

Review

Current Laboratory Biosecurity for Handling Pathogenic Viruses

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Abstract: The recent emergence of lethal viruses such as Ebola raises some concern about the possibility of the viruses being used as biological weapons. The application of pathogenic viruses as biothreat agents in the past is well documented. Although their use in warfare is currently prohibited by the Biological and Toxin Weapon Convention, their potential use in bioterrorism is a global concern. One of the requirements for conducting biological attack using viruses is successful acquisition of particular viruses for that action. Apart from natural sources, the laboratory can potentially be the source of viral biothreat agents. An effective strategy to avoid bioterrorism is to prevent bioterrorist action. Therefore, it is important in the laboratory handling of pathogenic viruses to implement biosecurity systems in order to prevent loss, theft, misuse, diversion, or intentional release that could lead dangerous viruses falling into unsafe hands. Viruses of biosecurity concern are those which particularly have an ability to cause large-scaled casualties and are easy disseminated. The level of biosecurity risk posed by the viruses being handled in the laboratory needs to be assessed in order to establish program at an appropriate level of laboratory biosecurity. A laboratory biosecurity system should include physical security, viruses control and accountability, personnel security, transport security and information security. In the laboratory, the implementation of a biosecurity system can be integrated to the biosafety plan. This review focuses on viruses of biosecurity concern, the principles of laboratory biosecurity, the assessment of laboratory biosecurity risk and how to render the biosecurity risk to an acceptable level. The review is intended to raise awareness among scientists and laboratory workers on the potency of the pathogenic viruses which can be misused and to develop secure and responsible scientific conduct involving pathogenic viruses.

Keywords: Biosecurity, Biosecurity Risk Assessment, Biosecurity Risk Management, Pathogenic Viruses

Introduction

Pathogenic Viruses of Biosecurity Concern

The emergence of life-threatening viruses such as Ebola, which has recently claimed more than 11,000 lives (WHO, 2016), raises concern of the possibility of such viruses being used maliciously. This fear is well-founded, as historically, the use of biological agents as bioweapons is well documented. For example, the British

distributed smallpox contaminated-blankets to a population of native American Indian tribes during the French-Indian War in the eighteenth century (1754-1767). The occurrence of this virus among the indigenous population persisted for more than 20 decades (Cenciarelli *et al.*, 2013). The ability to use viruses in warfare is currently prohibited by the Biological and Toxin Weapon Convention. However, their potential use in bioterrorism and biocrime remains of global concern (Jansen *et al.*, 2014).

Table 1: Viruses classified as bioterrorism agents by the U.S. Center for Disease Control and Prevention (Cenciarelli *et al.*, 2013; Das and Kataria, 2010; CDC, 2017)

Category	Virus	Disease
A	Variola major	Smallpox
	Filoviruses:	
	Ebola	Ebola virus disease
	Marburg	Marburg hemorrhagic fever
	Arenaviruses:	
	Junin virus	Argentine hemorrhagic fever
	Machupo virus	Bolivian hemorrhagic fever
	Lassa virus	Lassa fever
	Sabia virus	Brazilian hemorrhagic fever
Guanarito virus	Venezuelan hemorrhagic fever	
B	Alphaviruses:	
	Venezuelan equine encephalitis	Viral encephalitis
	Eastern equine encephalitis	Viral encephalitis
	Western equine encephalitis	Viral encephalitis
C	Nipah virus	Viral encephalitis
	Hantavirus	hemorrhagic fever with renal syndrome or hantavirus pulmonary syndrome

Pathogenic viruses of biosecurity concern include smallpox, Ebola, Marburg, Lassa, Machupo, Nipah, hantavirus, Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis etc. The US Center for Disease Control and Prevention (CDC) classified bioterrorism agents into categories A, B and C based on their ease of dissemination or transmission, severity of potential impact on public health, panic and societal disruption and the availability of public health response measures (Jansen *et al.*, 2014; Cenciarelli *et al.*, 2015; CDC, 2017). Viruses listed as bioterrorism agents by the US CDC are shown in Table 1.

Viruses belonging to the category A are agents of high-priority that would pose the highest risk because they can be easily disseminated or transmitted from person to person, have high mortality rates and are likely to cause mass casualties, public panic and social disruption and therefore require special preparation in public health policy. The category B viruses, which are the second highest priority, include those that are moderately easily spread, result in moderate morbidity rates and low mortality rates and require enhanced diagnostic capacity and disease vigilance. The third high priority agents include emerging viruses that could be engineered for mass dissemination in the future because of their availability, ease of production and dissemination and have the potential to cause high morbidity and fatality levels (CDC, 2017; Das and Kataria, 2010).

Viruses considered suitable as bioweapons are those having characteristics such as high morbidity and lethality, be highly infectious, which can be easily disseminated or transmitted from person to person, which might cause public panic and social disruption, which can be mass produced and stored without loss of pathogenicity

until use, are suited to available methods of delivery, are stable in the environment for long enough to infect humans, are suitable for improvement by genetic engineering and weaponization processes and which presently lack of prophylactic procedures (Jansen *et al.*, 2014; Tian and Zheng, 2014; Gunaratne, 2015).

Among the potential viruses suitable for bioweapons, Variola and Ebola viruses are the most likely candidates (Cenciarelli *et al.*, 2015). Variola major, the causative agent of smallpox, is recorded as the most deadly virus in human history. During the 20th century the virus caused 300-500 million deaths worldwide (Ristanovik *et al.*, 2016). Smallpox is considered as a suitable agent of bioterrorism because it does not present medical symptoms for several days giving the infected individuals time to spread infection from the initial site, making it difficult to track down point of infection. In addition, it has a very low infective dose and can be spread widely because it is stable in the form of an aerosol. It can also be disseminated simply by contact from person to person. Smallpox could potentially cause mass panic and social disruption (Hansen, 2012). It is important to note that since its eradication in 1980, mandatory smallpox vaccination ceased. This means that most of the world's population today is potentially susceptible to smallpox disease because of lack of immunity against this virus. Stocks of smallpox virus remain and are stored by the CDC and a Russian laboratory (Henderson and Arita, 2014).

Ebola virus cannot be easily obtained. However, if this virus is intentionally used as bioweapon it could cause devastation through fatal disease. Ebola virus causes severe hemorrhagic fever, with a high case-fatality rate and currently there is no fully-tested vaccine or approved therapy available (Cenciarelli *et al.*, 2015).

Based on ideal characteristics for a successful bioterrorism agent, Ebola virus is considered capable of being a successful bioterror agent, analogous to smallpox and anthrax. Ebola could even be a more suited bioterror agent compared to smallpox or anthrax considering its infectiousness and lack of proven prophylaxis (Gunaratne, 2015). The recent natural outbreaks of Ebola hemorrhagic fever in Africa have raised concern about accessibility to the virus and its misuse. The increase in Ebola outbreak and a possibility of a terrorist group recruiting experts to weaponize the virus is also considered as a potential risk to public health and security (Teckman, 2013). Marburg virus is another filovirus listed as category A because this virus can be lethal and potentially cause extreme public fear (Bray, 2003). Marburg virus is capable of causing severe hemorrhagic fever in all primates (CDC, 2014).

There are 5 viral species of the family *Arenaviridae* (Junin, Machupo, Guanarito, Sabia and Lassa virus) listed as category A bioterrorism agents due to their capability to cause fatal viral hemorrhagic fevers. They are zoonotic and can cause adverse public health impact if abused (Charrel and de Lamballerie, 2003). Populations of each of this virus is usually maintained in a specific rodent and aerosol transmission can occur where an individual inhales tiny particles soiled with virus-contaminating rodent urine or saliva. The Lassa and Machupo viruses, are reported to be associated with secondary person-to-person transmission (CDC, 2013). Other viruses such as Lujo, Chapare, Dengue, Rift Valley Fever virus and Crimean Congo Hemorrhagic Fever virus are also listed as category A agents by the US National Institute of Allergy and Infectious Diseases (NIAID) Biodefense Research (NIAID, 2016).

A number of alphaviruses are listed as bioterrorism agents category B. These include Venezuelan Equine Encephalitis (VEE), Eastern Equine Encephalitis (EEE) and Western Equine Encephalitis (WEE). All of these viruses can potentially be weaponized. They can attack the brain and cause severe disease in humans. VEE is considered to have the highest risk due to its lower infective dose. Other factors which make VEE attractive as a bioweapon are its potential to be spread through aerosol particles or weaponization through infected mosquitoes and its ease of production (Pappas *et al.*, 2006; Anderson and Bokor, 2012). Other viruses, such as Caliciviruses, Hepatitis A, West Nile, LaCrosse encephalitis, California encephalitis, Japanese Encephalitis (JE) and St. Louis encephalitis are also rated as category B agents by the NIAID (2016).

Nipah and Hantavirus fall into Category C of the Bioterrorism Agent according to the CDC. Nipah virus, a deadly zoonotic paramyxovirus, has a number of important characteristics which make the virus can potentially be weaponized. Its extreme pathogenicity, has

the potential to cause widespread panic and fear. The virus has caused considerable social disruptions and economic loss during its outbreak in Malaysia in 1998-1999 (Lam, 2003). Viruses in the genus *Hantavirus* cause two types of serious illness in humans: Hemorrhagic Fever with Renal Syndrome (HFRS) or Hantavirus Pulmonary Syndrome (HPS). Of the two diseases, HPS is more severe with a 40% fatality rate (Jonsson *et al.*, 2008). Other viruses such as influenza virus, rabies virus, Chikungunya, SARS-CoV, MERS-CoV, Hendra, Tick-borne encephalitis complex flaviviruses, Yellow fever virus etc. are also classified as Category C by the NIAID (2016).

The Southeast Asian region is also considered to have specific and increasing biosecurity risk. This region is considered as one of the hot spots of emerging viral diseases (Gronvall *et al.*, 2015). The circulation of pathogenic viruses such as Nipah (Lam, 2003; Looi and Chua, 2007), SARS (Goh *et al.*, 2006), H5N1 (Adisasmitho *et al.*, 2013), West Nile (Myint *et al.*, 2014), dengue (Sasmono *et al.*, 2015), Chikungunya (Kosasih *et al.*, 2013; Riswari *et al.*, 2016), Zika (Perkasa *et al.*, 2016) and coxsackievirus (Wiyatno *et al.*, 2016) has been reported in this region.

Laboratory Biosecurity Risk Assessment

Laboratory biosecurity is defined as the protection, control and accountability of valuable biological materials handled in the laboratory in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release (WHO, 2006). Risk is a function of the likelihood that an undesirable event will occur and the consequences of its occurrence. In the context of laboratory handling of pathogenic viruses, biosecurity risk is defined as the probability of theft of pathogenic viruses and other assets from the laboratory, the likelihood of malicious use and the severity of the consequences. The consequences of the malicious use of a virus depends on its specific characteristics and ability to affect health. The economic and psychological effects caused are also an important consideration (CBH, 2016). Work with pathogenic viruses in the laboratory carries biosecurity risk as the viruses can be stolen, misused, or intentionally released. Natural viruses with suitable properties to inflict harm are relatively rare, except during an outbreak. Individuals who wish to obtain such viruses, may seek them from laboratory where the viruses are handled and stored. Therefore, it is critical to assess the biosecurity risk associated with the handling of pathogenic viruses in the laboratory (Clevestig, 2009).

For a laboratory working with of dangerous viruses, biosecurity risk assessment is intended as a systematic procedure to evaluate the biosecurity threat posed by the laboratory activities involving the viruses. Biosecurity risk assessment is needed to develop systems to limit

undesirable events from occurring and to assure response preparedness to them (Cenciarelli *et al.*, 2015). The results of the biosecurity risk assessment are used to determine the appropriate levels of controls to secure the pathogenic viruses, other materials and relevant sensitive information. Biosecurity risk assessment is also used to properly allocate biosecurity resources and to determine which biosecurity components need to be prioritized (Gaudioso *et al.*, 2006; BMBL, 2009).

Assessing laboratory biosecurity risk is considered to be challenging because the risks are both potential and dynamic (Clevestig, 2009). The process can be started with the identification of viruses handled in the laboratory followed by review of their potential use for malicious purposes. Factors to be evaluated include strain suitability, ease of production, modes of dissemination, environmental stability and the availability of technical skills and knowledge to weaponize any particular virus. In addition, it is important to analyze the biochemical characteristics of the virus relevant to the potential hazard posed to the public. These include infectivity, incubation period, pathogenicity, virulence, transmissibility, lethality and the availability of preventive and treatment measures. The hazard characteristics of the viruses will dictate the potential consequences of their malicious use such as the number of fatalities or level of sickness and the economic and social impacts. For example, based on its biochemical properties, potential for weaponization and the consideration that the virus has been eradicated, Variola major is categorized as a viral agent of extreme risk (Gaudioso *et al.*, 2006).

Biosecurity risk assessment is a fundamental step to appropriately allocate limited biosecurity resources. As the biosecurity risk increases, the protection measures need to be strengthened. This is termed a "graded protection strategy", in that the level and cost of protection is proportional to the level of the risk (Gaudioso *et al.*, 2006). In general, the level of biosecurity risks corresponds to the Biosafety Level (BSL) designated. BSL1 and BSL2 facilities are generally considered to have a low to moderate biosecurity risk, BSL3 has a moderate to high biosecurity risk and BSL4 has a high biosecurity risk (Clevestig, 2009).

Apart from evaluating risk associated to the viral agents, the risk of each existing and the planned biosecurity component also needs to be assessed in order to identify the weaknesses of the biosecurity system. The unacceptable risks identified can then be reduced to an acceptable level. The risk from individuals in the laboratory operating environment that could pose threat to the viruses stored by the laboratory also needs to be identified. The individuals may be insiders that have authorized access to the laboratory or outsiders who are

without authorized access (Gaudioso *et al.*, 2006). Failure in controlling personal security could potentially cause a biosecurity failure. For example, a laboratory biosecurity incident was reported to occur in Canada in 2009 in which a former researcher at the National Microbiology Laboratory in Winnipeg, stole 22 vials of Ebola genetic material (Salerno and Gaudioso, 2015).

Biosecurity risks can also be posed by manipulation procedures in the laboratory. Work on pathogenic virus could bring about dual-use risk, in that the research intended for benefit could directly be misapplied to do harm. The rapid advances of genetic engineering, genome editing and synthetic biology have raised concern as to the possibility of the use of the techniques to deliberately generate viruses suitable for malintent. Genome editing is a powerful engineering tool that allows deliberate modification of a selected DNA sequence of the genome using site-specific nuclease with unprecedented accuracy. The technique has been revolutionized with the development of the more efficient and cost effective CRISPR/Cas9 system (Li *et al.*, 2015; Fears and Meulen, 2017). Synthetic biology can be used to synthesize biologically-based complex systems that display new functions, including those new to nature (Serrano, 2007). The above techniques can be used to modify, redesign, reconstruct or even synthesize a virus. This can potentially bring about new biosecurity risks as the novel virus is unprecedented in nature. One potential misuse of these techniques would be modification or recreation of known pathogenic viruses such as Nipah, SARS, influenza, Chikungunya, Ebola and smallpox in the laboratory so that it posed an increased biological threat (Tucker, 2010; Byrd *et al.*, 2013; Ahteensuu, 2017).

In 2012, scientists managed to manipulate avian influenza virus H5N1 in the laboratory to generate a mutant, which, in contrast to the wildtype, was transmissible between mammals (ferrets) through aerosol (Herfst *et al.*, 2012; Imai *et al.*, 2012). This was achieved by changing four amino acids in the host receptor-binding protein hemagglutinin and one amino acid in the polymerase complex protein basic polymerase 2 (Herfst *et al.*, 2012). The successful ferret-to-ferret transmission of H5N1 virus is considered as a reasonable model of how the virus may be transmitted between humans which is central to the risk of an influenza pandemic. This experiment, therefore, has raised serious biosecurity concerns as the virus constructs could be used for malicious purposes with the potential to cause a catastrophe (Jeggo *et al.*, 2012). In addition, the methods used to engineer virus to be more dangerous have a great potential to be misused (GEC, 2014).

Other work on pathogenic viruses that has raised biosecurity concern was the introduction of the interleukin-4 gene into the mousepox virus. A research

group in Australia (Jackson *et al.*, 2001), by accident, managed to increase the virulence of mousepox virus by adding interleukin-4 gene to the viral DNA. The aim of introducing the interleukin-4 gene into the virus was to induce infertility in mice, a major pest in Australia. Surprisingly, the manipulated mousepox virus could kill both mice that were naturally resistant to mousepox and mice that had been vaccinated against wildtype mousepox (Selgelid and Weir, 2010). As mousepox virus is closely related to human smallpox, it is feared that the same manipulation could potentially be used to increase the virulence of the smallpox virus and make smallpox virus resistance to standard protective vaccine (Tucker, 2010; Ahteensuu, 2017).

Laboratory Biosecurity Risk Management

The biosecurity risk associated with the handling of pathogenic viruses in the laboratory has to be managed to an acceptable level by implementation of a laboratory biosecurity program. Laboratory biosecurity refers to the principles, technologies and practices applied to secure pathogenic viruses, sensitive information and technology from unauthorized access, loss, theft, misuse, diversion or intentional release (Clevestig, 2009). The concepts of laboratory biosecurity are related to the concepts of laboratory biosafety. However, the two are not identical. In a laboratory handling pathogenic viruses, laboratory biosafety is intended to protect laboratory workers and the environment from exposure to pathogenic viruses, while laboratory biosecurity is dedicated to prevent loss of pathogenic viruses, information and other laboratory assets from the security-rated laboratories and denies laboratory access to individuals who could use them maliciously. In contrast with laboratory biosafety that addresses laboratory risks associated with accidental exposure and release of pathogenic viruses, laboratory biosecurity safeguards the viruses from intentional misuse, release or diversion. Adequate containment to prevent the infection of workers or virus release into the surrounding community, therefore, is critical for both laboratory biosecurity and laboratory biosafety. Biosecurity emphasis has also been placed in relation to the possession, use and transfer of viral agents having high adverse consequences to public health (BMBL, 2009; Clevestig, 2009).

The main components of laboratory biosecurity program include physical security, personnel security, material control and accountability, transport security, information security and program management. The implementation of each component is based on the results of a specific biosecurity risk assessment (Salerno and Gaudio, 2007). Although the objectives of laboratory biosecurity are different from that of laboratory biosafety, they usually have complementary

measures. They share a number of elements such as inventory control, access restriction, accountability and compliance, incident reporting and response, evaluation and revision, education and training (Clevestig, 2009). Therefore laboratories with good biosafety programs already fulfill many of the basic requirements of biosecurity. In some cases, biosecurity practices may conflict with biosafety practices and in those cases it is important to balance the biosafety and biosecurity considerations (BMBL, 2009).

Physical security is a very important component for creating a secure laboratory and is closely associated with laboratory design. In the laboratory working with pathogenic viruses, physical security is intended to reduce the risk of unauthorized access to the viral agents and other laboratory assets for unofficial purposes (WHO, 2006; BMBL, 2009). Generally, the biosecurity risk level corresponds to the biosafety level designation. Therefore, graded protection is commonly implemented in that higher levels of access restriction and more sophisticated surveillance systems are needed as the level of biosafety and biosecurity risk increases (Jansen *et al.*, 2014). Elements of physical security that can be used to control access include laboratory perimeter barriers such as locks, keypads, electronic card readers, biometric scanners, visual identification badges, guards and facility design. In addition, it is important to implement surveillance control, for example using closed circuit television cameras, motion detectors, guards etc (Clevestig, 2009).

Personnel security is a system of policies and procedures used to mitigate the risk of workers exploiting their legitimate access to the viruses in the laboratory. The intent of personnel security is to ensure that workers responsible for handling pathogenic viruses exhibit the appropriate level of professional responsibility for management of viruses and other research materials. In addition, personnel security is aimed to identify the roles and responsibilities of employees who handle, use, store and transport pathogenic viruses (BMBL, 2009). It is important to emphasize that the responsibility for laboratory biosecurity is shared by all laboratory workers, although the complete biosecurity overview of all activities are usually delegated to laboratory managers. Qualified personnel can be designated to oversee specific agents to ensure that all of the agents are accounted for at all times (Clevestig, 2009).

The material control and accountability component of laboratory biosecurity is a program established for the control and accountability of pathogenic viruses handled in the laboratory. Material control is intended to ensure that the viruses stay in the location where they are supposed to and are used for legitimate purposes. Material accountability is a procedure established to

track the inventory, storage, use, transfer and inactivation or destruction of pathogenic viruses when no longer needed. The objective of material control and accountability is to know which types of viruses exist in the laboratory, their location and the personnel responsible for them. This system addresses the insider threat especially from the workers granted access to the facilities. The emphasis of this administrative oversight is to discourage theft in order to protect the pathogenic viruses from potential misuse. Control and track of viral samples in the laboratory is also important for scientific purposes (Baldwin *et al.*, 2009; BMBL, 2009).

Transport security is intended to provide a measure of security during the movement of pathogenic viruses between laboratories, during shipping and receiving activities within or between institutions. Transport policies are required for appropriate documentation and material accountability and control procedures for viruses between locations. Transport security measures should ensure that appropriate authorizations have been received and that adequate communication between facilities has occurred before, during and after transport of viruses. External virus transportation requires trained personnel who is familiar with the regulatory and institutional procedures for proper containment, packaging, labeling, documentation and transport of viruses (WHO, 2006; BMBL, 2009).

Information security is intended to protect information from unauthorized release and ensure that the appropriate level of confidentiality is preserved. Information security establishes prudent policies for managing sensitive information on the viruses handled in the laboratory. Sensitive information may include laboratory details of security plans, virus inventory, storage location, entry code, *etc.* Information security should ensure that an appropriate level of information confidentiality is preserved by the system (Imai *et al.*, 2012; Jeggo *et al.*, 2012). Information and data security can be as critical as the security of viruses themselves. Sensitive information should be stored securely and the access to the information should be controlled. Loss of data can be devastating for a laboratory. It is recommended to store sensitive information electronically with backup hardcopies. Storing sensitive information on networked computers, storage peripheral (memory USB cards), or home computers, is not recommended. The dual-use issue applies to both pathogenic viruses as well as the sensitive information on them (Clevestig, 2009; NRCNA, 2011).

Program management is intended to guide and oversee implementation of the laboratory biosecurity program in order to ensure that each component of laboratory biosecurity is functioning optimally and in a coordinated manner. To achieve these goals, management should ensure the implementation of

biosecurity risk assessment processes. Based on the risk assessment results, decision is made on which risks to be prioritized and mitigated and then appropriate resources are allocated. Program manager needs to establish a biosecurity plan, incidence response plan and other documents required for the successful operation of laboratory biosecurity system. The laboratory biosecurity plan functions as a comprehensive guide outlining the biosecurity measures implemented in the laboratory. To ensure that personnel and external partners are familiar with laboratory biosecurity, training programs should be implemented. The main goal of biosecurity training is to educate personnel and other stakeholders on their roles, responsibilities and level of authority in relation to laboratory biosecurity (WHO, 2006; Salerno and Gaudioso, 2015).

Considering the potential threat posed by pathogenic viruses of biosecurity concern, research aimed at better understanding those viruses is obviously needed. This will provide a foundation to develop strategies to detect, prevent and treat the diseases caused, whether they emerge naturally or as a result of bioterrorism. It is worthy to note, that working on these viruses is challenging as the viruses used as biothreat agents are dangerous making the need for the research activity to be performed at high and maximum levels of biocontainment (biosafety level 3 and 4) (Tree *et al.*, 2015; Artika and Ma'roef, 2017). The unpredictability of future emergence and re-emergence of pathogenic viruses which pose biothreats demands development of local capacity to rapidly detect and characterize circulating pathogenic viruses (Ma *et al.*, 2011; Agustningsih *et al.*, 2016; Wiyatno *et al.*, 2016). In addition, local availability of diagnostic, therapeutic and preventive measures to protect and treat first responders and civilians from the consequences of bioterrorism with pathogenic viruses is critical. Therefore, development of countermeasures such as vaccines (Bowick and McAuley, 2011; Moise *et al.*, 2016) and antiviral agents (Byrd *et al.*, 2013; Artika *et al.*, 2013; Chang *et al.*, 2013) that will effectively prevent or diminish the impact of any viral attack is essential. Advances and revolution in bioscience must be taken into account for developing biodefense strategies (Garcia-Sastre and Mena, 2013; Fears and Maulen, 2017).

Conclusion

Pathogenic viruses with the potential to be used in bioterrorism continue to emerge throughout the world. Laboratory handling of pathogenic viruses should implement biosecurity measures to safeguard pathogenic viruses and other laboratory assets from unauthorized access, theft and misuse. Pathogenic viruses of biosecurity concern are those which are easy to

disseminate and cause severe diseases, high morbidity and mortality, economic loss and social disruption. Any manipulation conducted on pathogenic viruses in the laboratory may pose a biosecurity risk. The recent revolution in bioscience provides the possibility to engineer pathogenic viruses to create more detrimental next generation bioweapons. The level of biosecurity risk faced by a laboratory needs to be assessed in order to decide the level of measures to be implemented and properly allocate the resources needed. Biosecurity training for laboratory personnel and external partners is essential for effective implementation of any biosecurity program. Local capacity needs to be developed to rapidly identify and characterize viruses and elucidate the pathways by which they cause disease. The development of preventive and treatment measures for diseases caused by viral biothreat agents is critical. Advances and the revolution in bioscience must be contingent with any supporting biodefense strategy. The successful implementation of any laboratory biosecurity program is highly dependent on the level of commitment by top management in providing the resources needed.

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Author's Contributions

All authors equally contributed in this work.

Ethics

All authors have read and approved the manuscript and no ethical issues involved.

References

- Adisasmito, W., D.N. Aisyah, T.Y. Aditama, R. Kusriastuti and Trihono *et al.*, 2013. Human influenza A H5N1 in Indonesia: Health care service-associated delays in treatment initiation. *B.M.C. Pub. Health.*, 13: 571-576. DOI: 10.1186/1471-2458-13-571
- Agustiniingsih, A., H. Trimarsanto, V. Setiawaty, I.M. Artika and D.H. Muljono, 2016. Primer development to obtain complete coding sequence of HA and NA genes of influenza A/H3N2 virus. *B.M.C. Res. Notes*, 9: 1-6. DOI: 10.1186/s13104-016-2235-8
- Ahteensuu, M., 2017. Synthetic biology, genome editing and the risk of bioterrorism. *Sci. Eng. Eth.*, 23: 1541-1561. DOI: 10.1007/s11948-016-9868-9
- Anderson, P.D. and G. Bokor, 2012. Bioterrorism: pathogens as weapons. *J. Pharm Pract.*, 25: 521-529. DOI: 10.1177/0897190012456366
- Artika, I.M., Y. Budirahardja and A.L. Ekowati, 2013. Molecular cloning and heterologous expression of human interferon alpha2b gene. *Am. J. Biochem. Biotechnol.*, 9: 423-429. DOI: 10.3844/ajbbsp.2013.423.429
- Artika, I.M. and C.N. Ma'roef, 2017. Laboratory biosafety for handling emerging viruses. *Asian Pac. J. Trop. Biomed.*, 7: 483-491. DOI: 10.1016/j.apjtb.2017.01.020
- Baldwin, G.T., S.B. Rivera and R.M. Salerno, 2009. Material control and accountability for laboratory biosecurity. *Int. J. Risk Assess. Manage.*, 12: 368-379. DOI: 10.1504/IJRAM.2009.025927
- BMBL, 2009. Biosafety in Microbiological and Biomedical Laboratories. 5th Edn., U.S. Department of Health and Human Services: Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health.
- Bowick, G.C. and A.J. McAuley, 2011. Vaccine and adjuvant design for emerging viruses: Mutations, deletion, segments and signaling. *Bioeng. Bugs*, 2: 129-135. DOI: 10.4161/bbug.2.3.15367
- Bray, M., 2003. Defense against filoviruses used as biological weapons. *Antiviral Res.*, 57: 53-60. DOI: 10.1016/S0166-3542(02)00200-0
- Byrd, C.M., D.W. Grosenbach and D.E. Hruby, 2013. Antiviral options for biodefense. *Curr. Opin. Vir.*, 3: 537-541. DOI: 10.1016/j.coviro.2013.05.020
- CBH, 2016. Public health Agency of Canada. Canadian Biosafety Handbook
- CDC, 2013. *Arenaviridae.* <https://www.cdc.gov/vhf/virus-families/arenaviridae.html>
- CDC, 2014. Marburg hemorrhagic fever. <https://www.cdc.gov/vhf/marburg/index.html>
- CDC, 2017. Bioterrorism agents/diseases. <https://emergency.cdc.gov/agent/agentlist-category.asp>
- Cenciarelli, O., S. Rea, M. Carestia, F. D'Amico and A. Malizia *et al.*, 2013. Bioweapons and bioterrorism: A review of history and biological agents. *Defence S&T Tech. Bull.*, 2013: 111-129.
- Cenciarelli, O., V. Gabbarini, S. Pietropaoli, A. Malizia and A. Tamburrini *et al.*, 2015. Viral bioterrorism: Learning the lesson of Ebola virus in West Africa 2013-2015. *Virus Res.*, 210: 318-326. DOI: 10.1016/j.virusres.2015.09.002
- Chang, J., J. Guo, Y. Du and T. Block, 2013. Imino sugar glucosidase inhibitors as broadly active anti-filovirus agents. *Emerg. Microbe Infect.*, 2: 1-7. DOI: 10.1038/emi.2013.77
- Charrel, R.N. and X. de Lamballerie, 2003. Arenaviruses other than Lassa virus. *Antiviral Res.*, 57: 89-100. DOI: 10.1016/S0166-3542(02)00202-4

- Clevestig, P., 2009. Handbook of Applied Biosecurity for Life Science Laboratories. 1st Edn., Stockholm International Peace Research Institute, Sweden, ISBN-10: 9185114618, pp: 32.
- Das, S. and V.K. Kataria, 2010. Bioterrorism: A public health perspective. MJAFI, 66: 255-260.
DOI: 10.1016/S0377-1237(10)80051-6
- Fears, R. and F.T. Meulen, 2017. How should the applications of genome editing be assessed and regulated. Elife, 6: e26295-e26295.
DOI: 10.7554/eLife.26295
- Gaudio, J., R.M. Salerno and N. Barnett, 2006. Developing a risk assessment and management approach to laboratory biosecurity. Applied Biosafety, 11: 24-31. DOI: 10.1177/153567600601100105
- Garcia-Sastre, A. and I. Mena, 2013. Novel vaccine strategies against emerging viruses. Curr. Opin. Virol., 3: 210-216. DOI: 10.1016/j.coviro.2013.02.001
- GEC, 2014. Biosecurity — freedom and responsibility of research. German Ethics Council, Deutscher Ethikrat, Berlin.
- Goh, K.T., J. Cutter, B.H. Heng, S. Ma and B.K. Koh *et al.*, 2006. Epidemiology and control of SARS in Singapore. Ann Acad Med Singapore, 35:301-16. PMID: 16829997
- Gronvall, G.K., S. Ravi, T. Inglesby and A. Cicero, 2015. Singapore-Malaysia-Indonesia-US dialogue on biosecurity. Health Security, 13: 399-405.
DOI: 10.1089/hs.2015.0051
- Gunaratne, N.D., 2015. The ebola virus and the threat of bioterrorism. The Fletcher Forum World Affairs, 39: 63-76.
- Hansen, J.C., 2012. Smallpox: New perspectives regarding risk assessment and management. J. Bioterr. Biodef. S4: 002-002. DOI: 10.4172/2157-2526.S4-002
- Henderson, D.A. and I. Arita, 2014. The smallpox threat: A time to reconsider global policy. Biosecurity and Bioterrorism: Biodefense Strategy, Pract. Sci., 12: 1-5.
DOI: 10.1089/bsp.2014.1509.comm
- Herfst, S., E.J. Schrauwen, M. Linster, S. Chutinimitkul and E. de Wit *et al.*, 2012. Airborne transmission of influenza A/H5N1 virus between ferrets. Science, 336: 1534-1541. DOI: 10.1126/science.1213362
- Imai, M., T. Watanabe, M. Hatta, S.C. Das and M. Ozawa *et al.*, 2012. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. Nature, 486: 420-428.
DOI: 10.1038/nature10831
- Jackson, R.J., A.J. Ramsay, C.D. Christensen, S. Beaton and D.F. Hall *et al.*, 2001. Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. J. Virol., 75: 1205-1210.
DOI: 10.1128/JVI.75.3.1205-1210.2001
- Jansen, H.J., F.J. Breeveld, J. Stijnis and M.P. Grobusch, 2014. Biological warfare, bioterrorism and biocrime. Clin. Microbiol. Infect., 20: 488-496.
DOI: 10.1111/1469-0691.12699
- Jeggo, M., C. Butler, F. Jing, P. Weinstein and P. Daszak, 2012. EcoHealth and the influenza A/H5N1 dual use issue. EcoHealth, 9: 1-3.
DOI: 10.1007/s10393-012-0768-4
- Jonsson, C.B., J. Hooper and G. Mertz, 2008. Treatment of hantavirus pulmonary syndrome. Antiviral Res., 78: 162-169. DOI: 10.1016/j.antiviral.2007.10.012
- Kosasih, H., Q. de Mast, S. Widjaja, P. Sudjana and U. Antonjaya *et al.*, 2013. Evidence for endemic Chikungunya virus infections in Bandung, Indonesia. PLoS Neglect. Trop. Dis., 7: 1-9.
DOI: 10.1371/journal.pntd.0002483
- Lam, S., 2003. Nipah virus—a potential agent of bioterrorism? Antiviral Res., 57: 113-119.
DOI: 10.1016/S0166-3542(02)00204-8
- Li, W., J. Köster, H. Xu, C. Chen and T. Xiao *et al.*, 2015. Quality control, modeling and visualization of CRISPR screens with MAGeCK-VISPR. Genome Biol., 16: 281. DOI: 10.1186/s13059-015-0843-6
- Looi, L. and K. Chua, 2007. Lessons from the Nipah virus outbreak in Malaysia. Malaysian J. Pathol., 29: 63-67. PMID: 19108397
- Ma, M., Y. Huang, Z. Gong, L. Zhuang and C. Li *et al.*, 2011. Discovery of DNA viruses in wild-caught mosquitoes using small RNA high throughput sequencing. PLoS ONE, 6: 1-7.
DOI: 10.1371/journal.pone.0024758
- Moise, L., S. Beseme and A.S. de Groot, 2016. In silico vaccine design: Accelerating the response to biothreat and emerging infectious disease. IQT Quarterly, 7: 20-25.
- Myint, K.S.A., H. Kosasih, I M. Artika, A. Perkasa and M. Puspita *et al.*, 2014. West Nile virus documented in Indonesia from acute febrile illness specimens. Am. J. Trop. Med. Hyg., 90: 260-262.
DOI: 10.4269/ajtmh.13-0445
- NIAID, 2016. NIAID emerging infectious diseases/pathogens.
- NRCNA, 2011. Prudent practice in the laboratory: Handling and management of chemical hazard. National Research Council of The National Academies, The National Academies Press, Washington, D.C.
- Pappas, G., P. Panagopoulou, L. Christou and N. Akriditis, 2006. Category B potential bioterrorism agents: Bacteria, viruses, toxins and foodborne and waterborne pathogens. Infect. Dis. Clin. N. Am., 20: 395-421. DOI: 10.1016/j.idc.2006.02.002
- Perkasa, A., F.A. Yudhaputri, S. Haryanto, R.F. Hayati and C.N. Ma'roef *et al.*, 2016. Isolation of Zika virus from febrile patient, Indonesia. Emerg. Infect. Dis., 22: 924-925. DOI: 10.3201/eid2205.151915

- Ristanovik, E., A. Gligic, S. Atanasievska, V. Protic-Djokic and D. Javanovic *et al.*, 2016. Smallpox as an actual biothreat: lessons learned from its outbreak in ex-Yugoslavia in 1972. *Ann. Ist. Super Sanita*, 52: 587-597. DOI: 10.4415/ANN_16_04_21
- Riswari, S.F., C.N. Ma'roef, H. Djauhari, H. Kosasih and A. Perkasa *et al.*, 2016. Study of viremic profile in febrile specimens of chikungunya in Bandung, Indonesia. *J. Clin. Virol.*, 74: 61-65. DOI: 10.1016/j.jcv.2015.11.017
- Salerno, R.M. and J. Gaudioso, 2007. *Laboratory Biosecurity Handbook*. 1st Edn., CRC Press, Boca Raton, Florida, USA, ISBN-10: 1420006207, pp: 208.
- Salerno, R.M. and J. Gaudioso. 2015. *Laboratory Biorisk Management: Biosafety and Biosecurity*. 1st Edn., CRC Press, Boca Raton, Florida, USA, ISBN-10: 1466593644, pp: 264.
- Sasmono, R.T., I. Wahid, H. Trimarsanto, B. Yohan and S. Wahyuni *et al.*, 2015. Genomic analysis and growth characteristic of dengue viruses from Makassar, Indonesia. *Infect. Gen. Evol.*, 32: 165-177. DOI: 10.1016/j.meegid.2015.03.006
- Selgelid, M.J. and L. Weir, 2010. The mousepox experience: An interview with Ronald Jackson and Ian Ramshaw on dual-use research. *EMBO Rep.*, 11: 18-24. DOI: 10.1038/embor.2009.270
- Serrano, L., 2007. Synthetic biology: Promises and challenges. *Mol. Syst. Biol.*, 3: 158-158. DOI: 10.1038/msb4100202
- Teckman, A.M., 2013. The Bioterrorist Threat of Ebola in East Africa and Implications for Global Health and Security. *Global Policy Essay*.
- Tian, D. and T. Zheng, 2014. Comparison and analysis of biological agent category lists based on biosafety and biodefense. *PLoS ONE*, 9: 1-6. DOI: 10.1371/journal.pone.0101163
- Tree, J.A., E.D. Williamson, C.A. Rowland and L.M. Pitt, 2015. Vaccines and therapies for biodefence agents. *J. Immunol. Res.*, 2015: 1-2. DOI: 10.1155/2015/537319
- Tucker, J.B., 2010. Double-edged innovation: preventing the misuse of emerging biological/chemical technologies. Defense Threat Reduction Agency: Advanced System and Concepts Office. USA
- Wiyatno, A., U. Antonjaya, C.N. Ma'roef, S.F. Riswari and H. Djauhari *et al.*, 2016. Detection and identification of coxsackievirus B3 from sera of an Indonesian patient with undifferentiated febrile illness. *J. Infect. Dev. Ctries.*, 10: 880-883. DOI: 10.3855/jidc.7573
- WHO, 2006. *Biorisk management laboratory biosecurity guidance*. World Health Organization.
- WHO, 2016. *Situation report: Ebola viral disease*.