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Forensics for tracing microbial signatures: Biodefence perspective and preparedness for the unforeseen

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Biological weapons are assigned high priority in homeland security, defence, counterproliferation, nonproliferation, intelligence, and counterterrorist programmes. In order to strengthen active defense against development and use of these weapons, several comprehensive technological and forensic capabilities have been developed world over for investigative, intelligence, prosecutive, diplomatic and policy purposes. Microbial forensics is one of such strategies. It is a new discipline combining microbiology and forensic science. Microbial forensics associate the source of the causative agent with a specific individual or group by measuring molecular variations between related microbial strains. In this context, several advanced molecular techniques and practices including molecular phylogeny, whole genome sequencing, microarray analysis, DNA finger printing, etc., can be extremely valuable and effective analytical tools in a microbial forensic investigation. Results from such analyses may be related to the intentional use of microbial agents for bioterrorism or the accidental release of any offensive microorganisms or toxins of public health significance, specifically for the purpose of determining the origin. The new discipline of microbial forensics is an integration of an array of well established fields, such as microbial genomics, phylogenetics, forensic informatics and classical microbiology.

Keywords: Biological evidence, biological warfare, forensic microbiology, molecular phylogeny, molecular signature

Introduction

A number of bacteria, viruses and toxins pose serious health concerns to humans and can threaten country's economy and food supply and affect the environment. The potential to use any of these pathogenic agents as biological weapon has been demonstrated[1]. The fear and anxiety among people in many western countries due to intentional use of anthrax spores in postal mail envelopes confirmed their use as biothreat agents. Microorganisms make good weapons because they can be grown from single organism or cell and, unlike nuclear weapons, can be mass produced in a small establishment with low capital investment, and without the need of sophisticated instrumentation and skilled manpower. Microorganisms have been used as weapons by bioterrorists in criminal acts[2,3]. Although such "biocrimes" are few compared with other crimes, these acts pose questions about the ability to provide forensic evidence for criminal prosecution that can be used to identify the source of microorganisms used as a weapon.

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Microbial Forensics —A New Investigative Tool

Microbial forensics is a new discipline of microbiology and forensic science. Unlike public health investigations, microbial forensic investigation goes further to associate the source of the causative agent with a specific individual or group. Microbial forensics intend to measure molecular variations between related microbial strains and their use to infer the origin, relationship or transmission route of a particular isolate. The new discipline of microbial forensics is a conglomeration of an array of well established fields, such as microbial genetics, phylogenetics, forensic informatics and classical microbiology.

There are examples of well-developed practices for handling and analyzing pathogenic agents[4]. However, many of these assays address epidemiological concerns and do not provide sufficient information on the strain or isolate, or source of the pathogen to enable law enforcement to better identify the source or culprit. The source or origin of any pathogen and its relatedness to other strains or species offer considerable insight about its virulence. In this context, microbial signature becomes critical for tracing the source and relatedness. Phenotypic evidences have proved to be unreliable, as the phenotypic characteristics of any microbial agent may

vary according to the environmental circumstances. Though phenotypic evidences offer initial clues about the type of microorganism involved in the biocrime, they can not be used as forensic marker. Currently, more emphasis is being given to molecular signatures or molecular markers, i.e. finger prints and polymorphisms, which are reliable and quantifiable. 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[5].

Technologies of Microbial Forensics

Several methods are available to measure molecular variations between different strains to pinpoint origin and elucidate transmission routes of any isolate. Nucleotide sequencing and comparative evaluation of the sequence polymorphism is the classical approach to detect variations in signature sequences. Though many strains look very similar from phenotypic or physiological evaluation, comparative genome sequencing approach offers a more realistic solution for analyzing genetic variation within and between species and to resolve differences between them. Apart from whole genome sequencing approach, many a times, 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[6]. Though such information leads to clear understanding of molecular phylogeny of any given isolate[7,8], they fail to provide sufficient information which can be considered for criminal investigation.

With the advent of high throughput technologies belonging to the DNA-fingerprinting class, it has now become much easier to screen expression libraries and patterns. For instance, DNA microarray has gained popularity in diagnostics, specifically in detection of pathogens and study of their pathogenicity[9-11]. Such techniques are well established for their potential use in study of population structure, species evolution and acquisition of virulence[12,13]. Indiscriminate use of antibiotics and regional selection pressure has led many microorganisms to evolve much faster than their predicted rate. However, many strains are slow in progressive evolution and the rate of accumulation of genetic variation is quite slow.

Hyphanated technologies like MALDI-TOF (Matrix assisted laser desorption ionization time-of-flight), GC-MS (Gas chromatography-mass spectroscopy), LC-MS-MS (Liquid chromatography-mass spectroscopy) are becoming popular for detection of differences in protein or small-molecules. Potential use of MALDI-TOF-MS includes rapid microbial identification, rapid sub-typing and direct detection of bio-markers from samples. One of the profound advantages of this instrument is the speed of analysis. MALDI-TOF-MS could be applied directly to crude cellular fractions or cellular suspensions to produce chemotaxonomic signature profiles. It has been successfully used for analysis of bacterial RNA and DNA, detection of unknown protein and their characterization, bacterial proteomics, 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[14]. However, before this promising technique finds its possible routine application in microbial forensics, it is essential to generate a vast database of chemotaxonomic signature of as many weapon microorganisms as possible which can be either annotated or compared with the unknown sample using software programs.

Microbial forensics will be most effective if there is availability of sufficient basic scientific information concerning microbial genetics, evolution, physiology and ecology. A ‘Scientific Assessment’ on microbial forensics released by American Academy of Microbiology pointed out that simply studying the pathogen without understanding biotic and abiotic environmental backgrounds will lead to false confidence in our ability to detect it[15]. Strain subtyping analysis will be difficult to interpret if we do not understand some of the basic evolutionary mechanisms and population diversity of pathogens. The most recent advances have come over the last 14 years through the direct application of DNA fingerprinting to a wide range of human, plant and animal samples. This powerful tool and associated bioinformatics provide the criminal justice systems of many countries with a highly discriminating tool kit for the attribution and exclusion of sources of biological evidences. When forensic DNA is coupled with other informative analyses, such as highly sensitive analytical chemistry, microscopy, and pattern-matching techniques, the available evidence is often amplified and well represented.

Molecular microbiological techniques have been used for years to trace outbreaks of microbial

diseases, a practice called molecular epidemiology. In fact, there are currently surveillance systems that store and make available DNA fingerprints for microbes that are likely to be involved in nosocomial infections and food borne infections. PulseNet of the US Centre for Disease Control (CDC) is one of such surveillance system for tracking infections such as *Salmonella*. Although such surveillance systems are still only a few years old, they are rapidly growing and being constantly updated. Other than the molecular biology techniques, the classical approach of lipid profiling and, more recent, the total proteome analysis by MALDI-TOF has further sharpened the detection of molecular signatures. Protein microarrays could be of considerable value, but may be prone to loss of sensitivity due to conformational changes that occur during immobilization of protein to chips. Expression arrays could also be useful as a preliminary characterization of overall phenotype, but do not carry much importance so far as microbial forensics is concerned.

Stable isotope ratio as a tool in microbial forensics has also been explored recently[16]. Krueuzer-Martin *et al*[17,18] demonstrated that source of a micro-organism (spores of *Bacillus subtilis* used in the study) carry specific signature of the environment where in they were grown. It was demonstrated that microbial isotopic composition is a function of growth medium[17] and isotopic variation among different growth media can be used as a tool for sourcing origin of bacterial cells or spores[18]. Stable isotopes of carbon and nitrogen and their ratio in microbial cells carry a strong geographic signature. Stable isotope ratio analysis of pathogens may reveal information about the condition in which the agent was produced, complementing information obtained from genotyping and phylogenetic trace analysis. Kreuzer-Martin and his team successfully demonstrated that stable isotope ratios of oxygen and hydrogen in bacterial spores can potentially provide geolocation information by linking spores to the water source in which they were grown[16]. The growth medium in which the bacteria are mass produced for terrorism related applications can provide ample evidence for tracking the source. It is possible to derive general relationship between C, N, and H stable-isotopes in culture media and bacterial growth in them, and it is therefore possible to deduce information about both culture media and culture water from samples of suspect pathogen culture. The physiological differences between C_3 and C_4 (main photosynthetic paths) plants are the basic cause of isotope variability in biological material. This difference results in a distinct $\delta^{13}C$ values in their organic molecules which is incorporated into animal tissues when animals feed on these plant products. The isotope ratio values of bacterial culture media show the variations based on biological source of the media components (animal or plant origin). The range of variation of ^{13}C, ^{15}N, and ^{2}H content of bacteriological media can yield differences in microbe isotope ratios, which are readily measurable[18].

Biological weapons are assigned high priority in homeland security. In order to strengthen active defense against use of these weapons, it is becoming essential to establish a comprehensive technological and forensic capability for investigative, intelligence, prosecutive, diplomatic and policy purposes[19].

Proven Applications of Microbial Forensics

The application of molecular markers in forensic studies has led to some high profile discoveries. A forensic investigation in Florida to trace source and transmission of HIV led to the discovery that the deadly virus originated from a dentist and was passed to several patients. The case was solved by confirmative sequencing of polymerase chain reaction (PCR) amplified viral genes from the dentist and the infected patients[20].

An Arizona (USA) researcher recovered anthrax spores from a vial of slime, scraped 10 years ago from the walls of a Japanese doomsday cult's Tokyo headquarters, and pinpointed the specific strain harboured by the terrorists[21]. The same was confirmed by using multiple-locus variable number tandem repeat (VNTR) analysis and the Aum Shinrikyo *B. anthracis* bioterror strain was identified as Sterne 34F2, the veterinary vaccine strain[22].

The most challenging investigation was to define the cause of sudden outbreak of West Nile Virus in the northern USA in the year 1999. The forensic investigation pinpointed a dead goose in Israel as the source of the virus. The virus isolated from birds and human had great similarity to the strain isolated from the dead goose. Though suspected as a weapon attack, later it was confirmed to be a natural outbreak[23,24].

Comparative evaluation of full-genome sequencing of two related *B. anthracis* strains revealed comprehensive identification of genetic poly-morphism[25]. Investigation on Porton isolate of the

Ames strain and its comparision with an isolate from the index case in Florida, USA mail anthrax attack[26], led to introduction of a statistical model that distinguishes between true genetic polymorphism and random sequencing errors. It is emphasized that the markers to be selected for comparative evaluation are very critical for any forensic investigation. Comparison of any given set of markers will identify only a subset of the polymorphisms found within two strains/species, which may not be sufficient and conclusive. If the compared strains are closely related, then the fraction of polymorphisms will be underrepresented. This is supported and substantiated by the report of Keim *et al*[27]. They identified only two VNTR loci of *B. anthracis* for comparative evaluation of Porton and Florida strain, by using multiple-locus VNTR analysis[27]. In addition to increasing marker density, the distribution of each polymorphism must be established in a representative set of strains to determine the extent to which each marker varies in the population. The statistical power of this method will ultimately depend upon the number of markers and their allele frequencies. However, sequence polymorphisms observed in isolates maintained long in laboratory condition by routine passage are very different than the natural polymorphism. Growth in the laboratory imposes selective pressure on microbes, possibly leading to accumulation of mutations that are different from those in its natural environment. This needs to be considered while evaluating the generation and accumulation of genome sequence polymorphism[28].

Investigation of a Swedish rape case proved deliberate transmission of HIV-1 from the accused male to the female victim[29]. The investigation was carried out by comparing amplified HIV-1 *pol* and *gag* genes from virus obtained from the accused and victim. Similarly, later in the year 1998, phylogenetic analysis of amplified viral genes (HIV-1 *env* and *gag* gene sequences) from the blood samples collected from a French orthopedic surgeon and his patient proved transmission of HIV from the surgeon to his patient during surgical procedures[30]. In a more recent case study, molecular analysis of samples collected from a victim and accused confirmed attempts of second degree murder by intentional inoculation of HIV[31]. However, unlike the above, forensic evidence has also led to rejection of hypothesis of transmission of HIV from a surgeon of Baltimore to one of his patients[32]. Recently, inadvertent transmission of vaccinia virus infection by sexual contact has been traced to smallpox military vaccine. In the study, RT-PCR and electron microscopy were used to confirm the disease. Public health investigation and patient exposure interview were used as the basis for tracing the source of infection[33]. Transmission of vaccinia virus from an unusual source highlights the importance of epidemiologic tracing. These studies indicate an increasing numbers of criminal cases being solved by molecular trace analysis and phylogenetic evidence. Though their applications in solving problems related to bioterrorism are very limited, the proven track record of use of such microbial forensics analyses suggests possible successful application of the same for criminal investigation related to bioweapons and bioterrorism.

Future of Microbial Forensics in Applications of Bioterrorism

Microbial forensics is currently in its brooding stage. Time and experience are needed to develop a universal code of conduct and to define quality assurance guidelines for laboratory performing microbial forensics. This will help in avoiding complications and variability of data which can be challenged in legal proceedings. Current efforts need to be prioritized, specifically for pathogens and toxins that would most likely be used in biocrimes. Such intervention is expected to vary from country to country. Different criteria towards validation of methodologies used to characterize various threat agents need to be established that can be used forensically to attribute criminal acts. Regional microbial population dynamics need to be evaluated critically for developing better understanding of microbial population genetics. Availability of information in a format of globally accessible database will enhance analysis and validation. However, rational criteria for information database related to microbial forensics are yet to be developed. To be successful, any microbial forensics laboratory must rely on knowledge centre composed of databases on genomics, microbiology, forensic methods, associated material and related evidence assays, bioinformatics and standardized tools. The scientific working group on microbial genetics and forensics (SWGMGF) is one such group which has developed quality assurance guidelines for laboratories performing microbial forensics work, which is available online for open access[34].

Recommendations for Sample Collection, Preservation and Transportation

Correct sampling that can aid to forensic evaluation is important for the success of any investigation. Collection of specimens from appropriate locations is essential. In case of an anthrax attack *via* aerosols, it is expected that the spores will remain air borne for hours. In such cases, air samples and specimens from the surfaces of inanimate objects need to be collected. However, the lack of knowledge related to the immediate likely source of the spores during the first day of attack poses problem in deciding the sites for collection of samples. In special incidences wherein a bioweapon agent is intentionally released in a closed confinement, it is essential to collect wet swabs, wet wipes, vacuum sock, and air-filter samples throughout the room/confinement to characterize the extent of contamination. It is equally important to collect specimens from air vents and air handling units to ascertain the route of dispersal of biothreat agents. The isolation of organism or DNA is possible directly from the samples or after enrichment. A sterile rayon swab pre-dipped in phosphate buffered saline solution at *p*H 7.2 is generally used to swab a selected surface by moving the swab back and forth horizontally and then vertically. High-efficiency particulate air (HEPA) vacuum sock samples are also essential to be collected by pulling ~ 1000 L of air per minute though the vacuum nozzle. Air samples can be collected on to mixed cellulose acetate filters by sucking air (2-5 L min^{-1}). Air samples need to be collected for a longer duration to capture low density aerial microbial population. These samples can be subjected to standard microbiological evaluation for identification of the bioweapon agent, which can later be subjected to molecular typing or any other advanced analysis to trace strain, sub-type and source of the microorganism. Similar methodologies were followed for investigation of *B. anthracis* contamination and anthrax inhalation case in postal mail processing and distribution centre in New York and Washington DC[35].

Immunomagnetic separation is also a promising techniques for separation of target organism from a mixed population or directly from environmental samples. Immunomagnetic separation is the process of using small super-paramagnetic particles or beads coated with antibodies against surface antigens of target cells. Environmental sample collection is very important for relating the source of the bioweapon agent to any specific geographic locality. Other than sample collection from the affected person, which is to be carried out by trained medical personnel only, sample needs to be collected from the local areas, patient's home, workplace, and places that he/she frequented. Stagnant water reserves, swabs from the plant leaf surfaces, slime from walls, moss scrapings, amber or gums from the tree trunk, greasy deposits of exhaust or suction fans are few examples from where samples collection may offer some evidence. It is important to have well-established protocols for managing and accurately tracking each and every specimen. The site of collection of specimen is generally influenced by the previous experience of the investigator. Once the material is collected, it is required to be packed in aerosol free packages, sealed to avoid any kind of leakage or air contact. The packed materials/samples are to be shipped to reference laboratories. Large numbers of specimens collected randomly are to be subjected to primary screening procedures, i.e. PCR, time resolved fluorescence (TRF), etc. Specimens are then to be forwarded to respective specialized laboratory for isolation, confirmation and molecular typing (DNA fingerprinting) of the isolates. The random samples collected represent different environmental conditions. Samples collected may or may not yield any significant results but in over all totality represent the actual microbial situation of the sample collection site during the time of sampling.

The added responsibility of the investigator collecting specimens is to preserve the chain of evidences. The specimens must be stored in tamper-proof containers and a thorough record must be kept about when, where and by whom the specimens were collected. This is important to rule out the possibility of involvement of specimen contamination during the handling steps of the investigation. The collected specimens must be stored under conditions optimal for the preservation of the specimen for further testing. Spores are well known for their long survival in odd environmental conditions, but other non-spore forming microbes require special care during preservation. The real practical problem is that, during biological war related situation, it is expected that the first responder will be the police officer, medical personel or firefighter, who is not trained microbiologist. In such situation, careful and thorough execution of sampling protocol is essential.

Recommendation for Lab Preparation and Networking during Investigation

The general preparedness to deal with a biocrime or bioterrorism requires that laboratories have to have an awareness of the potential agents that may be used, laboratory techniques for the early detection of these agents, procedure for the management of the event, and knowledge of the safety precautions necessary to be undertaken for safe handling of the infectious agent. It is appropriate and most important to have the laboratory response network as initiated by Centres for Disease Control and Prevention (CDC), USA— a multi-level system that can link state and local laboratories with advanced-capacity, specialty laboratories. Stringent level of safety requirements, containment and technical proficiency is essential to perform the rule-out, rule-in and referral functions required for identification of the agents that could potentially be used as biological weapon. Biosafety level-3 (BSL-3) laboratory is essential for handling of microorganisms that can be used as biological weapons. BSL-3 is a level of containment and safety practice to be followed for handling of any infectious agents. The first responders not only have to collect materials or samples for evidence, but also need to maintain safety, avoid dissemination of the materials beyond the scenes, and avoid introduction of contaminating materials in to the scene. It is crucial to establish communication linkages between the public health and law enforcement communities. Law enforcement officials need to understand what is normal in medical and epidemiological investigations. The first responder to an investigation scene needs to understand what additional safety precautions are required to take to avoid infection, the spread of contamination, and to preserve the crime scene. Public health personnel need to understand what additional steps are required when a public health incident becomes a criminal investigation. Proper sample preservation, which can be later used as an evidence, can be achieved by proper coordination and communication among all these groups. To enhance cross-talk and interaction among these groups, it is essential to impart cross-discipline education programmes for all the concerned public health and law enforcement communities. Some first responders (a group of inter disciplinary people) should be identified in each community and need to be trained on standard forensic methodologies and practices. Other than the first responders, a team of experts need to be established, who have better understanding about the biology of microorganisms and likely to be used in any bioterrorist activity, and who could be called upon in crisis.

Common and established laboratory procedures/ protocols need to be followed for quick and smooth analysis of the sample. However, if the data generated is not sufficient to reach any conclusion, then it is advisable to follow advanced modern techniques. Though many forensic laboratories in the recent past have switched to rely more on advanced technologies for forensic evidence analysis, it is important to define standards and experimental criteria to rule out variability of any modern techniques. The advanced techniques followed are DNA finger printing, genetic profiling, SNP analysis, PCR, TRF, restricted fragment length polymorphism (RFLP), simple sequence repeat analysis (SSRA), amplified fragment length polymorphism (AFLP), expressed sequence tags (EST), random amplified polymorphic DNA (RAPD), etc.

Problems and Challenges

International bioterrorism that led to dissemination of *B. anthracis* spores *via* post mail raised questions about our competence to track the culprit in such unforeseen biocrimes. If a suspect is ever arrested and charged with perpetrating the anthrax attack, spores could be an important part of the physical evidence. It needs to be proved that spores isolated from the suspect's home or laboratories are the spores that were introduced into the envelopes and mailed. The problem is that the spores of *B. anthracis* are found widely in soil, especially farm soil at many places of the world. So the prosecution will have to prove that any spores submitted as evidence were the spores used in the attack and not simply that had been tracked into a house or laboratory from a nearby field. Proving such case will be tough. Spore forming strains of *Bacillus* sp. and *B. anthracis* look very much alike. Different strains of *B. anthracis* are also very similar to each other even at the genome sequence level. Given that the error rate for DNA sequencing is not zero, proving that a particular isolate of *B. anthracis* is the same as the strain used in the bioattack may be difficult.

The main hurdle with microbial forensics is how to establish standards for evidence collection and for analysis and interpretation of the plethora of new molecular tests. The first and foremost challenge is the collection of specimens at the attack site where the

release of infectious microbe is suspected. It is important to educate personnel who are expected to investigate bioterrorist activities to secure evidence, and confirm an outbreak by reliable field test kits. It has been suggested that at least three different strains of each pathogen and up to 20 for the highly pathogenic (highest risk) species should be sequenced, as the genomic information can help to pinpoint the source of the organism[36]. It is important to establish a team of microbiologist and forensic experts who understand the nature and biology of organisms that are likely to be used in a bioterrorist attack. The need of the hour is to develop systems that can integrate state-of-the-art analysis, shower-in-shower-out logistics and sterilization procedures for effective handling of microbial forensic samples. Investigating team's job will be to obtain samples both from victims as well as from the environment to identify the biothreat organisms and the source of the bioattack, and to preserve the evidence for a trial. Since it would not be clear about the route of exposure, samples should include background/ ambient/environmental materials. Standard protocol needs to be followed for sampling. In order to recognize that an attack is occurring, it is important to diagnose the disease as early as possible, preferably on-site. Such commitment requires strong and quick development of rapid and sensitive diagnostics, which can be used under field condition in any emergency. The major concern is the reliability, reproducibility, suitability and stability of such diagnostic tests. Proper analyses of the specimens collected by first responders and by microbiologists on site pose another challenge for effective implementation of microbial forensics. The most formidable challenge is rigorously validating each analytical method by establishing its limitation, its sensitivity, and its reliability. Also important is the robustness of any method. Above all, there is a growing concern that classifying scientific information and not sharing results about developed analytical methods will limit its effective and wide spread use in disease situations that are not bioattacks[37]. There is great demand and need to develop new methods based on DNA-fingerprinting which can offer very specific quantitative information for forensic application. Any new method must address issues like variability, reproducibility, robustness, economics, and ease of handling and simplicity for quick adaptation by various forensic laboratories[38]. Other than developing new methods, it is also important to develop standard operating protocols (SOP) and validation criteria for any new method. Strictly following SOP will rule out variability in results to a great extent between coordinating laboratories. To identify gaps and develop the field of microbial forensics, starting from sample collection to interpretation of evidences, a group of scientists from diverged discipline convened a meeting on 19-21 April 2004 at the Banbury Centre for Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. The specific gaps identified by the group have been published[39].

Indian Viewpoint

In the year 1994, the plague outbreak in Surat and several adjoining areas in India stirred a nation wide panic and virtually led to near international isolation[40,41]. Of late, India woke up to the threat of bioterrorism[42]. Several microbial isolates from the outbreak were examined and characterized[43]. However, the analysis results were not sufficient and due to lack of precise forensic evidence, definitive conclusion indicating any bioterrorist activity could not be proved. Many third world countries routinely experience natural outbreak caused by biological threat agents. Natural outbreak of Kyasanur Forest disease caused by KFD virus, a deadly hemorrhagic virus listed under group "A" biothreat agent, has occurred in Shimoga and adjoining forest areas in Karnataka state of India[44-46]. Several natural outbreak of anthrax cases have been reported from India and near by countries[47-50]. Recently, the mystery fever observed in Siliguri, India was attributed to Nipah virus, a well known haemorhhagic virus listed under group "A" biothreat agents[51,52]. Molecular typing data and detailed information are not available about such strains responsible for natural outbreaks in many developing countries. In the given circumstances, attribution or tracing source of infection and collection of reliable legal evidence for any bioterrorist activity by following microbial forensics becomes unrealistic rather difficult, though not impossible.

Conclusion

The full exploitation of the evidence of biological weapons can provide crucial information on which a response of any sort would be based, although such information would likely be not the only basis. Such biological and other associated forensic evidences are absolutely necessary to link "crime", "modus operandi" and "perpetrator". Knowledge base of microbial forensics is expanding due to the recent contribution of key scientists and laboratories working on identification or characterization of

microbes[16,22,27,53-55] and highly complex problems of microbial phylogenetics[56]. From microbial forensics viewpoint, it is very much essential to develop assays for identification of individual strains of microbial agents. Development of systems and methods to detect and track bioattacks will lead to greater safety and security of a nation. In a fundamental way, biocrimes are a public health concern. To lay a proper foundation for the field of microbial forensics, the Federal Bureau of Investigation (FBI) has led to the creation of the Scientific Working Group on Microbial Genetics and Forensics (SWGMGF). However, many more nations are yet to initiate such programmes to develop either competence to track bioterrorism events and biocrimes or preparedness for such unforeseen events. Nevertheless, aggressive research programmes are also needed for forensic tracking, micro-chemical analysis, trace evidence analysis coupled with microbial forensics, so that the new found discipline of microbiology and/or forensic science will grow in tandem and bear the fruit for national security.

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