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Concept of Forensic Microbiology and its Applications

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Abstract

In the recent years, Forensic Microbiology has been established as a new scientific discipline in order to strengthen the law enforcement response especially in a bioterrorism event. Microorganisms can indiscriminately and unlawfully be used as agents of biological warfare, bio-crimes and agro terrorism. The main goals of microbial forensics are to identify and prioritize biological threats, identify the vulnerable population, create an information database and develop protocols for identification which includes determining unique genetic signatures, protein signatures, develop programs for ensuring the validity of results and constantly update based on existing literature. This review article aims to shed some light on this upcoming discipline.

Keywords: Forensic Microbiology, biological warfare, agro terrorism

Introduction

Microbial forensics is a new and emerging type of forensic analysis defined as "the detection of reliably measured molecular variations between microbial strains and their use to infer the origin, relationships or transmission route of a particular isolate"[1]. In the recent years, it has been established as a new scientific discipline in order to strengthen the law enforcement response especially in a bioterrorism event [2]. Microorganisms can indiscriminately and unlawfully be used as agents of biological warfare, bio-crimes and agro terrorism. This review article aims to shed some light on this novel discipline.

Biological Warfare & Agro Terrorism

Bioterrorism as defined by the Centers for Disease Control is the "intentional or threatened use of bacteria, fungi or toxins from living organisms to produce death or disease in humans, animals and plants," and involves "intimidation of nations or people to accomplish political or social ends"[3]. Agro terrorism is the deliberate tampering with and/or contamination of the food supply with the intent of adversely affecting the social, economic, physical and psychological well-being of the society"[4]. Agro terrorism carries less risk for the terrorist outfits owing to the ease of being carried out more covertly and it does not require sophisticated methodology for weaponization[5]. Important vulnerabilities are intensive production practices, increased susceptibility of immunologically naive animal populations and rapid movement of animals & their products over long distances[6]. Attacks can result in disastrous economic losses due to eradication measures [mass culling], international trade embargos, loss of jobs, increased consumer costs, and may even cause difficulties in sustaining the food supply[7].

Biological warfare is an age old weapon which has been well documented but scientifically less advanced. References to biological warfare can be obtained from the Holy Bible which mentions about God's demand for the Israelite slaves to be freed by Pharaoh. When Pharaoh did not comply, God sent upon Egypt ten deadly plagues[8]. During the 6th

century BC, the Assyrians poisoned enemy wells with a fungus that would render the enemy delirious. In 1346, the bodies of Mongol warriors of the Golden Horde who had died of plague were thrown over the walls of the besieged Crimean city of Kaffa. Specialists disagree over whether this operation may have been responsible for the spread of the Black Death into Europe[9-12]. It has been claimed that the British Marines used smallpox in New South Wales in 1789[13]. Historians have long debated inconclusively whether the British Army used smallpox in an episode against Native Americans in 1763[14]. By 1900 the germ theory and advances in bacteriology brought a new level of sophistication to the techniques for possible use of bio-agents in war. Biological sabotage in the form of anthrax and glanders was undertaken on behalf of the Imperial German government during World War I (1914–1918), with indifferent results[15]. The Geneva Protocol of 1925 prohibited the use of chemical weapons and biological weapons. With the onset of World War II, the Ministry of Supply in the United Kingdom established a biological warfare program at Porton Down, headed by the microbiologist Paul Fildes. The research was championed by Winston Churchill and soon tularemia, anthrax, brucellosis, andbotulism toxins had been effectively weaponized. In particular, Gruinard Island in Scotland, during a series of extensive tests was contaminated with anthrax for the next 56 years. Although the UK never offensively used the biological weapons it developed on its own, its program was the first to successfully weaponize a variety of deadly pathogens and bring them into industrial production[16]. When the USA entered the war, mounting British pressure for the creation of a similar research program for an Allied pooling of resources, led to the creation of a large industrial complex at Fort Detrick, Maryland in 1942 under the direction of George W. Merck[17]. The biological and chemical weapons developed during that period were tested at the Dugway Proving Grounds in Utah. Soon there were facilities for the mass production of anthrax spores, brucellosis, and botulism toxins, although the war was over before these weapons could be of much operational use[18]. Shiro Ishii, commander of Unit 731, which performed live human vivisections and other biological experimentation. The most notorious program of the period was run by the secret Imperial Japanese Army Unit 731 during the war, based at Pingfan in Manchuria and commanded by Lieutenant General Shirō Ishii. This unit did research on biological warfare, conducted often

fatal human experiments on prisoners, and produced biological weapons for combat use[19].Although the Japanese effort lacked the technological sophistication of the American or British programs, it far outstripped them in its widespread application and indiscriminate brutality. Biological weapons were used against both Chinese soldiers and civilians in several military campaigns[20]. In 1940, the Japanese Army Air Force bombed Ningbo with ceramic bombs full of fleas carrying the bubonic plague[21]. Many of these operations were ineffective due to inefficient delivery systems [19], although up to 400,000 people may have died[22] During the Zhejiang-Jiangxi Campaign in 1942, around 1,700 Japanese troops died out of a total 10,000 Japanese soldiers who fell ill with disease when their own biological weapons attack rebounded on their own forces[23-24] During the final months of World War II, Japan planned to use plague as a biological weapon against U.S. civilians in San Diego, California, during Operation Cherry Blossoms at Night. The plan was set to launch on 22 September 1945, but it was not executed because of Japan's surrender on 15 August 1945[25-28] In Britain, the 1950s saw the weaponization of plague, brucellosis, tularemia and later equine encephalomyelitis and vaccinia viruses, but the programme was unilaterally cancelled in 1956.
In 1969, the UK and the Warsaw Pact, separately, introduced proposals to the UN to ban biological weapons, and US President Richard Nixon terminated production of biological weapons, allowing only scientific research for defensive measures. The Biological and Toxin Weapons Convention was signed by the US, UK, USSR and other nations, as a ban on "development, production and stockpiling of microbes or their poisonous products except in amounts necessary for protective and peaceful research" in 1972. However, the Soviet Union continued research and production of massive offensive biological weapons in a program called Biopreparat, despite having signed the convention[29]

Some other famous examples of biological warfare include the following: Tricothecenes, a type of Mycotoxin, was probably used in the famous yellow rain incident [30] which still is a subject of debate with no conclusive established proof. A case was documented in the US where a gastroenterologist was convicted of attempting a second-degree murder by injecting his former girlfriend with blood or blood-products obtained from an HIV-

(Human immunodeficiency virus-1) infected patient under his care[31] Other recent well publicized bio-crimes include use of “ricin” in Europe for the assassination of a Bulgarian exile, a laboratory worker intentionally infecting co-workers in Texas with *Shigella dysenteriae* [32] and use of *Salmonella Typhimurium* in Oregon by contaminating salad bars in local restaurants which was politically motivated[33] The most well known attack of bioterrorism in the present century is the use of anthrax spores in New York in October 2001[34] Biological warfare can also specifically target plants to destroy crops or defoliate vegetation. The United States and Britain discovered plant growth regulators (i.e., herbicides) during the Second World War, and initiated a herbicidal warfare program that was eventually used in Malaya and Vietnam in counterinsurgency operations. Scorched earth tactics or destroying livestock and farmland were carried out in the Vietnam war (cf. Agent Orange)[35].

In 1980s Soviet Ministry of Agriculture had successfully developed variants of foot-and-mouth disease, and rinderpest against cows, African swine fever for pigs, and psittacosisto kill chicken. These agents were prepared to spray them down from tanks attached to airplanes over hundreds of miles. The secret program was code-named "Ecology"[36].

Attacking animals is another area of biological warfare intended to eliminate animal resources for transportation and food. During the Mau Mau Uprising in 1952, the poisonous latex of the African milk bush was used to kill cattle[37].

Entomological warfare is a type of biological warfare that uses insects to attack the enemy. The concept has existed for centuries and research and development have continued into the modern era. It has been used in battle by Japan and several other nations have developed and been accused of using an entomological warfare program. Entomological warfare may employ insects in a direct attack or as vectors to deliver a biological agent, such as plague. Essentially, it exists in three varieties. One type of entomological warfare involves infecting insects with a pathogen and then dispersing the insects over target areas[38]. The insects then act as a vector, infecting any person or animal they might bite. Another type of entomological warfare is a direct

insect attack against crops; the insect may not be infected with any pathogen but instead represents a threat to agriculture. The final method uses uninfected insects, such as bees, wasps, etc., to directly attack the enemy[39].

Though there are many methods of creating biological weapons, a common approach would be to release a microorganism that is potentially pathogenic or may be attenuated to more pathogenic forms and then released into the community which makes it more lethal than any other weapon. With the use of genetic engineering, even an environmental commensal may be rendered pathogenic[40]. The Centers for Disease Control [CDC] in Atlanta have evaluated the priority of agents according to their relevance for national security due to ease of dissemination and transmission from person to person, high mortality rates, the potential for major public health impact, risk of public panic and social disruption and the requirement of special action for public health preparedness. Category A bio weapons includes the most dangerous agents: *Variola* major virus [smallpox], *Bacillus anthracis* [anthrax],*Yersinia pestis* [plague], *Clostridium botulinum* toxin [botulism], *Francisella tularensis* [tularemia], and viral hemorrhagic fever viruses[41]. As a preventive measure to stop the dangers of bio weaponry, BTWC (Biological and Toxin Weapons Convention) was established and now signed by more than 165 countries. This treaty denies the use and stockpiling of biological weaponry or its use[42]. However, nine of these countries are still suspected to possess offensive biological warfare programs[43].

Role of Microbial Forensics in Biological Warfare

Molecular techniques often have been used to trace outbreaks of microbial diseases, a practice called molecular epidemiology. In fact, there are currently available surveillance systems that store and make available DNA fingerprints for microbes that are likely to be involved in hospital-acquired infections and food borne infections[44]. The application of microbial forensics is to assist in resolving bio crimes, with a focus on research and education needs to facilitate the use of microbial forensics in criminal investigations and the subsequent prosecution of bio crimes, including acts of bioterrorism. The main goals of microbial

forensics are to identify and prioritize biological threats, identify the vulnerable population, create an information database and develop protocols for identification which includes determining unique genetic signatures, protein signatures, develop programs for ensuring the validity of results and constantly update based on existing literature [45,46]. Identifying and prioritizing the target is a difficult task. The organism that needs to be prioritized may either be totally unknown or may not be a human pathogen [47,48]. Identification of vulnerable population is equally challenging. Creating an information database would help in guiding towards the results, however on the contrary it can also help criminals in identifying those organisms which are not categorized and thus make way for the proliferation of an organism more difficult to identify. Authenticity of use of such a database should be clear for the same reasons. The database that is used should involve inputs from various classical fields inclusive of microbiology, genomics, forensic methods, chemistry and pure science [49].

Laboratory Aspects of Microbial Forensics:

The quality assurance and quality control program is an inevitable part of any laboratory analysis and microbial forensics is not an exception. Developing a protocol for identification may make use of routine diagnostic policies, for which quality guidelines exist. In addition, techniques that have not undergone validation may also have to be used, especially when the organism is unknown or very rarely encountered which is currently not recommended by any guiding documentations. Such results may not be highly reliable but can create clues for judging the possible organism. This is of special concern as the organism being dealt with may not be much known to the scientific world. Scientific Working Group on Microbial Genetics and Forensics (SWGMGF) establishes and sustains guidelines and/or standards for quality systems identifying processes and procedures, define criteria for knowledge systems and most importantly serve as an experienced resource on issues as they arise [46]. The SWGMGF defines the guidelines and updates it as and when required. The development of these guidelines helps the laboratory to perform various forensic analyses and host the results as valid and true to the best of scientific

knowledge available to that day. Additional stringent rules are required [49] compared to routine surveys as the issues involve legal matters and data will be relied upon heavily. An erroneous quality management or lack of quality control may mislead the final conclusion. The chain of custody should be impeccable to obtain perfect results, especially the biologic evidences obtained in this context. A standard operating procedure (SOP) may not be available always and often guidance from other institutions may be required and opinions considered. In addition constructing a validation plan and its execution will help the cause [49]. The development of rules should be based on standards of human DNA typing, clinical laboratories standards and International Standards Organization.

Laboratories involved in forensic microbiology analysis must be prepared to deal with chain-of-custody documentation, secure storage of evidence, tracking of individual items of evidence and their derivatives and all the legal requirements for handling evidence. Chain- of-custody protocols document the unbroken chain of records showing who had handled the evidence, where and under which conditions [temperature, time etc.] the material had been stored and whether access to the samples was restricted. The NATO document AEP-10 "Handbook for Sampling and Identification of Biological and Chemical Agents [SIBCA]", 2007, 5th Edition, Procedures and Techniques, Volume 1 provides practical guidelines how to sample select agents in the field even in a contaminated environment. These guidelines are used by NATO and Partnership for Peace [PFP] countries. Countries may have different national requirements, but general principles can be a guideline for Civilian-Military Cooperation [CIMIC] or purely civilian operational and forensic investigation teams. The European Guideline on Principles of Field Investigation "Biological Incident Response and Environmental Sampling" was published by the EU Commission, DG Health and Consumer Protection, Health Threats Unit in October 2006 and "describes the principles of response in the initial phase of a biological incident where the goal is to identify what has happened in order to initiate appropriate countermeasures". These documents underline the necessity of planning and pre-mission briefings as the environment may be life-threatening. Moreover, the quality of primary samples is critical for subsequent analyses.

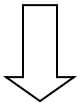
The personal protective equipment is also affecting personnel by limiting mobility, flexibilityand time available to work at the scene [50].

In clinical Microbiology, the sample matrix is important to decide, whether the analyses requested by the clinician are appropriate and which tests should be performed. Unfortunately, requests are not always justified by the clinical presentation and the sample matrix is sometimes conflicting. For example a microscopic examination of sputum samples will indicate, if the quality of the specimens is adequate. In forensic microbiology the same rules apply, but more detailed investigations may be necessary to obtain relevant information about the history of a specimen, environmental conditions, chemical and physical constitution of the matrix, presence of pollen etc [50]. This can be achieved by particle sizing, electron microscopy, analytical chemistry, isotope analysis and other techniques. However, several analyses will have to be performed outside appropriate laboratory safety containment and therefore, specimens will have to be inactivated. Especially when anthrax spores have to be killed the inactivation with chemical or physical techniques is quite aggressive. This treatment does not only denature the pathogens but will also cause changes of the matrix. It has to be demonstrated for each method that the inactivation process does not interfere with the subsequent tests [51].

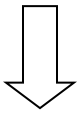
The knowledge of Microbial Forensics comes into play when investigators are presented with a suspected case with an unusual presentation or in a place where distribution of disease is unusual. In case of a bio-crime, generally the laboratory that obtains the sample as routine assay is the one to first raise a suspicion. If a strong suspicion is invoked it should be communicated to an investigative body or national reference centre, especially when a strain that looks genetically engineered or sample analysis shows multi-strains of possible aetiology [52]. The steps involved in the investigation are essentially the same as investigation of a natural outbreak. However, they are more demanding than the routine diagnostic or epidemiological assays [53]. The sample collection is of utmost importance. The samples to be collected include every material found in the scene which is labeled with time

Investigation of a suspected bioterrorist attack

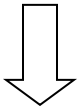
A suspected case of bio-crime with an unusual presentation or in a place where distribution of disease is unusual.



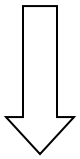
Samples to be collected include every material found in the scene which is labeled with time and site of collection.



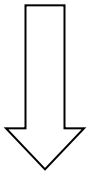
Each sample should be considered potentially hazardous and processed only in a well equipped laboratory, or ideally sent to a reference laboratory equipped with stringent bio safety levels.



Microbiological evidence can be obtained from viable samples of the microbial agent, protein toxins, nucleic acids, clinical specimens from victims, laboratory equipment, environmental samples, contaminated clothing etc.



Timely environmental sampling is of immense value as it may be rapidly destroyed and the evidence of intentional spread may be lost.



On the forensic front the method of collection should be sensitive, reliable and robust to clinch the presence of possible organism or the toxin.

and site of collection. The name of the person who has collected the sample should also be mentioned. The code of practice should be the same irrespective of the type of the sample from a community or individual. Microbiological evidence could include; viable samples of the microbial agent, protein toxins, nucleic acids, clinical specimens from victims, laboratory equipment, dissemination devices and their contents, environmental samples, contaminated clothing, or trace evidence specific to the process that produced and/or weaponized the biological agent. On the forensic front the method of collection should be sensitive, reliable and robust to clinch the presence of possible organism or the toxin [54]. Timely environmental sampling is of immense value as it may be rapidly destroyed and the evidence of intentional spread may be lost [49]. Each sample should be considered potentially hazardous and processed only in a well equipped laboratory, or ideally sent to a reference laboratory equipped with stringent bio safety levels [53].

Techniques used in elucidating the causative agent(s)

The identification of microbial agents – as defined by the SIBCA handbook - can be provisional [presumptive], when immunological methods, nucleic acid detection or cultivation and metabolic assays have been tested positive. Identification is confirmed by the combination of at least two of the above mentioned criteria. Unambiguous identification requires cultivation and *in vivo* studies [animal models] that prove the pathogenicity of the agent. However, animal models should be avoided for ethical reasons whenever possible. Biological agents can be difficult to cultivate due to sample contamination, low number of bacteria or pretreatment of patients with antibiotics. Some bacteria are fastidious [*F. tularensis*, *Brucella* spp.] and require special nutrient media, and some need prolonged cultivation times [*Brucella* spp.]. Phenotypical characteristics such as antibiotic susceptibility and biochemical reaction profiles, susceptibility to specific phages, colony morphology and others are not always reliable. Mutations of agents can be induced or engineered, but naturally occurring atypical strains have also been found e.g. among *Bacillus anthracis* and *Yersinia pestis* isolates which can result in misidentification and treatment failure [55]. Commercial biochemical identification systems are not optimized for these agents and can result in misidentification. Multiple antimicrobial resistances can occur

through natural horizontal gene transfer or by genetic manipulation. Natural resistance to a multitude of antimicrobials is typical for *Burkholderia pseudomallei*. *Francisella tularensis* is naturally resistant to penicillins and cephalosporines. A very dangerous multidrug resistant strain of *Yersinia pestis* has been isolated from a patient with bubonic plague in Madagascar. This strain carries a self-transmissible plasmid with a genetic backbone also prevalent among *Escherichia coli*, *Klebsiella* spp. and *Salmonella* spp. conferring high-level resistance to streptomycin, tetracyclin, chloramphenicol, and sulfonamides [56]. These facts underline the importance of cultivation and the assessment of antimicrobial susceptibility in addition to more rapid diagnostic tools. A polyphasic approach for identification and typing will help to avoid problems due to atypical genotype and phenotype, inhibition, or lack of specificity or sensitivity of assays. Handling of select agents is highly dangerous and cumbersome and restricted to laboratories with biosafety-level 3 containment. Biosafety-level 3 laboratories have to be operated according to special regulations that require e.g. a sophisticated ventilation system and personal protective equipment [e.g. FFP3 masks, overalls, face shields, gloves etc.].

1. Nucleic Acid Amplification Techniques

Many real-time PCR assays are highly specific and sensitive and shorten the time required to establish a diagnosis in comparison with conventional PCR protocols, cultivation, and biochemical identification methods. Therefore, real-time PCR assays have been developed for the identification of *Bacillus anthracis*, *Brucella* spp., *Burkholderia mallei* and *Burkholderia pseudomallei*, *Francisella tularensis*and *Yersinia pestis* [57]. PCR results can be false negative due to inadequate quality of clinical samples, low number of bacteria in samples, DNA degradation, inhibitory substances and inappropriate DNA preparation.

2. **Serology**

Seroconversion may prove the exposure to a certain agent in the past. However, seroconversion can be expected only after several days or weeks and is of little use for rapidly diagnosing infections caused by highly pathogenic agents. It will be difficult to organize serological investigations [including follow-up tests] when a terrorist attack causes mass

casualties that need medical treatment or when the situation is complicated by civil unrest, war or natural catastrophes at the same time. Various immunological assays have also been used to identify pathogens in samples of patients and environmental samples. Hand-held test kits can be used as bed-side tests and are useful under field conditions, but clinical validations hardly exist and most tests are "for scientific use only". Immunochromatographic lateral-flow assays have been developed e.g. for brucellosis, tularemia, and plague [58-61]. Limitations of these immunological assays are that they are frequently not available commercially, not specific enough, or have not been validated and licensed for use in humans or animals. Moreover, cross-reactions may cause false positives and modified or missing antigenic structures can cause false negatives.

3. Typing and strain identification:

Differences among microbes have to be assessed to determine whether strains are from the same source or lineage or from a different origin. The accuracy and precision will depend on the typing method, expected mutation rates, and other characteristics of the organism. In court scientists may need to quantify the reliability of a relationship among strains determined using molecular phylogenetic analyses. This will establish the probability of association to a certain source of infection [62]. Techniques for forensic microbiology can be very similar to those being used for phylogenetic and epidemiological investigations e.g. for food-borne outbreaks.

Molecular-epidemiological tools used for genotyping are most promising and have been applied in the past to elucidate the origin of biological agents. Especially whole genome sequencing and bioinformatic tools for comparison of genomes are potent tools, but technical complexity and costs are still prohibitive for routine application. In several chapters of the highly recommendable book "Microbial Forensics" by Bruce Budowle and many other "founders" of this new scientific discipline it was demonstrated that only highly specialized knowledge of microbial genetics will allow an assessment of the relevance of typing results obtained by Multi-locus Sequence Typing [MLST], Variable Number of Tandem Repeats [VNTR], Single Nucleotide Polymorphisms [SNPs] analysis or other typing tools [51]. Validation of typing assays and data of large collections of strains from all over the world are crucial for microbial

forensic investigations. Typing methods should be reproducible, stable during the study period, applicable to every isolate, discriminating among isolates, and discrimination should be concordant with the epidemiological picture [63]. DNA sequence-based data are robust, portable, easy to compare and amenable to computerised analysis for phylogeographical and epidemiological studies. However, the quality of open access sequence databases depends on the accuracy of submitted sequences and is consequently sometimes not reliable [51].

Indian scenario

To the best of our knowledge, there is no documented case of biological warfare in India. This may be interpreted as no case occurring or as lack of forensic microbiology work up. Keeping the latter possibility in mind, microbial forensics has a vast potential in India. As a country which is commonly threatened by terror attacks, there is no doubt that biological weapons will be made use of by various terrorist outfits in the near future. Establishment of a national organization which integrates expertise of specialists from various fields of science will prove to be beneficial despite the cost that will be incurred in creating and maintaining such a team. Three components will be absolutely essential to establish a fully functional National Microbial Forensic Laboratory. The first would be a knowledge centre composed of databases on genomics, microbiology, forensic methods, SOPs, evidence assays such as fingerprinting, bioinformatics and standardized tools. The second component will be maintenance of strong partnerships between the existing government, the laboratory in charges, scientists and investigating agents. The third component will be quality control and validation of newer assays [64].

Conclusion

Microbial forensics is a scientific discipline dedicated to analyzing evidence from a bioterrorism act, biocrime or inadvertent microorganism/toxin release for attribution purposes. The unlawful use of biological agents poses substantial dangers to individuals, public health, the environment, the economies of nations, and global peace. A national and international

collaborative approach can be used to handle the menace of bioterrorism by setting up a national and international reference laboratory thereby ensuring transparency of analysis and strict action against all bio crime perpetrators. Considering all the scientific facts already discussed, “Microbial Forensics” should be an ideal requirement in India.

References

[1] Cummings CA and Relman DA (2002) omics and microbiology. Microbial forensics--"cross-examining pathogens". Science. 296(5575):, 976 9

[2] Budowle. B, Schutzer SE, Einseln A, Kelley LC, Walsh AC, Smith JA et al (2003) Public health. Building microbial forensics as a response to bioterrorism. Science. 301(5641), 1852-3

[3] Bioterrorism: An Overview: Centers for Disease Control, Atlanta, USA.

[4] Levin J, Gilmore K, Nalbone T and Shepherd S (2005) Agroterrorism workshop: engaging community preparedness. J Agromedicine. 10(2), 7-15.

[5] Wilson TM, Gregg DA, King DJ, Noah D. Perkins LE, Swayne DE et al (2001). Agroterrorism, biological crimes, and biowarfare targeting animal agriculture. The clinical, pathologic, diagnostic, and epidemiologic features of some important animal diseases. Clin Lab Med. 21, 549 91

[6] Crutchley TM, Rodgers JB, Whiteside HP, Vanier M.Jr, and rndrup T.E (2007) Agroterrorism: where are we in the ongoing war on terrorism? J Food Prot. 70, 791-804

[7] McEwen SA, Wilson TM, Ashford DA, Heegaard ED and Kournikakis B (2006). Microbial forensics for natural and intentional incidents of infectious disease involving animals. Rev Sci Tech. 25(1), 329-39.

[8] Thomas J. Johnson (2008) A History of Biological Warfare from 300 B.C.E. to the Present. Available from: http://www.aarc.org/resources/biological/history.asp. Accessed on 2015 Nov 23.

[9] Wheelis, Mark (2002) "Biological warfare at the 1346 siege of Caffa". Emerg Infect Dis (Center for Disease Control). 8(9), 971–5.

[10] Barras V and Greub, G (2014) "History of biological warfare and bioterrorism".Clinical Microbiology and Infection. 20(6), 497–502.

[11] Andrew GR, Laura JR (1995) "From asps to allegations: biological warfare in history," Military medicine. 160(8), 369-373.

[12] Rakibul H (2014) "Biological Weapons: covert threats to Global Health Security." Asian Journal of Multidisciplinary Studies. 2(9), 38.

[13] Christopher, Warren (2013) "Smallpox at Sydney Cove - Who, When, Why". Journal of Australian Studies.

[14] Fenn, Elizabeth A.(2008) "Biological Warfare in Eighteenth-Century North America: Beyond Jeffery Amherst". Journal of American History. 86(4), 1552–80.

[15] Koenig, Robert (2006) The Fourth Horseman: One Man's Secret Campaign to Fight the Great War in America. Public Affairs.

[16] Prasad, S.K (2009) Biological Agents, Volume 2. Discovery Publishing House. p. 36.

[17] Covert, Norman M (2000) "A History of Fort Detrick, Maryland", 4th Edition.

[18] Guillemin J (2006) "Scientists and the history of biological weapons: A brief historical overview of the development of biological weapons in the twentieth century". EMBO; Reports 7 (Spec No). S45–S49.

[19] Williams P and Wallace D (1989) Unit 731: Japan's Secret Biological Warfare in World War II. Free Press. ISBN 0-02-935301-7.

[20] Hal Gold (1996) Unit 731 testimony, p.64-66

[21] Barenblatt, Daniel (2004) "A Plague upon Humanity". HarperCollins. pp. 220–221.

[22] Hudson, Christopher (2007) "Doctors of Depravity". Daily Mail.

[23] Chevrier MI, Chomiczewski K, Garrigue H, Granasztói G, Malcolm R.D and Pearson GS (2004)The Implementation of Legally Binding Measures to Strengthen the Biological and Toxin Weapons Convention: Proceedings of the NATO Advanced Study Institute, held in Budapest, Hungary, 2001. Springer Science & Business Media: p.19.

[24] Croddy E and Wirtz JJ (2005) Weapons of Mass Destruction: Chemical and biological weapons. ABC-CLIO. p. 171.

[25] Naomi Baumslag (2005) Murderous Medicine: Nazi Doctors, Human Experimentation, and Typhus, p.207.

[26] "Weapons of Mass Destruction: Plague as Biological Weapons Agent" (2014) GlobalSecurity.org. Retrieved 21 December.

[27] Amy Stewart. "Where To Find The World's Most 'Wicked Bugs': Fleas" (2011) National Public Radio, 25th April.

[28] Russell Working (2001) "The trial of Unit 731". The Japan Times, 5th June.

[29] Alibek K, Handelman K and Biohazard (1999) The Chilling True Story of the Largest Covert Biological Weapons Program in the World Trade From the Inside by the Man Who Ran It. New York: Random House.

[30] Robert W. Wannemacher and Stanley L Wiener. Trichothecene mycotoxins. http://www.au.af.mil/AU/AWC/awcgate/medaspec/Ch34electrv699.pdf

[31] Metzker, ML., DP. Mindell, XM. Liu, RG. Ptak, RA. Gibbs, and DM. Hillis. Molecular evidence of HIV-1 transmission in a criminal case. Proc. Natl. Acad. Sci. USA 2002; 99:14292-14297.

[32] Chutzer SE, Budowle B and Atlas RM (2005) Biocrimes, Microbial Forensics and the Physician . PLoS Med 2 (12):e337.doi:10.1371/journal.pmed.0020337

[33] Török TJ, Tauxe RV, Wise RP, Livengood JR, Sokolow R, Mauvais S, Birkness KA, Skeels MR, Horan JM and Foster LR (1997) A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. JAMA. 278 (5), 389-95.

[34] P. Aggarwal, A.K, Chopra,S, .Gupte, S and Sandhu, S (2001). Microbial Forensics – An Upcoming Investigative Discipline. J Indian Acad Forensic Med. 33(2):, 63-165.

[35] "Vietnam's war against Agent Orange" (2010) BBC News. 14 June 2004. Retrieved 17 April.

[36] Kenneth Alibek and S. Handelman (1999) Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World – Told from Inside by the Man Who Ran it. Delta (2000) ISBN 0-385-33496-6 [2].

[37] Verdourt, Bernard; Trump, EC.; Church, ME (1969) "Common poisonous plants of East Africa". London: Collins. p. 254

[38] "An Introduction to Biological Weapons, Their Prohibition, and the Relationship to Biosafety" (2013) The Sunshine Project, April 2002. Retrieved 25 December 2008. Archived May 12 at the Wayback Machine

[39] Lockwood Jeffrey A (2008) Six-legged Soldiers: Using Insects as Weapons of War, Oxford University Press, USA, pp. 9–26, ISBN 0195333055.

[40] Chakrakodi NV, Kuruvilla TS and Furtado Z (2012) Microbial Forensics-Past, present and future. Int J Biol Med Res. 3(2), 1546-1549.

[41] Rotz LD, Khan AS, Lillibridge SR, Ostroff SM and Hughes JM (2002) Public health assessment of potential biological terrorism agents. Emerg Infect Dis. 8(2), 225-30.

[42] The Biological and Toxins weapons convention website (2015) http://www.opbw.org/. Accessed on Nov 21.

[43] "26 Countries' WMD Programs; A Global History of WMD Use - US - Iraq War - ProCon.org" (2009) Usiraq.procon.org. May 29.
.

[44] Centers for Disease Control and Preventions's Pulse Net site (the U.S. National Molecular Subtyping Network for Foodborne Disease Surveillance).

[45] Sharad Jain, Ashish Kumar, Pratima Gupta and Ramjee Prasad (2005) Microbial forensics: A new forensic discipline. JIAFM, 27 (2).

[46] Defining a New Forensic Discipline (2015) Microbal Forensics Bruce Budowle Laboratory Division, Federal Bureau of Investigation, Washington, DC, U.S.A. March 2003. www.promega.com. Accessed on Nov 20.

[47] Whitby S and Rogers P (1997) Anticrop biological warfare—Implications of the Iraqi and US programmes. Defense Analysis. 13, 303–318

[48] Whitby SM (2001) The potential use of plant pathogens against crops. Microbes Infect. 3(1), 73–80.

[49] Budowle, Quality sample collection, handling, and preservation for an effective microbial forensics program. Appl. Environ. Microbiol.; 72:6431-6438

[50] Budowle B, Schutzer SE, Burans JP, Beecher DJ, Cebula TA, Chakraborty R et al (2006) Quality sample collection, handling, and preservation for an effective microbial forensics program. Appl Environ Microbiol. (72), 6431-8.

[51] Tomaso H and Neubauyer H (2011) Forensic Microbiology. In: Vieira D.N (ed), Forensic Medicine-From old problems to new challenges, Intech.

[52] Treadwell TA, Koo D, Kuker K and Khan AS (2003) Epidemiologic clues to bioterrorism. Public Health Rep; 118:92–98.

[53] Ronald M (2004) Atlas. Microbial Forensics- Taking Diagnostic Microbiology to the Next Level. Clinical Microbiology Newsletter. 26, 13

[54] National science and technology council (2015) National strategy to support research in microbial forensics attribution investigations and national security.http://www.whitehouse.gov/files/documents/ostp/NSTC%20 Reports/National%20MicroForensics%20R&DStrategy%202009%20UNLIMITED%20DISTRIBUTION.pdf. Accessed on Nov 24

[55] Wilmoth BA, Chu MC and Quan TJ (1996) Identification of Yersinia pestis by BBL Crystal Enteric/Nonfermenter Identification System. J Clin Microbiol. (34), 2829-30.

[56] Welch TJ, Fricke WF, Mc Dermott P.F,White DG, Rosso ML, Rasko DA et al (2007) Multiple antimicrobial resistance in plague: an emerging public health risk. PLoS One. (2),e309 EOF

[57] Jones SW, Dobson ME, Francesconi SC, Schoske R and Crawford R (2005) DNA assays for detection, identification, and individualization of select agent microorganisms. Croat Med J. (46), 522-9.

[58] Chanteau S, Rahalison L, Ralafiarisoa L, Foulon J, Ratsitorahina M, Ratsifasoamanana L et al (2003) Development and testing of a rapid diagnostic test for bubonic and pneumonic plague. Lancet. (361), 211-6

[59] Mizanbayeva S, Smits HL, Zhalilova K, Abdoel TH, Kozakov S aand Ospanov KS (2009) The evaluation of a user-friendly lateral flow assay for the serodiagnosis of human brucellosis in Kazakhstan. Diagn Microbiol Infect Dis. (65), 14-20.

[60] Splettstoesser W, Guglielmo-Viret V, Seibold E and Thullier (2010) Evaluation of an immunochromatographic test for rapid and reliable serodiagnosis of human tularemia and detection of Francisella tularensis-specific antibodies in sera from different mammalian species. J Clin Microbiol. (48), 1629-34

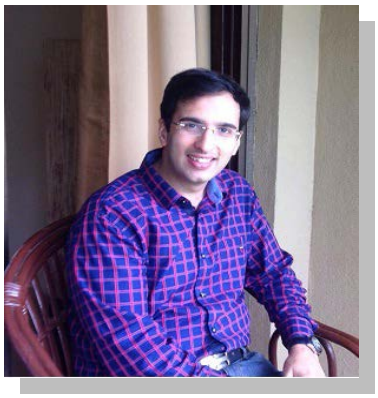
[61] Tomaso H, Thullier P, Seibold E, Guglielmo V, Buckendahl A, Rahalison L et al (2007) Comparison of hand-held test kits, immunofluorescence microscopy, enzyme-linked immunosorbent assay, and flow cytometric analysis for rapid presumptive identification of Yersinia pestis. J Clin Microbiol, (45):3404-7.

[62] Gonzalez-Candelas F, Bracho M.A, Moya A (2003) Molecular epidemiology and forensic genetics: application to a hepatitis C virus transmission event at a hemodialysis unit. J Infect Dis. (187), 352-8

[63] Van Belkum A, Tassios PT, Dijkshoorn L, Haeggman S, Cookson B, Fry N.K et al (2007) Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin Microbiol. (3), 1-46

[64] Bruce Budowle (2008) Criteria for validation of methods in microbial forensics, Applied And Environmental Microbiology. Vol. 74, No. 18, P. 5599–5607.

Authors Column



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