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New Molecular Methods for Detection of Bioterorism Agents

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Abstract
In this study we reviwed the newly and featured molecular methods which are increasingly used in bioterrorism surveillance. Some cornerstone devolopments in the detection of bioterrorizm agent are; diversities of mainly emergent disease agent, phage engineering as an effective means to detect or eliminate biological agents, PCR and pre-PCR processing which an important tool for surveillance, biological agent detection kits, mass spectrometric detection and Metagenomics which the genetics subfield studying the combined genomes of a sample are now mainly quickly growing fields.

Key words: **Bioterrorism, disease detection, Metagenomics, biothreat agents.**

INTRODUCTION

Bioterrorism is a term used for terrorism incident using biological agents, such as pathogen microorganizms and biological toxins such as ricin or botulinum. Bioterrorism outbreak works like a natural outbreak and we cannot foretell which disease may spread and when it will begin. Recently we have seen that *Ebola* outbreaks cases first confirmed in Sierra Leone following an outbreak in Guinea, unfortunately there is no cure or vaccine for Ebola which one of the world's deadliest viruses. MERS outbreaks also threatened the human beings in Middle East and The bad side of the situation there is no any treatment for these disease too. İn the MERS outbreaks camels are thought to be the main carriers of the virus that has killed at least 200 people mainly in the world, but researchers now believe other animals could also be spreading the infection. We must learn to live with this indefiniteness and found ways to protect ourselves against any disease outbreaks. In the field of diagnostic microbiology, rapid molecular methods are critically important for detecting pathogens. With rapid and accurate detection, preventive measures can be put in place early, thereby preventing lifes and further spread of any disease. For this necessity we have reviewed featured melecular methods devolopments in bioterrorism studies.

Some Cornerstone Devolopments in Detection of Bioterrorizm Agent

Agent diversity works

When we look at the prominent studies, it is thought that *Bacillus anthracis*, the ethiological agent of anthrax which relatively common throughout the world, can be used as an agent of bioterrorism. For naturally occurring outbreaks senarios or criminal release of this pathogen a fast and accurate diagnosis is crucial to an effective response and threat mitigation [1]. Microbiological forensic studies and epidemiologic investigations progressively using molecular markers, such as polymorphisms in DNA sequence, to obtain reliable information regarding the identification or source of a suspicious strain. Recently significant research efforts have been undertaken to develop genotyping methods with increased power to differentiate *B. anthracis* or other emergent strains. A growing number of DNA markers have been identified and used to survey any biological agent's diversity in the world, leading to rapid advances in our understanding about the pathogens [1,2,3].

Phage engineering as an effective means to detect and eradicate threats

Microorganisms can cause widespread desease otbreaks and some of those can be used as agents of bioterrorism. From a biosecurity standpoint, the capacity to detect become key challenges [4]. scientist turned to phage engineering as a potentially highly effective means to both detect and mitigation of threats originating from emergent

bacterial strains [4]. For example they developed technologies allowing us to modify multiple regions of the sequence in a gene while conserving intact the remain, so reversibly interrupt the lytic cycle of T4 within its host. They carry out efficient insertion with homologous recombination and reactivate the lytic cycle and this lead to the production of engineered infective virulent recombinant progeny. This allows the production of very large, genetically engineered lytic phage banks. Screening of such a bank may allow the rapid isolation of recombinant T4 particles capable of detecting, infecting, and destroying hosts bacteria [4,5,6].

PCR processing

Diagnostic DNA analysis using polymerase chain reaction (PCR) has become a valuable tool for rapid detection of biothreat agents. However, analysis is often challenging because of the limited size, quality, and purity of the biological target [7]. Pre-PCR processing is an integrated concept. sampling methods must maximize target uptake and minimize uptake of extraneous substances that could impair the analysis with PCR inhibitors which must be known [7]. In sample treatment extensive purification leads to lossing of DNA. A cornerstone of pre-PCR processing is to apply DNA polymerase-buffer systems that should tolerant to specific sample impurities, so lowering the need for costly purification steps and maximizing DNA we have. İneffective sample processing give way to costly and ambiguous results. This stuation may hinder the decision process in a bioterrorism crisis. Quality control methods for pre-PCR processing to simplify the analysis of various biothreat agents is important [7-13]. Several quantitative PCR assays also have been developed for biopreparedness which targeting different agents. this methods employ new detection and identification principles. In one, the dissociation pattern of the DNA markers generated with universal primers studied through high-resolution melting and enabled the differentiation of 16S rRNA gene from different microorganisms, so identify 100 relevant bioterrorism agents [22-26)].

Detection kits

Many different methods based on both immuno assays and PCR-based detection systems have been developed to help detect toxins. Detection kits are commercially available for the detection of different toxins in suspicious samples. While reports does not evaluate the performance of commercially available tests. Such as ricin that is extracted from seeds of the *Ricinus communis* is the most encountered biothreat agent. The seeds yields a highly toxic product that has been used as threats to commuity. We have rapid detection assays such as lateral flow assays (LFAs). But we have complicating factors associated with LFAs. Such as the incorporation of antibodies of poor specificity that cross-react with near-neighbors or lectins which are cause nonspecific cross-linking for the antibodies [14,15].

Mass spectrometric detection

Mass spectrometric detection of protein-based toxins such as botulinum neurotoxin, anthrax toxins and ricin which are among the most toxic substances is important. They are composed of 2 polypeptide chains responsible for cell uptake and enzymatic function with the ability to destroy basic cellular functions [16]. Traditionally, large molecules like proteins have been detected using immunological techniques. Although sensitive, these methods suffer from some drawbacks, such as the risk of false-positives due to crossreactions and detection of inactive toxin. İn Recent years, instrumental methods based on mass spectrometry for the reliable detection of botulinum neurotoxins, anthrax toxins and ricin developed. İdentification of a protein based toxin can be carried out by mass spectrometry based amino acid sequencing. Furthermore, in combination with antibody affinity preconcentration and biochemical tests with mass spectrometric detection demonstrating the toxin's enzymatic activity, very powerful analytical methods have been described. The advent of sensitive, easily operated mass spectrometers provides new possibilities for the detection of protein-based toxins[16]. The use of synthetic positive control templates containing small modifications outside the primer and probe regions is essential to ensure all aspects of the assay are functioning right. But, a general PCR assay is suffer from differentiating products which generated from positive controls and biological samples because the fluorescent probe signals generated from each type of amplicon are similar. To solve this problem scientists also have developed a new application of electrospray ionization mass spectrometry to rapidly differentiate qPCR amplicons generated with positive biological samples from those generated with synthetic positive controls. The method supports sure determination of the presence of a biothreat agent [16-18].

Metagenomics

It is important to quickly detect and confirm the specific infection for effective outbreak control. İt is estimated that a one week delay in implementing control measures for a respiratory syndrome resulted in a 2.6 fold increase in the epidemic size and an extension of one month. The traditional culture based, immunologic and nucleic acid based methods suffer from drawbacks when dealing with new organisms. There are no culture based methods for all organisms and sometimes methods have poor specificity also. Immunological and nucleic acid based methods have a high specificity, so unable to detect all organisms. But in metagenomics the combined genomes of all organisms present in a sample are analyzed that requires no prior knowledge of the target also. This can be of crucial importance when encountering a new target, as it removes the initial delay caused by identification process. Metagenomics is a growing field in mapping interactions in a microbial community and you aren't need for culturing [19-20].

RESULT and DISCUSSION

Not only anthrax, plaque, botilinum toxins or ricin which we are accustomed in the past but also newly emergent deseases such as ebola or MERS can be used for intentionally contamination of the people. In the stuation of bioterror event, rapid molecular methods are critically important for detecting pathogens or toxins. With rapid and accurate detection, preventive measures can be put in place early, thereby preventing loss of life and further spread of any disease. When we look at the prominent studies Over the past decade, significant research efforts have been undertaken to develop genotyping methods to differentiate *B. anthracis* strains which was used in US Following the terrorist attacks of September 11 and the anthrax attacks in 2001. Scientist survey B. anthracis diversity in nature, leading to rapid advances in our understanding of the global population of this pathogen [1,2,3]. Phage

engineering as a effective means to detect and eradicate threats is a newly introduced field that we could benefit future in mitigation of bioterrorist event's potential devastating results [4,5,6]. Diagnostic DNA analysis using PCR has become a valuable tool for rapid detection of biothreat agents. We now can use this commonly used tool but when it comes to this bioterrorism topic this tool must be more portable and fast. Because they will not have time to wait a few days long labratuary process [7]. Many different methods based on both immuno assays and PCR based detection systems have been developed and evaluated to help detect toxins and diagnose toxin poisoning in patients but they should devolop more. The sampling method should maximize target uptake and minimize uptake of extraneous substances that could impair the analysis [7-13]. Detection kits have also been developed and are commercially available for the detection of bioterrorist agents. Mass spectrometric detection of toxins is also important introduction. İt especially useful for proteins so may be used for the bacterial toxins such as botulinum and anthrax as well as the plant toxin ricin which are all protein based toxins [14,15]. Metagenomics gaining importance in studying the combined genomes of a sample and their interaction. İt does offer a rapid high throughput method for mapping interactions in a microbial community and circumvents the need for culturing of an agent in a bioterrorism event survey. The use of wholegenome sequencing techniques deserves attention, as it will be an important tool in future bioterrorism and also normal surveillance research. Whole-genome sequencing can be used to detect any microorganism in a sample with one analysis, thus making it truly a technique to detect the unexpected agents[19,20]. It can be foreseen that the introduction of whole-genome sequencing, not only in the field of bioterrorism, but also in the fields of public human health and animal health or as a diagnostic tool used by hospitals and general practitioners, will greatly advance treatment of infectious diseases and human health [21].

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