BARRIERS TO BIOLOGICAL WEAPONS DEVELOPMENT:
Potential Implications for Pathway Disruption

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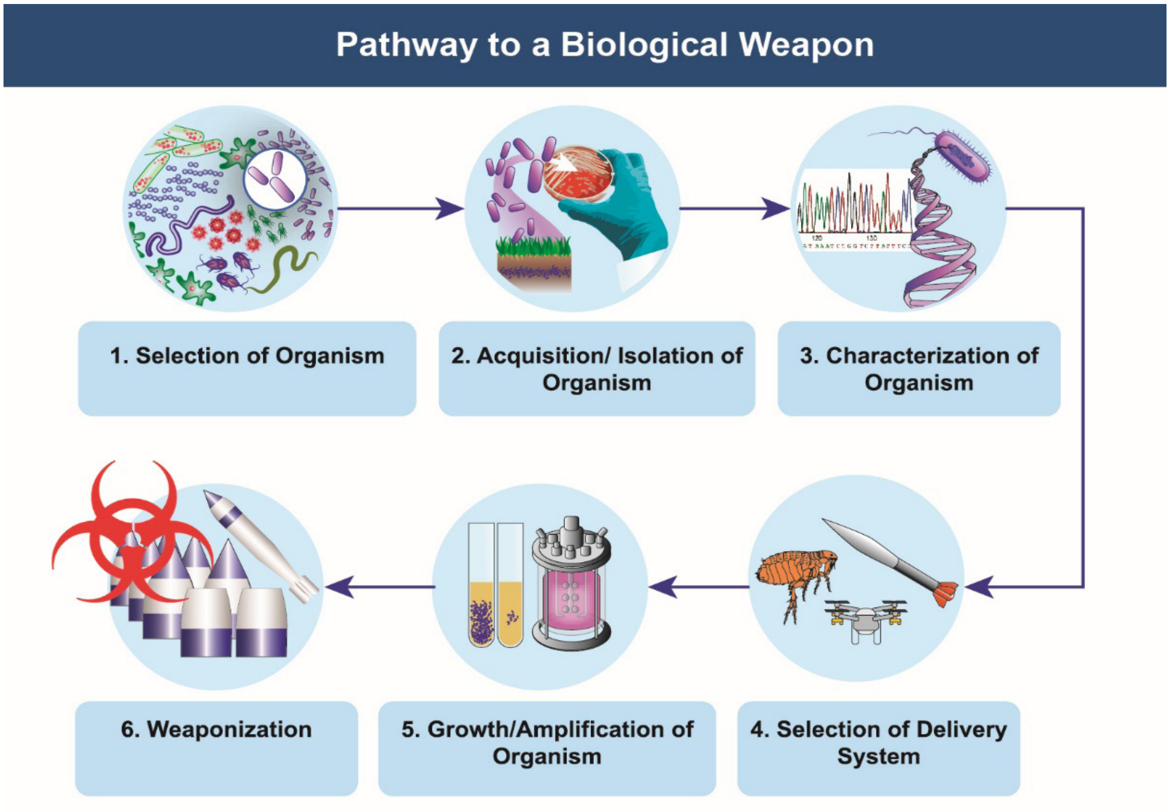
Introduction

Biological weapons (bioweapons) are a highly complex and diverse group of threat agents.[1] They can be derived from any organism that can cause disease. The complexity of bioweapons arises from the fact that they are living organisms with the ability to grow, replicate, mutate, and evolve.[2] Consequently, bioweapons are more difficult to control and regulate than chemical agents or nuclear weapons.[3] Additionally, they can be produced and distributed with minimal financial and material investment in a largely clandestine manner. [4] A recent example of this fact was provided by the discovery of an undercover biomedical laboratory in Reedley, California in 2023.[5] This facility was found to be storing illegal samples of several of the most infectious diseases including SARS-CoV2, rubella, malaria, dengue, chlamydia, hepatitis, and HIV. It was being operated by a Chinese national with ties to the communist party. Concerningly, local government entities did not know the Reedley lab existed until it was discovered through the chance observations of a local city code enforcement officer. [5] It is also important to recognize to the trained eye, grocery and hardware stores may offer a potential adversary access to nontraditional equipment and reagents that are capable of being used in the production and dispersal of biological weapons. For example, ricin is a biological agent that is capable of rapidly causing death in affected individuals. It is derived from the seeds of *Ricinus communis*, a species of flowering plant that is used to make castor oil (a common laxative).[6] These seeds can be readily procured at a hardware or horticulture store, and if they are used in a home garden setting, they can enable the production of more seeds that can be mashed and processed to extract the ricin in a home laboratory.[7] Despite the ease of and economy of producing these agents in a surreptitious manner, biological weapons are not commonly used during armed conflict. This is because there are numerous barriers to the development, production, and delivery of effective biological weapons.[8] However, the risk of a biological weapons attack will continue to increase as technology develops and knowledge of the life sciences improves and is distributed. Weapon development barriers can be exploited to interrupt the pathway for the production and use of these weapons by potential adversaries. These barriers typically fall into six categories. These can be best described as resulting from the selection of the organism, the acquisition or isolation of the organism, the characterization of the organism, selection of a delivery system, the growth, or amplification of the organism, and weaponization.



ABOVE: The six-step process to bioweapon development. The process begins with the selection of the organism for weaponization and proceeds through the steps of acquisition, characterization, delivery system identification, growth of the organism and final weaponization and combination with the delivery system. Each of these steps represents a barrier to weapon development and key nodes in the development process that can be targeted during counter-weapons of mass destructions operations. (Illustration produced by Ronald Pettit, MSMI)

Selection of an Organism as a Barrier to Bioweapon Development

The first barrier that must be overcome in the pathway toward the development of a bioweapon is the selection of an appropriate organism for the weaponization process. This task is non-trivial as history has shown that sub-optimal selection can lead to a failure in weapon development.[9] Bacterial pathogens such as *Bacillus anthracis*, the causative agent of the disease known as anthrax is the most common agent that has been developed and used as a biological weapon by both state and non-state actors.[10] Bacteria have been described as collections of autonomous biotic systems that are endowed with the ability to self-replicate and self-engineer.[11,12] Correspondingly bacterial growth tends to approximate an exponential function in which one bacterium inoculated into a flask of nutrient broth can produce billions of progeny bacteria within a few hours of growth time if it is given optimum nutrition ideal temperature, and proper gas exchange.[13] This rapid and autonomous reproduction rate was a major factor in the selection of bacteria for bioweapon development by state governments in the early 20th century as it reduced the cost and labor involved in production and enabled large amounts of material to be produced with minimum investment. However, the development of a bacterial pathogen into a bioweapon requires the ability to overcome numerous operational barriers. The first barrier is imposed by the diversity of the bacteria themselves. Bacteria are a tremendously diverse group of organisms. Recent estimates suggest that there are between 800,000 and 1.5 million prokaryotic operational taxonomic units (distinct types of bacteria) worldwide.[14]

To initiate a bioweapons program both state and non-state actors will necessarily have to down select a subset of this diversity for weaponization. This selection must be carried out in such a way as to select organisms that possess a set of predetermined desired characteristics. The most important characteristics of a bacterial bioweapon include rapid growth rate, (to facilitate production and dispersal), a minimal growth media requirement (to ensure economical amplification), low mutation rate (to ensure stability during storage and amplification), stability in dry form (to allow powder formation and aerosol dispersion), and thermal stability (to allow dispersion in the presence of sunlight and dispersion by low-yield explosive). In some cases, this down-selection has inadvertently been performed by academic or industrial scientists and the open-source literature can be utilized to identify strains with the desired characteristics. However, there is diversity within each of the strains themselves that will require expertise in the areas of microbiology and/or biochemistry to ensure that the appropriate organism has been selected, the desired characteristics are present, and that the strain can be grown in large enough quantities to allow tactical or strategic use.

An example of how strain selection can serve as a barrier to weapons development can be found by revisiting the case of the attempted biological attack on Kameido, Japan by the Aum Shinrikyo cult in 1993.[9] In this case, non-state actors attempted to aerosolize anthrax by spraying a liquid suspension containing the organism into the street from the roof of their headquarters near Tokyo. This attack failed to produce the desired effect since the strain of anthrax that they were able to acquire was lacking in a genetic element (that is necessary for pathogenicity).[9] The enormous diversity of bacteria, even within a single group, was not fully appreciated. This lack of understanding led to the selection of an unsuitable agent for weaponization. As a result, the attempt at biological terrorism failed.

Acquisition of Biological Agents as a Barrier to Bioweapon Development

Once an organism has been selected for weaponization the next barrier in the pathway toward converting it into a bioweapon is the acquisition of the organism. The magnitude of this barrier can range from minimal to substantial depending upon the nature of the organism in question. Bacteria and viruses can be acquired from a variety of sources. In some cases, they can be purchased from biological supply companies or acquired from non-profit culture collections.[15] They can also be obtained from academic or industrial research laboratories under the guise of legitimate research and collaboration. Since bacteria are autonomously replicating organisms, large numbers of organisms can be readily produced from a small sample. Actions as simple as furtively touching a bacterial culture growing on the surface of a nutrient plate with the tip of an ink pen can provide ample material for weaponization.[16] Both bacteria and viruses can be cultured from the environment or from infected patients. For example, anthrax is ubiquitous throughout the world, and it can be isolated directly from contaminated soil. Published data concerning regions of the world with high anthrax contamination is available in the open literature.[17] However, there are numerous barriers to isolating bacterial or viral agents from the environment. Using anthrax as an example, these barriers would include determining the form of the agent to be isolated and obtaining the reagents and equipment necessary to initiate the process. First, a decision would have to be made as to whether bacterial spores (the dormant form of the bacteria that has historically been used in aerosol-delivered weapons) or vegetative (actively growing) organisms are to be isolated and then a method would have to be identified to selectively isolate agent from the soil. To give an illustration of the complexity of this process consider that direct spore isolation would require the collection of soil in some type of sample bottle, followed by mixing with a deflocculant (chemical that breaks up large soil particles) followed by shaking and centrifugation at low speed to remove bulk soil.[18] The remaining liquid would then need to be centrifuged a second time at increased speed to collect the spores which will settle at the bottom of the tube. The isolation of vegetative anthrax cells is also a complex process in which a selective nutrient media will be required.[19] Once the soil is plated on this media, it would need to be grown in an incubator capable of maintaining a stable humidity, atmosphere, and temperature for the growth of the organism and then collection of the organism for further expansion would need to be performed in a biological safety cabinet by personnel wearing the appropriate safety equipment to prevent staff contamination.[20]

It is worth noting that techniques have been developed that allow the construction of artificial bacterial genomes that can be inserted into a host cell that has been purged of its native genome.[21] These techniques have been utilized to produce partially synthetic bacterial cells capable of functioning on a minimal genome. In theory, this could allow genes encoding toxin production, environmental stability, spore production, and other factors of pathogenicity to be added onto the minimal genome backbone. These techniques may eventually allow the construction of designer biological weapons that can be tailored to target populations and environmental factors without the need to acquire the organism from an environmental or commercial source. However, this level of biological engineering is accompanied by numerous barriers and would require specific expertise and technical capabilities to include specialized expertise in the fields of bioinformatics, biochemistry, and molecular genetics.[22]

Characterization of Potential Biological Agents as a Barrier to Bioweapon Development

The characterization of a potential bioweapon represents another barrier in the pathway of weaponizing biological agents. This is again due to the extraordinary diversity that can be found in the biological world. Biological organisms (particularly bacteria and viruses) are notoriously difficult to identify and distinguish from one another. Bioweapon development requires specific strains or subgroups of bacteria and viruses that can deliver a pathogenic effect on a given population to achieve a strategic or tactical objective. In the laboratory setting, bacteria are either grown on small circular plates containing a nutrient substrate or they are grown in a liquid broth.[23] Without microbiological expertise, the appropriate equipment, and an appropriate reagent set, identifying strains with weaponization potential would difficult if not impossible.[24] This fact was clearly demonstrated in the Aum Shinrikyo case mentioned above. [9] Microbiological expertise and a well-equipped laboratory would have been necessary for the cult members to determine that the strain of anthrax that they acquired did not have the genetic material for toxin production. They then would have had to select another strain (which would also have to be characterized) or attempt to modify the strains that they had available to confer the desired level of pathogenicity. Characterization of this agent could have been completed by using a selective nutrient media specific for anthrax to verify that they had the correct species of bacteria. This would have been followed up by a confirmatory evaluation to demonstrate the correct microscopic morphology of the bacteria. The presence or absence of the appropriate genetic material could have then been verified by a molecular biological technique known as a polymerase chain reaction that functions by producing numerous copies of the bacterial genome allowing specific characteristics (such as the presence or absence of pathogenicity markers) to be rapidly identified. A more advanced technique known as DNA sequencing would be necessary for complete characterization (determination of all pathogenicity and environmental stability associated genes) of the bacterial genome. This is a technically complicated procedure that relies on a skilled laboratory staff as well as on computational expertise and the availability of networked computer access.[25] It is worth noting that the complexity of characterizing biological agents has decreased in recent years. This is mostly due to the near-ubiquitous availability of DNA sequencing technology, new molecular techniques, and the widespread distribution of bioinformatics tools and analysis pipelines.[26]

Selection of a Delivery System as a Barrier to Bioweapon Development

It has been demonstrated that biological weapons can be delivered using insect vectors, aerosol dissemination, human to human transmission, or by the contamination of food or water.[27] Depending on the characteristics of the organism the selection of a delivery system can represent a barrier to weaponization. Recent experience with anthrax has shown that aerosol dissemination is one of the most likely methods of dispersion to be used by an adversary.[28] This is because an aerosol method would enable maximum casualty generation with minimum resources. Indeed, it has been estimated that a line-source release of 50 kilograms of anthrax spores over two kilometers can potentially lead to 95,000 deaths and 125,000 incapacitations. [27] Aerosol delivery can be accomplished by the release of vegetative (actively growing) cells or spores. As mentioned above, anthrax spores tend to have a higher tolerance for extreme environmental conditions and are therefore the form most likely to be used as strategic or tactical biological weapons. [28] This material can be delivered by industrial sprayer in a fixed location (point-source dissemination), from a moving aircraft such as an airplane or drone (line source dissemination) or by the detonation of an explosive device (point source dissemination).[29] In the Aum Shinrikyo case, industrial sprayers were used by the terrorist cult. However, they proved to be ineffective since the correct strain of anthrax was not selected, characterized, and processed into a usable form. [29] The selection of delivery system will most likely depend upon a rational calculus that will be developed and employed by potential adversaries in such a way as to increase the chance of achieving specific tactical and strategic objectives. Industrial sprayers disseminating a bacterial agent will most likely be used for tactical effect, line source dissemination from a drone or an airplane will most likely be used to generate large numbers of casualties for strategic impact and point source dissemination from explosive devices can be used in both a strategic and tactical manner.

Amplification of the Organism as a Barrier to Bioweapon Development

Agent amplification or expansion can be a significant barrier to weaponization. To produce a viable weapon, it is necessary to produce large quantities of agent for dispersal or to fill munitions. This activity requires knowledge of the growth characteristics of the agent, the selection of appropriate growth media, and the acquisition of the equipment required for growth and containment. In the case of anthrax, growth of the organism in a laboratory setting would require the acquisition or development of a specific growth medium.[30] Typically, such media consists of a liquid suspension of glucose (carbon and energy source), amino acids (carbon and nitrogen sources), and other compounds capable of supporting robust bacterial growth. The optimization of the growth conditions for large-scale production of bacteria in the laboratory setting requires the use of a fermentation system.[31,32] Such devices typically consist of an autoclavable glass reactor vessel (capable of holding several liters of liquid), a bio-controller unit capable of maintaining the pH and temperature of the culture, a motor control unit capable of controlling the activities of a thermo-circulator for the maintenance of aeration, gas exchange and nutrient circulation, and a sterile air source. Production begins by inoculating sterile culture media in the bioreactor with either an overnight bacterial culture or with bacterial spores that will germinate within the system. Growth can be carried out for several hours at a predetermined temperature and monitored with the use of a spectrophotometer.[33] If a bacterial agent is being produced for aerosol dispersion it will be necessary to generate spores. Since spores are the dormant inactive forms of bacterial cells that can survive in conditions of temperature, humidity, and nutrient deprivation that would kill actively dividing cells, they are ideal for aerosol delivery. The acquisition of the required equipment and the development or acquisition of protocols and procedures to use this equipment to produce the quantities of agent necessary for a biological attack will continue to be a rate limiting step in bioweapon production.

Weaponization as a Barrier to Bioweapon Development

With respect to biological weapons, the term weaponization refers to the process by which a biological organism is converted from its native state into a form that can be used to inflict mass casualties, stored for future use, and combined with a delivery system for dispersal. The barriers to this process begin with the conversion of the organism into a form that can be widely distributed in the environment and remain stable long enough to infect the target population. Again, the use of anthrax as a historical precedent for bioweapons development can provide insight into the barriers posed by this process. The spore form of anthrax is the preferred form for aerosol delivery. Producing a concentrated suspension of spores can be technically challenging and require the use of specialized equipment and reagents. This process involves the growth of the selected anthrax strain on nutrient media in a controlled temperature, humidity, and atmosphere environment for at least 24 hours, followed by the growth in a bioreactor of a small portion of the 24-hour culture for amplification and the initiation of spore formation by nutrient deprivation.[35] The resulting spores can be collected from the bioreactor and purified through a series of wash steps and a process known as density gradient centrifugation in which the spores are passed through a medium that allows the separation of the spores from the nutrient media and other contaminants.[36] The purified spores then need to be freeze-dried in a process called lyophilization and then they can either be used without modification or they can be treated with a variety of reagents to alter their electrostatic properties to increase the range of aerosol dispersion.[37] The final step in weaponization is the combination of the biological agent with a munition or delivery system. High concentrations of spores are needed for this purpose. This was demonstrated in 2001 when a series of anthrax-laced letters were sent out to various media personalities and government officials. These events were called the “Amerithrax” attacks and they appeared to have been conducted by someone familiar with biological weapons development. Laboratory analysis indicated that the spore preparations used in the letters contained average of 100 billion spores per gram of material. [37] These attacks were highly successful and even with a suboptimal delivery system (the postal system) they resulted in 5 deaths and 17 non-lethal infections. However, it should be noted that the choice of delivery system will depend upon the goals of the biological attack (strategic versus tactical), and it will not always be chosen for psychological impact or terrorism purposes. For tactical effect, point source or line source dissemination of the agent from a low-yield explosive munition or from a commercial sprayer might be employed. For strategic effects, intercontinental ballistic missiles containing anthrax-filled submunitions might be employed. The primary barrier to the use of strategic biological weapons might be inconsistent shelf-life. Anthrax has been found to lose the plasmids necessary for toxin production during long term storage.[38] Therefore, it might not be possible to stockpile effective biological agents in the same way that strategic nuclear weapons are stockpiled.

Barriers to the Production of Viral Bioweapons

Viruses have been developed as bioweapons in the past. This was illustrated in the late 1970s in the Soviet Union in a case in which approximately 400 grams of a virus known as *Variola* major was released into the atmosphere through a low-yield explosive munition. This release resulted in the death of a laboratory technician working on an unrelated project 15 kilometers from the release site.[39] Significantly, the acquisition and development of viruses for bioterrorism presents several challenges which make their use as bioweapons particularly difficult. Viruses are obligatory intracellular parasites requiring a living cell for replication, this makes their acquisition and mass production more challenging than bacteria since they cannot be readily isolated from the environment, and they require specific animals or animal-derived cells for production. Potentially weaponizable viruses include members of the filoviruses such as Marburg and Ebola, which have a high mortality rate, require low numbers of viral particles for infection, display rapid dissemination, and lack an effective treatment or prophylactic vaccine.[40] The acquisition of these highly virulent viruses is, by itself, an obstacle due to currently limited knowledge regarding their geographic distribution and the identification of all existing animal reservoirs. In addition, there is a limited stock of these viruses in highly regulated and secure BSL-4 laboratories. In fact, the Aum Shinrikyo cult attempted to acquire Ebola virus from the Democratic Republic of the Congo in the early 1990's but they were unsuccessful.[41] Although actual acquisition, propagation, and dissemination of these viruses may be difficult, the mere threat of their use can be employed to incite widespread fear and panic and this threat alone may be an effective psychological weapon.[42] However, it should be noted that it is now possible to synthesize viruses directly from a set of chemical precursors. This was demonstrated in 2002 when researchers were successful in constructing a synthetic polio virus genome resulting in the production of infective virus in animal cells.[43] As this technology is refined, it may become possible for state and non-state actors to develop tailor-made viruses without the need to acquire them from an outside source. There are numerous barriers and challenges to this approach including the acquisition of bioinformatics expertise and the necessary laboratory infrastructure.

Pathway Disruption

At the state level, one of the most common methods of pathway disruption is the development of international treaties as a means of discouraging the production and use of bioweapons by potential adversaries, in 1972 a total of 87 countries signed onto the Biological Weapons Convention (BWC) This treaty initiated an international ban on the production and use of biological weapons development and provided for the disarmament of biological stockpiles.[44] Another means of preventing weapons development is to deny the enemy the expertise necessary to succeed and to divert their interests away from weapons development to public health improvements and other peaceful pursuits. Once a nation-state or terrorist is on the path toward weapons development the focus for disruption must shift to the disruption of the technical aspects of the pathway. This can begin with the six steps outlined in this paper. Organism selection can be disrupted by blocking access to databases and websites containing data on biological agent properties, pathogenicity, and growth characteristics. The acquisition of an organism can be blocked by preventing the shipping of an isolate from commercial source to the group or nation of concern. The isolation of an organism may be blocked by restricting access to websites or databases containing instructions for pathogen isolation, media components, atmospheric conditions, or growth times. It might also be possible to block the purchases and shipping of media, media components, bioreactors, and incubators. Agent characterization is largely a bioinformatics driven effort, and this can be disrupted by blocking access to bioinformatics expertise, bioinformatics software and tools (local or online), and by blocking the acquisition of the necessary computer equipment to carry out the required analytical process to characterize an agent. Defensive cyber operations might be effective in identifying computer systems with bioinformatics capability and removing or interfering with these functions. The ability to grow and amplify a biological agent can be interrupted by restricting access to bioreactors, centrifuges, culture media, culture reagents, or the components to produce these items. From the cyber perspective, it might also be possible disrupt the function of bioreactors and centrifuges using computer worms or other forms of malware targeting the control system. This would be particularly effective during this stage of weapon development since precise growth conditions are required for the large-scale production of an effective agent. The final step in the biological development pathway (weaponization) can be interrupted by preventing access to the components necessary to produce sprayers, missiles, low yield munitions, or submunitions. It can also be interrupted by preventing the storage of biological material by blocking freezer acquisition or interfering with freezer function through cyber operations to prevent the storage of the agent.

Conclusion

Biological weapons are deceptively simple. A cursory review of the literature suggests that they can be easily produced by both state and non-state actors with minimal expertise and material resource investment. While it is true that biological agents are cheaper to produce and easier to acquire than the materials needed for fission-based weapons, there are significant barriers that must be overcome to design, develop, and deploy a reliable biological weapon. These barriers include the selection of an appropriate agent, the acquisition of the agent, the characterization of the agent, delivery system selection, amplification of the agent, and final weaponization. The significance of these barriers is underscored by the fact that effective biological attacks are not common on the modern battlefield and widespread biological terrorism has not been effectively carried out by non-state actors. However, it should be noted that as technology increases and access to knowledge and expertise becomes more universal, these barriers will begin to degrade. Understanding the process of biological weapon development and the barriers to weaponization will be essential to developing tactics, techniques, and procedures for discouraging biological weapons development and disrupting the weaponization pathway. ■

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