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Laboratory biosafety for handling emerging viruses

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ABSTRACT

Emerging viruses are viruses whose occurrence has risen within the past twenty years, or whose presence is likely to increase in the near future. Diseases caused by emerging viruses are a major threat to global public health. In spite of greater awareness of safety and containment procedures, the handling of pathogenic viruses remains a likely source of infection, and mortality, among laboratory workers. There is a steady increase in both the number of laboratories and scientist handling emerging viruses for diagnostics and research. The potential for harm associated to work with these infectious agents can be minimized through the application of sound biosafety concepts and practices. The main factors to the prevention of laboratory-acquired infection are well-trained personnel who are knowledgable and biohazard aware, who are perceptive of the various ways of transmission, and who are professional in safe laboratory practice management. In addition, we should emphasize that appropriate facilities, practices and procedures are to be used by the laboratory workers for the handling of emerging viruses in a safe and secure manner. This review is aimed at providing researchers and laboratory personnel with basic biosafety principles to protect themselves from exposure to emerging viruses while working in the laboratory. This paper focuses on what emerging viruses are, why emerging viruses can cause laboratory-acquired infection, how to assess the risk of working with emerging viruses, and how laboratory-acquired infection can be prevented. Control measures used in the laboratory designed as such that they protect workers from emerging viruses and safeguard the public through the safe disposal of infectious wastes are also addressed.

1. Introduction

Emerging viruses is a term used to describe the appearance of viruses whose presence has increased over the past twenty years or whose presence threatens to increase in the years to come. Emerging viruses include those that have been diagnosed in the civil population as a new or that may have been present before but are now rapidly increasing in their global range [1,2]. A number of viruses that meet this definition include the highly pathogenic avian influenza (HPAI) virus of subtype H5N1, severe acute respiratory syndrome (SARS), Nipah, Ebola, Chikungunya, Japanese encephalitis, hantavirus, the Middle East respiratory-syndrome coronavirus (MERS-CoV), Zika, West Nile *etc*. Diseases caused by emerging viruses threaten human and animal health [1–4]. Most of emerging viruses are zoonotic. Their appearance is believed to be driven by a number of factors such as socio-economic, environmental and ecological changes [4]. More local interaction with wildlife in undeveloped countries, greater levels of global travel and trade, and different land use have also been identified as contributing factors for their rapid emergence [5]. Such factors, together with a substantial increase of human population over the past five decades, and enormous urbanization in developing countries, have contributed to the increased chance of viral diseases emergence and re-emergence [3]. Virological

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factors which can increase the transmission potential of emerging human viruses have been analyzed [6].

Parts of the Asian region can be viewed as a hot spot of new viral infections. This area is one of rapid social and environmental change [7]. For instance, SARS emerged in this region in 2003. From 11th March up to 6th June 2003, some 1750 infections were diagnosed in Hong Kong, with 286 mortalities during the same period. Previously, Guangdong Province in Central China underwent an extensive epidemic of SARS. This epidemic was made up of 1511 cases with 57 deaths. In the months of April to June 2003, cases of SARS were recorded in other provinces and cities of Mainland China. Mainland China recorded 5329 cases with 336 fatalities [8].

The Southeast Asia region is vulnerable to emerging viral diseases, especially the overpopulated and economically backward countries. Over the past ten years, there have been intermitten outbreaks of a number of emerging and reemerging zoonotic viral diseases in Southeast Asia. Importation of emerging virus infection from this region into Europe [9], Canada [10], Sweden, Denmark and Australia [11] have been reported. The incidence of Nipah virus (NiV) in Malaya from the month of September 1998 to the month of May 1999 resulted in 265 cases and 105 fatalities [12]. In 2001–2002, the outbreak of NiV in Malay pig farmers led to several fatalities. Fruit bats are considered to be the reservoir for NiV [13]. Avian influenza is a major public health threat due to its high mortality rate of the disease together with its ability to produce novel forms of influenza virus which can cause pandemics [14]. By 9th May 2016, a total of 850 cases had been reported internationally with 449 fatalities. Of these, the Indonesian archipelago, together with Egypt in Africa, have diagnosed more cases than other countries. The total number of cases diagnosed in Indonesia by the same time was 199, or 23% of the global diagnosed cases. Globally, the mortality rate linked to influenza A H5N1 infection is 53% (449/850). In the Indonesian archipelago the mortality rate is 84% (167/199), while in Egypt it is 33% (116/350) [15]. In addition, major outbreaks of dengue disease were reported in Indonesia in 1998 and 2004 [16]. The circulation of other emerging viruses such as West Nile [17], Chikungunya [18], Zika [19] and coxsackievirus [20] have also been recently reported in Indonesia.

In 2015, an epidemic of Zika virus occurred in South America, Central America and in the Caribbean. One substantial concern associated with this outbreak was an apparent increase in microcephaly in babies from mothers who were infected with Zika virus [21]. In Brazil, the Zika virus outbreaks were reported to occur with co-circulation of other arboviruses [dengue virus (DENV) and Chikungunya virus]. The Zika infection was an ongoing virus outbreak in Camaçari city, in Bahia Province, Brazil. The symptoms were maculopapular rash, fever, myalgias/arthralgia, and conjunctivitis [22]. The Zika virus was discovered and isolated in 1947 from a sentinel rhesus macaque monkey's blood after the animal was placed in the Zika Forest in Uganda [23].

Similarly, Sub-Saharan Africa is also prone to the emergence of pathogenic viruses. A large outbreak of Ebola virus disease occurred from March 2014 in West Africa. As of March 11th, 2015, the outbreak had involved 24282 reported cases and 9976 reported deaths. As the outbreak was occurring in some of the poorest and least accessible parts of the world, the actual numbers are predicted to be significantly higher [24]. The outbreak is a major health concern in the African Sub-Saharan region. The illness was characterized by fever, severe diarrhea, vomiting, and high mortality (30%–90%) [25]. The genus *Ebolavirus* is one of three members of the Filoviridae family (filovirus), along with the genus *Marburgvirus* and the genus *Cuevavirus*. The genus *Ebolavirus* is made up of five distinct species: *Zaire ebolavirus* (EBOV), *Bundibugyo ebolavirus*, *Sudan ebolavirus* (SUDV), *Taï Forest ebolavirus* and *Reston ebolavirus* [26]. Three ebolavirus species have been recorded which cause substantial outbreaks in the African sub-Saharan region: EBOV, SUDV, *B. ebolavirus*. Potential sources of Ebola virus are fruit bats of the species *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*. They are found in large areas of West Africa, indicating the likelyhood that the Ebola virus has been circulating silently in this area over a period of time. The incidence of Ebola in Guinea signifies the possibility of EBOV outbreaks in the greater area of West Africa [27].

2. Laboratory acquired emerging virus infection

Laboratory workers all over the world are at risk of viral infection when working with emerging viruses. Accidental viral infections of workers in hospitals or research laboratories are an emerging threat mainly due to the increasing amount of virological research being carried out involving the Risk Group 3 or 4 [28]. Even though the risk of infection after an exposure to a virus lacks precise definition, infections due to the bloodborne emerging viruses such as hepatitis C and HIV are the commonest diagnosed viral infections [29]. Laboratory-acquired infection by other emerging viruses such as SARS, Marbug [29], dengue [30], vaccinia [31,32], Crimean-Congo hemorrhagic fever, Western equine encephalitis [28], Ebola [33], West Nile virus [34], Zika [35,36] have also all been reported. The common ways of infection in a laboratory environment include inhalation, ingestion, contact with mucous membrane, self inoculation, and direct contact with animal or insect vectors [37].

With respect to the work with emerging viruses, the laboratory-acquired infection is a reality that cannot be ignored. The risk of laboratory-acquired viral infection is illustrated by a number of case reports. Two cases of laboratory-acquired West Nile virus infections were recorded in the USA in 2002 through percutaneous inoculation. In the first case, occurring in the month of August 2002, a laboratory microbiologist undertook a necropsy on a bird in a biosafety cabinet under biosafety level 2 (BSL2) conditions and lacerated a thumb when using a scalpel to isolate the brain of the bird. In a second case, in October 2002, a microbiologist pierced a finger with a contaminated needle in a laboratory when harvesting West Nile virus-infected mouse brains in a biosafety cabinet under biosafety level 3 (BSL3) conditions [34].

On September 3rd, 2003, a microbiology student in Singapore was admitted to hospital, with fever and later confirmed to be infected by SARS-associated coronavirus (SARS-CoV). Over the month of July and August 2003, he had worked with a non-attenuated West Nile virus strain in a BSL3 laboratory. In the same institute, research on SARS-CoV, dengue virus, and Kunjin virus was being conducted [38] resulting in his SARS infection.

In 2011, Britton *et al.* [30] published data on a DENV infection of a laboratory scientist undertaking infection and

transmission experiments with mosquitos in Brisbane, Australia. A few days before admission to hospital, the scientist had undertaken experiments on the primary infection of a group of mosquitoes with DENV-type 2 (DENV-2). During the experiments the scientist had worn the recommended personal protective equipment. The scientist, however, was bitten by an escaped non-blood-fed mosquito. Ten days after the onset of a fever, a DENV infection was diagnosed by the presence in the scientist's serum of specific DENV-2 nucleic acid and anti-DENV-2 immunoglobulin M antibodies. Another laboratory-acquired dengue virus infection was reported in South Korea in 2014, when a laboratory worker was infected with dengue virus when conducting virus filtering [39].

Infection of vaccinia virus in a laboratory was also reported by Lewis *et al*. [31]. The infection was acquired by an unvaccinated graduate student experimenting on various strains of vaccinia. The student acquired infection of a novel strain being manipulated partly outside a biosafety cabinet. Similarly, on November 23rd, 2013, an immunized laboratory worker at an academic institution in Massachusetts was infected by a wild type vaccinia virus due to a needle stick injury [32].

Pedrosa and Cardoso [28] reviewed 35 scientific articles relating to 219 laboratory-viral infections. They found that most (84%) of arboviral infections that took place in a laboratory were airborne while the rest (16%) were acquired percutaneously. Aerosolic inhalation was found to be the cause of most of the lymphocytic choriomeningitis cases, hanta and coxsackievirus infections. However, inhalation of infected droplets was the leading cause of infection for severe acute respiratory syndrome coronavirus and mucocutaneous infection was the leading cause of infection by influenza B. In the laboratory, most (77%) of blood-borne viral infections were found to be due inhaling infected aerosol.

A number of infections in laboratories occur due to careless laboratory workers that can lead to unsafe procedures such as undertaking procedures which generates aerosol outside of a biosafety cabinet. The best ways to avoid laboratory-acquired infection are knowledgable personnel who are trained in biohazards, who have an understanding of possible routes of transmission, and who are professional in their laboratory safety practices [40].

3. Biological risk assessment for working with emerging viruses

What is biological risk (biorisk) assessment? Biorisk is a combination of the likelyhood of harm and the level of severity of that harm where the source of harm is a biological in nature. Biorisk assessment is the process of evaluating biorisk(s) which may arise from a biohazard(s), assessing the adequacy of predetermined controls, and concluding whether or not a particular biorisk(s) is acceptable [41].

Before any experimentation with emerging viruses in a laboratory environment, health and environmental-related risks associated with their manipulation must be assessed. The assessment of biological risk in working with emerging viruses focuses mainly on the prevention of laboratory-acquired infections and unintended release of a virus. Biorisk assessment needs to be undertaken by scientists who are familiar with the specific characteristics of the viruses being experimented with, the level and suitability of equipment and procedures to be used, animal models to be used, and the containment facilities available [42]. Determination of which mitigation measures should be applied to manage the specific laboratory risks should be dependent upon the assessment of risk. This should be conducted using standardized and systematic procedure which allows it to be repeatable and comparable [43].

The biorisk assessment is often considered difficult due partly to the lack of information associated with the characteristics of the viruses and systematic reports on the infection caused. Biorisk assessment is however a very important process to determine the appropriate biosafety measures for the safe experimenting with infectious agents in a laboratory environment. Typical outcomes of biological risk assessment are the identification of risks that have to be properly managed as well as the determination of appropriate biosafety levels to be implemented [44]. When working with emerging viruses, there may not be sufficient information to make an informed assessment of biorisk. In early stages of any emerging disease, the level and nature of associated biorisk will be uncertain, and uncertainty causes fear. Laboratory workers are at risk from infection by a disease with novel and frightening properties, for which, there may be unproven treatments or none at all. The level of risk to laboratory workers should be assessed using the best-available information of the emerging viruses such as the route of spread, stability in the environment, presence in various body sites and sample types, and the number of cases likely to be encountered. Risks to laboratory personnels should be reduced to the minimum by the provision of appropriate equipment, personal protective equipment (PPE), procedures, and an adequate level of training [24]. Immediate communication with the scientific community has also been found to be helpful in the biorisk assessment and management of exposure to emerging viruses which do not have approved treatment procedure or any post-exposure prophylaxis treatment [33].

Emerging viruses have affected, and will continue to affect, the health care system. The laboratorium is the only place where potentially infectious material is deliberately sent. Emerging viruses, such as Ebola virus, SARS coronavirus, with less-well-studied and potentially more-diverse routes of transmission, present new risks that may not be effectively mitigated by standard laboratory practices. In addition to assessment of engineering and biological risk, quantitation of risks should involve an assessment of the epidemiologic context, such as the number of samples handled [24]. Increased biorisk is faced by workers when handling emerging viruses with high viral loads and which involves aerosol-generating methodology [28]. Laboratory-specific issues of biorisk concern include sample collection and handling, the kind of tests and instruments used, sample disposal and storage, and disposal of biohazardous waste. Risk of exposure is also faced during decontamination and repair of instruments [24].

Traditionally the relative hazardous organisms, including viruses, have been grouped into four levels of risk. Risk Group 1 are agents with low risk to individual and the community; Risk Group 2 are agents with moderate risk to individuals and low risk to community; Risk Group 3 are agents with high risk to individuals, low risk to the community; and Risk Group 4 are agents with high risk to individuals and a high risk to the community. Emerging viruses generally belong to either Risk Group 2 such as hepatitis C, and dengue virus; Risk Group 3 such as the HPAI H5N1, West Nile virus, Japanese B encephalitis virus; or Risk Group 4 such as Ebola virus, Marburg virus [45,46].

Three major steps in the process of biorisk assessment include the identification of the biological agent, the determination of the likelyhood that such a biohazard will cause an undesired event or consequence, and the management of the biorisk through established control measures. With respect to emerging viruses, the pathogenicity as well as the virulence of the virus will affect the outcome. Viruses with an increased host range will result in an increased overall probability that an infection might occur upon exposure. Successful biorisk assessment depends on the knowledge and information available. At the minimum, the biohazard has to be identified and characterized and the activities conducted, as well as procedures applied, have to be defined [44].

In addition to information of risk groups, good starting points for biosafety information on emerging viruses are the agent summary statements in the BMBL guidelines [45] that provide risk-related information for handling the particular viruses and recommendations on biorisk management. With regard to SARS-CoV, following the 2003 outbreak, two publications exist on infections of staff in research laboratories in Singapore and in Taiwan [40]. However, no staff-infection cases have been linked with any routine analysis of diagnostic specimens. The risk of SARS-CoV to the laboratory community is not fully understood. The mechanism of transmission in nature is not understood. It seems likely that SARS-CoV is transmitted by close personal contact. Airborne transmission of SARS virus has been shown [47,48]. SARS may also be spread through droplets, aerosols and possibly fomites. The original source of SARS-CoV is not known. SARS-CoV may be detected in the respiratory system, blood, or faeces. The precise transmission mechanism of SARS-CoV-laboratory-acquired infection has yet to be elucidated. Experiments requiring any manipulation of non-inactivated specimens should be undertaken in BSL2 facilities with the application BSL3 practices. All aerosol-generating procedures should be carried out in BSC, and the necessary PPE needs to be worn. Cultivation of SARS-CoV in cell culture must be carried out in a BSL3 facility using BSL3 practices and procedures. Currently, there is no anti-SARS vaccine available [40,45].

In the case of rabies virus, the disease caused, rabies, is characterized by acute, progressive, fatal encephalitis. Rabies-laboratory-acquired infections are very rare. The hosts of rabies virus in nature are a number of bat species and terrestrial carnivores, but most mammals are infectable. The saliva of infected animals is highly infectious, and biting is the commonest method of transmission. The highest viral concentrations are to be found in the central nervous system (CNS) tissue, salivary glands, and saliva. The most likely sources for infection are accidental parenteral inoculation, cuts, or pricking by needles using contaminated laboratory equipment, biting by infected animals, and exposure of mucous membranes or broken skin to contaminated tissue or fluids. BSL2 and/or animal BSL2 practices and facilities are recommended for all experimentation using known or potentially infectious materials or animals. Vaccination for Rabies is a necessary precaution for all workers prior to experiments on the rabies virus or Rabies-infected animals [45]. Rabies is lethal to humans [49]. However, recovery from the disease by subject not receiving any treatment has been reported [50].

Nipah virus and Hendra virus are two closely related and rare paramyxoviruses that cause severe disease and are lethal to humans and a variety of animals. They first appeared in Malaysia and Australia, respectively [51]. Nipah virus and Hendra virus are Risk Group 4 due to the high mortality of the viruses in humans [52]. Presently, no therapies or vaccines for Nipah or Hendra virus exist [51], although a vaccine for Hendra virus in horses was recently reported [53]. Viruses belonging to the Paramyxoviridae family appear relatively labile and are readily killed with currently available detergents. Advice has been given concerning hand-washing after the handling of infected materials with soap plus water [54].

For the HPAI, the virus is a cause of influenza, an acute disease of the respiratory tract. This virus can be found in respiratory tissues or secretions of humans and animals or birds carrying the infection. Additionally, this virus can be found in the intestines and cloacae of infected birds species. Laboratory workers are at risk of inhaling the virus from aerosols produced by infected animals or by the aspiration, dispension, mixing, centrifugation or other forms of manipulation of virus-infected samples. Laboratory workers may also be infected directly by inoculation of mucus membranes via virus-contaminated gloves after the handling of tissues, feces or other secretions of infected animals. Work involving HPAI viruses in laboratories requires increased caution because of the likelyhood of infection of laboratory personnels. BSL3 practices and protocols with adequate facilities are recommended. High-efficiency particulate air (HEPA)-filtered respirators or powered air-purifying respirators (PAPR) are essential for the safe handling HPAI viruses which have the potential of human infection [45].

With respect to the Ebola virus, this virus is highly infectious and a fatal case associated with laboratory acquired Ebola virus infection has been documented [33]. Strict precautions must be implemented when experimenting with the Ebola virus or diagnostic materials. Laboratory tests on the non-inactivated virus present an extreme level of biological risk. Adequate precautions and facilities must be applied at all times, in line with the biorisk issues identified in the assessment of each procedure. The Ebola virus should only be isolated in a maximum containment BSL4 laboratory. The inactivation of specimens, which depends on the detection protocol being used, should be carried out under BSL3 conditions. If specimens have been inactivated, testing is then can be undertaken at a BSL2 laboratory. Appropriate PPE should be used when handling the specimens before inactivation [55].

Risk groups of certain emerging viruses and recommended precautions for handling them are shown in Table 1.

A major concern associated with the risk of working with emerging viruses in the laboratory is the generation of aerosols from various laboratory activities which are not immediately be recognizable and may affect other scientific personnels. The dynamics of accidental infection risk in research laboratories is dominated by infective aerosols and, to a smaller extent, percutaneous infection [28]. Aerosols are suspensions in the air (or other gaseous medium) of solid or liquid particles which are small enough for them to remain airborne for long periods because of their low-settling rate. The sizes at which particles exhibit aerosol behavior also depends on the median diameters at which they become deposited in the lower respiratory tract after inhalation [58,59]. Four characteristics of virus–host relationship have been proposed to determine aerosol transmission which include the amount of available virus, virus or virus aggregate particle size, level of mucosal inflammation, and efficiency of viral replication in susceptible mucosa [60]. For example, the importance of aerosols in the transmission of influenza virus has been indicated [61,62].

Table 1
Risk group and recommended precaution of certain emerging viruses*.

Virus	Risk Group	Recommended precaution	Reference
Hantavirus	3	BSL2 for diagnostic specimen; BSL3 for virus propagation	[45]
Hendra virus	4 (animal:3)	BSL4 for all work	[45,52]
Nipah virus	4 (animal:3)	BSL4 for all work	[45]
HIV	3	BSL2 for diagnostic specimen; BSL3 for large volume or preparation	[45]
HPAI H5N1	3	BSL2 for diagnostic specimen; BSL3 for virus propagation	[45]
Ebola	4	BSL4 for all work	[45]
West Nile	3	BSL2 for diagnostic specimen; BSL3 for virus propagation	[45]
Chikungunya	3	BSL3	[45]
Zika	2	BSL2	[45]
Japanese encephalitis	3	BSL3 for all work	[56]
Dengue	2	BSL2 for all work	[45]
SARS-CoV	3	BSL2 for diagnostic specimen; BSL3 for virus propagation	[45]
MERS-CoV	3	BSL2 for diagnostic specimen; BSL3 for virus propagation	[57]

*:Biological safety levels are distinct from risk group levels. A proper risk assessment for emerging viruses must always be conducted before establishing a biological safety level.

Although particular emerging viruses such as the Ebola virus are not usually transmitted by the aerosol route, a malfunctioning instruments have the potential to generate aerosols or droplets that could place multiple laboratory workers under the risk of exposure [24]. Aerosols may be produced by using unprotected or malfunctioning high-energy equipment like centrifuges and homogenisers. Aerosol-generating-accidents can occur by dropping glassware or plates, or by catastrophic equipment failures such as centrifuges exploding. The subsequent release of the pathogenic viruses may lead to infection by aerosolic inhalation. Therefore, equipment used in microbiological laboratories needs to be designed to prevent the release of aerosols. However, accidents can still arise [63].

Biorisk associated with genetic modification of emerging viruses must also be assessed before starting the work. Prior to modifying the genome of an emerging virus in the laboratory, risks for health and environment associated with this genetic manipulation must be assessed [64]. The first stage in the assessment of risk of a genetically-manipulated emerging virus should be the identification of its potentially harmful properties resulting from the genetic modification. Elements that need to be taken into account include pathogenicity and infectious dose, the route of transmission, host range, stability and persistence of the virus in the relevant environment, the availability of effective prophylaxis and effective therapy [65]. Special attention should be given to the genetic modification of emerging viruses that become modified in increased transmissibility in humans, and the potency to cause human pandemic threats [66].

4. Preventing exposure and infection by emerging viruses

The biorisk in working with emerging viruses must be managed to an acceptable level. A fundamental focus of a good biosafety program for handling emerging viruses lies in the containment of the potentially-harmful materials in order to minimize or remove viral exposure of laboratory personnels and other workers, and the external environment. Any comprehensive approach to biosafety needs to be based on a combination of administrative controls, standard operating procedures, engineering controls, and personal protective equipment [45,67]. In a high containment (BSL3) facility, the essential elements for containment include good microbiological techniques, specialized safety practices and procedures, safety equipment and containment devices (often called primary barriers) with the design of laboratory facilities (often called secondary barriers) to protect persons inside and outside the facility [67].

The most important element of containment is the adherence to standard microbiological practices and techniques. Scientists undertaking work with emerging viruses must be aware of the potential hazards, and must be competent in the practices and techniques needed to work with pathogenic viruses safely. Appropriate training of personnel should be provided [45].

A part of the safety practices and procedures is personal protection which is critical to the prevention of exposure and infection by emerging viruses of the laboratory workers. Some recommended practices related to personal protection include: 1) Wearing laboratory coveralls, gowns or uniforms during work in the laboratory; 2) Wearing appropriate gloves for all procedures that may involve direct or accidental contact with potentially biohazardous materials and removing gloves aseptically after use followed by washing hands; 3) Washing hands after handling infectious materials before leaving the laboratory working areas; 4) Wearing safety glasses, face shields to protect the eyes and face when necessary; 5) Not wearing protective laboratory clothing outside the laboratory; 6) Not wearing open-toed footwear in laboratories; 7) Not eating, drinking, smoking, applying cosmetics and handling contact lenses in the laboratory; 8) Not storing human foods or drinks in the laboratory; 9) Not storing used protective laboratory clothing in the same compartments as street clothing [42]. Vaccination may provide a higher level of personal protection [45].

Safety equipment includes biosafety cabinets (BSC), enclosed containers, and other engineering controls designed to eliminate or reduce exposure to emerging viruses. The BSC is the main safety device used to provide containment of infectious droplets or aerosols generated by many manipulative procedures. In addition, safety equipment may also include items for personal protection. The enclosed centrifuge cup designed to prevent the release of aerosols during centrifugation is another safety equipment functioning as primary barrier for virus containment [45].

PPE is an effective control measure when exposures in the laboratory cannot be eliminated [68]. PPE, such as impermeable gloves, coats, gowns, cuffed gowns or disposable coverall suits, long-sleeved shoe covers, boots, face masks, eyes protection, or

goggles, respirators, are generally used in the handling emerging viruses [24,55]. The use of respirators is an important consideration in diagnostic and research settings where aerosols pose a high risk of infection by emerging viruses to workers. To ensure sufficient personal protection, a properly fitting respirator is imperative. Performance of the respirators depends on the type of the respirator and the proper donning of the respirator. Respirator fit-test is important because workers often fail to achieve sufficient protection with their respirators [69].

Traditionally the level of biosafety is classified into four biosafety levels according to combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Biosafety level 1 (BSL1) represents a minimal containment level which relies on standard microbiological practices. Biosafety level 2 (BSL2) is for laboratories that handle emerging viruses requiring containment level 2 such as HIV, dengue virus, rabies virus, that are not generally transmitted by airborne routes. Biosafety Level 3 (BSL3) applies to the laboratory activities involving emerging viruses requiring containment level 3, typically those with a potential for respiratory transmission, often having a low infectious dose to cause serious or fatal illness. SARS-CoV, HPAI H5N1, Japanese encephalitis, West Nile virus are representative of the emerging viruses assigned to BSL3 facilities. The high containment BSL3 emphasizes additional primary and secondary barriers to reduce or eliminate the release of infectious organisms into the immediate laboratory space and the general outside environment. Additional measures to prevent transmission of viruses are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access [45,70].

Biosafety level 4 (BSL4) is the maximum containment level and is designed to be used for manipulating emerging viruses with the highest level of risk. BSL 4 practices, safety equipment, and facility-design and construction are suitable for working with the most pathogenic and virulent emerging viruses, which pose a high individual risk of acquiring life-threatening disease, which can be transmitted via the aerosol route and for which there is no effective vaccine or therapy. Emerging viruses, with a near or identical antigenic relationship to BSL4 viruses, need to also be handled at this maximum level. Very virulent viruses like Ebola, Marburg, Congo-Crimean hemorrhagic fever, Junin, Lassa, Hendra are manipulated at BSL4 [37,45,52].

A fundamental biosafety principle is that contaminated materials be decontaminated before their disposal. Infectious laboratory waste can be effectively decontaminated in the autoclave. Chemical disinfection is usually used for the decontamination of surfaces and equipment which is not autoclavable, such as specimen containers and other items removed from containment, and for the decontamination of spills of infectious materials, infected rooms and animal containment areas (pens), and a variety of other items for which high temperature treatment is not possible. The initial choice of a chemical disinfectant is related to the resistance of the viruses being handled. Enveloped viruses are the most susceptible to chemical disinfection. Nonenveloped viruses are the least susceptible. Consideration needs also be given to practicability, stability and compatibility with materials and the health hazards. Gaseous decontamination of working areas is only required at BSL3 and BSL4 and then only in particular cases. Incineration is the traditional method for disposing anatomical biomedical waste and animal carcasses [45].

Correct disinfection is essential for interrupting the environmental spread of emerging viruses in the laboratory. Some virus species are resistant to harsh environmental conditions and are being able to remain infectious on surfaces over long periods of time and thereby presenting high resistance to disinfection. Potassium hydroxide- and sodium hydroxide-based alkaline detergents, peracetic acid- and acetic acid-based disinfectants, and gaseous hydrogen peroxide were shown to have capacity to inactivate several viruses. They have been demonstrated to offer virucidal efficacy and can therefore provide for a very high level of protection against viral contamination [71]. Zika virus is killed by potassium permanganate at 0.5%, 24 h of contact with ether, and temperatures above 60 °C but is not inactivated by 10% ethanol [72]. For decontamination of BSC, the primary methods are the use of formaldehyde gas, the vapor phase of hydrogen peroxide, and chlorine dioxide gas [73].

The virus inactivation mechanisms of several common virucidal agents have been reported. Treatments with ultraviolet (UV) radiation, singlet oxygen, and hypochlorous acid usually destroy the viral genome, whereas chlorine dioxide and heat interrupt the process of host cell recognition for virus binding [74]. Due to the presence of essential lipids in their envelope, enveloped viruses are considerably more susceptible to virucidal chemicals. Among the nonenveloped viruses, those with a smaller particle size are less susceptible than those of a larger size [75]. Other studies, however, showed that closely related viruses can exhibit different kinetics for disinfection when the same biocidal agents were used [74].

5. Biosecurity for working with emerging viruses

In addition to a biosafety program, it is important to have a biosecurity plan in place when handling emerging viruses. In this case, the goal of the biosecurity implementation is to prevent emerging viruses falling into unsafe hands. Work involving emerging viruses may have dual-use potential. Any biosecurity plan needs to be tailor-built for each facility, and types of activity conducted, as well as the local environment. Specialist working groups should be involved, which include science directors, principal investigators, laboratory personnels, general administrators, safety officers, security staff, facility maintenance staff, officers and law enforcement agencies where and when it is appropriate. Risk assessment is the primary component to any biosecurity plan. Risk assessment for biosecurity should review and make an inventory of the relevant assets, define the relevant threats, outline the particular vulnerabilities, and determine the countermeasures or mitigation strategies which are specific to each facility [45]. Good laboratory biosecurity system involves a multi-faceted approach that includes physical security, personnel security, biohazard material control and accountability, transport security, and information security [76].

Emerging viruses have the potential be used as bioweapons and agents of bioterrorism. Among the reasons which make biological agents attractive for these purposes is their low cost. Viruses can multiply in the host organism and can be transmitted to new hosts, generating unpredictable effects on the population, both in terms of number of victims and geographical spread [77]. Although the threat of biological warfare seems remote to most industrialized and developing nations, the threat of bioterrorism by extremists is a matter of current concern. Bioterrorism, and its effects, can impose heavy demands on the public health care

system which will be needed to handle the consequences. Generally, there are five phases of activities in dealing with a bioterrorist attack. These include a phase for preparedness, a phase for early warning, a phase for notification, a phase for response and a phase for recovery. Laboratories with good capacity that can provide quick laboratory support are critical for public health emergency preparedness and adequate responsiveness to bioterrorist attack [78].

The classification of biological agents such as emerging viruses is a fundamental element for both biosafety and biosecurity. The classification of emerging viruses is therefore based on two measures: laboratory biosafety and biosecurity considerations. For biosafety assessment, the main consideration is the ability of viruses to cause local or widespread disease from laboratory accidents. In biosecurity, especially in assessment for biodefense, the main concern is the potential for viruses to be used as weapons, in terrorism and to cause harm associated with their unauthorized release [79].

Emerging viruses of biosecurity concern include Ebola, Marburg, Lassa, and Junin virus (categorized group A) for their ability to cause large or widespread casualties and a need for broadly-based preparedness in public health; Venezuelan, Eastern, and Western equine encephalomyelitis viruses (categorized group B) for their potential of large scale dissemination, however which usually cause milder illness than viruses placed in category A; Nipah virus, Hantaviruses (categorized group C) which are not currently thought to present a high bioterrorism risk to public health, but which capable of becoming threats in the future [80].

6. Conclusion

The recent outbreaks of Zika virus following the Ebola crisis reveals how vulnerable to the threat of emerging viral disease we are in this global, interconnected world. This also highlights the complexity of the system that leads to the emergence of viral outbreaks. Emerging viral threats need to be met with deliberative actions such as improved surveillance and outbreak response measures. Speedy identification of emergent of disease-causing viruses is an essential component of any responsive program for control. To successfully control emerging viruses, knowledge of many key aspects of their pathogenicity, molecular characteristics and information on factors causing efficient person-to-person spread, viral immunology and immunogenetics is critical. This is a challenging task, and the roles of the laboratory in diagnosis and research of emerging viruses are indispensable. To be confident in handling pathogenic emerging viruses, it is vital to develop and implement biosafety principles for safe handling of the viruses in the laboratory and preventing laboratory viral exposure and infection. Biosafety programmes should, therefore, be in place. Keys to the biosafety programmes are an assessment process for biorisk and a biorisk management by implementation of containment systems. As emerging viruses might be of dual-use concern, a biosecurity system is also important. Increased capacity in the safe handling of emerging viruses will in turn improve surveillance and strengthen preventive and control procedures. Although a tendency in the emergence of pathogenic viruses is increasing, prediction in this area remains difficult for the occurrence of future viral diseases and the size of the public health burden and economic threats posed. Development and implementation of laboratory biosafety principles is therefore critical as part of a response for preparedness to future outbreaks of emerging viruses.

Conflict of interest statement

We declare that we have no conflict of interest.

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