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*REVIEW PAPER*

# Biological Warfare Agents

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**ABSTRACT**

There is a long historic record of use of biological warfare (BW) agents by warring countries against their enemies. However, the frequency of their use has increased since the beginning of the twentieth century. World war I witnessed the use of anthrax agent against human beings and animals by Germans, followed by large-scale field trials by Japanese against war prisoners and Chinese population during world war II. Ironically, research and development in biological warfare agents increased tremendously after the Geneva Protocol, signed in 1925, because of its drawbacks which were overcome by Biological and Toxin Weapons Convention (BTWC) in 1972. Biological warfare programme took back seat after the 1972 convention but biological agents regained their importance after the bioterrorist attacks of anthrax powder in 2001. In the light of these attacks, many of which turned out to be hoax, general awareness is required about biological warfare agents that can be used against them. This review has been written highlighting important biological warfare agents, diseases caused by them, possible therapies and other protection measures.

**Keywords:** Biological warfare agents, biological warfare, weapons of mass destruction, BTWC, Biological and Toxin Weapons Convention, biodefence, bacterial agents, viral agents, toxins

## 1. INTRODUCTION

Biological warfare agents have received greater attention after the episode of terrorist attack on World Trade Centre in USA on September 11, 2001 followed by the use of anthrax spores as means of bioterrorism. One must acknowledge that no moral, technical or legal barriers can protect the world from the use of deadly biological agents. So this is becoming an emerging threat, not only from the public health point of view but also for the stability of a country, as it will have serious effects on the economy.

The history of use of biological warfare (BW) agents dates back to 14$^{th}$ century when Tartar forces used the plague-infected bodies of their own soldiers to create disease and havoc in the walled city of Kaffa (now in Ukraine) which ultimately led to the downfall of Kaffa[1]. In another incidence in the 18$^{th}$ century, British forces distributed smallpox-infected handkerchiefs and blankets to the native American tribes which were opposing the British rule in America[2]. Subsequently in world war I, Germans used *Bacillus anthracis* and *Burkholderia mallei* against livestocks transported from Romania to USSR. Although use of BW agents was very less in world war I but chemical weapons were used extensively, which lead to the formulation of Geneva Protocol in 1925. This Protocol states, "Prohibition of the use in war of asphyxiating,

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poisonous or other gases, and of bacteriological methods of warfare". Although the Geneva Protocol prohibited the use of biological weapons, it did not prohibit research and development, production and/ or storage of biological weapons[1]. As a result, later in world war II, Japanese conducted extensive field trials of *Bacillus anthracis, Neisseria meningitidis, Shigella, Vibrio cholerae* and *Yersinia pestis* against prisoners of war. They also used these agents against Chinese, inflicting more than 10,000 casualties, including death of around 1700 Japanese soldiers itself [1]. After this, the world community realised the importance of biological warfare agents as weapons of mass destruction (WMD) and several countries started research and production of biological warfare agents. Ultimately in 1969, the then US President insisted on disarmament of biological weapons which resulted in 1972's Biological and Toxin Weapons Convention (BTWC). This is called 'Convention on the Prohibition of Development, Production and Stockpiling of Bacteriological and Toxin Weapons and their Destruction'. At this time, most of the western countries stopped public funding for biological warfare research and development except a few. The anthrax outbreak of 1979 in Sverdlosk (USSR) resulted in 66 deaths, affecting the human population up to 4 km downwind. Later, the then Russian President in 1992 admitted the outbreak by accidental release of anthrax spores from one of its offensive biological warfare production facilities[3].

In 1975, US ratified the 1925 Geneva Protocol and BTWC of 1972. Since then, the Convention has been ratified by more than 140 countries to which India is also a signatory. But this Convention still has certain flaws and the main flaw is that it does not contain provisions for monitoring and inspection of biological warfare agents, and enforcement of the treaty[4].

The term NBC refers to nuclear, biological and chemical weapons. Nowadays, people are mainly concerned with B and C, ie, biological and chemical weapons because unlike nuclear, these can be used covertly by the rogue countries and terrorist organisations. Chemical Weapons Convention (CWC) is in force for prohibiting the use of chemical weapons but there is no such legally binding treaty for biological weapons. The major difference between the two types of agents is that chemical weapons are nonliving poisons made artificially while biological agents are living, natural and reproduce inside the host to cause incapacitating or fatal diseases[5]. So a very small quantity of a biological agent may be sufficient to cause morbidity and mortality. Some biological agents are highly infectious, eg, plague and smallpox, which can be spread to large population within few days, causing epidemics and sometime pandemics also. However, toxins like botulinum and others share features common to chemical warfare agents as well[6]. There are many other characteristics of biological warfare agents which make them attractive choice as weapons of mass destruction. Specialised equipment and huge infrastructure is not required for the production of biological warfare agents. A small microbiological laboratory can produce them because both literature and equipment are available in public domain without any distinction for use in offensive or defensive purposes. Since a very small laboratory is sufficient for biological warfare agents' production, so it is very easy to hide the production facility as well. Further, a small amount of pure culture is required to initiate production which was quite easy to obtain commercially until recently. Since biological warfare agents produce delayed symptoms, and unlike chemical warfare agents are odourless and colourless, so it is difficult to pinpoint the origin of attack. These biological warfare agents are very specific and damage is done only where it is intended. So, all these features alongwith difficulty in detection make biological warfare agents as weapons of choice for mass destruction.

## 2. BIOLOGICAL WARFARE AGENTS

Biological warfare agents are microorganisms (or toxins produced from them) which cause diseases in man, animal or plant, or which can cause the deterioration of material, eg, petroleum-eating bacteria. Use of such biological agents to cause lethal or incapacitating effects on target population or plants is called biological warfare. These biological warfare agents are broadly classified into four groups, viz., bacteria, viruses, fungi, and toxins.

- *Bacteria*: Bacteria are prokaryotic microorganisms, usually single-celled and most of which have

a characteristic type of cell wall while a few are wallless, eg, *Bacillus anthracis*, *Yersinia pestis*, etc.

- *Viruses*: Viruses are noncellular submicroscopic obligate pathogens which consist of proteins and nucleic acids (DNA or RNA), eg, Variola (small pox) virus. These can replicate only in specific host cells by utilising the host cell machinery. These host cells may be of animal, microbial or plant origin.

- *Fungi*: Fungi are unicellular, multicellular or coenocytic, heterotrophic eukaryotic microorganisms which do not contain chlorophyll and which characteristically form a rigid cell wall containing chitin and/or cellulose, eg, *Coccidioides immitis*.

- *Toxins*: Toxins are secondary metabolites usually of microbial origin which, when present in low concentrations in cells or tissues of a higher multicellular organism, can cause injury by interfering with the structural and functional integrity of these cells or tissues. Toxins can be produced by various bacteria[7] (bacterial toxins, eg, botulinum toxins), algae[8] (phycotoxins, eg, saxitoxin), fungi[9] (mycotoxins, eg, trichothecene) and plants[10] (phytotoxins, eg, ricin).

The infective dose of these biological warfare agents vary considerably from one agent to another (Table 1). Some of the important BW agents are listed in Table 2 and described briefly in the following text.

## 2.1 Bacterial Agents

### 2.1.1 *Bacillus anthracis*

*Bacillus anthracis* is Gram-positive, facultative anaerobe, non-motile, encapsulated, spore former, and its cells are arranged in chains (Fig. 1(a)). Extreme resistance of spores to environmental stress makes it the most sought after biological warfare agent. Sporulated cells can survive for years in water and soil. Live fire tests of anthrax bombs by the US and Allies in 1943 were conducted at Gruinard island which led to the long-term contamination of the Island. The decontamination could only be done after more than 40 years using formaldehyde as disinfectant[11]. Anthrax, a disease primarily of animals, caused by *Bacillus anthracis*, is of three types: (i) Cutaneous anthrax occurs when spores come in contact with skin due to handling of infected animals; (ii) gastrointestinal anthrax is caused by ingestion of food and meat contaminated with spores; and (iii) respiratory anthrax is caused by the inhalation of spores present in the environment.

Among these types of anthrax, respiratory anthrax is the most severe with the mortality rate of more than 80 per cent, if untreated. According to an estimate made by World Health Organization (WHO),

**Table 1. Infective dose (aerosol) of important biological warfare agents**

| Agent | Infective dose (per man) | Mortality rate (%) |
|---|---|---|
| *Bacillus anthracis* | 8000-50000 spores | 5-80 |
| *Yersinia pestis* | 100-500 organisms | 50-100 |
| *Burkholderia mallei* | Low | 50-100 |
| *Brucella* species | 10-100 organisms | 5 |
| *Francisella tularensis* | 10-50 organisms | 40-60 |
| Variola virus | 10-100 plaque forming units (PFU) | 10-30 |
| Venezuelan equine encephalitis (VEE) virus | 10-100 PFU | 1 |
| Ebola virus | Low | 65-80 |
| Marburg virus | Low | 35 |
| Botulinum neurotoxin | 70-100 ng | -* |
| Staphylococcal enterotoxin B (SEB) | 2-6 µg | Rare |
| Tricothecene (T2) toxin | 60-84 mg | -* |
| Ricin toxin | 150-210 µg | -* |

* Statistical data not available

**Table 2. Important biological warfare agents**

| Agent | Disease | Effect | Vaccine available | Potential for epidemic spread | Therapeutics |
|---|---|---|---|---|---|
| Bacteria | | | | | |
| *Bacillus anthracis* | Anthrax | Lethal | Yes | Negligible | Penicillin, Ciprofloxacin |
| *Yersinia pestis* | Plague | Lethal | Yes | High | Tetracycline, Chloramphenicol |
| *Burkholderia mallei* | Glanders | Lethal | No | Negligible | Sulphadiazine, Chloramphenicol |
| *Brucella* species | Brucellosis | Incapacitant | Yes | Negligible | Streptomycin, Tetracycline |
| *Francisella tularensis* | Tularemia | Lethal and incapacitant | Yes | Negligible | Streptomycin, Chloramphenicol, Tetracyclin |
| Viruses | | | | | |
| *Variolla virus* | Smallpox | Lethal | No | Very high | Cidofovir (proven for *in-vitro* effectiveness) |
| *Venezuelan equine encephalitis* (VEE) *virus* | Venezuelan equine encephalomyelitis | Lethal and incapacitant | Yes | Possible | No specific therapy available |
| *Ebola virus* | Hemorrhagic fever | Lethal | No | Possible | No specific therapy available |
| *Marburg virus* | Hemorrhagic fever | Lethal | No | Possible | No specific therapy available |
| Fungi | | | | | |
| *Coccidioides immitis* | Coccidioidomycosis | Lethal and incapacitant | No | Rare | Amphothericin B, Fluconazole |
| Toxins | | | | | |
| *Botulinum neurotoxin* | Botulism | Lethal | Yes | No | Antitoxins |
| *Staphylococcal enterotoxin type* B (SEB) | Food poisoning | Incapacitant | No | No | No |
| *Trichothecene* (T2) *toxin* | Alimentary toxic aleukia (ATA) and blisters on skin | Lethal and incapacitant | No | No | Phenobarbital, Clofibrate found beneficial in animal models |
| *Ricin toxin* | Ricin toxicity | Lethal | No | No | No |

if 50 kg anthrax spores are released from an aircraft, a total of 5,00,000 people will be affected, out of which there will be 1,00,000 deaths[12].

Though the description of detection methods for individual biological warfare agents is beyond the scope of this article but detection methods for *Bacillus anthracis* in view of its importance have been described. A variety of methods are in practice for detection of anthrax bacteria/spores like polymerase chain reaction (PCR)[13], enzyme-linked immunosorbent assay (ELISA)[14], and immunofluorescence assay[15]. As low as 1-1000 spores have been detected by these methods which is the acceptable limit of detection[16]. However, the detection of spores from environmental samples is difficult and fluorescence microscopy coupled with pre-concentration has been proved as a fast, easy, and specific method[15]. A few advance portable devices like rapid analyte measurement platform (RAMP)[17] and biohazard detection system (BDS)[18] have also been developed for detection of *Bacillus anthracis* spores from environmental samples.

Live-attenuated and protein-based vaccines are in use for human beings by different countries but these vaccines also have side effects and protect

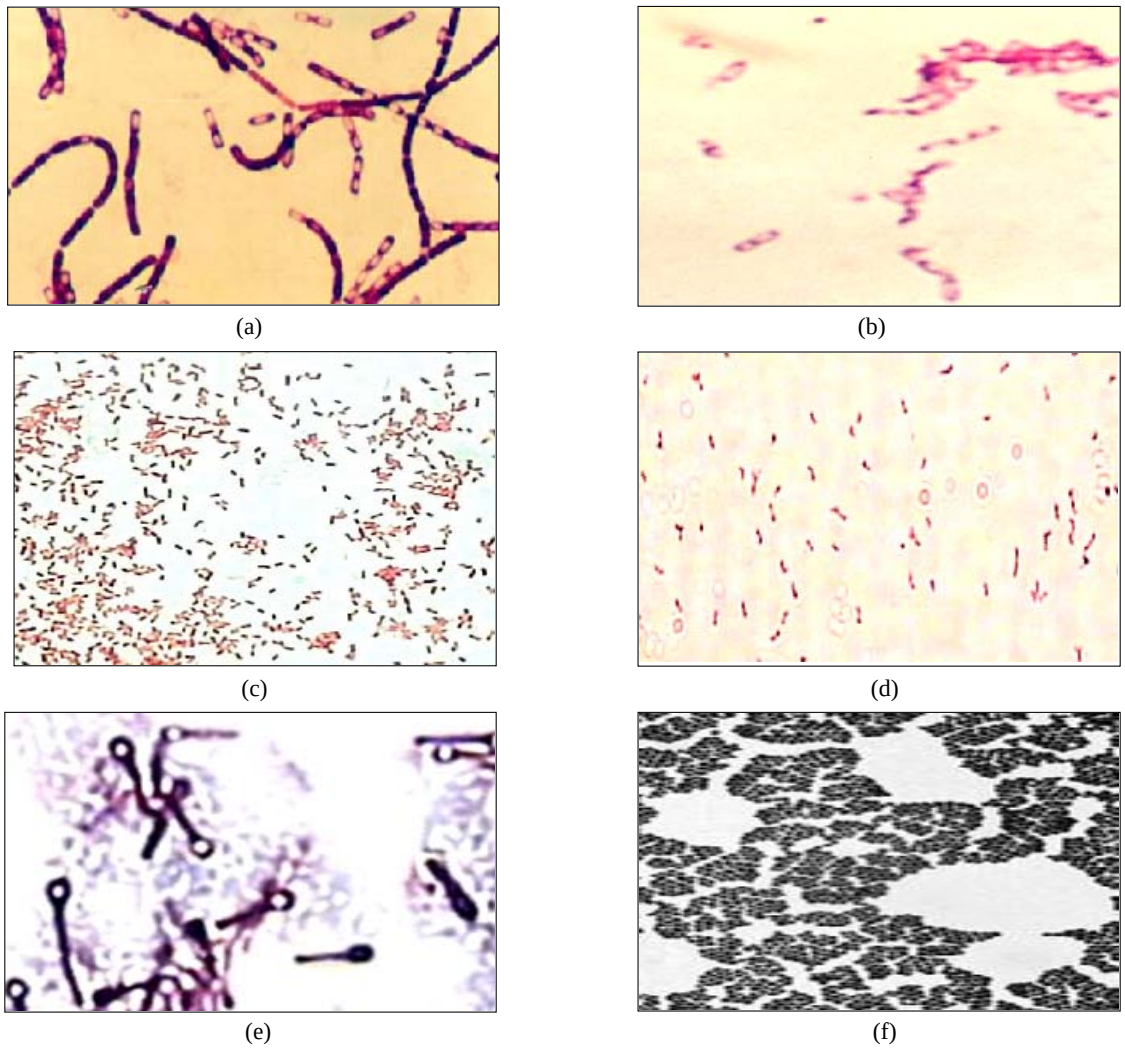


**Figure 1. Morphological features of some important biological warfare agents: (a) *Bacillus anthracis,* (b) *Yersinia pestis (c) Burkholderia mallei,* (d) *Brucella abortus* (e) *Clostridium botulinum,* and (f) *Staphylococcus aureus.***

the individual only for a short duration. Common antibiotics like tetracycline are largely effective during the early course of infection but treatment becomes largely ineffective if onset of symptoms have occurred.

### 2.1.2 *Yersinia pestis*

It is Gram-negative, non-motile, rod shaped, facultative anaerobe that shows bipolar (also called safety pin) staining with Geimsa stain (Fig. 1(b)). *Yersinia pestis* is the agent of plague, a zoonotic disease of rodents and other animals that is usually transmitted to humans via flea bites. This route of infection results in bubonic form of plague characterised by the inflammation of lymph nodes in groin and armpits referred to as bubos. Pneumonic plague occurs as a result of hematogenous spread of plague bacilli from bubos to lungs or after inhalation of the organisms, the most likely route of infection as a result of bioterrorist attack. The pneumonic form of the plague is highly contagious and can spread from person to person via airborne droplets. Administration of streptomycin, gentamycin or tetracycline early in the course of the disease is an effective treatment[19]. The mortality rate for untreated cases is more than 50 per cent.

### 2.1.3 *Burkholderia mallei*

*Burkholderia mallei* is a Gram-negative rod shaped bacterium [Fig. 1(c)] which is the causative

agent of glanders, a febrile illness that attacks equine population. Human beings are their accidental hosts and the disease has been reported among individuals handling the infected animals. Because of low infective dose, lack of effective vaccine and therapy, *Burkholderia mallei* is considered as a potential biological warfare agent. Glanders occurs in four forms: (i) an acute localised form, (ii) a septicemic and fatal form, (iii) an acute pulmonary form, and (iv) a chronic form.

Aerosol infection can cause any of these four forms. Incubation period of glanders ranges from 10-14 days post-exposure, and symptoms include fever, sweat, myalgia, headache, chest pain, swelling in lymph nodes, pustular eruptions, etc. The mortality rate for septicemic form is 100 per cent, if untreated but person to person transmission is rare[20].

#### 2.1.4 *Brucella Species*

*Brucella* are slow growing Gram-negative coccobacilli which can survive in the environment for several weeks [Fig. 1(d)]. Brucellosis, caused by *Brucella* species is a zoonotic disease contracted by humans as a result of contact with animals infected with *Brucella*[21]. There are four species of *Brucella* pathogenic to human beings and the disease-causing species in the decreasing order of severity are *Burkholderia melitensis* (infect goats), *Burkholderia suis* (infects swine), *Burkholderia abortus* (infect cattle) and *Burkholderia canis* (infects dogs). Infection in human beings by these species can be established via cutaneous, respiratory or gastrointestinal routes. Like most other biological warfare agents, these are also extremely infectious via aerosol route and as low as 10-100 organisms are sufficient to cause the disease. The incubation period of brucellosis is extremely variable, ranging from 5-60 days and symptoms include fever, chills, headache, sweating, depression, etc. The mortality rate is only 5 per cent, if untreated and hence, it is considered as an incapacitating type of disease.

#### 2.1.5 *Francicella tularensis*

These are Gram-negative coccobacilli which cause the disease called tularensis. This is primarily a zoonotic disease and in human beings, it is caused by animals, or from the bites of infected insects. Though the organisms are susceptible to heat but may persist for long duration in the environment and in the animal products. Under natural conditions, infection by inhalation or ingestion is less common in human beings but exposure by aerosol (biological warfare attack) would cause pneumonic tularemia. As few as 10-50 organisms can cause the disease in human beings which makes *Francicella tularensis* an attractive biological warfare agent. Its incubation period ranges from 2-10 days. Symptoms of pneumonic tularamia caused by inhalation include fever, headache, fatigue, weight loss, non-productive cough, and pneumonia. Tularemia can be cured with antibiotics, but if untreated, the mortality rate[5] may be 35 per cent to 60 per cent.

### 2.2 Viral Agents

#### 2.2.1 *Smallpox* (*Variola*) *Virus*

The smallpox virus is the largest of all animal viruses. Virus particles are brick to ovoid shaped. Human beings are the only natural host of smallpox virus, and infection is spread from person to person. After about 12 days' incubation period, it causes fever and headache. As the virus spreads to the skin, it forms pus-filled vesicles throughout the body. The mortality rate for immunised individuals is approximately 3 per cent, while for non-immunised, it increases to 30 per cent. Aerosol exposure to individual may cause malaise, fevers, rigours, vomiting, headache, and backache. The smallpox virus is considered a much higher threat for the following reasons[5]:

- A larger population of the world is unimmunised as the process of vaccination against smallpox was stopped in 1980.
- High infectivity through aerosol
- The relative ease of culturing the virus.

After the declaration of World Health Organization in 1980 that smallpox has been eradicated from the earth, the vaccination for general public has been stopped. As a result, the whole unimmunised human population has become susceptible to the infection of *Variola* virus, if encountered through bioterrorism

or by any other mean. There is no effective treatment for smallpox virus and the vaccine is not available in the world except in the USA.

#### 2.2.2 *Venezuelan Equine Encephalitis (VEE) Virus*

It is an arthropod-borne virus and the disease is caused by mosquito bite. Animals like horses, mules, and donkeys serve as natural reservoir of this virus. Besides, this virus is highly infectious via aerosol. The incubation time ranges from 2-6 days and symptoms include malaise, spiking fever, headache, light sensitivity, etc. Some patients may consequently develop neurological complications at the later phase of the disease[20]. It is largely considered as an incapacitating agent and the mortality rate is very low (1%).

#### 2.2.3 *Ebolla and Marburg Viruses*

These are the two major viruses among others which cause viral haemorrhagic fever. Person to person transmission of these viruses occurs through direct contact with infected bodily fluids and organs. The high mortality rate (30-90 %), low infective dose, and lack of treatment make these viruses desirable for use as biological warfare agents. The incubation period for viral haemorrhagic fever ranges from 4–21 days. Symptoms are fever, muscular pain, headache, vomiting, and diarrhoea[5].

### 2.3 Fungal Agents

*Coccidioides immitis* is a dimorphic spore-forming fungal pathogen that causes a disease called coccidioidomycosis. Most often, the disease causes mild flu-like symptoms. The disease often begins as a benign, inapparent or mild upper respiratory infection that usually resolves rapidly, but if infection is stabilised, the disease may progress as a chronic pulmonary condition or as a systemic disease involving the meninges (lining the brain), bones, joints, and subcutaneous and cutaneous tissues[22]. In untreated cases, the mortality rate is as high as 50 per cent. The disease can be cured with amphotericin B, ketoconazole or itraconazole.While most fungi do not generally cause fatal disease in healthy human beings, they can be used to destroy crops[23-25].

### 2.4 Toxins

There are mainly three microbial toxins apart from ricin (phytotoxin) which have potential for use as biological warfare agents:

#### 2.4.1 *Botulinum Toxins*

These are the causative agents of a diseased condition called botulism, and are produced by the bacteria, *Clostridium botulinum* (Fig. 1(e)). These toxins are released upon the death and lysis of the organism. Botulinum toxins are the most poisonous substances ever known. As little as five picogram ($5 \times 10^{-12}$ kg) of botulinum toxin is enough to kill a laboratory mouse. According to an estimate, 39.2 g of botulinum toxin is sufficient to kill the whole human world population of six billion. The toxin is 100 million-times more toxic than cobra venom and 1,00,000-times more toxic than the most dreaded chemical warfare nerve agent, sarin[7,20]. Botulinum toxin blocks the release of the neurotransmitter, acetylcholine, and thereby prevents transmission of nerves impulses. Classically, botulism in human beings occurs as food poisoning which does not involve fever, although it causes difficulty in breathing and problems with vision (the pupils may become fixed). Death is caused by respiratory arrest within 24 h of ingesting the toxin. With effective supportive therapy, the mortality rate from botulism can be reduced to 10 per cent.

Surprisingly, very little work has been done on this toxin by Indian scientific institutions and bacterial cultures/isolates are not available anywhere in India. However, Defence Research and Development Establishment (DRDE), Gwalior, has succeeded in isolating *Clostridium botulinum* from Gwalior region which produce different types of botulinum toxins.

#### 2.4.2 *Staphylococal Enterotoxin (Type B)*

Most people encounter the bacterium, *Staphylococus aureus* [Fig. 1(f)] and its enterotoxin, Staphylococal enterotoxin (type B) (SEB), at some point in their lives from food poisoning. As a potential biological weapon, aerosolised SEB could cause 80 per cent or more of targeted personnel to become extremely ill within 3-12 h. The toxin can withstand

boiling temperature and is resistant to acids and alkalies to a great extent. These properties are of concern in terms of a possible attack using SEB in water and food supplies. It might take up to 1-2 weeks for human beings to recover from SEB poisoning, and higher concentrations of SEB could even cause septic shock and death[5].

#### 2.4.3 *Trichothecene Mycotoxins*

Trichothecene mycotoxins (T2 toxin) are produced by a number of fungi like *Fusarium*, *Trichoderma*, *Myrothecium*, *Stachybotrys*, etc. These cause a diseased condition called alimentary toxic aleukia (ATA). Typical ATA symptoms include vomiting, severe skin irritation, and internal bleeding[20]. T2 toxin is unique among biological warfare agents in that it is a skin-damaging agent of great potency– several hundred-times more potent than the chemical warfare agents, mustard or lewisite[9,26]. It is able to injure the eye in microgram quantities, which again indicates that it is more potent than mustard gas.

### 2.5 Ricin Toxin

Castor plant *Ricinus communis* is the producer of ricin toxin and is grown world over. Seeds of the plant are the source of the toxin. Ricin, consisting of two peptide chains (A and B), is quite stable in the environment and is toxic when ingested, injected or even inhaled. Widespread availability and production ease are the main reasons for the toxin to be used as biological warfare agent. Symptoms of ricin toxicity appear within 18-24 h after exposure which include fever, cough, breathlessness, nausea, joint pain, etc. Ricin is suspected to cause respiratory failure when inhaled[20]. It also causes severe gastrointestinal symptoms followed by vascular collapse and damage, if ingested, and multiple organ failure, when injected. Presently, no vaccine is available as preventive therapy for ricin toxin.

## 3 BIODEFENCE

Biodefence is the defence against biological warfare agents including toxins. It has two integral components–detection and protection.

### 3.1 Detection

Most of the biological warfare agents are contagious except toxins and a few pathogens, so they will readily spread to targeted/ non-targeted human population causing panic in general public in case of a biological warfare attack. Therefore, their prompt detection is of utmost importance, and microbiological laboratories with their state-of-the-art detection and identification tools will be at the helm of affairs. Detection can be of general nature involving laser sensors for remote surveillance of the environment, indicating the increase in concentration of microorganisms in a particular locality. This should be a routine exercise and will work as an early warning for a possible biological warfare agent attack[27]. In the event of a bioterrorist attack, one needs to specifically identify the organism to the species level, and that too within a short period of time, so that countermeasures can be applied. Therefore, any detection system should have properties of rapidity, sensitivity, and specificity.

Detection and identification methods can be classified as conventional microbiological methods and rapid, specific and sensitive methods.

#### 3.1.1 *Conventional Microbiological Methods*

These include culture and growth of microorganisms and their subsequent identification by biochemical and serological tests[10,28-31]. But these are too slow for early countermeasures; however, these can be used for final confirmation of the biological warfare agent.

#### 3.1.2 *Rapid, Specific, and Sensitive Methods*

These methods include polymerase chain reaction (PCR), DNA probes, immunosensors, flow cytometry, and gas chromatography coupled with mass spectrometry (GC-MS)[32, 33]. These are the basic tools being employed in the development of more sensitive and robust detection systems like bio detector (BD), rapid analyte measurement platform (RAMP), microbial identification system (MIS), and biohazard detection system (BDS)[16-18,34]. These are state-of-the-art online detection systems composed of air samplers and biosensors that can pinpoint the presence of specific organism in the aerosol. Most of these systems are

capable of detecting two or more biological warfare agents simultaneously.

Bio detector, developed jointly by M/s Smith Detection and United States Army Medical Research Institute of Infectious Diseases (USAMRIID), is an automatic device capable of operating[34] continually for 14 h. When a threat is detected, it generates audio-visual alarm and specifies the type of organism and its concentration. The system is immuno-based and uses light addressable potentiometric sensor. BD is capable of detecting eight different biological warfare agents simultaneously within 15 min.

Rapid analyte measurement platform is a platform technology which consists of two components, a disposable test cartridge that houses an analyte specific immunochromatographic strip, and a portable fluorescence reader that quantifies antigen-antibody complexes[17]. The system is able to perform environmental tests for detection of a number of biological warfare agents including *B. anthracis*, botulinum, and ricin toxins. Some of the test systems available in India for detection of important biological warfare agents are listed in Table 3.

Defence Research and Development Establishment (DRDE), Gwalior, is also working for the development of diagnostics for different bacterial, viral, and toxin agents. A fast method for the detection of anthrax spores from environmental samples has been developed. This involves concentration/ enrichment of spores, followed by detection using immunofluorescence microscopy. The method is very specific and the test is completed within two hours[15].

Further, toxigenic strains of *Bacillus anthracis* have been detected by nested polymerase chain reaction (PCR)[13]. A rapid agglutination-based test for ricin[10] with a sensitivity of 9 µg/ml, and an enzyme-based assay for cyanobacterial toxins have also been established. A duplex PCR for differentiation of toxigenic and non-toxigenic *Vibrio cholerae* is a routine practice[35]. Use of immunomagnetic beads for the enrichment of environmental and clinical samples followed by duplex PCR is very effective during the cholera outbreaks.

**Table 3. Detection tests for BW agents available in India**

| Disease/ BW agent | Detection test type | |
|---|---|---|
| | Indigenous | Imported |
| Anthrax | ELISA, immunofluorescence microscopy | ELISA |
| Plague | ELISA, agglutination | ELISA |
| Brucellosis | ELISA | ELISA |
| Hanta virus | No | ELISA |
| Q-fever | No | ELISA |
| Ricin toxin | Agglutination | No |
| T2 toxin | No | No |
| Botulinum toxin | ELISA | No |
| SEB | ELISA | ELISA, agglutination |
| Small pox | No | No |
| Ebola virus | No | No |
| Marburg virus | No | No |

In addition to the laboratory-based methods for the detection of biological warfare agents, DRDE, Gwalior, has also developed various field-based detection kits for different pathogens, eg, plague, anthrax, dengue, typhoid, brucella, malaria, leptospira, etc. Besides, kits for detection of different bacterial and phytotoxins are at different stages of development.

### 3.2 Protection

Protection is to adopt various control measures to prevent the disease after exposure to harmful concentrations of biological warfare agent. These include physical protection, decontamination, and medical management. Physical protection equipments include protective gowns, boots, gloves, and face masks fitted with HEPA filters to prevent entry of live biological warfare agent into the human body. These are personal protective equipment but collective protection equipments are also important in the event of biological warfare agent attack, and include water purification plants, shelters fitted with HEPA filters for supply of germ-free air[36,37]. The other important area of protection is decontamination of personnel, equipments, and surrounding environment. Personal decontamination is carried out by taking a bath with soap and water[38,39]. Vehicles, clothing,

and equipment can be decontaminated using chemical reagents like detergents (for clothing), and formalin, glutaraldehyde, alcohol, and hypochlorite (for equipment and biological samples). The contaminated terrains can best be decontaminated using formalin solution (0.5 %) in the form of foam or froth.

Among the medical management practices, the most important aspect is the use of antibiotics and drugs to save the life of patients[30,40]. The common antibiotics used for different biological warfare agents are listed in Table 2. However, in cases where no antibiotic treatment is available, post-exposure prophylactic treatment is given, eg, in case of botulinum toxin, antitoxin is used for curing the patient alongwith life supportive devices like artificial respiration.

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