamaret S, Guetard D ,Montagnier L; Molecular evidence for nasocomial transmission of human immunodeficiency virus from a surgeon to one of his patient, J Virol, 1998; 72: 4537-4540.

25. Schoolnik GK; Microarray analysis of bacterial pathogenecity , Adv. Microbiol Physiol., 2002; 46: 1-45.
26. Straub TM,Chandler DP; Towards a unified system for Detecting Waterborne pathogens, Journal of microbiology Methods, 2003; 53:18 3-197.
27. Budowle B, Schutzer SE, Einseln A, Kelley LC, Walsh AC, Smith JA, Marrone BL, Robertson J, Campos J; Building microbial forensics as a response to bioterrorism, Science, 2003; 301: 1852-1853.
28. Striebel HM, Brich-Hirschfeld E, Egerer R Folders-Papp Z; Virus diagonostics on microarray. Curr pharma, Biotechnol, 2003; 4: 401-415.

Source of support: Nil **Conflict of interest:** None declared