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Genetic Engineering and Biosecurity: An Analysis of Literature and Anticipated Future Developments

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Annotation: The discovery of DNA as the fundamental material for heredity and biology has led to the development of various techniques for manipulating genetic material, allowing for the modification of organisms to benefit society and provide essential materials in the agricultural, industrial, and medical sectors. The emergence of CRISPR technology has further facilitated genetic modification, leading to significant advancements. However, these rapid developments in genetic engineering have raised concerns regarding the potential misuse of biotechnology for the production of biological weapons. Consequently, there has been a notable emphasis on developing effective detection methods for such weapons, culminating in the establishment of international agreements, such as the Geneva Convention, aimed at preventing the use and proliferation of biological weapons.

Keywords: Biological Weapons, Geneva Protocol, Biological And Toxin Weapons Convention, Synthetic Biology (Synbio).

1. INTRODUCTION:

Throughout the discovery of DNA as the fundamental unit of heredity and the elucidation of its role in molecular biology, the scientific community has devoted significant effort to comprehending the mechanisms through which DNA governs heredity[1]. The advent of molecular biology tools, such as restriction endonucleases, DNA sequencing, and DNA cloning, spurred inquiries into the manipulation of chromosomal DNA in cells. Genetic engineering encompasses a spectrum of targeted techniques employed to modify organisms with the specific objective of generating chemicals that the organism does not naturally produce, while also enhancing existing biological processes[2]. The process typically entails isolating the target DNA fragment or gene from a donor organism, subjecting it to a series of laboratory manipulations, and subsequently introducing it into a genetic vector for transfer into the recipient strain. The methods of gene transfer vary depending on the type of organism and can be categorized as viral and non-viral techniques. Transformation, transfection, transduction, and conjugation represent common approaches for gene and DNA transfer, each tailored to specific organisms[3]. Consequently, it is imperative to discern between cells that have undergone gene transfer and those that have not, as no gene transfer method can universally alter every cell in a population. Genetic engineering finds applicability in diverse domains such as medicine, research, industry, and agriculture, catering to a broad spectrum of plants, animals, and microorganisms. The initial genetic manipulation of bacteria facilitated the incorporation of plasmid DNA-carrying genes for synthesizing drugs or essential enzymes. The versatility of genetically engineered bacteria extends to applications encompassing biofuel production, remediation of oil spills and hazardous waste, as well as detection of contaminants in drinking water[4]. Furthermore, plants undergo genetic modification to acquire traits such as insect and herbicide resistance, increased nutritional value, and the ability to produce edible vaccines.The emergence of CRISPR, a genetic engineering tool that modifies DNA sequences in prokaryotes, has substantially impacted the field. Derived from DNA fragments of bacteriophages, CRISPR sequences play a pivotal role in prokaryotic defense against viruses[5]. Despite its wide-ranging applications, genetic engineering necessitates adherence to bioethical principles, especially in light of ethical and safety concerns surrounding the technology. Notably, genetic engineering has raised significant apprehensions due to its potential to alter the genetic makeup of organisms, prompting deliberation on the ethical implications[6]. Biological weapons, comprising pathogenic organisms and other biological substances, have garnered attention as potential agents of harm[7]. The convergence of computer science, engineering, life sciences, and chemistry has facilitated the manipulation of living systems, leading to not only positive developments in the biotechnology and biopharmaceutical industries, but also the potential for malicious applications[8]. Synthetic biology, since the identification of DNA in the 1950s, has made substantial progress in modifying and engineering biological systems, reducing the time and cost required for the development of biological weapons. The modular nature of genetic material in organisms facilitates rapid adjustments to environmental changes, enabling targeted gene removal and incorporation of foreign genes into host organisms[9][10]. This modularity forms the basis of ongoing research in the realms of biosecurity and military medicine, aimed at developing a "molecular toolkit" for designing and producing biological agents[11].

2. TYPES OF BIOLOGICAL WEAPONS

2.1 Anthrax

Anthrax represents an infectious condition with zoonotic potential, caused by the bacterium Bacillus anthracis. Predominantly, approximately 95% of anthrax cases manifest as cutaneous infections. Inhalational anthrax, by nature, presents as highly fatal, with symptoms typically emerging several days subsequent to exposure. Patients may exhibit manifestations related to the

integumentary, respiratory, or gastrointestinal systems[12]. Exposure may result from direct contact with infected animals, manipulation of contaminated animal products such as wool, hair, or hides, inhalation, or consumption of tainted meat. Cutaneous anthrax typically presents 2-5 days post-exposure, characterized by the formation of lesions on the infected skin. Oropharyngeal anthrax, marked by neck enlargement and elevated body temperature, occurs in the presence of oral lesions or wounds. Intestinal anthrax features nonspecific abdominal pain with fever and may include symptoms such as nausea, vomiting, malaise, anorexia, hematemesis, bloody diarrhea, and/or dysentery. Inhalational anthrax emerges abruptly 1-3 days post-exposure and follows a biphasic pattern[13].

2.2 Botulinum toxins

The botulinum toxin is a neurotoxin produced by Clostridium botulinum, an anaerobic bacterium that exhibits a positive response to the Gram stain. This bacterium is prevalent in various environments including soil, aquatic habitats, and the gastrointestinal tracts of animals. Initially recognized for its efficacy in addressing strabismus in humans, the application of this neurotoxin has since gained approval for the management of spasticity and other medical conditions[14]. Botulinum toxin is produced as inactive, single polypeptide chains with a molecular weight of approximately 150 kDa. Its mechanism involves interference with neuronal signaling by inhibiting the release of acetylcholine, the principal neurotransmitter at the neuromuscular junction. Intramuscular administration of botulinum toxin induces muscle paralysis by impeding acetylcholine release from presynaptic motor neurons. The toxin selectively and irreversibly binds to high-affinity receptors located on the presynaptic membrane of cholinergic neurons. This toxin-receptor complex is subsequently internalized via endocytosis. By blocking the transmission of alpha motor neurons at the neuromuscular junction, the toxin weakens skeletal muscles, making it beneficial for conditions characterized by excessive muscle activity, such as dystonia. The onset of its effect requires a timeframe of 24-72 hours, and its impact typically persists for 8-12 weeks[15].

2.3 Cholera

Cholera toxin, originating from Vibrio cholerae, is a potent virulence factor that leads to severe diarrhea and dehydration in the human body[16]. This toxin is categorized under the AB5– subunit family and consists of one A subunit responsible for ADP-ribosylation, and five B subunits that bind to cell surface receptors, enabling transmembrane transport. Cholera toxin, along with its pure A component, is employed for investigating signal transduction pathways and functions as an adjuvant by stimulating B cells[17]. The B subunit, which lacks inherent adenylate cyclase activity, interacts with cells through its binding to ganglioside GM1.8, making it an effective marker for microglial cells, but not for oligodendrocytes or astrocytes. Furthermore, the B subunit serves as a highly efficient tracer for studying axonal transport using immunohistochemical techniques, and is commonly utilized as an indicator of membrane lipid rafts associated with cell signaling and protein trafficking [18].

2.4 Clostridium perfringens

Clostridium perfringens, a gram-positive bacillus, is associated with severe gastrointestinal ailments such as diarrhea, necrotizing enterocolitis, and myonecrosis. Its pathophysiology involves tissue necrosis mediated by toxins, pore-forming toxins, and glucose fermentation, resulting in cellular swelling and eventual death[19].

2.5 Q fever

Q fever, a zoonotic disease, is primarily found in cattle, sheep, and goats. Humans are mainly exposed to the disease through the inhalation of aerosolized particles containing the causative bacteria, which are released by infected animals. The prevalence of Q fever varies across countries due to differences in epidemiological factors and reporting practices. In regions where the disease is endemic, Q fever typically presents as sporadic cases, often associated with high-

risk activities such as agricultural work or rural tourism[20].

2.6 Ricin is a toxic substance

Ricin, classified as a Type II ribosome-inactivating protein (RIP), is composed of multiple protein chains interconnected by a disulfide bond. Its toxicity stems from the cleavage of the N-glycosidic bond of an adenosine residue present in the ribosomal RNA of eukaryotic cells, thereby impeding protein synthesis [21]. Cellular uptake of ricin occurs through receptor-mediated and adsorptive-mediated endocytosis, vesicular transport, and involvement of the Golgi apparatus. Calreticulin, a chaperone protein, facilitates the transportation of ricin to the endoplasmic reticulum, where it undergoes partial unfolding and traverses the membrane via the Sec61p translocon[22].

2.7 Rift Valley fever

Rift Valley fever (RVF) is a zoonotic disease transmitted by vectors and caused by a phlebovirus belonging to the Phenuiviridae family. Its initial identification dates back to 1931, during an epidemic in the Rift Valley region of Kenya. The disease is characterized by a high incidence of miscarriages in pregnant ewes and sudden mortality of newborn lambs[23].

RVF virus (RVFV) is classified as a phlebovirus under the order Bunyavirales and the family Phenuiviridae. Its genome consists of three segments: a short (S) segment, a medium (M) segment, and a large (L) segment, all comprising single-stranded RNA with negative or ambisense polarity. The S segment encodes the nucleoprotein (N), while its anti-genomic RNA encodes the non-structural NSs protein, which significantly affects virulence. The M segment contains genetic information for the synthesis of glycoproteins precursor Gc and Gn, as well as the nonstructural proteins NSm and the proteins P78, P14, and P13. The L segment contains genetic information for the viral RNA-dependent RNA polymerase (RdRp) [24].

2.8 Smallpox

Smallpox, classified as a member of the poxvirus family, specifically the orthopoxvirus genus, is caused by the variola virus. This virus possesses a unique genome comprising double-stranded DNA, which encodes the necessary proteins for its replication within the cytoplasm of host cells [25].

3. UTILIZING GENETIC ENGINEERING TO CREATE BIOLOGICAL WEAPONS

The convergence of advancing DNA synthesis capabilities, computational power, and information accessibility is poised to enable a wider pool of individuals to potentially engage in the production of bioweapons. Notably, the conversion of DNA nucleotides (adenine, cytosine, guanine, and thymine) into a binary code of ones and zeroes signifies a pivotal shift in genetic engineering, rendering it a process of electrical manipulation and consequently reducing the associated costs. This transformation has the potential to democratize the manufacturing of bioweapons, as it simplifies the process through the use of easily obtainable viruses, affordable equipment, and knowledge of chemistry and biology commonly taught at the college level [26].

1. Binary biological weapons entail the incorporation of plasmids, small fragments of bacterial DNA, into the DNA of diverse bacteria, with the objective of amplifying the potency or other pathogenic characteristics of the host bacterium [27].

2. The European Bioinformatics Institute has cataloged the genetic sequences of 3139 viruses, 1016 plasmids, and 2167 bacteria, a number of which have been publicly disseminated on the internet and are thus accessible to the general public [28]. Leveraging the current access to complete genomes and the progress in gene synthesis, scientists are nearing the capacity to manipulate diseases by creating artificial genes, synthetic viruses, and even entirely new organisms [27].

3. Gene therapy, involving the permanent modification of an organism's genetic composition by repairing or replacing a specific gene, holds potential for the development of bioweapons through the substitution of harmful genes with existing ones [27].

4. Concealed viruses, presenting as viral infections that lay dormant within cells until externally activated to induce illness, harbor the capacity for wide dissemination across populations. Deliberate postponement of their activation could be deployed as a coercive or blackmail tactic in the context of warfare [27].

5. Prospects for customized bioweapons include the development of a pathogen capable of selectively targeting an individual's unique genetic sequence. Such a disease could propagate within groups with minimal or imperceptible symptoms while posing a fatal threat to the specific individual it targets [25].

In 1997, the JASON group underscored six emerging biological threats warranting vigilant surveillance with respect to biological warfare, encompassing binary weapons, designer genes, gene therapy, viral evasion, virus mobility, and designer diseases. The advent of advanced synthetic biology techniques has markedly increased the likelihood of encountering one or more of these perils to an almost inevitable degree [11]. The field of synthetic biology, also known as SynBio, has revolutionized molecular engineering by empowering scientists to concoct synthetic organisms boasting precise biochemical traits. The successful chemical synthesis of the entire poliovirus genome by the State University of New York at Stony Brook in 2002 epitomizes the transformative potential of synthetic biology [10]. These endeavors have been facilitated by advancements in molecular engineering techniques [9], enabling insight into complex and interconnected biochemical reactions constituting vital biological metabolism. Deliberate acquisition of specific biological traits in organisms has been successfully achieved by coupling conventional molecular and cellular laboratory methods with cellular selection techniques. Notably, in 2005, scientists reconstructed the 1918 pandemic influenza virus, demonstrating the feasibility of crafting a disease-causing agent using the modular structure of a viral genome [10]. Subsequently, a Canadian research group, in 2016, achieved the recreation of the contagious horsepox virus by synthesizing its genetic data sourced from a publicly accessible database. In 2017, Lithuanian researchers devised a method aimed at enhancing the transfer of genetically modified sequences in microorganisms. While this innovation can be harnessed for peaceful purposes, it also harbors the potential for exploitation in the development of modified biological weapons, exemplifying the susceptibilities associated with nefarious intent [5]. Projections stipulate that by 2025, bioengineering and molecular technology advancements are slated to revolutionize the provision of vital resources across medical, industrial, and military domains. From a military standpoint, bioengineering has bestowed several critical capabilities, including the deployment of portable biosensors for detecting specific molecules in the immediate surroundings, the production of vaccines to fortify immune responses against diseases in remote or arduous settings, the creation of health sensors for continuous monitoring of soldiers' well-being in the face of chemical or biological threats, and enhancement of soldiers' combat effectiveness and endurance to sustain performance in demanding conditions.

4. THE IMPACTS OF USING BIOLOGICAL WEAPONS

4.1. Health:

The potential use of biological weapons for the purpose of biological warfare or bioterrorism is a matter of increasing concern. An extensive range of microorganisms and toxins suitable for use as biological weapons can be readily obtained and manufactured in large quantities. Dissemination of aerosols containing these biological pathogens could lead to a high number of casualties. If employed by a terrorist group, such weapons possess the capability to overwhelm our existing public health infrastructure. Common choices for potential biological agents include Bacillus anthracis (anthrax) and botulinum toxin. The release of these agents may go unnoticed for an extended period, ranging from a few hours to several days, followed by widespread illness

among the population, necessitating an immediate response from the public health sector. Conducting a timely epidemiological investigation to ascertain the nature of the illness outbreak is imperative to minimize the number of casualties. While medical treatments are available for many biological agents, they may not be effective for all, emphasizing the importance of preparedness and response measures to mitigate the impact of such events [29].

4.2. Biodiversity:

To effectively manage human disease epidemics resulting from plague and tularemia bioweapon attacks, it is essential to consider the potential animal reservoirs and insect vectors once the initial outbreaks among humans have been brought under control. In impacted regions, efforts to eradicate endangered or uncommon species populations may be necessary due to their role as disease reservoirs[30]. As a result, the endangered Stephen's Kangaroo Rat (Dipodomys stephensi) faces a significant threat of extinction because of its limited presence in small, isolated populations within heavily urbanized areas. It is important to highlight that a notable number of endangered and threatened species are now confined to habitats located within or near US military installations and training ranges, which could be targeted in bioweapon assaults. Over 220 species, officially classified as threatened or endangered, are known to inhabit or traverse areas owned by the US military. Despite military lands representing only about 3% of the total area of US federal lands, they play a crucial role in providing significant habitat for endangered plant and animal species[31].

4.3. ECONOMY:

Biological weapons have both immediate and indirect impacts on the economy. Immediate outcomes include increased healthcare costs, heightened demand for medical supplies, and economic setbacks caused by a reduced workforce. Indirect consequences encompass disruptions in commerce due to travel and transit restrictions, the closure of public facilities and tourist attractions, and infrastructure damage [30].

5. PREVENTIVE MEASURES AND SAFEGUARDS

5.1. Methods employed in the detection of biological weapons:

A. Particle sizers are instruments designed to quantify the quantity and size distribution of particles within a specified range, typically around 0.530 μm. The High-Volume Aerodynamic Particle Sizer (HVAPS) is a commonly employed apparatus for this purpose. Its operation entails exposing particles to a continuous, highly concentrated air stream. As the particles traverse the aerosol, they experience differential rates of acceleration based on their size, with smaller particles undergoing greater acceleration. This technology leverages laser-based measurement equipment to attain precise data on particle quantity, dimensions, and morphology. However, it should be noted that this method does not possess the capability to distinguish between biological and non-biological aerosols [31].

B. Fluorescence-based systems harness the properties of naturally occurring fluorophores to detect and differentiate biological organisms through bioluminescence. This technique involves exciting molecular components commonly found in biological substances, such as the aromatic amino acid tryptophan, using light waves, typically in the ultraviolet (UV) range. By leveraging the emission of a commonly available fluorophore, these methods can be applied to detect living organisms in unknown materials without the need for specific targeting. The Fluorescent Aerodynamic Particle Sizer (FLAPS) stands out as the leading device utilizing fluorescence measurement[32].

C. Molecular biology methodologies, notably the polymerase chain reaction (PCR), serve as prevalent tools for amplifying minute quantities of genetic material. This technique facilitates the identification of viable microorganisms such as bacteria, bacterial spores, or viruses, as these agents contain genetic material [33]. A key prerequisite of this approach is the comprehensive

understanding of the target biological entity, as it necessitates the utilization of specific primer sequences for nucleic acid amplification. It is important to note that the majority of PCR reactions are tailored to target a singular agent, except for multiplex PCR, which allows for the concurrent analysis of multiple compounds [34]. PCR can be bolstered by incorporating specific probes as an adjunctive element, enabling the identification of a specific genetic sequence within the specimen through sequence interactions. This methodology is presently widely employed in DNA microarrays [35].

D. Immunoassay technologies enable the detection of biological agents by utilizing the unique interaction between antigens and antibodies, leading to the formation of a detectable complex [36]. These tests typically yield a prompt response, although their sensitivity may vary depending on the sample medium, suspected agent, and specific instrument [34]. Hand-held immunochromatographic assays (HHAs) are single-use kits that leverage the principle of antigen/antibody interaction to produce color-based results. They can provide a combination of qualitative and partially quantitative feedback for a specific chemical. These devices are highly user-friendly and have proven to be crucial during emergency situations such as anthrax outbreaks due to their applicability for screening purposes [37].

5.2. Security measures in biological research centers:

Physical Security Measures:

1. Access Control: Restrict access to laboratories to authorized personnel only. Implement stringent access regulations, including the use of key cards for entry.

2. Surveillance Systems: Utilize closed-circuit television (CCTV) cameras and motion detectors for continual monitoring of designated areas.

3. Secure Storage: Store pathogens and sensitive materials in highly secure containment facilities, such as biosafety cabinets or secure freezers.

Information security:

1. Data encryption involves the application of encryption techniques to protect sensitive data from unauthorized access.

2. Access Control is the practice of restricting access to confidential information based on the principle of need-to-know.

3. Cybersecurity measures include the deployment of firewalls, intrusion detection systems, and regular security audits to mitigate potential cyber threats.

4. Confidentiality Agreements ensure that all personnel are fully informed and agree to maintain the confidentiality of sensitive information [38].

5.3. prevention strategy:

The implementation of preventive measures involves the dissemination of knowledge to scholars and practitioners regarding potential hazards. It also entails involving researchers from academic institutions and industry in endeavors to strengthen the Biological Weapons and Toxin Convention. Prevention efforts also encompass the establishment of protocols to address the appropriate scientific response to research that could be utilized for bioweapons, as well as supporting initiatives aimed at engaging former bioweapons experts in peaceful pursuits. However, current preventive measures are insufficient in ensuring the complete avoidance of biological weapons. Therefore, the infectious disease (ID) community should take the following actions: increasing awareness and providing education to ID professionals; enhancing laboratory diagnostic capabilities; establishing systems for the distribution of therapeutics and assessing hospital responses; and conducting scientific research to develop new strategies for diagnosis and prevention [39].

6. CONVENTIONS AND INTERNATIONAL REGULATIONS REGARDING BIOLOGICAL WEAPONS

6.1. The Geneva Protocol on Biological weapons

The Geneva Gas Protocol, established in 1925 by a majority of nations, is an internationally recognized legal instrument. It prohibits the use of chemical and biological weapons in armed conflict as a measure to prevent the atrocities witnessed during World War I. The Protocol explicitly prohibits the use of bacteriological and other poisonous agents in warfare, but it does not explicitly address the creation, production, or accumulation of such weaponry. Subsequent agreements such as the Chemical Weapons Convention (CWC) of 1993 and the Biological Weapons Convention (BWC) of 1972 further strengthened the prohibitions. While many nations ratified the Protocol before World War II, the United States did not officially approve it until 1975. Notably, during conflicts, some nations, including the United Kingdom, France, and the Soviet Union, expressed their intent to use prohibited weapons for retaliatory purposes. The Protocol also did not address the creation, storage, testing, and transportation of these weapons, allowing countries like the United States and the Soviet Union to amass substantial quantities of harmful agents. Despite its shortcomings, the Protocol remains a crucial framework for international treaties addressing the threat posed by chemical and biological weapons[40].

6.2. Biological and Toxin Weapons Convention

The Biological and Toxin Weapons Convention (BTWC), also known as the BWC, is an international treaty that prohibits the development, production, acquisition, transfer, stockpiling, and use of biological and toxin weapons. Officially titled the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction, it was the first multilateral disarmament treaty to outlaw an entire category of weapons of mass destruction upon its adoption on March 26, 1975. It is worth noting that the convention does not have a specified duration. As of February 2023, 185 states have ratified or acceded to the treaty, while eight states have neither signed nor joined the treaty and four states have signed but not ratified it. The impact of the BWC in establishing a robust and universally accepted global standard against the use of biological weapons is widely recognized. This is articulated in the treaty's preamble, which emphasizes the moral repugnance of biological weapons. Notably, no state openly acknowledges possessing or pursuing biological weapons, nor advocates their use in warfare. Daniel Gerstein, an authority in biodefense, has emphasized the significance of the Biological Weapons Convention (BWC) as the preeminent accord for regulating weapons in the 21st century, particularly in light of the rapid advancements in biotechnology. However, the convention's effectiveness has been undermined by the absence of a formal verification mechanism to monitor compliance and the inadequate institutional support.

7. CONCLUSION

The proliferation of bioengineering, facilitated by advancements in applied sciences, has revolutionized the scientific understanding of biological systems and enabled a wide array of experiments through the integration of diverse technologies. This has led to practical applications in agriculture, industry, military, and medicine. However, these advancements also engender significant risks, particularly in the context of illicit use, such as the development of biological weapons. The ability to manipulate genetic material and advancements in DNA manufacturing technology have empowered both state and non-state actors to engineer biological agents, raising considerable biosecurity concerns. Given the dual-use nature of genetic engineering, stringent measures are essential to mitigate the proliferation of such weapons. The production and deployment of biological weapons pose serious threats to human health, industry, and the global economy, necessitating the implementation of robust detection methodologies and the enhancement of security protocols in biological research facilities. While international agreements like the Geneva Gas Convention and the Biological and Toxin Weapons Convention

encompass provisions aimed at preventing and addressing the spread of biological weapons, challenges persist due to varying levels of commitment between nations and inadequate enforcement. Strengthening international agreements and fostering cooperation is imperative to mitigate the hazards associated with biological weapons. Given the significant potential and contributions of bioengineering in fields such as medicine, agriculture, and industry, comprehensive oversight and international monitoring are crucial to ensure the ethical and responsible utilization of these capabilities. This research study offers a comprehensive analysis of the influence of molecular engineering on biosecurity, emphasizing advanced methodologies such as CRISPR, CAS9, and synthetic biology, which have not previously been extensively discussed. The study also underscores sophisticated tools for identifying and averting biological hazards. Furthermore, it accentuates international accords and security protocols, thereby contributing to the progress of biosecurity.

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