**Bioterrorism and Biological Warfare: An Emerging Challenge**

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**Abstract**

The deliberate use of pathogens and toxins, typically of microbial, plant, or animal origin to cause illness and death in people, animals, and crops is known as biological warfare. The ease of access to a variety of disease-producing biological agents, the low production costs, the non-detection by standard security systems, and the simplicity of transportation from one location to another are all factors that make bioweapons attractive for use in war and in terrorist attacks. Furthermore, new and widely available technologies encourage the development of such weapons, which have effects on regional and worldwide security. Through international consultation and cooperation, the necessity for leadership and example in developing preventative and protective methods has been highlighted in order to counter such dangers, secure the culture of peace, and defend it. Adherence to the Biological and Toxin Weapons Convention is a crucial and essential step in minimizing and eliminating the threats of biological warfare and bioterrorism. It is supported by confidence-building measures and maintained by use of monitoring and verification methods.

**Keywords – Bioterrorist, Anthrax, Covid, Biowarfare, Bio agents, Bioweapons**

**I. Introduction**

Bioterrorism (BT) is a term used to describe the intentional or deliberate use, spread or release, dissemination, or production of living organisms, toxins, and chemicals of animal or plant origin to produce such diseases that can harm or cause the death of humans, animals, plants, and even the environment as a whole. Bioweapons and biowarfare are other terms that are sometimes used interchangeably. Terrorists can transmit it through the air, water, soil, and food and it may take them many days to have an impact. It covers a wide spectrum of living organism (bacteria, viruses, and diseases), as well as chemical toxins, which are employed to disseminate fear, danger, risk, and threat [1]. The deliberate use of microbial organisms or their toxins as weapons for political advantage is known as bioterrorism, and it continues to pose a serious concern worldwide due to the ease with which terrorists can utilize these substances against innocent civilian targets. The destruction of economic stability and hindering of target nation progress is one of the key objectives of biological warfare. Furthermore, such warfare can always be carried out under the pretext that such traumatic occurrences are the result of natural circumstances that lead to outbreaks of diseases and disasters of either endemic or epidemic proportions. Although, exposure to biological threat agents carries a significant risk of adverse effects, there is no way to foresee whether someone will be exposed to these dangers. The magnitude of bio-attack depends on how unexpected and malicious minds worked behind such attack. As a result, it is impossible to calculate "risk" in relation to BT precisely. However, in order to lessen the impact of an attack, medical practitioners must be aware of the diagnostic and therapeutic approaches to illnesses brought on by agents of BT due to the possibility of catastrophic sequelae. Any non-state agent who employs or intends to employ biological agents in support of a political, religious, ecological, or other ideological cause without regard for the cause's moral or political justice is considered a bioterrorism [2]. This may be carried out by someone acting in self-interest or as retaliation, but it may also be supported by a government as part of a political agenda. Due to their invisibility and seeming weightlessness, these weapons are impossible to detect and verify. This is a reality that biological warfare plays out on future battlefields, national security decision-makers, defense professionals, and security personnel will have to prepared to counter that (Schneider and Grintner, 1995). Smallpox and anthrax illnesses were once exploited as a source of bioterrorism in the West, and anthrax is still a very popular agent for bioterrorism. The agents used in this form of terrorism are the most accessible, do not produce sound or blast (unless they involve chemicals), may traverse international borders, and are most common because they can exterminate the entire population [35]. The term "bioterrorism" is defined in three major categories. The categories (A, B, and C) are determined by the threat level, which ranges from greatest to moderate to gradual. The disease-causing agents in Category A are those that can be quickly released and can transmit from one person to another. Because people can get sick and not attend to work, school, or conduct business, which eventually has a negative social impact, they can become a public health issue. Examples include viruses like filoviruses (cause Ebola) and toxins like *Clostridium botulinus* (causes botulism), as well as bacteria like *Yersinia pestis* (causes the plague) and *Bacillus anthracis* (causes anthrax). The agents in category B impact some persons but are not fatal and are only moderately easy to spread like alpha viruses (which cause encephalitis) and the bacterial species Salmonella (which contaminates food). The viruses in Category C are those that are being genetically modified to have an effect in the future and have the potential to cause a significant number of deaths, *Naegleria* and *Nipah* viruses, taken as an example [1]. In the following sections of this chapter the various aspects of bioterrorism will be discussed.

**II.** **History of Bioterrorism**

Modern Bioterrorism has been referred to as the oldest form of terrorism ever committed. However, science was advancing and new strategies were being developed to quickly conquer the planet and vanquish the enemy. Therefore, the simplest strategy was to disseminate diseases that could weaken the adversary. Most people think that some sailor from an opposing nation brought the plague to Europe, starting the plague epidemic there. Later, when bioterrorism took on the form of chemical warfare, it was frequently used during world wars. The people used to be poisoned with food, viruses were disseminated through prostitution, mustard gas was used to blind the adversary, and poison was sprayed into freshwater supplies to kill the target population.

In the fourteenth century, plague-infected human corpses were hurled into the besieged city of Kaffa in the Black Sea (biowarfare). In the fifteenth century, the conquistador Pizarro accidentally introduced smallpox into a naive population in South America and increased the infection rate by exposing the local population to infected clothing. In the 1930s, China conducted experiments with anthrax that proved fatal on humans and then examined the autopsies, The death rates and pathogenicity of some of the most frequently thought agents that are: anthrax, plague, and smallpox are widely documented. In 1984 United State of America, 751 people were harmed by the *Salmonella typhimurium* contamination of salad bars which was apparently done for local political reasons.

During the course of first Gulf War of 1991, it became clear that some nation had created and stored enormous amounts of *aflatoxin, botulinum toxin*, and anthrax spores. Later USA and European research teams searched for weapon of Mass Destruction (WMDs) but never discovered them; it is assumed that they were destroyed on the spot.

The growth of microbiology in the early 20th century provided the scientific foundation for the development of biological weapons, and some governments, or so-called state actors, started to create program as part of their combat arsenal. For instance, Germany established a biological weapons program during World War I and carried out unproven attacks against animals (such as horses, mules, lambs, and cattle) being transported to the allies by neutral nations. During the First World War, chemical weapons (asphyxiating, poisonous or other gases) had been used by both alliances and the outcomes were horrible for mankind. The Geneva Protocol prohibited the use of these bacteriological methods of warfare. This agreement was created and signed in 1925 under the auspices of the League of Nations, and it became effective in 1928. As of March 15, 2013, 137 State Parties had ratified, acceded to, or succeeded to implement the protocol, which prohibited the use of biological weapons. Additionally, there was no provision for verification, and compliance was optional. In Japan 1995, during the investigation of the *Aum Shinrikyo* cult's attack on the Tokyo underground system, it has been found that (neurotoxin sarin gas) attacker visited Zaire to get Ebola virus strain for BT purposes. Further, they were also found unsuccessful in spread of *Clostridium botulinum* toxin.

 In the USA in 1996, food was tainted with *Shigella dysenteriae* in the staff lavatory of a laboratory. Twelve people were sick, but no one died. Later in 2001, 22 cases were reported about 10 gm of *anthrax* spores supplied through mail by an unidentified person. News media and government employees were the targets. *Anthrax meningitis* affected the index case, twenty of the patients worked in the mailroom, and one of them likely unintentionally contracted an infection from indirect letter-to-letter communication [3].

**Table: 1 Recorded History of Bioterrorism: -**

|  |  |
| --- | --- |
| **Year** | **Incident** |
| 190 BCE | Threatening enemy ships with poisonous snakes, Hannibal hurls |
| 400 BCE | Scythian archers employ arrows that have been covered with blood, waste products, or dead bodies. |
| 1346  | Mongols toss plague-infected bodies over their adversaries' defenses. |
| 1405 | French soldiers are served wine that has been contaminated by leprosy patients by the Spanish. |
| 1650 | Hollow shell casings are filled with the saliva of crazed dogs by Polish soldiers. |
| 1710 | Russian invaders in Estonia throw dead victims with the plague over their defenses. |
| 1763 | In Pennsylvania, British officers distribute smallpox victims' blankets to American Indians, sparking a terrible smallpox epidemic. |
| 1860s | War attempt to ship garments and bedding used byyellow fever victims to New YorkDuring the American Civil War, Confederate supporters attempt to transfer clothing and bedding used by yellow fever victims to New York. |
| 1863 | In order to contaminate the water supply for Union forces during the American Civil War, Confederate soldiers placed dead animals in wells and ponds. |
| 1972 | Ratification of Biological and Toxin Weapons Convention prohibiting offensive bioweapons |
| 1992 | Debriefing reveals a sizable bioweapons program as Dr. Ken Alibek flees the former USSR. |
| 1984 | 750 individuals became ill when ten restaurants in Oregon had tainted salad bars. |
| 2001 | Attacks by Bacillus anthracis occur through the US Postal Service |

**Table: 2 List of Epidemics of the world:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diseases** | **Year** | **Origin/Country** | **Reported Causalities** | **References**  |
| Black Death Pandemic | 13-15 century | Europe | 25 million reported deaths | [36] |
| Small Pox Virus | 1600s | North America | 400,000 Causalities | [37] |
| Severe Acute respiratory syndrome (SARS) | 2002-2003 | Hong-Kong/China | 298 reported | [38] |
| Avian Influenzas | 2006 | Azerbaijan, Cambodia, China, Egypt, Djibouti, Iraq, Thailand | 150 million affected birds resulted in 335 confirmed cases | [39] |
| Equire | 2007 |  |  |  |
| Swine Flu | 2009/2014 | Worldwide | 203,000 deaths | [40] |
| Tuberculosis | 2016 | Worldwide | 10.4 million affected people | [41] |

**III. Agents of Bioterrorism**

Biological agents are living things or the toxins they produced by living things. These agents can be employed to harm humans, animals, or crops. Chemical agents, on the other hand, are man-made materials that are lethal or incapacitating poisons. The Centers for Disease Control and Prevention (CDC) have published a list of probable bioterrorist agents with a high probability. These have been ranked in order of priority based on their ease of dissemination, transmissibility, mortality, ability to have a significant impact on public health, likelihood of inciting public anxiety and causing social unrest, and need for specific preparation for public health.  Clinical latency is a feature of BT agents that may make it difficult to detect transmission during this time. Current biodefense tactics have been influenced by the US Centers for Disease Control and Prevention's (CDC) classification of BT agents [2].

A biological agent is one that is easily created, stored, and highly contagious. They ought to be reliable and appropriate for outdoor use. They should be able to create a disease against which the target population has only little immunity. The majority of biological warfare agents are colorless, odourless, or toxin-producing microbes that travel through the air as aerosols or through food or drink. The Two main categories of microorganisms from which a biological warfare agent is most likely to be derived are as follows.

 **a. Pathogens:** Microorganisms that cause disease in the natural world are known as pathogens. Numerous pathogens exist, such as bacteria, viruses, fungi, and parasites. The pathogens *Bacillus anthracis*, which causes anthrax, and *Yersinia pestis*, which causes plague, are among those frequently considered as potential biological agents. Pathogens reproduce on their own since they are living things. Even a small amount of exposure to an organism can cause serious symptoms or even death. The ID50 for pneumonic plague is therefore thought to be less than 100 *Y. pestis* organisms, whereas 8–10,000 *B. anthracis* spores induce inhalation anthrax. Only a selected few virus can spread from person to person. For example, a person with pneumonic plague can distribute *Y. pestis* germs to others, posing a major threat to the spread of an epidemic. Bubonic plague, in contrast, is typically only contagious if someone is exposed to pus from an infected individual. On the other hand, those who are exposed to the released B*. anthracis* spores are likely to get anthrax, which is not contagious. Before signs of infection arise, pathogens require an incubation time. The incubation period can range from a few days for some diseases to many weeks for others. The incubation period for Q fever (produced by the *Coxiella burnetii* organism) is two to three weeks, depending on the magnitude of the dose, as opposed to the typical 3-5 days for inhalation of anthrax [4]. Following are some important classes of pathogens with the potentiality to be an agent of bioterrorism.

**Table: 3 Description of Pathogens abused in Biowarfare:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pathogens** | **Description** | **Biological Agents** | **Fatality rate (%)** | **Reference** |
| Virus | A virus is a microscopic infectious agent that can only replicate inside the live cells of other species. All kinds of life, including bacteria, as well as animals, plants, and microbes, can be infected by viruses. Since viruses are not cells, they lack the normal structures found in cells. Since viruses are immune to antibiotics, there is no specific treatment for them. | Variola major | 30% | [44] |
| *Filoviridae* | 90% | [43] |
| *Arenaviridae* | 15-30% | [45] |
| Bacteria | Bacteria are microscopic, free-living, unicellular prokaryotic organisms. Which are easy to reproduce in the lab. They differ from the cells of other species because they have simple, non-membrane-enclosed nuclei. Under poor circumstances, some bacteria convert into spores, which are inactive microorganisms. When suitable or favorable conditions exist, the spores become active. As a result of the bacteria's ability to endure adverse environments for extended periods of time even years it is thought to be a defense mechanism. | *Bacillus anthracis* | Cutaneous:<1%Respiratory: 75%Gastrointestinal: 25% - 60% | [44] |
| *Clostridum botulium* | Foodborne: 3-5%Wound and Intestinal:15% | [47] |
| *Yersinia pestis* | 8-10 % |  [47] |
| *Francisella tularensis* | Subspecies tularensis:2%Subspecies holarctica: fatal cases are rare |  [46] [47] |
| Fungi | A fungus (plural fungi) is any organism that belongs to the class of microorganisms, including yeasts and moulds. Most fungi can exist as resistant spores or in a yeast-like form. Mycotoxins, which are produced by fungi, are significant BW agents | *Aflatoxins**Aspergillus flavus* or *Aspergillus parasiticus trichothecene* |  | [48][49] |
| Rickettsia | These are primarily parasites of insects, and they affect people and other animals as a secondary effect. The intestinal cell lining and other tissues of insects typically contain the bacteria. They are present in a specific subset of blood-sucking insects, including ticks, mites, lice, and fleas. Some rickettsia can produce serious illnesses that are frequently fatal. They respond well to antimicrobial therapy. Like viruses, they can only grow within live cells. |  |  |  |

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 **b. Biological Toxins:** Technically, toxins are chemicals, but because they come from living things, biotoxins are frequently referred to as biological weapons. Several poisons are on the CDC's list of possible bioterrorism agents. In addition, a number of additional poisons have the potential to be utilized as bioterrorist agents.

Toxins can be employed as bioweapons in the past by poisoning food supplies or by dispersing them as aerosols. The poison needs to be between one and three microns thick for the best aerosolization in order to be used as an airborne agent. Toxins are dangerous compounds created by living things. Among the most well-known are ricin, which is derived from the castor bean plant's seed, and botulinum toxin, which is created by the bacteria *Clostridium botulinum*. Toxins, unlike infections, do not self-replicate, therefore the agent released is the only cause of their physical consequences. Toxins and chemical agents have many similarities, but there are also some key differences. The most dangerous chemical agents are not the only toxins that are toxic. Injection-based botulinum toxin has a lethal dose (LD50) of 0.001 micrograms per kilogram of body weight. The LD50 for VX (VX is an extremely toxic synthetic chemical compound in organophosphorus category specially, a thiophosphonate), possibly the deadliest chemical agent, is 15 micrograms per kilogram of body weight [28]. Contrary to many chemical agents, toxins are not volatile; therefore, they do not by nature create a chronic hazard. In general, toxins are not dermally active, which means that just coming into touch with the skin is not enough to cause illness. The substance must instead enter the body by a hole in the skin, consumption, or inhalation [9].

**c.**  **Mycotoxins:** Fungi can produce hundreds of different types of toxins, the most dangerous of which being *trichothecenes* and *aflatoxins*. *Aflatoxin* contamination of harvested food is frequently caused by *Aspergillus flavus* or *Aspergillus parasiticus*. Aflatoxin-bound DNA and cellular proteins are damaged. Numerous funguses, such as *Fusarium, Stachybotrys, Trichoderma, Myrothecium,* and *Cephalosporium*, create *trichothecenes*. T2, a *trichothecene,* and *deoxynivalenol*, a vomitoxin, are the two most likely trichothecenes to be utilized as bioweapons. T2 irritates or pain the skin, can be ingested or absorbed through the skin, and when it enters the bloodstream, it binds to peptidyl transferase, which stops protein synthesis. Monoamine oxidase and DNA polymerase are also affected by it. The GI tract, bone marrow, and skin are the first organs to be damaged, along with any tissues where coagulation and the Krebs cycle-related proteins are impaired. Serotonin, epinephrine, and norepinephrine breakdown are also hindered. T2 appears as a yellow droplet. If exposure occurs, decontamination steps include removing jewellery and clothing, washing the skin with soap and water, and isolating any contaminated clothing [4, 9].

 **d. Toxicants: -** Any poisonous material, whether created or found naturally, is referred to as a toxicant. Toxicants are harmful chemicals causing diseases, injury, birth defects or death in living organism. E.g. DDT, Mercury, Snake venom etc. They are generally synthesized in laboratory. A toxin, on the other hand, is a poison that an organism naturally produces.  system level impacts that a population exposed to a chronic or acute dose of toxicant exhibits. The analytical analysis is performed on a three-dimensional model of a toxicant and a population that includes state variables such as population biomass, toxicant concentration in an organism, and toxicant concentration in the environment connected by a linear dose-response function. [42]

**Fig.1: Classification of Toxin**

**IV. Characteristics of Ideal Bioterrorism**

Microorganisms are frequently used as agents of bioterrorism. Some of these organisms are considered non-persistent because they are so sensitive to changes in temperature, humidity, and sunshine. Some of these species can only develop and reproduce in the right environments. Some agents like anthrax, are persistent and have a high level of climate resistance. Most biological warfare agents are colorless, odorless bacteria or toxins made of microbes that travel through the air as aerosols or in food or drink. The following are some significant and fundamental traits of BW agents: (i) Capability of the agent to be easily disseminated, (ii) Ability to be transmitted from person to person, (iii) Potential to cause significant morbidity and mortality, (iv) Capacity to cause public panic and social disruption.

 **a. Infectivity: -** The capacity of germs to spread disease is referred to as their infectivity. Fewer microorganisms are needed when the infectivity is higher. It refers to how quickly the bacteria can penetrate the target's body.

 **b. Virulence:** Diseases of varying degrees of severity can be produced when a sufficient number of microorganisms penetrate the body. The most virulent strain has more immediate or severe effects and is more effective as a BW agent.

 **c. Less Incubation Period:** The incubation period is the length of time between the ingestion of enough infectious microorganisms into the body and the onset of illness symptoms. Typically, it lasts no less than 24 hours.

 **d. Transmissibility:** Disease caused by some microbes, such as the plague, may spread from person to person and result in an epidemic. Others microbes like Anthrax may have less transmission rate. The effectiveness of the BW agent is directly linked with its transmissibility.

 **e. Lethality:** If the target population lacks immunity, some microorganisms will cause diseases that are typically fatal (such as smallpox). Others will cause illnesses (like influenza) that are incapacitating rather than fatal [5].

**Fig:2 Characteristics of Ideal Bioterrorism**

**V. Mass Production of Bio-Agent**

Since financial investments are not as significant as those needed to produce chemical and nuclear weapons, the production of biological weapons has a higher cost-efficiency index. In contrast to the substantially higher numbers of the dead that arise from the employment of invisible and microgram payloads of biological agents, larger payloads of chemical and nuclear weapons cause fewer casualties. The application or delivery methods used for biological agents are significantly different from those used for chemical and nuclear weapons. Systems for transferring diseases between people and animals include the use of live vectors including insects, pests, and rodents as well as aerosol sprays of dried spores and infectious powders. Plant disease spreads among plants by using propagation tools like contaminated seeds, plant and root tissue culture materials, organic carriers like soil and compost dressing, and water from tainted garden reservoirs [6].

 **a. In Laboratory: -**Bioweapons have a conflict between their potential dual uses. A bioweapons production facility is just a standard, everyday microbiological laboratory on a smaller scale [6]. Variety of laboratory facilities, such as clinical laboratories, animal laboratories, plant laboratories, environmental laboratories, military laboratories, and forensic laboratories are required to respond to human, animal, and plant biothreat agents. Due to their pathogenic properties, the majorities of biothreat agents which are also select agents according to the CDC and Animal and Plant Health Inspection Service (APHIS) need different biosafety thresholds. There are a number of different laboratory levels, including BSL-2, BSL-3, BSL-4, as well as BSL-2 and BSL-3 animal facilities. Biosafety Level 4 standard (BSL-4) is required for agents like *Variola* major (smallpox) and viral hemorrhagic fevers (*Ebola*, *Marburg*, etc.). BSL-3 laboratories are needed for other agents as *Yersinia pestis*, *Fransicella tularensis*, and *Bacillus anthracis*. The most recent edition of the biosafety manual was released by the World Health Organization (WHO) in 2004 (WHO, 2004). The WHO continued to work after the publication of this document, and in 2006 they released the Bio risk Management: Laboratory Biosecurity Guidance (WHO, 2006). Both biosafety and biosecurity are combined in this manual. The Laboratory Bio risk Management Standard CWA 15793:2008 was issued by the European Committee for Standardization/Comité Européen de Normalization (CEN) in 2008. In order to guarantee that organizations are well-prepared to respond in the event that biological agents are discharged or go missing, the bio risk management standard offers guidelines to an organization on how to detect, monitor, and control laboratory biosafety and biosecurity [10].

 **b. Through Genetic Engineering: -** Genetic engineering techniques could be purposefully used to produce vaccine-resistant strains for terrorist or military purposes. These genetically engineered pathogens can nullify the effect of vaccine and can cause to damage of unmeasurable magnitude. In conclusion, the inability to clearly distinguish between offensive and defensively focused research and development efforts regarding infectious diseases and toxins is the root cause of the dual-use dilemma. The development and improvement of infectious organisms as bioweapons is increasingly susceptible to misuse of genetic engineering and information. Such misuse might result in the emergence of bacteria that are resistant to antibiotics and increased invasiveness and pathogenicity in commensals [6]. Antibiotic-resistant strains of bacteria could be used in a bioweapons attack to start the occurrence and spread of communicable illnesses like anthrax and plague on either an endemic or epidemic scale. Gene-designed organisms have the potential to develop a wide range of bioweapons, including:

* organisms functioning as microscopic factories producing a toxin, venom or bioregulator
* organisms with enhanced aerosol and environmental stability
* organisms resistant to antibiotics, routine vaccines, and therapeutics
* organisms with altered immunologic profiles that do not match known identification and diagnostic indices
* organisms that escape detection by antibody-based sensor systems

**VI. Classification of Bio-Agent**

The potential of bioweapons prioritized depending up on the Centers for Disease Control (CDC), Atlanta, USA, has divided to ease of dissemination, transmissibility, mortality, potential for major public health impact, potential to cause public fear and social disruption, and requirement of special action for public health preparedness [8]. Based up on these criteria the centers for disease control (CDC), Atlanta, USA has divided BT agents in to following these criteria (table-3).

 **a. Category A:** Microorganisms in this category include those BT agents which are a threat to national security due to their ease of dissemi8nation or person-to-person transmission, high mortality rate, potential for significant public health impact, potential for widespread panic and social unrest, and need for special public health preparedness measures.

 **b. Category B:** The BT agents that are fairly simple to spread, have moderate morbidity and low fatality, and necessitate specialized improvements to the CDC's diagnostic capabilities and expanded disease surveillance are among the second-highest priority agents.

 **c. Category C:** A further Category C list has been compiled by the CDC Emerging pathogens are the third-highest priority agents because of their availability, ease of production and spread, potential for high morbidity and mortality, and significant adverse health effects [8].

**Table: 3 Categoriesof BT Agents As Per CDC Classification**

|  |  |  |  |
| --- | --- | --- | --- |
| **Category** | **A Agents:** | **B Agents** | **C Agents:** |
| **Level** | Highest | Moderate | Gradual (future use) |
| **Impact** | Large Population | Small Population | Potential to affect Large Population |
| **Examples** | AnthraxBotulismPlagueSmall PoxTularemiaViral hemorrhagic fevers (*filoviruses* e.g., *Ebola, Marburg*) and *Arenaviruses* e.g., Lassa, Machupo | *Brucella* speciesFood Safety Threat (*Salmonella* species, *E Coli* O157:H7, *Shigella dysenteriae*)*Burkholderia pseudomallei*Q fever – *Coxiella bumetii*Viral encephalitis (alphaviruses e.g., Venezuelan, encephalitis)*Staphylococcus* enterotoxin BTyphus fever (Rickettsia Prowazekii) Ricin Toxin from Ricinus communis (castor beans) | *Nipah virus* and *Hantavirus**Flavi viruses**Mycobacterium tuberculosis* |

The medical literature frequently offers scant data on which to base clinical judgments because many diseases have been completely eradicated and are extremely uncommon in the industrialized world. As a result, consensus-based guidelines have been developed by public health professionals to assist clinicians in making such decisions. As constantly updated sources of such material, the CDC's (www.bt.cdc.gov), the AHRQ's (www.bioterrorism-uab.ahrq.gov), and a number of other institutes (such as www.bioterrorism.uab.edu) bioterrorism websites are beneficial.

**VII. Identification of Bioagent**

 **a. Through Symptoms by Medical officer: -** Local medical officers and pathologists are the first responding officer in the case of bioterrorism. Medical personnel in association with local authorities and public information systems have to aware the general public about the any doubtful scenario regarding sudden unexpected outbreak of particular disease. In current scenario it is of the utmost importance that medical personnel should must have training in handling such scenario. With the aid of a hospital-based command center, local authorities, medical personnel, hospital management, and the public information officer can all work together to plan the proper release of information to the public. Through this coordination, public health organizations can help with triage, supplies, and diagnosis while law enforcement can help with crowd control and isolation or quarantine [11].

 **b. Serological Techniques: -** Biological products which disrupt metabolism and whose antigenic structure has been specially genetically changed to evade detection by antibody-based detection methods, generic and polyvalent immunosensors have been developed. Other bio-detection devices that serve as early warning/alert systems include laser eyes and electronic noses with built-in alarms that can detect biological particle concentrations [12,13].Such systems place more emphasis on early warning capabilities, which are an effective tool in thwarting the threat of bioterrorism in regular, peaceful situations, rather than on the identity of the biological agent.[14] Depending on the agent, several approaches has been developed for the laboratory diagnosis of BT agents. The gold-standard diagnostic assay for the majority of bacterial agents is still standard culture. Alternative techniques include modified light microscopy staining, motility testing, gamma phage lysis, capsule production staining, hemolysis, wet mounts, staining for spores, slide agglutination, direct fluorescent antibody, enzyme-linked immunosorbent assay (ELISA), and rapid immunochromatography. Standard tests for viral agents include ELISA, plaque reduction neutralization, hemagglutination inhibition, neuraminidase activity, tissue culture, growth in eggs, direct and indirect immunofluorescence, immunodiffusion in agar, electron microscopy, modified staining, and light microscopy. Immunohistochemistry and pathologic evaluation of tissues are crucial in the diagnosis of BT agents [2].

 **c. Molecular Analysis: -** In comparison to serologic assays, the sensitivities and specificities of molecular assays are nearly 100%, making them the new gold standard for BT detection. These assays use target nucleic acid isolation and amplification, followed by pathogen identification to identify infectious agents in humans. Different BT agents have been detected multiplexed using a variety of technologies and techniques. Although they are currently in the research and development stages, DNA micro fluidic platforms will probably become widely utilized diagnostic platforms in the future. These approaches can theoretically be used to unprocessed materials in field settings. Since, they are sensitive and specific. These techniques eliminate the need for time-consuming microbe isolation processes. However, a number of issues, such as sample issues, data processing, development of particular probes, quality control, cost containment, automation, performance, and integration, must be resolved before such approaches may replace the conventional ones [2].

**VIII. Method Involves in Bioterrorism**

When an agent is not contagious, as is the case with many infections and poisons, a dissemination mechanism must be used to transmit the agent to the intended target. While it is conceivable to infect people by injecting them one at a time with biological pathogens, the majority of terrorists are unlikely to find this strategy appealing. A terrorist will more likely look for a method to infect the entire target at once, whether it is people, cattle, or crops. Following are the prominent means through which BT agent may be dispersed.

 **a. Through Aerosol dissemination:** The potential that a terrorist could release biological agents as an aerosol cloud is the biggest concern. The aerosol cloud for biological warfare should have particles that are 1 to 5 microns (one-millionth of a meter) in size. Since the upper respiratory tract filters out particles larger than 5 microns, they do not enter the lungs. Additionally, they frequently descend from the air rather swiftly. Smaller particles, on the other hand, are usually exhaled and cannot remain in the lungs [15]. Concern about aerosol delivery is due to a number of factors. It is unsafe to get many infections in this way. Thus, even if antibiotic treatment is very efficient, cutaneous anthrax, which is contracted through the skin, has a case fatality rate of 5 to 20%. In contrast, anthrax that is inhaled frequently results in death and cannot be treated if discovered quickly. Similar to this, *Y. pestis* is the cause of a variety of diseases, including bubonic and pneumonic plagues. Bubonic plague, which is typically spread by flea bites, has a case fatality rate of 50 to 60 percent if left untreated, but it typically responds to medical care. Untreated pneumonic plague typically results in death. To save individuals who are infected, early treatment is crucial. All of the agents on the Category A list have the potential to spread through a fine particle aerosol with particles between 1 and 5 mm in size. This can be absorbed and transported to the alveoli without filtration or capture since it is invisible and small enough to do so.[16] The agents are dispersed via a variety of strategies, such as:

* Paint-sprayers
* Fogging machines that are used to disseminate insecticides
* Hand-held perfume atomisers
* Hand-held drug delivery devices (like asthma inhalers)
* Airplanes, as for crop-dusting

 **b. Through Water contamination:** Water-borne organisms like *Vibrio cholerae*, which causes cholera, and *Salmonella typhi*, which causes typhoid fever, have a considerable negative influence on human life. Additionally, harmful materials, including poisons, can be injected into water systems. It follows that some terrorist organizations interested in biological weapons have attacked urban water systems. Water systems are, fortunately, less susceptible than is frequently believed [17]. Municipal water systems are made to get rid of contaminants, particularly pathogens. Communities utilize filters as part of this procedure to remove particulates from the water and chlorine to kill any remaining organisms. Furthermore, the ID50 for infections transmitted through water is frequently very high. According to one experiment, only 50 percent of those exposed to 107 (10 million) *S. typhi* organisms felt ill [18]. It would take "trainloads" of botulinum toxin to contaminate the water system in New York City, according to a Department of Defense biological warfare analyst, just because of how much the toxin is diluted. For all of these reasons, it is challenging to intentionally contaminate water supplies in order to infect a large population [19].

 **c. Through Food contamination:** Biological agents have also been distributed by terrorists through food contamination. Only raw or incorrectly stored food is typically at risk because the heat from cooking easily kills the majority of bacteria and poisons. This suggests that a terrorist would have to target common uncooked foods or those that can become tainted after being cooked. The terrorists would instead need to use a poison that can withstand cooking. Due to fundamental adjustments made to food delivery systems, the risks associated with purposeful food contamination have most likely increased. Due to the centralized nature of the food processing business, contamination introduced at a single site can have a significant impact on a broad population. Additionally, as more food is imported, there is a greater chance that criminals acting out of state could taint food consumed in the United States [20].

 **d. Direct application:** Injecting a subject with the pathogens that cause a disease is the most effective technique to infect them. The majority of the technical issues related to the spread of biological agents are avoided by this method. Toxins can be employed in similar ways. Furthermore, some poisons might hurt even when administered to the skin.

 **e. Insect vectors:** Insects naturally spread a lot of diseases. For instance, the *Aedes aegypti* mosquito, which also carries yellow fever, transmits plague, whereas *Pediculus humanus corporis*, a type of body louse, spreads typhus. Thus, it is not unexpected that experts in biological warfare have thought of insects as potential biological weapon vectors. The Japanese biological warfare program put a lot of work into this channel of distribution. The Japanese are known to have employed plague-infected fleas to spread the disease on at least a few times. The US stated using mosquitoes to spread certain substances and set up a laboratory to breed the necessary mosquitoes. Problems with insect vectors are difficulty in control and the possibility that their usage would result in the establishment of disease reservoirs in the area where the insects were discharged [21].

**Table:4 Mode of administration of Biological Agents:**

|  |
| --- |
| 1. **Dispersal Systems for inhalational exposure**
 |
| a. Mail/Packagesb. Commercially available spray device/ crop dusterc. Fire extinguisherd. Smoke generatorse. Air conditioning systemsf. Street air freshers |
| 1. **Contamination of food or water supplies**
 |
| a. Individual consumption itemsb. Food chain contamination |
| 1. **Injection**
 |
| a. Contaminated needlesb. Projectiles/Contaminated shrapnel |
| 1. **Direct contact by infected persons/animals**
 |
| 1. **Military unitions**
 |

**IX. Preventive Method**

 **a. Build Manpower: -** The hospital staff should be anticipated to experience high levels of persistent physical and psychological stress when dealing with bioterrorism or a widespread infectious disease outbreak. Employees will be exposed to infectious pathogens while working long hours under added stress. Healthcare personnel may become unwell in these conditions, as was the case during the Covid outbreak in 2020 [8]. Staff members and doctors may feel more confident in their ability to maintain personal safety while carrying out their professional responsibilities with the help of appropriate planning and education activities. Hospitals must deal with equipment and supply challenges in addition to potential staffing problems. Even a modest number of extra patients who need to be isolated or given mechanical ventilation could be too much for most hospitals to handle. Medication, cleaning supplies, disposable medical equipment, and other protective gear may be quickly used up. Although the Strategic National Stockpile, under the CDC's management, has ready access to "push packs" of medications and other supplies for bioterrorism, it is crucial that hospitals assess their requirements for additional supplies and equipment from other sources in order to maintain the necessary level of preparedness for mass casualties. A supply of community-based supplies for catastrophes is available to urban hospitals through interactions with the Metropolitan Medical Response System and their local or state Emergency Management Association. It is crucial to establish early contact with local, regional, state, and federal government and public health agencies [8].Hospital staff must collaborate with public health and public safety to preserve and maintain a chain of evidence in order to prove causality and identify offenders. Therefore, identifying covert leaks is a critical task for health experts. New, emerging, or re-emerging illnesses may potentially be the cause of previously unknown symptoms. New infections would be ones that were either previously unknown or known in animals but not to impact humans (it is likely that this is how all significant human infections first appeared, via transmission from animal to man). Infections that have been previously described but had low rates of natural occurrence and incidence are now spreading more widely. Re-emerging illnesses were once thought to be extinct or to have a low enough natural incidence to not constitute a risk to humans, but it has now been discovered that they are occurring more regularly. These descriptions and definitions are provided to show how public health authorities are preparing both themselves and the larger network of medical and paramedical staff [3].

 **b. Public Awareness: -** There has been an increase in public awareness of the bioterrorism concern in the USA [22]. The coordination of on-site care for the injured and sick, immediate decontamination of the affected area, identification of the type and character of the biological agent, and its immediate isolation and neutralization are the main objectives of national preparedness and emergency response. The emergence of bioterrorism as a top concern for international cooperation and concern is now reflected in the development of verification processes to prevent violations of the Biological and Toxin Weapons Convention and in efforts to institutionalize a desired and critically needed state of preparedness. International conferences and seminars emphasize the Convention on Biological Weapons and the peaceful application of biotechnology. Additional procedures are in place to keep an eye on the creation and usage of bioweapons [6].

 **c. Proper Surveillance: -** There is a surveillance system in place before laboratory work starts, which entails gathering information through surveys or observation and afterwards relaying it to the laboratories. Given that bioterrorism is a public health issue, information should be gathered from local clinic and hospital reports in order to monitor and manage the problem. This type of surveillance system is also known as conventional or traditional surveillance; however, modern surveillance systems, which include nationwide automated surveillance for disease-related syndromes and analysis of routinely collected clinical, administrative, pharmacy, and laboratory data, have also been adopted by many countries around the world to counter bioterrorism [23]. The expense, social obstacles, and environmental ethics are other crucial factors that should be taken into account when conducting surveillance. Therefore, it makes no difference which sort of monitoring is used because it is determined by the political, social, and economic circumstances of the nation. As it affects the entire population, there must also be a system of effective management and a chain of command that is overseen and regulated by the government [11,24].

**R & D**

**R & D**

**R & D**

**R & D**

**As per WHO Guidelines**

**Role of Media**

**Fig:3 Preventive Measures Against Bioterrorism**

 **d. Research and Development Activities: -** The development of relevant techniques will be aided by collaborative R&D efforts between first responders, forensic institutions, and public health officials. However, this requires infrastructure for laboratories and strategic planning. Much R&D work is done in a particular industry, including public health, animal health, food safety, or law enforcement. Joint diagnostic techniques have been developed in recent years to combat bioterrorism. However, a lot of research has been conducted without asking the various diagnostic end-users at the local, regional, or national level. R&D activities have involved a wide range of techniques, including electron microscopy [25], novel molecular techniques [26], automated testing and screening [27], immunoassays for toxins (2008, microarray and multiplexing), and nanotechnology methods [28]. Many diagnostic techniques are still dependent on immunoassays, ELISA, and PCR, due to its high specificity and sensitivity [29].

**X. Conclusion**

Under the false pretense of natural disease outbreaks, biological warfare can be practiced with impunity and be used to obliterate economically important crops and livestock as well as to systematically eradicate human populations. Bioterrorism may or may not be state-sponsored but it is always against a nation to disturb the economic development of that particular nation. The development of biosensors with specialized antibodies to identify respiratory pathogens that are likely to be disseminated by aerosols and air-cooling systems is the current focus of research on bioweapon defense. Additionally, contract research focuses on the applications of biotechnology to clean environmental areas that have been contaminated by radioactive materials, heavy metals, herbicides, pesticides, and other toxic wastes. Recent advancements in the field of bioinformatics have facilitated the genetic screening of human disorders and drug discovery [30].The "Sherlock Holmes' dog that doesn't bark" theory, states that the sensor's silence signals the existence of a biological agent, reflecting the goal of such research in designing more advanced sensors for the prompt identification and neutralization of biological weapons [31]. The rapidity of intervention by trained anti-terrorist personnel, which includes microbiologists, doctors, hospital staff, psychologists, military or law-enforcing forces, and public health personnel is essential for the efficient development of national preparedness and emerging responses to biological agents, in the case of bioterrorists attack. Due to the huge destructive potential of bioterrorism [32].It is of utmost importance to spendmoney on public health surveillance helps to improve domestic readiness for dealing with bioterrorism, emerging diseases, and foodborne infections.

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