BIOTERRORISM AND BIOLOGICAL WARFARE: AN EMERGING CHALLENGE

Abstract

The intentional use of pathogens and biological toxins, typically of microbial, plant, or animal origin to cause socio, medico, and economic harm to a nation is known as bioterrorism or biological warfare. Bioterrorist attack on a nation can be imparted by a terrorist organization or state sponsor organization of enemy country. This biological agent is easy to use and difficult to trace. Furthermore, new and widely available technologies encourage the development of such bioweapons which, have potential of mass destruction that's why it included in the category of weapon of mass destruction. Although, there is Biological and Toxin Weapons Convention which is for development of international consultation and cooperation to combat the threat of bioterrorism but developing preventative and protective methods are of utmost importance for any nation. Besides that, it is necessary to take confidence-building measures at international level which is inclusive of all the nations. This chapter is going to highlight the various aspects of bioterrorism and means through which it can be negotiated.

Keywords : Bioterrorist, Anthrax, Covid, Bioterrorism, Biological agents, Bioweapons

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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. The bioterrorism can be spread widely through various medium such as soil, water, air and food. Since there is an incubation period involve with the micro organism of harmful effect so it is really cumbersome to trace the actual time of attack. Biological agents used for bioterrorism encompasses a broad range of living organisms (bacteria, viruses, and diseases), as well as chemical toxins, which are used to spread anxiety, danger, risk, and threat among the population of targeted country [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. Additionally, as bioterrorist attack resembles natural epidemic it is really difficult to claim it as a deliberate attack. 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 trained in such a way that they may able to differentiate between normal illness and BT attack. The medical professional must be efficient in handling the diagnostic and therapeutic pressure brought on by agents of BT and thus avoid the possible of catastrophic consequences. 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 requital, but it may also be supported by a government as part of a political concern. Due to their invisibility and seeming weightlessness, these weapons are impossible to detect and verify. Therefore, National security legislators, defense experts, and security personnel will need to be ready to combat this reality if biological warfare occurs on future battlefields (Schneider and Grintner, 1995). Smallpox and anthrax illnesses were once exploited as a source of bioterrorism in the west. The anthrax is still a very popular agent for biological terrorism. The advantage of bioweapons over traditional weapon is that bioweapons are the most accessible, do not produce any noise or shockwaves (unless they involve chemicals), may traverse international borders, and are most lethal because they can exterminate the entire population [35]. The term "bioterrorism" is defined in three major categories by Centers for Disease Control and Prevention's (CDC). These 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 which 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 causes the developmental halt to target nation. BT agents may be any pathogens including 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 category B agents have some effects on people but are not lethal and propagate relatively slow compared to alpha viruses (which cause encephalitis) and the bacterial species Salmonella (which contaminates food). The BT agents in Category C are those whose modifying genes will have an impact in the future and could potentially result in a large number of fatalities, *Naegleria* and *Nipah* viruses, taken as an example [1]. In the following section of this chapter the various aspects of bioterrorism will be discussed.

II. HISTORY OF BIOTERRORISM

History of Bioterrorism is as old as civilization. There are various historical account which indicated possible use of bioweapons (Table-1). 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. List of epidemic exposed around the world describe in Table: 2.

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, Conquistador Pizarro unintentionally spreaded smallpox to a naïve population in South America by exposing the locals to contaminated 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 1991, it became clear that the 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 programmes as part of their combat arsenal. For instance, Germany established a biological weapons programme 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 by yellow fever victims to New York During 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	European Country	25 million reported deaths	[36]
Small Pox Virus	1600s	North America (Western Hemisphere)	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]
Swine Flu	2009/2014	Worldwide	203,000 deaths	[40]
Tuberculosis	2016	Worldwide	10.4 million affected people	[41]
COVID-19	2020	First detected in China	276118561 reported Cases, 2248538 confirmed deaths	[52]

III. AGENTS OF BIOTERRORISM

Biological agents are either living like bacteria, virus or non-living like the toxins released for the pathogens. 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 categories of microorganisms from which a biological warfare agent is most likely to be derived are mentioned in Table-3.

1. **Pathogens:** Microorganisms that cause disease in the natural world are known as pathogens. Numerous pathogens exist, such as bacteria, viruses, fungi, and parasites. Among those usually thought of as potential biological agents are the pathogens *Yersinia pestis*, which causes the plague, and *Bacillus anthracis*, which causes anthrax. 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 selected few viruses 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 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 potentially to be an agent of bioterrorism.

Table: 3 Description of Pathogens abused in Biowarfare

Pathogens	Description	Biological Agents	Fatality rate (%)	References
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.	*Bacillus anthracis*	Through skin (Cutaneous):<1% Through lungs (Respiratory): 75% Through Gastrointestinal: 25% - 60%	[44]
		Clostridum botulium	Through Foodborne: 3-5% Wound and Intestinal:15%	[47]
		Yersinia pestis	8-10 %	[47]
		Francisella tularensis	Through Subspecies tularensis:2% Subspecies holarctica: fatal cases are rare	[46] [47]

	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.			
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]

2. **Biological Toxins:** Although, toxins are chemicals but biotoxins are commonly referred to as biological weapons because they originate from living organisms. There are several toxins which has been included in the CDC's list of potential BT agent (Table-4). Besides that, several poisons are on the CDC's list of possible BT 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 toxins are botulinum toxin, which is produced by the bacteria Clostridium botulinum, and ricin, which is generated from the seed of the castor bean plant. Toxins, unlike infectious microbes, do not self-replicate therefore, the toxin used as BT agent only causes single short halt due to its physical and chemical natures. Toxins and chemical agents have many similarities, but there are also some key differences in their lethal dose (LD50). Usually LD 50 of toxins is on significantly lower side in comparison to chemical poison. As for example, the lethal dose (LD50) of injection-based botulinum toxin is 0.001 micrograms per kilogram of body weight where as the lethal dose (LD50) for VX (very poisonous synthetic chemical compound belongs to the category of organ phosphorus, namely a thiophosphonate) is 15 micrograms per kilogram of body weight [28]. Contrary to many chemical agents, toxins are not volatile; therefore usually they do not causes 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. To be effective, the toxin must enter the body by any mean like skin wound, through ingestion or inhalation [9]. The general classification of Toxins are shown in Figure 1.

3. **Mycotoxins:** Fungi can produce hundreds of different types of toxins, the most dangerous of which being *trichothecenes* and *aflatoxins*. *Aflatoxin*. The contamination of harvested food is frequently caused by *Aspergillus parasiticus* or *Aspergillus flavus*. Aflatoxin-bound DNA and subsequently, cellular proteins become mutated. Numerous funguses, such as *Stachybotrys, Fusarium, Cephalosporium, Trichoderma,* and *Myrothecium*, are included in *trichothecenes.* The two most likely trichothecenes to be used as bioweapons are the trichothecene T2 and the vomitoxin deoxynivalenol. T2 causes irritation or pain in the skin, can be ingested or absorbed through the skin, and when it enters the bloodstream, it binds to peptidyltransferase, which interferes with protein synthesis. Monoamine oxidase and DNA polymerase are also affected by T2. The gastrointestinal tract, bone marrow, and skin are the first organs to be damaged, along with any tissues where coagulation and the Krebs cycle-related proteins become impaired. Serotonin, epinephrine, and norepinephrine pathways are also affected due to the presence of T2 in blood stream. T2 appears as a droplet of yellow color. Decontamination procedures after exposure include taking off jewellery and clothing, cleaning the skin with soap and water, and segregating any contaminated clothing [4, 9].

4. **Toxicants:** Toxicants are the chemically synthesized analogue of natural toxin (Fig: 1). Toxicants are harmful chemicals causing diseases, injury, birth defects or death in living organism. e.g. DDT, Mercury, Snake venom etc. In an organism a three-dimensional model comprised of population biomass, toxicant concentration, and dose-response function is examined for analytical investigation [42].

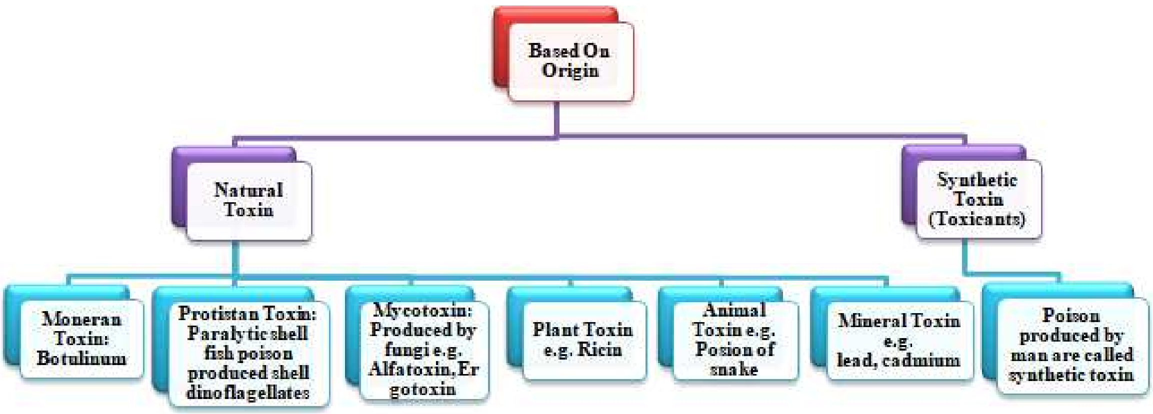

Figure 1: Classification of Toxin

IV. CHARACTERISTICS OF IDEAL BIOTERRORISM

Microorganisms constantly used as agents of BT. These organisms are considered non-persistent because they are so sensitive due to changes in temperature, humidity, and photosensitivity. Some of these species can develop and reproduce in the right environments. Agents like anthrax, are persistent and have a highly resistive of climate change. Most biological warfare agents are colorless, odorless like bacteria or toxins that reaches the body through food, water, and air as aerosols. The following are some significant traits of BW agents (Fig:2): (i) Spreads quickly among population, (ii) Ability of easy transmittance, (iii) Potential of increasing death and morbidity, (iv) Able to create panic situation.

1. **Infectivity:** The capacity of microbes to spread disease is referred to as their infectivity. Low number of microbes are required when the infectivity is higher. It refers to how quickly the pathogens can intrude into the target's body.

2. **Virulence:** When a sufficient number of microorganisms enter the body to cause illness its intensity depends on the increase in degree of severity. The most virulent strain has more immediate or severe effects and is more effective as a BT agent.

3. **Less Incubation Period:** The latency period is the length of time taken by an organism between causing infection and onset of illness symptoms. Typically, it lasts no less than 24 hours.

4. **Transmissibility:** Some bacteria can cause illness that can spread from person to person and trigger epidemics, like the plague. Some microorganisms, such as anthrax, may have less transmission rate. The effectiveness of the BT agent is directly linked with its transmissibility.

5. **Lethality:** If the target population has less 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].

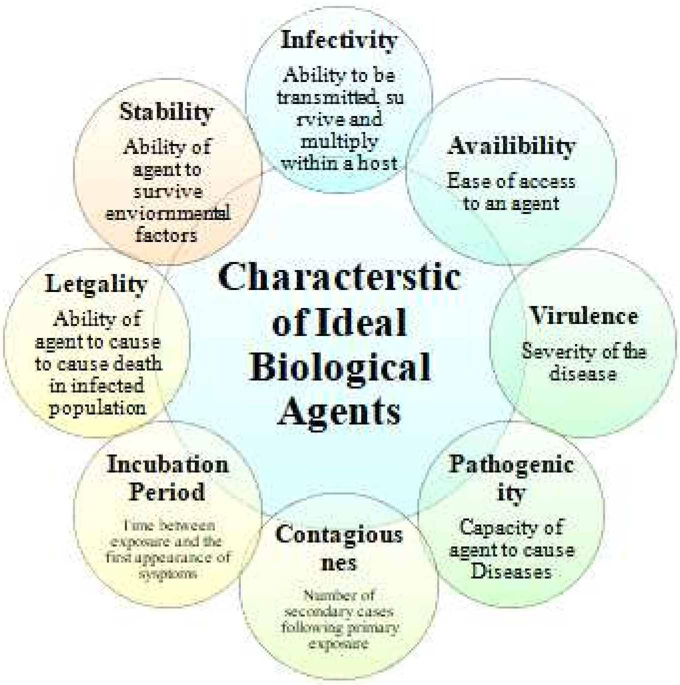

Figure 2: Characteristics of Ideal Bioterrorism

V. CLASSIFICATION OF BIO-AGENT

In accordance to the regulations set by Centers for Disease Control (CDC), Atlanta, USA, the potential of these bioweapons has been prioritized on the basis of their ease of dispersal, transmissibility, mortality, impact on public health, and capability to cause public fear and social disruption. The class of bioweapons also depend upon the need of special action for the protection of the public [8]. Based up on these criteria the Center for Disease Control (CDC), Atlanta, USA has divided BT agents into following these criteria (Table-4).

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

2. **Category B:** The second-highest priority agents are those that are relatively easy to spread, have a moderate morbidity and low fatality, and require for specialized enhancements to the CDC's diagnostic capabilities and increased disease surveillance.

3. **Category C:** The CDC has established another Category C list. Due to their accessibility, ease of production and dissemination, the potential for high morbidity and mortality, and major negative health effects, emerging pathogens are the third-highest priority agents [8].

Table: 4 Categories of 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	Anthrax Botulism Plague Small Pox Tularemia Viral hemorrhagic fevers (*filoviruses*e.g.,*Ebola, Marburg*) and *Arenaviruses*e.g., Lassa, Machupo	*Brucella* species Food 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 B Typhus fever (Rickettsia Prowazekii) Ricin Toxin from Ricinus communis (castor beans)	*Nipah virus* and *Hantavirus* *Flaviviruses* *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, (www.bt.cdc.gov) the CDC's, the AHRQ's (www.bioterrorism-uab.ahrq.gov), and a number of other institutes (such as www.bioterrorism.uab.edu) bioterrorism websites are beneficial.

VI. MASS PRODUCTION OF BIO-AGENT

Since financial investments are not as significant as those needed to produce chemical and nuclear weapons, the production of bioweapons are cost effective and with prolonged effect. 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 as well as 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].

1. **In Laboratory:** Bioweapons production laboratories are generally like standard, everyday microbiological laboratories on a smaller scale and hence difficult to locate or identify [6]. To combat human, animal, and plant biothreat agents, a variety of laboratory facilities, including medical labs, animal laboratories, plant laboratory settings, environmental testing facilities, military research centers, and forensic science laboratories are established which may be easily utilized as the site of mass production of BT agents. Due to their pathogenic properties, the mass production of majorities of biothreat agents which are also select agents according to the Animal and Plant Health Inspection Service (APHIS) and the CDC need different biosafety thresholds. Biosafety Level 4 (BSL -4) standard is required for handling 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. The bio risk management standard provides instructions to an organization on how to detect, monitor, and control laboratory biosafety and biosecurity in order to ensure that organizations are ready to respond in the event that biological agents are released or disappear [10].

2. **Through Genetic Engineering:** Genetic engineering techniques could be purposefully used to produce vaccine-resistant strains of pathogens for terrorist or military purposes. These genetically engineered pathogens can nullify the effect of vaccine and may cause 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 [6]. Antibiotic-resistant strains of bacteria could be used in a bioweapon attack to start the occurrence and spread of communicable illnesses like anthrax and plague on either an endemic or epidemic scale. The transgenic micro organisms have the potential to develop into a wide range of bioweapons, including: 1. organisms acting as micro factories to create toxins, venom, or bioregulators, 2. organisms with improved environmental and aerosol stability, 3. organisms that are resilient to medicinal products, common vaccinations, and antibiotics, 4. organisms with altered immunologic profiles that do not match known identification and diagnostic indices, 5. organisms that are undetectable by antibodies-based sensor systems.

VII. IDENTIFICATION OF BIOAGENT

1. **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 dutiful 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 centre, 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].

2. **Serological Techniques:** Generic and polyvalent immunosensors have been created to detect substances that disrupt metabolism and whose antigenic properties get altered through genetic modification which subsequently, prevents detection of this modified antigen by antibody-based detection methods. Laser eyes and electronic noses with built-in alarms that can detect biological particle concentrations are other advance biodetection tools that act as early warning/alert systems [12,1,3]. Instead of focusing on the identification of the biological pathogen, such systems emphasize greater attention on the improvement of early warning capabilities.[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. 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 are among the common methods used to detect viral agent. Immunohistochemistry and pathologic evaluation of tissues are crucial in the diagnosis of BT agents [2].

3. **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. To identify infectious agents in human beings, these tests isolate and amplify target nucleic acids before identifying the pathogens. 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 because they are sensitive and specific. These techniques eliminate the need for time-consuming microbe isolation processes. However, before such approaches can displace the conventional ones, a number of challenges, including sample issues, data processing, creation of specific probes, quality control, cost containment, automation, performance, and integration, must be taken care off [2].

VIII. METHOD INVOLVES IN BIOTERRORISM

When an agent is not contagious, in such cases many infections, 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 attractive. Following are the prominent means through which BT agent may be dispersed.

1. **Through Aerosol Dissemination:** The potential that a terrorist could release biological agents as thr form of 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 filter out particles larger than 5 microns, So they do not enter the lungs. 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 contacted through the skin, has a case fatality rate of 5 to 20%. 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, but it generally responds to medical care. Otherwise untreated pneumonic plague results in death. 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, Devices that mist the area to spread pesticides, Hand-held perfume atomizers, and Portable drug delivery systems, such as inhalers for asthma, Airplanes, as for crop-dusting.

2. **Through Water Contamination:** The safety and well-being of human life is significantly impacted by water-borne pathogens including *Vibrio cholerae*, which causes cholera, and *Salmonella typhi*, which causes typhoid fever. Additionally, harmful materials, including poisons, can be injected into water systems. Fortunately, water systems are less susceptible than typically believed [17]. Municipal water systems are designed to remove impurities, especially pathogens. Communities utilize filters as part of this procedure to remove particulates from the water and chlorine to kill any remaining organisms. So that LD50 for infections transmitted through water is frequently very high [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].

3. **Through Food Contamination:** Biological agents have also been spread 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. 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 different countries [20].

4. **Direct Application:** The pathogens that cause a disease is the most effective technique to infect the public by injecting microorganisms. 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. So, some poisons might hurt even when administered to the skin.

5. **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].

IX. PREVENTIVE METHOD

1. **Professionally Trained Manpower:** 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].

2. **Public Awareness:** The public's awareness of the biological warfare threat in the USA has increased [22]. The primary goals of national preparedness and emergency response are the coordination of on-site care for injured and ill people, rapid decontamination of the affected region, identification of the type and character of the biological agent, and quick isolation and neutralization. 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].

3. **Proper Surveillance:** There is a surveillance system in place before laboratory work starts, which entails gathering information through surveys or observation and afterward relaying it to the laboratories. Given that bioterrorism is a public health hazard, data from regional clinics and hospital reports should be acquired in order to track and manage the situation. This kind of surveillance system is also referred to as conventional or traditional surveillance; however, contemporary surveillance systems have also been adopted by many nations worldwide to combat bioterrorism. These systems include nationwide automated surveillance for disease-related syndromes and analysis of regularly collected clinical, administrative, pharmacy, and laboratory data. [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].

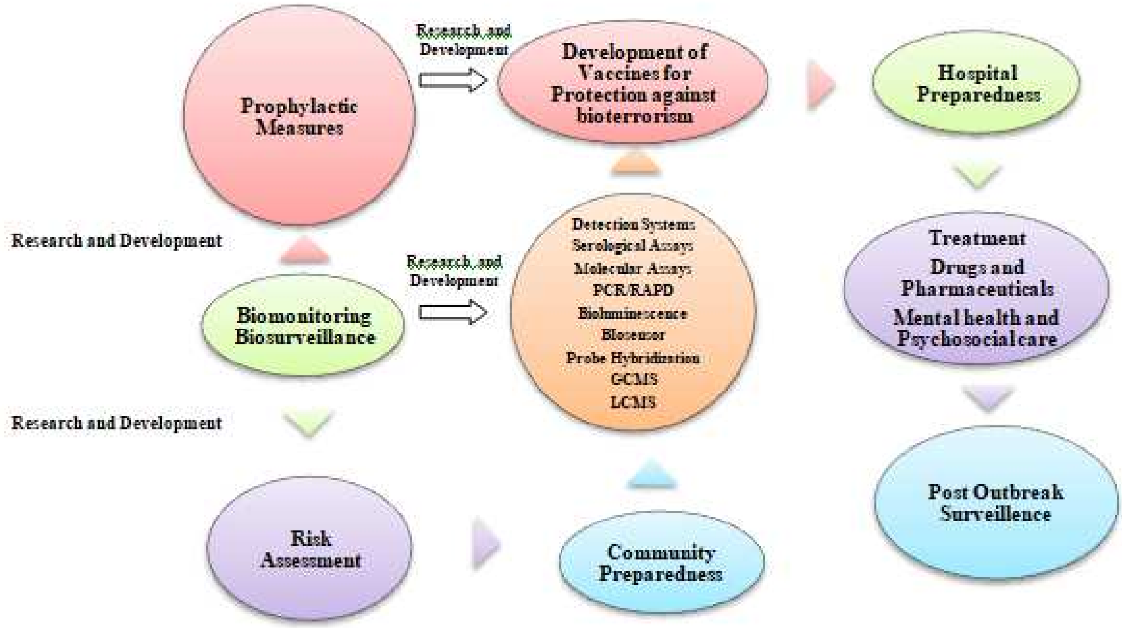

Figure 3: Preventive Measures against Bioterrorism

4. **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 their high specificity and sensitivity [29].

X. CONCLUSION

Bioterrorism is the oldest form of terrorism in the history of mankind. Biological warfare can be easily mistaken as a natural disease outbreak. Bioterrorism may or may not be state-sponsored but it is always against a nation to disturb the socio-economic progress 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 thrust area of research on bioweapons' defense. With the advent of new tools and techniques now it is possible to develop a novel strategy to detect BT Agents present in the environment and food items. The rapid development and production of new prophylactic and preventive means are now possible against these BT agents [30]. The "Sherlock Holmes's 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]. For the effective development of the country's preparedness and emerging responses to biological agents, in the case of a bioterrorist attack, the rapidity of intervention by trained anti-terrorist staff which includes microbial scientists, medical professionals, healthcare workers, professionals in psychology, the military or law-enforcing forces, and public health personnel, is essential because bioterrorist agents have such a great potential for destruction [32]. It is of utmost importance to spend money on surveillance of public health helps to improve domestic readiness for dealing with biological warfare, emerging diseases, and infections transmitted through food.

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