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## **Microbial Agents in Terrorism, Biomarkers, and Public Health Challenges**

Olukayode Olugbenga Orole\*, Aleruchi Chuku, Kayode Olawuyi, Eno Chongs Mantu,  
Alexander Phillips, Chukwuma Okoli

Department of Microbiology, Faculty of Science, Federal University of Lafia, Nasarawa State, Nigeria

### **ABSTRACT**

Bioterrorism agents are mostly microorganisms with the capacity to deal explosive and lethal harm to humans, animals, and food crops. These microorganisms spread in the form of gases, whole organisms, or products of secondary metabolism of microorganisms. Classification of the agents is into three categories based on the ease of dissemination and end effects on a living population. While most health institutions are equipped to take care of sick people and treat suspected cases of infections, these institutions lack basic means of identifying bioterrorism acts. Special diagnostic equipment to identify causal organisms or agents is not available. Lack of training on what to do when terrorists strike using biological agents can cumulatively increase the lethal effects of such agents. Molecular techniques of identifying microorganisms to species level are as promising as they are time-consuming, while technical expertise and a conducive environment for managing such equipment are mostly not available in the African setting. The governments in Africa as a matter of urgency should provide an atmosphere where the teeming population of people without jobs are employed, while hospitals are adequately equipped, and training of health workers on what to do immediately after cases of terrorism are reported. The review highlights these agents and the diagnostic tools necessary to facilitate early response to bioterrorism.

**Keywords:** Bioagents, Bioterrorism, Classification, Identification, Spread.

### **INTRODUCTION**

Bioterrorism is the deliberate release or threat of release of a biological weapon to a civilian population with the intent of causing serious illness or death to animals or humans and destruction to food crops [1]. Bioweapons, which could be insects, microorganisms, or toxins, are engineered with the ultimate aim of influencing Government conduct or policy; this could be due to religious, political, or ideological reasons with the ultimate goal of spreading fear and panic within the population. Bioterrorism is a planned threat of discharge of pathogenic microorganisms and their products (bacteria, fungi, toxins, and viruses) to cause morbidity and mortality among the designated human population, food crops, and livestock. Microorganisms are target agents of bioterrorism and have proven to be effective due to their potential of producing disease responses with significant clinical consequences that may lead to death or illness in the target host. The resultant effects are to create an atmosphere of fear, anxiety, and panic among the public [2]. Bioterrorism agents are weapons of mass destruction because of their potential to spread

within a short time and create devastating consequences. These microorganisms are directly employed or modified to increase their virulence (causing disease conditions in man and animals) and resistance (against anti-bioterrorism agents) [3].

Classification of bioterrorism agents is according to the risk they pose to State and National security. The risks are defined based on the ability of these agents to be easily transmitted from person to person or from their source to the intended target. Classification of the agents is also on their public health impact to cause high mortality, creating panic among people, and the level of public preparedness required for mitigating it. Microorganisms adapted for bioterror attacks are pathogenic and weaponized. These agents differ in the level at which they cause infections, morbidity, and mortality as consequences of exposure to the organisms. Microorganisms could be genetically altered and conferred with significantly higher virulence and capacity of thriving and maintaining themselves in the environment indefinitely with potential ease of transmission and spread in the population within a short period after release. Other bioterrorism agents include products of the metabolism of microorganisms that kill or incapacitate hosts. The most targeted host of bioterror attacks are humans, commercial plants, and environmental systems.

\* Correspondence address:

**Olukayode Olugbenga Orole**

Email : orolekayode@gmail.com

Address : Federal University of Lafia, FHF4+CHQ, 950101,  
Lafia, Nigeria

Microorganisms are manipulated for bioterrorism because the cost of production is usually low, making it readily available, and the relative ease of isolation and mass production within a short period. Other reasons are ease of transportation from place to place without detection by the routine security system; ease of spread over large areas by wind, water, insects, animal, and humans; and ease of the agent to establish a viable community in the target area for a long time. Bioterrorism agents are transmitted in aerosols and incorporated into food and water as poison. In Africa, little is done to mitigate the problem of terrorists due to varying factors relating to religion, ethnicity, corruption, dearth of infrastructures, incompetence, and other such factors. The review is to highlight bioterrorism and its related challenges to healthcare provision in Africa.

#### **CATEGORIES OF BIOLOGICAL AGENTS OF BIOTERRORISM**

Microorganisms used in terrorism attacks are categorized into three groups labeled A, B, and C based on the relative ease of dissemination. The level of risk resulting from exposure to the agent is determined by morbidity and mortality rates in the target hosts.

**I. Category A Agents** - are the most lethal agents presenting the highest risk to national security and public health, characterized by hemorrhagic fever and its associated syndrome. They spread effortlessly, resulting in death and threat to public safety. Their effects result in general fear among the public and require measures for public health alertness [4]. Microbial agents in this group are *Francisella tularensis* (Tularemia), *Bacillus anthracis* (anthrax gas), Smallpox (*Variola major*), *Yersinia pestis* (Plague), *Clostridium botulinum* (toxin), Viral hemorrhagic fevers, Lassa virus.

**II. Category B Agents** - in this category, microbes or microbial products are at moderate risk of exposure to humans and animals. They cause moderate morbidity and low mortality but can spread easily from host to host. Agents in this class are *Brucella* species (Brucellosis), Epsilon toxin of *Clostridium perfringens*, food poisoning caused by *Salmonella* species, *Escherichia coli* O157:H7, *Shigella*, and *Staphylococcus aureus*.

**III. Category C Agents** - are classified as emerging infectious disease agents, mostly with zoonotic attributes. They are relatively lower risk agents compared to categories A and B agents. Hantavirus, Coronavirus, MERs, influenza pandemic, and Nipah virus are examples of category C agents [5].

#### **Characteristics Peculiar To Bioterrorism Agents**

The characteristics that define the hazardous potential of bioterror agents are that; the agent should be highly infective at low doses to establish a disease condition with the desired effect [6]; the capacity of the organism to cause disease conditions by evading the host's innate defense system (pathogenicity); ability to easily infect a healthy individual from a diseased patient. The agent should be resistant to treatment remedies and possess relative ease of mass production. These agents should be stable and viable in any environment dispersed. Dispersal should be relatively easy and efficient without altering the stability of the agent and must be virulent, toxic, and lethal at low concentrations or doses.

#### **MANIPULATING MICROORGANISMS FOR TERRORISM**

Microorganisms have the attribute of easily being manipulated hence, their use in terrorism. One of the best progression and accomplishments in gene manipulation using biotechnological techniques opened the way for effective adjustment of microbes into new microbes with lethal attributes that are untreatable and wild. The knowledge of molecular biology and biotechnology made it possible for genetic manipulation of biological agents to resist treatment to be able to cause harm to life. Genetic engineering involves a deliberate intervention to transfer genes (DNA) between different/same biological entities to create a new organism with novel characteristics. The new organism created with new characteristics has a higher degree of survival, infectivity, virulence, and drug resistance.

Alteration of naturally occurring pathogens into deadly genetically modified pathogens like the insertion of an alien gene into *B. anthracis* constitute a threat to humans as a bioweapon [7]. Genetically engineered bioterrorism agents are classified based on the technique of creation into a binary biological weapon, designer gene and life forms, gene therapy, host swapping diseases, and designer diseases.

**I. Binary Biological Weapons:** are a two-part component system made-up of an autonomous protected part and another part that exists separately but works better to produce a greater result. An example is a blend between Hepatitis B and D. Hepatitis D after infection of Hepatitis B uses the proteins expressed by hepatitis B to increase the severity of the infection, as it alone cannot cause disease. Binary weapons have great possibilities for future application due to their benign properties, making them easy to be preserved and to be manipulated. Transportation from place to place is easy since the parts are not separately dangerous. It additionally makes tracing more troublesome due to its potential and capacity to be stored secretly away for a long time [8].

**II. Designer Genes:** Available data of already sequenced viruses, plasmids, bacteria, fungi, and animals could now be engineered using recombinant DNA gene-splicing techniques to alter an organism's genetic properties. These data and information in the wrong hands are prone to abuse for the creation of genetically designed microorganisms that are intolerant to antimicrobials and vaccines and increase virulence suited for bioweapons. With these data, it would be feasible to make sicknesses utilizing create agents that could clear out a whole populace. Designer gene is one of the greatest breakthroughs in biotechnology, as adopted strategies are refined [8]. Despite the advantages of this biotechnology, the downsides cannot be ignored because quality can be customized into an irresistible expression that could undoubtedly be changed into a biological weapon [8].

**III. DNA/multigene Shuffling:** *In-vitro* atomic development encouraged research on the effectiveness with which a wide variety of genetic successions can be determined. The capacity to create new DNA successions was an aid to deliver enormous cascades of DNA that are exposed to evaluation or determination for the scope of wanted characteristics. The technique aided antibiotic production from bacteria and other microorganisms [9].

**IV. Gene Therapy:** a very important concept in treating patients with genetic abnormalities. Transfer of healthy genes requires a vector, usually a virus that is modified which predisposes the technology to abuse. Gene therapy was utilized in

animal and human clinical preliminary examinations with promising outcomes [10]. Modification and enhancement will continue as the technology gain more acceptance and could be adopted for creating bioweapon [8].

**V. Stealth Viruses:** the introduction of a viral agent into the body system using a vector. The viral agent is dormant until set off by an inward or outward trigger for it to cause disease or harm to the target host. With this innovation, a malignant (cancer) growth causing the embedded infection into humans made lethargic until set off to trigger the disease condition. Whenever the sign is initiated, the cells become unusual and could quickly produce strange cell development prompting growth and eventual death. Stealth viruses can become a candidate bioweapon [11].

**VI. Host Swapping Diseases:** Instances abound where a pathogenic agent peculiar to a reservoir or host switches to another host. They become resistant and deadly, creating a new emerging threat. Disruption of the harmony between the host and resident pathogen could create viruses that are destructive or harmless, and this occurs when a virus leaps out and move to an alternate host animal where it can make or become new viruses by transforming or getting different genes unintentionally [11].

#### **BIOMARKERS AS RELEVANT TOOL FOR EVALUATING EFFECTS OF BIOTERRORISM**

Biomarkers are evaluated based on their roles as descriptors of the measurement of biological systems [12]. Biomarkers are tools that can widen our understanding of prediction, cause, diagnosis, pathogenic processes, or pharmacological responses to medication. For a biomarker to be useful in the identification of biological agents, it should be easily obtainable from blood, saliva, urine, or any other body fluid or tissue. The biomarker test should be fast, and results should be available within minutes. Another factor considered is the method of detection, which should be accurate and easy to carry out. Biomarkers for the detection of bioterrorism agents should be sensitive and specific to the organism in question. It should be consistent between different strata and environments. Biomarkers are organic particles found in blood and body liquids or tissues, resulting from typical or unusual interactions,

conditions, or illnesses [13,14]. Biomarkers are grouped based on the suitability of the classifying body and applicability. Accordingly, any phase from the beginning to recuperation or severity of illness is fitting to a particular distinguishing marker.

**I. Diagnostic Biomarker:** These classes of markers affirm the presence of an illness or ailment. These detect specific biomolecules related to a specific disease. They aid in the identification of people with a type of disease. An example is a rheumatoid factor in serum as a diagnostic marker to diagnose and differentiate rheumatoid arthritis from other types of arthritis. Beyond the vital role of diagnostic tools, these markers are useful in prognosis and prediction of the outcome of treatment [15].

**II. Monitoring Biomarkers:** These evaluate the presence, status, or degree of sickness or ailment [16]. They assess the impact of clinical or environmental agent exposure in a targeted host. It is adopted in a known disease condition to monitor the effect of medical intervention. This biomarker overlapped with other types of biomarkers. It also surveys the restorative reaction by contrasting the progressions in biomarker articulation or fixation when treatment has been administered.

**III. Pharmacodynamic/Response Biomarkers:** They verify and evaluate the dosing regimen, checking whether a medication follows up on its key objective. They are also referred to as drug activity biomarkers whose role is to measure the effect of the therapeutic agents. They are classified as efficacy biomarkers, mechanism biomarkers, and toxicity biomarkers, which all indicate therapeutic effect, mechanism of action of drugs, and toxicological effect, respectively [16]. Response biomarkers assume a fundamental part in clinical preliminary decisions taking processes giving pertinent data about the clinical advantage of the medication required.

**IV. Predictive Biomarkers:** These identify susceptible individuals based on associated risk factors.

**V. Prognostic Biomarkers:** These biomarkers show disease progression without drug intervention. Prognostic markers help to foresee the event of clinical occasion like death, sickness movement, infection repeat, or development of another ailment [17].

**VI. Susceptibility or Risk Biomarkers:** The primary contrast between these classes of markers is the way susceptibility/risk markers estimate in people without introducing sickness. In this manner, these markers identify well before the presence of illness and are not valuable to portray the reaction to a particular therapy [18]. The choice of biomarkers for determining the effects of terrorism is based on the following criteria:

- Providing reliable and consistent outcomes or results
- Obtained results must be accurate and dependable
- Markers should be sensitive at very low concentrations
- The method and results should be reproducible
- Ease of sampling
- The test/marker should be reproducible

#### **EPIDEMIOLOGICAL IMPORTANCE OF BIOMARKERS**

The utilization of biomarkers is the key in epidemiological examinations because the data are used to foresee the advancement of sickness and to carry out infection control programs. Biomarkers estimate reactions taking place in human and animal hosts due to the introduction of environmental or manipulated stress inducers. In environmental epidemiology, disease transmission is monitored and tracked using markers, which address subclinical/reversible changes.

Appropriately, biomarkers in the epidemiology of disease transmission are going through a fast turn of events and development and are becoming one of the most encouraging areas of environmental examination [19]. Biomarkers can signal the impact of therapy on diet, confirm the presence of sickness, and determine how an infection might start in a singular case, no matter what the sort of therapy (prognostic marker). Among the many limitations of biomarkers is that only microorganisms and toxins are translated into weapons to induce fear and threat [20].

Mitigating biological threats requires the adoption of proficient preventive measures with quick and precise techniques to identify the threat/agent amongst environmental samples in the targeted location [21]. The result of the challenge of separating agents from environmental and clinical specimens is the need to design probes for specific biomarkers to remove the burden of

large sampling sizes and produce biomarkers that are reproducible and specific to bioterrorism agents [22,23]. In the event of a bioterrorism attack, the first and most important step is to identify the agent used for the attack. Closely, it is followed by epidemiologic surveillance, management of the affected individuals, and prevention of attack. Biomarkers are very important in epidemiology when they relate to disease distribution and risk determination. It is a mediator of disease and could be targeted to prevent and treat diseases, which is very important in epidemiology [24]. The epidemiological study design usually assists in identifying and characterizing the bioterrorism trends.

#### **TYPING OF AGENTS OF BIOTERRORISM**

Diagnosis to determine agents of bioterrorism involves the use of throughput technology. Typing techniques depend on certain features when special machines investigation, for example, metabolites are adopted. A technique or method for evaluating a type of system should possess the following characteristics. I) Typeable: ability to obtain a clear positive result for the isolates tested. II) The typing tests must give repeatable, unambiguous, clear findings that are simple to understand. III) Reproducible: capacity to provide the same result when the same strain is tested or when the same strain is repeatedly tested. IV) High power of discrimination: distinguishing between unrelated strains. V) Ease of use: a typing method should be extensively helpful and broadly relevant to a variety of microorganisms, as well as simple to conduct and readily available (inexpensive). Typing methods employed in the diagnostic laboratory differ and are classified as follows.

##### **I. Phenotypic Typing Methods**

These methods are for detecting qualities that a microbe exhibits or expresses. The parameters include biochemical properties, size, staining qualities, shape, and antigenic properties are all phenotypic characteristics that are independent of the genome. Phenotyping is sensitive to environmental changes in an isolate. Phenotyping group organisms based on their similar characteristics. Common phenotypic methods employed are:

- a. **Multilocus Enzyme Electrophoresis (MLEE):** evaluates differences in electro-

phoretic motilities of a collection of metabolic enzymes in isolates. It is simple to use and offers a high level of repeatability. Most strains are classified using this technique though it has limited discriminating power.

- b. **Serotyping:** it gives reliable, reproducible results when employed. Stable testing conditions and preparation methods are very important. Commonly used serological techniques are the complement fixation test, serum agglutination test, and Rose Bengal test [25]. Serotyping is easy to replicate and interpret, as the majority of strains are typeable. Some serotyping methods are difficult to master, and some strains that are autoagglutinable (rough) are untypeable because of the vast number of serotypes and antigen cross-reactions. It has limited discriminatory power [26].

##### **II. Genotypic Typing Methods**

These are procedures adopted to determine and evaluate the composition and homology of DNA samples. The techniques are also useful in identifying the presence or absence of target genes and plasmids in DNA samples. Genotypic typing technique measure disparity in bacterial isolates genetic makeup of an individual organism by matching it with another organism sequence. This method shows different alleles a human being inherits from their parents.

- a. **Nucleotide Sequence Analysis** - DNA (or RNA) nucleotide-base sequences are for determining genotypic information about an organism. PCR DNA-based methods are species-specific techniques that utilize primers from specific polymorphic sections on the DNA. Sequencing of RNAs is either by converting them to DNA or by sequencing the DNA gene that produced the RNA. The results are repeatable and easy to interpret, and the technique works on any strain, though it is labor-intensive and costly.
- b. **Southern blot analysis of RFLPs** - Southern blot assays, unlike restriction endonuclease analysis (REA) of DNA, only identifies a single restriction fragment. The endonuclease digests the DNA, and gel electrophoresis is used to separate the fragments, which are then transferred to

nitrocellulose membranes. Labeled DNA probes detect the fragments carrying certain sequences. If the DNA sequences of organisms exhibit less than 98 percent homology, it is classified as distinct species, and if there is less than 93 percent identity between the sequences, they are classified as different genera. The 16SrRNA gene is extremely valuable due to its high conservation. They are repeatable, easy to read, and typeable for all strains. It is expensive, labor-intensive and the discriminatory power dependent on the probes used.

- c. **Multiplex PCR typing** – to identify species and biovar levels exploiting polymorphism that arises from species-specific localization of the genetic element. Multiplex PCR simultaneously amplifies multiple primer sets of different targets in a single reaction tube to obtain amplicons with different DNA sequences [27]. The technique targets more than one specific DNA sequence in an isolate from the resulting amplicons obtained from the PCR mixture. The design of the primers is specific to the isolate and determines the success of the multiplex PCR co-amplification.
- d. **RAPD-PCR (Random Amplified Polymorphic DNA)** - RAPD uses primers composed of short sequences (oligonucleotides) to identify complementary sites on the genome of the target DNA. The procedure is easy. The nucleotides (size range of 8 to 15 in length) primers hybridize to multiple regions in an organism's chromosomal DNA, thus aiding rapid detection of genomic polymorphism. It has poor reproducibility from place to place. It has low stringency that allows the oligonucleotides to anneal and eventually give rise to heterogeneous DNA products that are strain-specific [28].
- e. **Real-time PCR** - known also as quantitative PCR, is a rapid and sensitive method for detecting, quantifying, and typing microorganisms. It reduces incidences of contamination and false-positive result [29]. It amplifies and identifies the target sequence; as the reaction mix is run using interacting fluorescent dye or fluorescence-labeled probe. It has the capacity of detecting point

mutations and high throughput while reducing contamination. The machine is costly and requires well-trained personnel.

- f. **Restriction Fragment Length Polymorphism (RFLP)** - The technique identifies by analyzing specific variations in the DNA molecules in a chromosome. It uses restriction endonucleases, which target restriction sites on the DNA and cleaving to them. The method helps in differentiating specific strains, diversity, and relatedness of microorganisms using the pattern of DNA fragments [30]. It is easy to use, has high consistency, and is quick.
- g. **Single nucleotide polymorphisms (SNPs) typing** - it accurately probes the phylogenetic framework of an isolate at the polymorphic region [31]. Single nucleotide polymorphisms (SNPs) are for analyzing single-nucleotide base variation in organisms at the subspecies level. SNP are base pair variations that can alter a nucleotide by replacing the base pair in a genome; hence, one SNP is a difference in a single nucleotide.
- h. **PFGE TYPING (Pulsed-field gel electrophoresis)** - It is a discriminating and repeatable typing technique commonly used for characterizing bacterial isolates in outbreaks. It is a low-cost method with high type ability and intra-laboratory reproducibility. It is time-consuming, tedious, and does not give a good resolution of bands [32].

#### **CHALLENGES OF BIOTERRORISM AGENTS TO PUBLIC HEALTH**

Bioterrorism constitutes a major threat to public health worldwide. In the natural setting, infectious diseases are one of the leading causes of morbidity and mortality both in humans and animals [33]. Consequently, the intentional release of highly virulent pathogenic microorganisms to cause disease and death within the human, animal, and plant populations is of great concern that calls for concerted efforts and deliberate preparedness [34]. The impact of bioterrorism spreads across all spheres of human endeavors. When outbreaks of known or unknown diseases occur within a defined geographical location, it is pertinent to investigate the source, as it could be the initial stage of a bioweapon attack [35]. The adoption of biological

agents as a weapon is on the ease of obtaining and manipulating them without detection. The technology adopted for mass production of these deadly agents is the same as those used in the mass production of other medical and household everyday substances for human use and survival.

The limitation of biomarkers in forestalling bioterrorism in a population is that bioterrorism agents are not easily distinguishable from normal biological microbes causing infection. Anytime there is an attack, it takes some time to design the probes and develop specific biomarkers during which casualties would have been recorded. The cost implication of producing biomarkers and huge sampling size constitute issues as they lead to incurring huge expenses. Another factor limiting the use of biomarkers relates to acceptability. Acceptability borders are on ethical considerations, beliefs, and convictions of a people and the target human host. The Covid vaccine rejection by different religious sects is a ready example. The incubation period and the inception of symptoms provide a terrorist with the window period of escape [36]. It necessitates high throughput technology and early warning and rapid detection systems that can detect aerosolized bio-agents as early as possible [37].

Bioterrorism agents are transmitted through water, air, or food, which poses a challenge as they cannot easily be detected, consequently causing illness after the initial exposure until too late [38]. Inadequate training of the first responders and other healthcare workers to recognize and react to diseases caused by biological agents is another challenge. Unpreparedness by different first responder agencies was charged with taking action when bioterrorism acts take place. These agencies sometimes lack improved detection and data gathering equipment and cannot provide contingencies when it matters most. From a public health perspective, it is vital for timely surveillance, media awareness, and publicity.

#### **PUBLIC HEALTH COUNTER-TERRORISM RESPONSE**

Counterterrorism is political and military efforts carried out or aimed to prevent or deter terrorist acts. It encompasses law enforcement and intelligence agencies that employ techniques, policies, and strategies to combat terrorism. Responding immediately to biological weapon strikes in combatting terrorism and biological

warfare is critical in protecting life against lethal disease outbreaks. Surveillance of infectious diseases is a major task of any public health institution [35]. Hence, steps employed by public health agencies to prevent emerging infectious diseases are applicable in the prevention of bioterrorism agents. An effective surveillance system such as the syndrome surveillance requires an effective communication system, and adequate epidemiological and laboratory provisions to give timely discovery of outbreaks by exploring existing health data to alert public health agencies [35,39]. Local and state-level developmental plans (such as immunization) against bioterrorism agents will help in saving lives and reduce costs [40]. As part of readiness to counter bioterrorism, health departments will require up-to-date laboratory facilities and competent health workers.

To address the bioterrorism challenge from a public health perspective, it is needful to adopt a broad-based approach and tackle the problem from its root. Bioterrorism is a threat to our corporate existence, and therefore all hands must be on deck to counter the menace. It is pertinent to analyze the social determinant associated with terrorism, such as the high rate of joblessness, political isolation, poverty, incorrect philosophy, and inequality. These are factors driving young people into crime. Hence, the provision of a meaningful standard of living to the growing youth population is fundamental to avoiding radicalization and the tendency to become victims of social vices. Therefore, there must be global concerted efforts through a partnership among public health experts, law enforcement agencies, and redirection of government policy towards the universal basic income concept/proposal to lift many out of poverty, promote human dignity and avert the evil consequences of bioterrorism acts [41]. Furthermore, to counter bioterrorism, it is pertinent to invest more in public health infrastructure to aid in rapid detection and prompt diagnosis of agents of bioterrorism [36,42].

#### **CONCLUSION**

Activities of terrorist groups are increasing all over the world with ferocious sophistication in their methods of operation. Countries with civil wars reportedly use chemicals as a means of putting out perceived sovereign enemies. The threat of the use of biological agents by terrorists is real and



concerted efforts at frustrating it needed be put in place. Considering the advantages these agents possess, managing them, storing and transporting them make it easy to be deployed at the least expected of places with ease. To this end, the best method of preventing serious carnage and destruction is to adequately educate the first-line responders in the health sector in communities, and secondly, the people. Training on what to do should be paramount while the government provides a conducive environment that can deter people from joining terrorist groups.

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