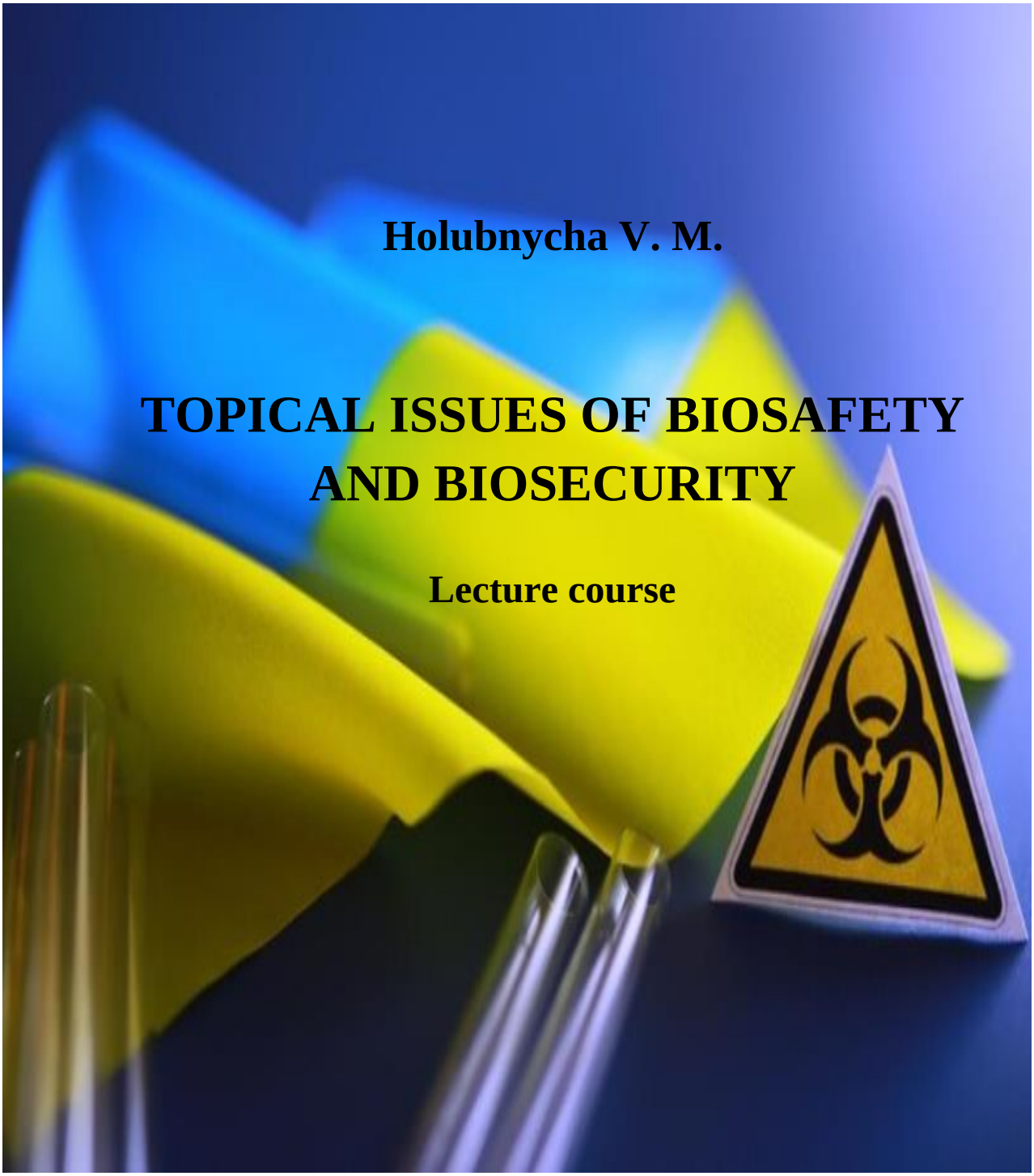
Ministry of Education and Science of Ukraine

Sumy State University



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TOPICAL ISSUES OF BIOSAFETY AND BIOSECURITY

Lecture course

Sumy
Sumy State University
2023

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TOPICAL ISSUES OF BIOSAFETY AND BIOSECURITY

Lecture course

for students of specialty 222 “Medicine”, 221 “Dentistry”, 229 “Public health” of full-time course of studies

Approved at the meeting of the department of Public Health as lecture course on the discipline “European regulations in biosecurity and biosafety considering global challenges ”. Minutes № 12 of 12.04.2023.

Sumy
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2023

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Department of Public Health

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Introduction

The technological revolution has led to the changes in the international security situation, the progression of globalization, and the constant development of biotechnology. Risks and challenges posed by major infectious diseases and bioterrorism to the international community are also increasing. Meanwhile, the development of technologies, the availability of increasingly complex scientific tools and effective methods are not able to protect humanity from a possible biological threat.

The public expects scientists, laboratory and plant employees act responsibly, requires that the environment not be exposed to biohazards and comply with health and safety regulations (biosafety) related to methods that help securely and safely store work results and materials, and adhere to bioethics. That is why biological safety is relevant not only for a narrow circle of laboratory workers, but for all mankind issue facing the scientific community is the implementation of the definite rules and techniques into the teaching process of biomedical faculties.

Raising awareness of biosafety and biosecurity among students and young scientists who work with biohazardous substances will contribute to the reduction of individual and social risks. The proposed lecture course aims to promote knowledges of basic infection control practice among the population and is designed to disseminate best practice in biomedical settings.

Topic 1. Biosafety

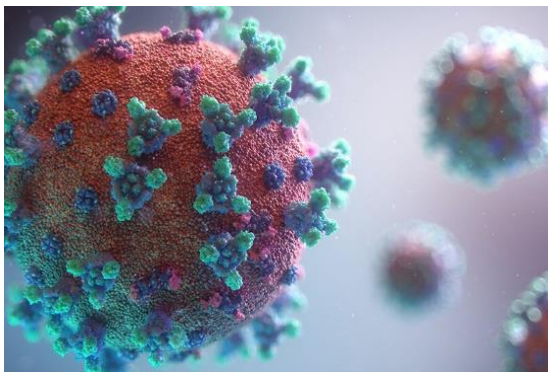
Learning objectives :

1.1 Biosafety: definition, history, and impact on human society

1.2 Biosafety regulations and containment principles

1.3 Classification of biohazardous materials and biosafety levels

1.4 Risk assessment



1.1 Biosafety: Definition, History, and Impact on Human Society

Since the middle of the last century concerns about biosafety issues have become permanent among the broad public. Different resources and organizations are involved in the monitoring, prevention, and eradication of the biological threats. Thus, there are several definitions of biosafety. In general, **biosafety** is the combination of practices, procedures, and equipment that protect public health workers, the public, and the environment from the infectious agents and their products.

The initial attempts to set up the biosafety practices in different fields of social life were done in the USA. The first person who launched the assessment of the biological threat in the laboratories was Dr. Arnold G. Wedum (Fig 1). As a director of the Industrial Health and Safety Institute in Fort Detrick (1943-1969) he established the first Biological Safety Conference in 1956. He also provided guidance on biosafety programs and practices as well. The scientists from the Fort Detrick biological laboratories established the American Biological Safety Association (ABSA). This institution made huge contributions to biosafety development in these years. It included occupational health programs, risk assessment, development of decontamination protocols, evaluation of microbial hazards and HEPA filter efficacy.

Figure 1 – The founder of biosafety Dr. Arnold G. Wedum

From 1957, the US Department of Agriculture (USDA), National Institutes of Health (NIH), Center of Diseases Control (CDC), and other American organizations took part

in biosafety conferences that led to raise awareness about biosafety among the broad public. By 1966, ABSA included representatives from universities, private laboratories, hospitals, and industry. In the mid-1970s the rules for shipment of the microorganisms, toxins, and the classification of the biological hazards on 4 groups were implemented in common practice.

After terrorist attacks in the USA in 2001 the attention to this field was increased worldwide. The authorities and researchers have developed regulations and guidelines that describe containment measures and working instructions, especially for agents and toxins that could be used in acts of bioterrorism and pose a severe threat to society.

Nowadays biosafety is related to several fields such as ecology, agriculture, medicine, chemistry, exobiology, and synthetic biology. Apart from this, biosafety is a critical area in biomedical research. Medical research and practice are estimated as the most dangerous activities in the frame of biological safety. It involves the handling of infectious agents and their vectors. The WHO indicates that human error and poor technique are the main reasons for the mishandling of biohazardous materials. Biosafety provides containment strategies to keep the dangerous pathogens efficiently isolated and ensure the safety of job-related activity for various groups of public healthcare workers.

1.2 Biosafety Regulations and Containment Principles

Biosafety as a discipline sets the standards, policies, and procedures required to personnel protection in handling microorganisms and microbiological products in various institutions and fields. There are a lot of national and international regulations and guidelines that are applied today. We focus on some of them that were created in the USA, and in the EU.

1 Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, 2009. This document contains guidelines for microbiological practices, safety equipment, and facilities.

2 National Institutes of Health Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules. This document comprises guidelines for constructing and handling recombinant or synthetic nucleic acid molecules and organisms containing those molecules.

3 Bloodborne Pathogens. This regulation consists of the combination of engineering controls, work practices, and training to reduce the risk of infections that are transmitted via the human blood and other potentially infectious materials.

4 EU Directive 2003/85 on Community measures for the control of hand-foot-and-mouth disease. The document includes measures provided for control and eradication in the event of an outbreak of hand-foot-and-month disease.

5 EU Regulation 1069/2009 and Commission Regulation 142/2011 implementing Regulation (EC) No. 1069/2009 of the European Parliament and the Council establish sanitary regulations for by-products and products of animal origin not intended for human consumption.

6 EU Directive 2001/18, Commission Decision 2002/263, Commission Decision 2002/811 are established to guide a deliberate release into the environment of genetically modified organisms.

7 Council Regulation 428/2009 setting out a Community regime for the control of export, transfer, brokering and transit of dual-use items.

8 EU Regulation 1829/2003, EU Regulation 1830/2003, Directive 2001/18/EC, and Commission Regulation 1981/2006 are related to the handling of genetically modified foods.

9 EU Directive 2000/54 on the protection of workers from risks related to exposure to biological agents at work.

10 Council Decision 2002/628 concerning the conclusion, on behalf of the European Community, of the Cartagena Protocol on Biosafety.

The principles and components of biosafety can be described by the term **containment**. It describes safe methods for managing infectious agents in the environment where they are being handled or maintained. The purpose of the containment is to reduce or eliminate exposure of workers, other people, and the outside environment to potentially hazardous agents. There are two types of containment: **Primary containment** is the protection of the people inside the facility. It is provided by good microbiological techniques and the use of appropriate safety equipment. **Secondary containment** is the protection of the external environment from exposure to infectious materials. It comprises a combination of facility design and operational practices. The elements of containment are administrative controls, work practices, protective equipment, and facility design.

Administrative control comprises the policies and procedures for safe laboratory work practices and sets the standard for behavior within the laboratory. Their strict implementation is compulsory for all staff working in the laboratory or public health institutions that work in compliance with biosafety rules.

Work practices include Good Laboratory Practices (GLP), Standard Operating Procedures (SOP), Current Good Manufacturing Practices (CGMP) and Hospital Infection Control Program.

GLP is a system of management controls for research laboratories and organizations to ensure the uniformity, consistency, reliability, reproducibility, quality, and integrity of products designed for human or animal health. The use of GLP ensures safety, consistency, high quality, and reliability of chemicals, medical devices, food additives, food packaging, and other biological and electronic products in the process of non-clinical and laboratory testing.

SOPs are procedures developed by each individual laboratory to provide biosafety. SOPs include laboratory-specific rules for manipulating microorganisms, methods of exposure controlling, decontamination and waste-handling requirements.

CGMP is developed by the Food and Drug Administration (FDA) in the USA and it is applied by research laboratories that produce gene therapy products.

Hospital infection control programs facilitate the prevention and control services to healthcare personnel and prevent transmission of infections between healthcare personnel and others.

Protective equipment includes specialized engineered controls and personal protective equipment that keeps workers safe and minimizes the exposure to biological agents. It is specific for every institution that operates with microbes or their products. Their facility design should correlate with the type of agent being used or stored.

Biosafety cabinets (BSCs) are a very important component of protective equipment that allows to protect the staff, environment, and product. Biosafety cabinets can be divided into several classes.

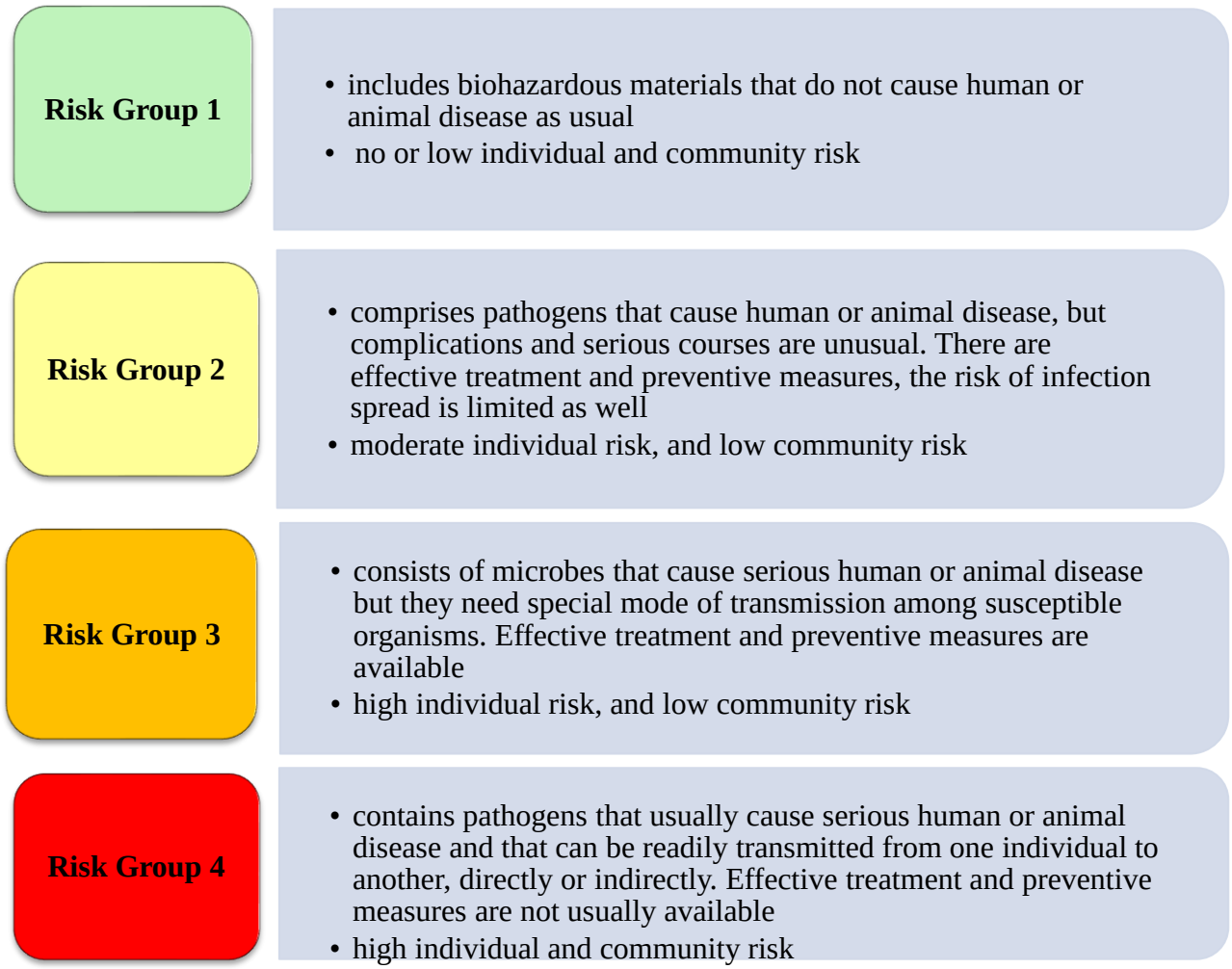
Personal Protective Equipment (PPE) is another very important component of biosafety equipment in the laboratory. Personal protective equipment includes gloves, masks, lab coats, and other wearable equipment (such as safety glasses and respirators) that protect laboratory workers from infectious agents and toxins in the laboratory.

Biosafety equipment can also include the equipment used in building design to prevent the release of infectious agents and toxins. Examples of protective features in buildings include double doors and negative air pressure rooms. Air filtration and waste management systems can also be very important for biosafety.

Needles, centrifuges, glass instruments, and other tools can all pose unique hazards to laboratory workers. Over time, the design of this equipment has changed to improve safety and reduce risks. Biosafety innovation is an ongoing process of hazard identification and design changes to address new or newly recognized risks.

1.3 Classification of Biohazardous Material and Biosafety Levels

CDC and NIH classify biohazardous materials into four groups taking into account pathogenicity and epidemiological features of the microbiological agent:



Biosafety Levels

According to CDC guidelines, there are four biosafety levels (BSL) for working with infectious agents and experimental animals. Although this classification was developed initially for work in laboratories, nowadays, it is relevant for different fields and sectors of society. It is based on combinations of equipment, procedures, techniques, and laboratory design appropriate for the features of the laboratory and used infectious agents.

BSL-1 includes activities that are not associated with infections.

BSL-2 practices include handling of the infectious agents that are not transmitted via aerosols (e.g., HBV, HIV, enteric pathogens, and staphylococci). These agents are associated with a moderate health hazard.

BSL-3 is recommended when working with agents that are highly infectious and are transmitted via aerosols (e.g., *M. tuberculosis*, *Brucella* spp., and *Coccidioides immitis*).

BSL-4 practices are required when working with unusual agents that cause life-threatening infections that are frequently fatal: there are no treatments or vaccines. Two examples of such microbes include Ebola and Marburg viruses.

1.4 Risk Assessment

In general, **risk** is a function of the likelihood of an adverse event iassociated a particular hazard and/or threat will occur and its consequences. **Biorisk** is defined as a combination of the probability of occurrence of harm and the severity of that harm where the source of harm is a biological agent or toxin. There is a huge list of biological risks. It includes individuals, community, and environment risks.

Risks are classified based on the levels of intentionality as natural, unintended, and deliberate. The naturally occurring risks comprise the naturally occurring infectious diseases of humans, animals, and plants. The occurrence of unintended biological risks is caused by staff mistakes and mishandling of biological hazards. The intentional use of pathogens for harmful purposes is the last type of the risk spectrum.

Biological risk assessment is one of the key principles to reduce the impact of the risks on individuals and communities. **Biorisk assessment** is a process of evaluating the biorisk(s) arising from a biohazard(s), considering the adequacy of any existing controls, and deciding whether or not the biorisk(s) is acceptable. It resulted with development of the guide for the selection of appropriate biological safety and security measures to reduce negative impact of biohazardous factors.

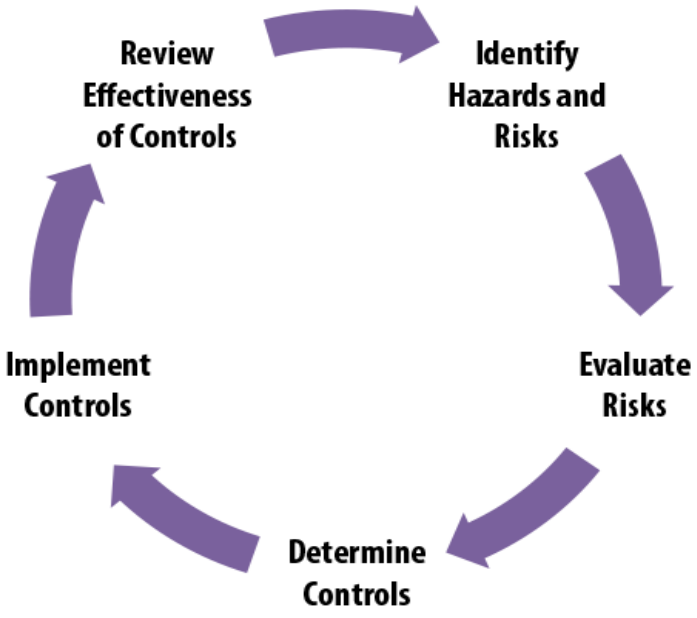


Figure 2 – Biological risk assessment methodology

The basic risk assessment process resembles a circle from several steps (Fig 2).

Step 1. Identify hazards and risks.

This is a critical step of the biosafety risk assessment process. Employees must answer the questions: What, where, and how is the work occurring? Who is involved

in it? The main component of this step is identification of the hazard type and the biological agents that you work with.

Step 2. Evaluate the risks.

In this step you will answer the questions: What could go wrong? How likely is it to happen? What are the consequences? Initially you need to classify all biological agents you are working with according to their risk group.

For this, you need to identify the biological features of each agent, its pathogenicity, mechanism of transmission, existence of therapeutic and prophylactic preparations. On the other hand, you need to characterize the features of the hosts (susceptible person). At the end characteristics of the work environment must be identified. Thus, you need to assess all three chains of epidemiological processes in combination.

This is followed by characterizing the risks as a function of likelihood and consequences by using a standard set of criteria. The likelihood component of risk includes factors that influence the probability of the incident happening. They are reflected in Table 1.

Table 1–A List of factors influencing on probability of the incident

Factors	**Influence on Probability**
Agent	1 Stability of the agent in the environment (e.g., ability to produce spores, resistance to disinfectants) 2 Potential routes of transmission (direct mucosal contact, inhalation, ingestion, injection) 3 Endemicity of biological agent in the local environment and population (e.g., endemic or exotic) and host range 4 Life stage / form of the biological agent (e.g., dimorphic fungi, antigenic shift)
Host	1 Competency of personnel, level of training 2 Following safe work practices 3 Stress, risk perception, risk tolerance 4 Behavioral aspects
Environment	1 Physical infrastructure and existing controls: type of facility,

Continuation of Table 1	
Factors	**Influence on Probability**
	presence of engineering / safety controls, type of equipment used, functioning / reliability of ventilation systems, use of sharp objects; amplification of the biological agent by culturing and types and complexity of procedures performed. 2 Procedural factors: existence of administrative controls such as policies and training; availability of appropriate PPE, generation of aerosols and use of sharps, amplification of the biological agent by culturing, and the types and complexity of procedures performed.

Risk consequences are characteristics that describe the severity of an incident. To evaluate the consequences after an undesirable incident means to assess the characteristics of the hazard (s) or biological agents, the health and immune status of the laboratory / testing personnel, and the availability of vaccines, prophylaxis, or therapies.

Table 2–A list of factors influencing the consequences of the incident

Factors	**Influence on Consequences**
Agent	1 Virulence factors: adhesion, invasiveness, toxicity, production of exoenzymes, antigenic variation, resistance to antibiotics, tissue tropism, multiple replication sites within the host, ability to elicit autoantibodies against the host) 2 High communicability 3 Infectious dose 4 Severity of infection / disease (morbidity / mortality rate)
Host	1 Health and immune status of the staff: immunocompetent or immunocompromised, pregnancy, pre-existing medical conditions, allergies, age, large susceptible population 2 Behavioral aspects 3 Willingness to accept vaccines 4 Adherence to safe work practices and proper use of PPE

Continuation of Table 2

Factors	**Influence on Consequences**
Environment	1 Availability of vaccines, prophylaxis, therapeutic interventions, and emergency response procedures 2 Administrative controls.

Risk assessment results should be documented via the report. Each risk should be prioritized, and acceptability of every risk should be assessed. In case of unacceptable risk, the assessment procedure is followed with determination of the necessary mitigation measures to make risk acceptable. Any risk can be reduced and even eliminated if the work is provided with some unharmful surrogates.

Steps 3–4. Determine and implement control measures.

Implement risk mitigation measures in your professional activity on all levels (engineering controls, administrative and work practice controls, and use of PPE).

Steps 5. Review the effectiveness of controls.

The effectiveness of risk control measures must be evaluated again.

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6 Canadian Biosafety Standard (CBS): 2nd Ed. / Ottawa : Public Health Agency of Canada, 2015. 188 p.

Topic 2. Biosecurity

Learning objectives :

2.1 Biosecurity: Definition, Goal, Impact

2.2 Biosecurity legislation

2.3 Dual use research of concern and gain of function research (GOF)

2.4 The principle of biological harm prevention at the European University

2.1 Biosecurity: Definition, Goal, Impact

Globalization of economics, changing agricultural practices, human ecology and behavior, as well as development of biotechnology, have caused the impact of biosecurity to grow. The term “biosecurity” has multiple definitions. In some countries it is used instead of the term “biosafety”. The National Research Council (2009) gives such a definition for them: “Biosafety is about protecting people from bad bugs biosecurity is about protecting bugs from the bad people”. The WHO defined **biosecurity** as a protection, control, and accountability for valuable biological materials agents and toxins within facilities to prevent their loss, theft, misuse, diversion of unauthorized access, or intentional unauthorized release.

Historically, some events led to increased biosecurity impact and legislative changes. Since 2001, there have been a variety of national and global initiatives to increase biosecurity. It was promoted with some events in the scientific world such as synthesis of recombinant horsepox virus with using segments of mail-order DNA (2017, Canada); detection of unsecured smallpox samples in FDA cold-storage room (2014); identification of the botulinum neurotoxin (BuNT/H) (2013); theft of vials of pathogens by the Canadian Food Inspection Agency researcher in 2012 and a former researcher at the National Microbiology Lab in Winnipeg in 2009. Some cases caused higher impact on biosecurity. For instance, in September 2011, scientists created the modified variant of the H5N1 avian influenza virus that was transmissible via aerosol between ferrets and could cause a deadly global pandemic. Apart this, severe acute respiratory syndrome (SARS) (2004, 2009), Ebola (2014), Covid (2019) and bovine spongiform encephalopathy (BSE) (1986) outbreaks confirmed the importance of biosecurity issues in real world.

In the frame of biosecurity, the WHO introduced the term of valuable biological materials (VBM). They are biological materials that require (according to their owners, users, custodians, caretakers, or regulators) administrative oversight, control,

accountability, and specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm. VBM may include pathogens and toxins as well as non-pathogenic organisms, vaccine strains, foods, GMOs, cell components, genetic elements, and extraterrestrial samples. Moreover, the knowledge obtained from working with these materials may be misused to threaten public and animal health, food security, or the environment. Biosecurity strategy and approach encompass the policy and regulatory frameworks for analyzing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment.

In the public health field term biosecurity is more often associated with laboratory biosecurity. However, the general principles of laboratory biosecurity could be applied to various branches of public health. Development of the biosecurity strategy comprises inventory of VBM, physical security of equipment, informational security, transport security, access control, administrative control (staff management, training and education), permanent use of the security-specific policies and procedures, internal audits and external inspections.

The biosecurity impact could be physical and psychological. Physical impacts are characterized as numbers of death or disease in human and animal populations. Psychological impacts are usually related to public fear. Total impact includes the impact on public health, animal health, or the organization. Owing to prevent the biological threat, we must assess its impact on all elements. It can be expressed as a value ranging from 1 to 5, where 5 is very high and 1 is very low. The entire value of the impact is estimated as the highest of the three element assessed values.

2.2 Biosecurity legislation

Governments from different countries have started to harmonize and rationalize biosecurity policies, legislation, and core roles with the goal to optimize efficiency of protective measures. Last decades various international organizations and conventions with relevance for biosafety and biosecurity were established. The World Health Organization (WHO), the World Trade Organization (WTO), the World Organization for Animal Health (WOAH), the Food and Agriculture Organization (FAO), the International Plant Protection Convention (IPPC), the Convention on Biological Diversity (CBD), Cartagena and Nagoya Protocols, and the Biological Weapons Convention (BWC) are among them. They set up the objectives, principles, and requirements on biosecurity and biosafety worldwide.

EU legislation on biological agents and genetically modified microorganisms is not specific enough to ensure harmonization leading to difficulties in implementation

for most laboratories. In the same way, biosecurity is a relatively new concept, and a few EU Member States are known to have introduced national laboratory biosecurity legislation.

A biosecurity policy framework sets out a broad course of action to address biosecurity risks in public health, and food industry. It provides a common basis for assessing biosecurity risks and priorities for action and gives direction and guidance to all the parties concerned. The International Health Regulations (IHR) is an agreement designed to prevent the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with international traffic and trade.

2.3 Dual Use Research of Concern and Gain of Function Research (GOF)

Concerns about risks affiliated to research appeared in the last several decades. This has caused the discussion on dual-use life science because any research can take unexpected results and lead to unanticipated discoveries. **Dual Use Research of Concern** (DURC) is life sciences research that can provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security. Research that involves any of the 15 listed agents and one or more of the seven categories of experiments listed below require DURC oversight.

Due to this, scientists should implement specific safeguards to mitigate the risks when they do the planning of any experiments. These safeguards may include all issues related to biological hazard handling and the information on how to prepare these materials, such as methodological protocols or genomic sequences of pathogens. However, such limitations may affect the research progress in a particular area.

Agents and toxins required DURC oversight:

1 Avian influenza virus (highly pathogenic)
2 *Bacillus anthracis*
3 Botulinum neurotoxin
4 *Burkholderia mallei*
5 *Burkholderia pseudomallei*
6 Ebola virus
7 Foot-and-mouth disease virus
8 *Francisella tularensis*
9 Marburg virus
10 Reconstructed 1918 Influenza virus

11 Rinderpest virus

12 Toxin-producing strains of *Clostridium botulinum*

13 Variola major virus

14 Variola minor virus

15 *Yersinia pestis*

Categories of experiments requiring oversight of DURC that

1 Enhance harmful consequences of the agent or toxin.

2 Disrupt immunity or the effectiveness of immunization against the agent or toxin without clinical and/or agricultural justification.

3 Impart the agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin or facilitate their ability to evade detection methodologies.

4 Increases the stability, transmissibility, or the ability to disseminate the agent or toxin.

5 Alter the host range or tropism of the agent or toxin.

6 Enhance the susceptibility of the host population to the agent or toxin.

7 Generate or reconstruct the eradicated or extinct agent or toxin listed above.

There were several experiments with dual use potential in past two decades. They are reflected in Table 3.

Table 3–Examples of research with dual-use potential

Year	**Type of Experiment**	**Dual-Use Potential**
2001	Creation of a genetically engineered mousepox virus for pest control resulted in significantly increased virulence	The same approach could be used for modification of the smallpox or other poxvirus that infects humans and could lead to the formation of a vaccine-resistant virus
2002	Artificial synthesis of infectious poliovirus in vitro with promulgation of the poliovirus genome structure	A virus could be resurrected through assembly of oligonucleotides based on genomic information
2002	Molecular engineering of a Variola virus protein led increased virulence of the vaccinia virus	The data could be used to the changing of the Vaccinia virus virulence or an the smallpox virus effectiveness

Continuation of Table 3		
Year	**Type of Experiment**	**Dual-Use Potential**
		of the vaccines against
2005	Reconstruction of the influenza A (H1N1) virus caused Spanish flu pandemic in 1918	The reconstructed virus and research information could be misused
2011	Creation of highly pathogenic strains of A/H5N1 avian influenza virus with enhanced transmissibility in mammals	An increased risk of accidental release of a pathogenic virus from the laboratory and mishandling of test results
2013	Development of immunological sterility in mice by introducing the ZP3 protein into the ovarian oocytes of female mice. Scientists inserted the gene encoding the immune regulator interleukin 4 (IL-4) into the mousepox virus. The virus then expedited its potential and became uniformly lethal. Moreover, even mice vaccinated against mousepox were killed by the new engineered virus	An increased risk of using this model to modify the human smallpox virus
2013	Genetic modifications of the wild-type H5N1 variant followed by the creation a new version of the virus that could spread through the air between ferrets	The increased risk of transmissibility of avian influenza virus to mammals
2015	The validity of the CRISPR/Cas9-based gene drive approach was tested in a proof-of-concept study using Drosophila fruit flies and Anopheles mosquitoes	Ecological consequences of the accidental release of modified insects into the wild, and the potential misuse of this new technology with malicious intent

All these experiments could be named as experiments with gain of function. Term "**gain-of-function"** is related to any modification of a biological agent that confers new or enhanced activity.

Potential benefits of GOF research

• Help define the fundamental nature of human–pathogen interactions.

• Enable assessment of the pandemic potential of emerging infectious agents.

• Inform public health and preparedness efforts.

• Further medical countermeasure development.

Potential risks of GOF research

• Involve generating novel engineered pathogens that could pose a pandemic threat if they were to be accidentally or intentionally released.

• May generate information that could be misused to threaten public health or national security.

• Risks would increase as more labs perform this type of research.

There are two United States policies on dual-use research of concern. One is the United States Government Policy for Oversight of Life Sciences DURC. The other is United States Government Policy for Institutional Oversight of Life Sciences DURC, which was released by the United States government on September 24, 2014.

2.4 The principle of biological harm prevention at the European University

Each university working with biohazards has its own biosafety and biosecurity program that ensures safe handling biological agents by all members of the university community. Staff and students receive sufficient and appropriate training (both theoretical and practical) to enable them to identify biological risks and to know and practice preventive and protective measures to minimise them. Safety rules include tools of approaches to minimize damages in case of contact with dangerous microbes. They comprise information about:

- general safety rules,
- personal behavior, hygiene rules, personal protection,
- cleaning of the working space,
- use of chemical, biological and physical agents,
- use of glass materials,
- handling of apparatus and equipment,
- safety warning sings,

- waste management,
- emergency actions.

Safety rules in the biological teaching laboratory ensure that practices are performed properly. They ensure the safety of the students and other users in case of potential accidents.

Students are responsible for the correct implementation of the safety rules. The training on biosafety is compulsory for everyone and it is followed with assessment of student knowledge. According to common approaches students are responsible for the safety of the materials used in classes. They must report immediately if any accidents or personal circumstances occur.

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Topic 3. Natural Epidemics

Learning objectives :

3.1 Endemic, Epidemic, Pandemic – definition and history

3.2 Preventing a pandemic.

3.3 The WHO phase program for identifying potential pandemics

3.4 Natural disasters and infectious disease outbreaks

3.5 Challenges to disease eradication

3.1 Endemic, Epidemic, Pandemic – Definition and History

The biological risk linked with natural occurrences could cause many different infections, which follow humanity from the beginning of its existence. Some pathogens disappeared, other arise and some other still go with humans, animals, and plants. Infectious diseases are classified into common, emerging / re-emerging and chronic. Emerging and re-emerging infectious diseases are a main global concern, since many of them involve highly hazardous pathogens or a very large population. The CDC estimates that three-quarters of the "new or emerging" diseases that infect human beings have originated in wild or domesticated animals. These new appeared infections were spread in human population and caused the endemic, epidemic or pandemic. Term **endemic** describes a disease that is present permanently in a region or population. The area where endemic diseases are spread is closely connected with particular animals that could be a source of infection or its transmitter. Classical examples of such diseases are malaria, yellow fever, Lyme disease and others. **Epidemic** infection is an outbreak that affects many people at one time and can spread through one or several communities. It occur when an agent has gotten a genetic change (mutation) resulting from a mutation or introduction of new pathogens to a host population. **Pandemic** is the term used to describe an outbreak that has spread globally. The WHO declares a pandemic when a disease shows exponential growth, and the infection rate increases significantly every day.

The speed of infection spreading depends on the contagiousness of the disease. It is called the basic reproduction number / rate also known as R0 or "R naught." This number tells us how many susceptible people, on average, each sick person will infect. This term is very important for disease prevention and eradication. Measles tops the

list, being the most contagious with an R0 range of 12–18. This means that a single person can infect, on average, 12 to 18 people in an unvaccinated population.

The history of pandemics includes a long list of infections caused by bacteria and viruses. The most famous and trilled infection is **plague.** It killed by far the most people around the world. There have been at least six known plague pandemics in the history of mankind existence. Approximately 330 million people passed away because of plague. It still kills 100 to 200 people a year. **Cholera** epidemics is the second famous bacterial infection that has caused seven pandemics in the world, and the last one has not accomplished yet. It killed more than 1 million people in Asia and Europe. Cholera is transmitted by consuming contaminated food or drinking water and can develop quickly if sanitation systems are disrupted. Cholera still affects many people today, with WHO reporting 1.3 million to 4 million cases annually. The **Russian typhus** epidemics led to death about three million people in the period of 1914–1922, with the peak of the epidemic in the Soviet territory.

The most dangerous viral pathogen in history was the causative agent of **smallpox**. It caused the deaths of 30 % of infected people. There were two types of smallpox: variola major and variola minor. The last naturally occurring case of smallpox was diagnosed in October 1977, and WHO declared its global eradication in 1980. **Spanish flu** or influenza was caused by a deadly subtype of the H1N1 virus in 1918–1920. It is considered the worst in modern history, killing an estimated 50 million to 100 million people in just 18 months. This Spanish flu pandemic affected young adults and people without immunological disorders. **Viral hemorrhagic fevers** (VHF) are the group of infections (yellow fever, Ebola, dengue fever) that are contagious and mostly lethal. VHF outbreaks occurred in Mexico between 1545 and 1548 and killed an estimated 5 million to 15 million of the native population. It was called "cocoliztli" or the Great Pestilence. **Human immunodeficiency virus** (HIV) causes a range of illnesses in those infected, leading to acquired immune deficiency syndrome (AIDS). About 37 million people have died from AIDS or HIV-related illnesses since the first reported case in 1981. Some countries of South Africa have almost 50 % of the population infected with HIV.

Last several decades WHO declared the pandemics of HIV infection, influenza virus infection, coronavirus infection and others. There have been three outbreaks caused by the **coronovirus,** one of them even turned into a pandemic. Severe acute respiratory syndrome (SARS) caused by the highly infectious coronavirus SARS-CoV started in Singapore and quickly spread to 37 countries between 2002–2003. The SARS epidemic had a mortality rate of 10.9 %. Middle East respiratory syndrome (MERS) is a respiratory illness that is caused by coronavirus too. It was first reported in Saudi

Arabia in 2012 and has spread to several other countries, including the United States. Most people infected with MERS-CoV developed severe respiratory illnesses, including fever, cough, and shortness of breath. Approximately 35 % of patients with MERS have died. New type of Coronavirus appeared in Wuhan, China, in late 2019 and caused a pandemic. There is not only one theory of its appearance. Some scientist assume wildlife origin of this novel coronavirus. The bat and pangolin are natural sources of SARS-CoV-2. There is also a suggestion that SARS-CoV-2 was the result of a laboratory accident, or it was intentionally engineered for gain-of-function research and had been previously studied with bat SARS-like coronaviruses to understand the risk of cross-species transmission. On May 4, 2020, scientists from Switzerland, Germany and Russia reported that they had successfully pieced together synthetic viral gene fragments using the published SARS-CoV-2 sequence. They reconstructed the active novel coronavirus harboring green, fluorescent signal in the sequence by using a well-established yeast-based gene combination platform. The novel coronavirus is less dangerous than its predecessors (mortality rate of 3.4 %) the high speed of spread and mutation activity makes it dangerous.

Swine flu is caused by the H1N1 influenza virus and appeared in central Mexico in March 2009. By April, it had reached California, infected a 10-year-old, and then quickly spread around the world. The virus genome contained the bird, swine and human flu virus components, a combination that has never been detected. CDC estimated that 151,700-575,400 people worldwide died from (H1N1) pdm09 virus infection during the first year the virus circulated. Globally, 80 % of (H1N1) pdm09 virus-related deaths were estimated to have occurred in people younger than 65 years of age. This differs greatly from typical seasonal influenza epidemics, during which about 70 % to 90 % of deaths are estimated to occur in people of 65 years and older.

The **Ebola** outbreak that erupted in West Africa between 2013 and 2016 was the most famous one. First, the Ebola virus was detected and described in 1976 near the Ebola River in the Democratic Republic of Congo. Since then, the virus has emerged periodically and infected people in several African countries. The West African Ebola outbreak started in Guinea in December 2013, and the virus spread in West African countries such as Liberia and Sierra Leone. It was assumed that the virus originated from forest bats. It spread to seven more countries: Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States. Later, secondary infection, mainly in a healthcare setting, occurred in Italy, Mali, Nigeria, and the United States. Two and a half years after the first case was discovered, the outbreak ended with more than 28,600 cases and 11,325 deaths.

Many common diseases have the potential to become emerging or re-emerging as biological agents evolve and adapt to new conditions, but also the environment and human actions play a major role in these events. Among the multiple factors influencing this process, the following ones are the main:

- High mobility of population contributes to the spread of biological agents, as they travel with people as hidden hosts.
- Urbanization leads to close contact between humans and wild animals and increases the probability of transmission microorganisms.
- Climate changes in temperature and precipitation lead to the spread of the vector born infections to new territories that causes the spread of endemic infections.
- Congestion means more contacts, which in turn entails a higher risk of transmission.

3.2 Preventing a Pandemic

To prevent epidemics and pandemic, a set of policies and preventive measures (containment) are used to limit the spread of any infection. These measures include:

- **Controls** of border to limit / prevent movement of individuals to and from the affected areas.

- **Improved detection** by increasing the rise of the public awareness of symptoms and risk factors, wide testing coverage, and tracking contacts with infected individuals.

- **Quarantine** - separate of an individual suspected of being infected from contact with others for a certain period that covers the incubation period of the disease. The practice of quarantine was launched in Italian ports when arriving ships remained at anchor for 40 days before the over went ashore to prevent the spread of the plague.

- **Isolating** is the separation of an individual who has been identified as infected from contact with other people.

- **Protection** is the use of appropriate equipment to protect healthcare workers who cannot avoid contact with infected individuals. *Social distancing* minimizes contact among individuals. Maintaining a distance of about 2 meters from another person reduces the incidence of most respiratory infections. *Self-isolation* is a required action to stop spreading of the infection.

3.3 The WHO Phases Program for Identifying Potential Pandemics

The WHO developed the pandemic phases for identification of the influenza pandemic in 1999 and revised in 2005. The phases are applicable to the entire world

and provide a global framework to aid countries in pandemic preparedness and response planning. WHO proposed to use a six-phased approach for easy incorporation of new recommendations and approaches into existing national preparedness and response plans. Phases 1–3 correlate with preparedness, including capacity development and response planning activities, while phases 4–6 clearly signal the need for response and mitigation efforts.

Phase 1, no viruses circulating among animals have been reported to cause infections in humans.

Phase 2, an animal influenza virus circulating among domesticated or wild animals is known to have caused infection in humans and is, therefore, considered a potential pandemic threat.

Phase 3, an animal, or human-animal influenza reassortant virus has caused sporadic cases or small clusters of disease in people but has not resulted in human-to-human transmission sufficient to sustain community-level outbreaks. Limited human-to-human transmission may occur under some circumstances.

Phase 4 is characterized by verified human-to-human transmission of an animal or human-animal influenza reassortant virus able to cause "community-level outbreaks".

Phase 5 is characterized by human-to-human spread of the virus into at least two countries in one WHO region, while most countries will not be affected at this stage.

Phase 6, the pandemic phase, is characterized by community level outbreaks in at least one other country in a different WHO region in addition to the criteria defined in Phase 5.

3.4 Natural Disasters and Infectious Disease Outbreaks

Natural disasters are catastrophic events with atmospheric, geologic, and hydrologic origins. They include earthquakes, volcanic eruptions, landslides, tsunamis, floods, and drought. The potential impact of communicable diseases is often presumed to be very high in the chaos that follows natural disasters. The risk of outbreaks is associated with the size, health status and living conditions of the population displaced by the natural disaster. Crowding, inadequate water and sanitation, and poor access to health services, often characteristic of sudden population displacement, increase the risk of communicable disease transmission. The dead bodies do not pose a risk of outbreaks following natural disasters. It is overemphasized by health officials and the media, leading to panic, confusion and sometimes to unnecessary public health activities.

In fact, the impact of natural disasters on a community may be the consequence of the collapse of health facilities and healthcare systems, the disruption of surveillance and health programs (immunization and vector control programs), the limitation or destruction of farming activities (scarcity of food / food insecurity), or the interruption of ongoing treatments and use of unprescribed medications.

The risk factors for increased infectious diseases transmission and outbreaks are mainly associated with the after-effects of the disasters rather than to the primary disaster itself or to the corpses of those killed. These after-effects include displacement of populations (internally displaced persons and refugees), environmental changes and increased vector breeding sites. Unplanned and overcrowded shelters, poor water and sanitation conditions, poor nutritional status or insufficient personal hygiene are often the case.

Although the overall risk of communicable disease outbreaks is lower than often perceived, the risk of transmission of certain endemic and epidemic-prone diseases can increase following natural disasters. There are a few situations, such as deaths from cholera or hemorrhagic fever epidemics, that require specific precautions, but families should not be deprived of appropriate identification and burial ceremonies for their dead relatives from disasters.

The most documented and commonly occurring after natural disasters diseases are water-borne diseases (diarrheal diseases and Leptospirosis). Diarrheal diseases cause over 40 % of the deaths in disaster and refugee camp settings. Epidemics among victims are commonly related to polluted water sources (fecal contamination), or contamination of water during transportation and storage. Diarrheal epidemics are frequently reported following natural disasters in developing countries. For instance, floods in many African countries lead to a significant increase in diarrheal disease incidences. Following the 2005 earthquake in Pakistan, an estimated 42 % increase in diarrheal infections was reported. In Thailand, the 2004 Indian tsunami also contributed to a significant increase in diarrheal disease incidences.

Diseases associated with crowding are measles, meningitis, acute respiratory infections. Crowded living conditions are common among people displaced by natural disasters. They facilitate transmission and necessitate even higher immunization coverage levels to prevent outbreaks. A measles outbreak in the Philippines in 1991 among people displaced by the eruption of Mt. Pinatubo involved more than 18,000 cases. Acute respiratory infections four folded in Nicaragua in the 30 days following Hurricane Mitch in 1998, and as well as the tsunami in Aceh in 2004, and the 2005 earthquake in Pakistan.

Vector-borne diseases (malaria, dengue) increase after natural disasters. Malaria outbreaks in Costa Rica's Atlantic Region in 1991 were associated with changes in habitat after the earthquake.

Other diseases associated with natural disasters are tetanus and coccidiomycosis. These pathogens are soil related microbes associated with increased levels of exposure to soil and dust after natural disasters.

Prevention measures of communicable diseases following natural disasters are critical to reduce the impact of communicable diseases after natural disasters. They comprise:

- Ensuring uninterrupted provision of safe drinking-water.
- Providing the primary health-care services to ensure early diagnosis and treatment of diarrheal diseases and acute respiratory diseases.
- Rapid detection of cases of epidemic-prone diseases. In some situations, the threats may include rare diseases such as viral hemorrhagic fevers, plague, or tularemia.
- Mass immunization against measles, typhoid, hepatitis A, cholera reduce the probability of the natural epidemics caused with these microbes.
- Prevention of malaria and dengue is based on an informed assessment of the local situation, including on the prevalent parasite species and the main vectors, indoor residual spraying of insecticides, or the re-treatment / distribution of insecticide-treated nets preferably long-lasting insecticidal nets (LLIN), weekly monitoring of the case numbers, tracking the slide / test positivity rate, use the treatment with artemisinin-based combination (ACT) therapy.

3.5 Challenges to Disease Eradication

Healthcare improvements and understanding of the main sanitary hygienic aspects provided the huge decrease in the mortality caused by infections. Such dangerous infection as smallpox has been eradicated worldwide, but total eradication of other social important infections meets several challenges.

Influenza eradication is limited due to the high mutation potential of the influenza virus, and some animals infected by different strains of influenza could be the source of the infection to people and other mammals. So, vaccination cannot prevent new epidemics and pandemics.

Measles is a promising candidate after smallpox for entire eradication. This infection is easily diagnosed. The vaccination against measles is highly effective and needs to be carried out worldwide.

Tuberculosis possesses huge social importance because it remains the leading cause of infectious mortality and morbidity worldwide. Although targeted antimicrobial therapy and vaccine are available, they are not effective enough. The discovery of new diagnostic approaches and vaccine candidates could make eradication of the virus possible at some point in the future.

HIV infection was discovered forty years ago and became one of the most investigated types of viral pathology. It causes a pandemic and currently about 38 million people worldwide are infected. There is an effective cure, and the number of AIDS deaths per year is decreasing. HIV transmission can be reduced by raising awareness of best practices. Due to the nature of the pathogen and high speed of virus mutations the vaccine against HIV has not been created yet. The development of an mRNA vaccine against CoV-19 offers the hope for creation an effective HIV vaccine and its eradication.

Ebola is a deadly epidemic disease. In recent years, significant advances have been made in the containment and prevention of Ebola through the wide implementation of effective control measures and improve diagnosis and treatment of the disease. In 2015, MSD's vaccine was officially implemented in Guinea with 100 % efficacy. After that, Ebola becomes a preventable disease as all new outbreaks (2018, 2020) were taken under control. Entire eradication is limited only because the virus can re-enter human populations through contact with infected wild animals.

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Topic 4. Laboratory Biosafety and Biosecurity. Laboratory-Acquired Infections

Learning objectives:

4.1 Laboratory-acquired infections : definition, history, classification

4.2 Measures to reduce biorisks in laboratory (physical protection, personnel management, control and accounting of materials, protection during transportation, and protection of information)

4.3 Laboratory safety standards, standard operating procedures, and best practices

4.1 Laboratory- Acquired Infections: Definition, History, and Classification

Laboratory-acquired infections (LAIs) or occupational illness are infections acquired through laboratory or laboratory-related activities regardless of whether they are symptomatic or asymptomatic in nature.

First reports about infectious diseases acquired in a laboratory were done in 1890. However, the safety measures in laboratories that work with pathogenic microorganisms were implemented in North America and the United Kingdom only at the beginning of the 1970s. In 1976 Pike performed the survey of the 4079 LAIs and determined that ten agents caused infections accounting for 50 % of cases. Another survey in medical laboratories of Great Britain from 1979 to 1989 revealed that shigellosis, tuberculosis, and hepatitis were the three most frequently reported laboratory-acquired infections.

LAIs are classified based on the reason – works with the infectious agents, clinical specimens, autopsies, contaminated glassware and exposure to infectious aerosols, accidents. It is estimated that human error accounted for 78 % of the underlying causes of LAIs. The source of the laboratory-associated infection is not apparent in 20 % of the cases. Another classification includes route of exposure. There are four main groups of LAIs according to the mode of transmission – respiratory (inhalation), transmissible (percutaneous inoculation via needle and syringe, cuts or abrasions from contaminated items and animal bites), contact (contamination of the mucous membranes), and food-born (drinking or eating).

4.2 Measures to Reduce Biorisks in Laboratory

The link between LAIs and biosafety is obvious. Most risks from biological hazards can be reduced through the use of appropriate microbiological procedures and techniques, containment devices and facilities, and protective barriers. The risk factors associated with the pathogens include the pathogenicity of the microorganism and its specific biosafety level (BSL).

The reduction of the risk starts from the risk assessment. Personal risk factors variety comprises factors related to the immunocompetency of an individual as well as factors related to employee behaviors and attitudes. Personal immunocompetence includes presence of disease (neoplastic, immunodeficiency, autoimmune or infectious), use of immunosuppressive therapy, age, race, sex, pregnancy, and surgical interventions. Prophylactic immunization is performed to all at-risk laboratory workers with vaccines specific for each situation. Persons who follow to safety regulations, respect infectious agents, demonstrate safe work habits and have a low LAI. Men and younger employees are assessed as risk group with a higher rate of the accidents than women and older employees.

Risk management follows the risk assessment and consists of administrative activities, implementation of standard microbiological practices, safety equipment, engineering and facility design, employee health programs and training. To efficiently manage laboratory accidents and exposures to infectious agents and minimize or control accident outcomes, each laboratory must have a safety plan. All accidents and potential exposures are reported immediately to the appropriate individuals in the organization, usually the supervisor and safety officer. Follow-up accident investigation (identification of the source patient and risk factors in the case of blood-borne pathogens); confidential medical consultation with the employee to answer his or her questions regarding risk of infection, need of prophylaxis, potential transmission to family members, and future treatment and surveillance; and corrective action to prevent future accidents or exposures.

Depending on the infectious agent and features of the accident, the working algorithms are different. Small accidents can be handled immediately by cleaning up with a suitable disinfectant, while massive spills or aerosols may require disconnection of the ventilation system and decontamination of the entire room or laboratory.

Each laboratory must have a waste management plan that identifies potentially infectious material and provides guidelines for the proper handling, transportation, storage, and disposal of the waste. Infectious material should be separated from other waste at the point or source of origin by being placed into leakproof red bags or bags with a universal biohazard symbol. Sharps must be stored in leakproof, puncture-

resistant containers. The infectious waste should be autoclaved prior to disposal in a landfill or should be incinerated. Blood, serum, urine, feces, and other patient secretions and excretions may be carefully poured into a sanitary sewer. Alternative methods of sterilization include chemical treatments, microwaves, dry heat, radio waves, and infrared radiation.

4.3 Laboratory Safety Standards, Standard Operating Procedures, and Best Practices

The strategy for minimizing the occupational exposure of laboratory workers, and the surrounding environment to infectious agents is based on the concept of microorganism containment.

Primary containment provides physical separation of the infectious agent from the laboratory worker. Primary barriers include strict adherence to microbiological practices and techniques and use of safety equipment such as biosafety cabinets (BSCs), safety centrifuge containers, and personal protective equipment (PPE) (e.g., gloves, masks, face shields and glasses, coats, and gowns).

Secondary containment refers to the facility design and acts as a secondary barrier to protect all workers within the facility and to protect the outside environment. The type of the secondary barrier depends on the risk of agent transmission. The secondary barriers may comprise special separated or controlled access zones, decontamination equipment, handwashing facilities, specialized ventilation systems, air treatment systems, or even separate buildings or modules for physical isolation of the laboratory building itself.

There are four types of laboratories depend on the infectious agent biosafety levels according to CDC-NIH guidelines. Each BSL consists of combinations of equipment, procedures and techniques, and laboratory design that are appropriate for the type of laboratory (e.g., clinical, research, or industrial) and infectious agent handled.

BSL-1 laboratory is recommended for teaching activities with agents that are not associated with infections. A BSL-1 lab usually is not isolated from surrounding facilities. The activity in BSL-1 laboratory is performed on benches without the use of special contaminant equipment. There are used standard microbial practices (mechanical pipetting only – no mouth pipetting allowed); safe sharps handling; avoidance of splashes or aerosols; daily decontamination of all work surfaces when work is complete and immediate decontamination after spills; hand washing; prohibition of food, drink, and smoking materials in lab setting. Personal protective equipment includes eye protection, gloves, and a lab coat or gown. Infection materials

are decontaminated prior to disposal, generally through the use of an autoclave. Biohazard warning signs must be present on the door and equipment (Fig. 3).

Figure 3 – Biohazard warning signs (from low to high risk)

BSL-2 laboratories use practices to manipulate agents associated with human diseases (i.e. pathogenic or infectious organisms) that pose a moderate health hazard. Examples of agents typically worked with in a BSL-2 include equine encephalitis viruses and HIV, as well as *Staphylococcus aureus.* BSL-2 laboratories maintain the same standard microbial practices as BSL-1 labs, plus controlled access, decontamination of all waste, protective clothing, and a baseline serum specimen. Biological safety cabinet (BSC) or other containment device are used for all manipulations of infectious agents, all necessary personal protective equipment (PPE). The following practices are required in a BSL-2 lab setting:

- Appropriate personal protective equipment (PPE) must be worn, including lab coats and gloves. Eye protection and face shields can also be worn, as needed.

- All procedures that can cause infection from aerosols or splashes are performed within the BSCs (Fig. 4).
- An autoclave or an alternative method of decontamination is available for proper disposal.
- The laboratory has self-closing, lockable doors.
- A sink and eyewash station should be readily available.
- Biohazard warning signs.

Figure 4 – Class II biological safety cabinets

Access to a BSL-2 lab is far more restrictive than to a BSL-1 lab. Outside personnel, or those with an increased risk of contamination, are often restricted from entering when work is being conducted.

BSL-3 laboratory is recommended when working with agents that are highly infectious and can cause serious or potentially lethal disease through inhalation (e.g., *M. tuberculosis*, *Brucella spp*., yellow fever, West Nile virus, and *Coccidioides immitis*) and for large-scale work with BSL-2 agents.

Common requirements in a BSL-3 laboratory include:

- Standard personal protective equipment must be worn, and respirators might be required, change to protective clothing before entering, and shower on exit.

5 – Class III biological safety cabinets

- Solid-front wraparound gowns, scrub suits or coveralls are often required.
- All work with microbes must be performed within a Class III BSC (Fig. 5) or other BSC in combination with a full-body, air-supplied positive-pressure suit for all procedures BSL-3 plus separate building or dedicated systems.
- Access hands-free sink and eyewash are available near the exit.
- Sustained directional airflow to draw air into the laboratory from clean areas towards potentially contaminated areas (Exhaust air cannot be re-circulated).
- A self-closing set of locking doors with access away from general building corridors.

BSL-4 laboratory is the highest level of biological safety that is required when working with unusual agents that cause life-threatening infections and come without treatment or vaccines (for instance Ebola and Marburg viruses). There are 59 BSL-4 facilities in operation around the world (Fig.6).

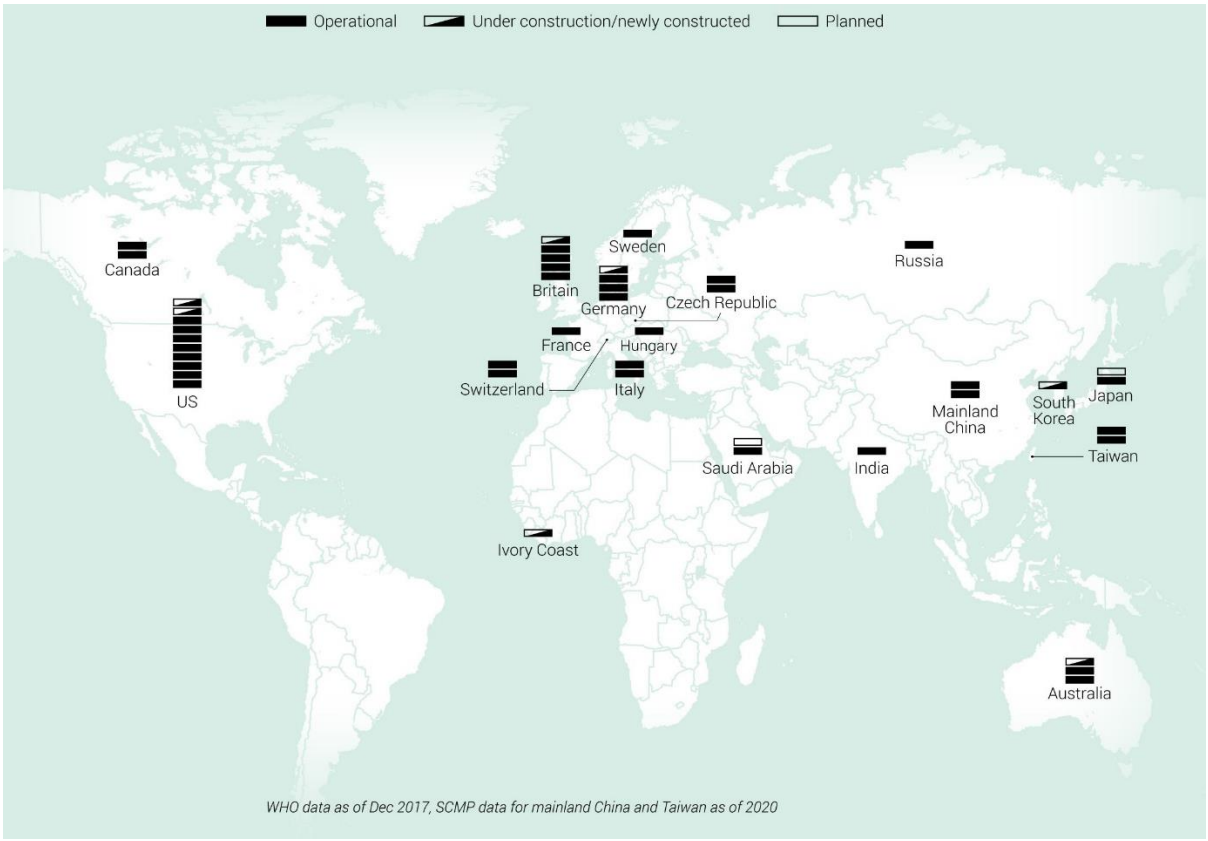

Figure 6 – BSL-4 facilities in operation in the world

BSL-4 laboratory comprises all requirements applied to BSL-3, and it is also extremely isolated, often located in a separate building or in an isolated and restricted zone of the building. The laboratory also features a dedicated supply and exhaust air, as well as vacuum lines and decontamination systems (Fig. 7).

Figure 6 – BSL-4 laboratory

The standard microbiological practices

Federal, state and local regulatory agencies often regulate exposure to infectious agents, licensing of laboratory, and disposal of biohazardous materials. Agencies and associations that set safety standards include the Joint Commission on Accreditation of Healthcare Organizations, the College of American Pathologists, the National Committee on Clinical Laboratory Standards (NCLS), Center of Diseases Control (CDC), and National Institute of Health (NIH). The laboratories must follow these recommendations.

The standard microbiological practices include general rules for the laboratory equipment and work organization. The laboratories should be easily cleaned and should contain hand-washing sinks, an autoclave or other decontamination equipment, and eyewash stations. Bench tops should be impervious to liquids and resistant to chemicals. Access to the laboratory should be limited to authorized personnel only. The laboratory ventilation system should produce a negative pressure with respect to outside corridors that should have approximately 10 to 15 air changes per hour and should be vented to the outside.

To avoid hazards associated with the syringe and needle, the staff must apply the covering of the needle and rubber stopper with a disinfectant-soaked swab and cleaning the inoculation site, the use of needle-locking syringes or needleless systems. The mandatory disposal of needles and syringes in labeled, sealed and puncture-resistant containers has decreased the number of needle and syringe injuries among healthcare workers (HCW).

The use of a microbiologist's loop includes the use of well-formed loops on a short shaft, disposable plastic loops, glass spreaders and electric incinerators, and the use of a biosafety cabinet when working with hazardous organisms.

Pipetting should be performed with disposable plastic pipettes, prohibiting pipetting by mouth, blowing out the last drop and mixing with a pipette (use a test tube mixer); work on mat moistened with disinfectant.

The centrifuge is provided using centrifuge tubes, without defects; ceiling the centrifuge tube in a protective beaker can protect laboratory workers from microbial exposure. The use of blenders, homogenizers, shakers, sonicators, and mixers should be manipulated in a BSC or with the use of an instrument that compresses material inside a sealed plastic bag.

Personal protective equipment and procedures

The use of appropriate personal protective equipment (PPE) and techniques is designed to minimize exposure of pathogens. Generally, PPE includes gowns,

laboratory coats, disposable gloves, face shields and goggles, splatter shields, and masks and respirators.

Protective gloves must be worn whenever a laboratory worker may have contact with potentially infectious material or surfaces and equipment. They are changed when torn, punctured, or visibly soiled. Hands should be washed after removal of the gloves. The protective goggles, face shields, and masks are used for eye and mouth protection when there is a possibility of accidental exposure to sprays and aerosols during manipulations with infectious material. Respiratory protection is performed in areas with a risk of aerosol formation, and the type of PPE (mask, respirator, positive air pressure suite) depends on the type of agent. Protective clothing such as laboratory coats, gowns, and aprons must be worn to protect the laboratory worker's skin from contamination by infectious material (Fig.7).

Figure 7 – Personal protective equipment (PPE) for laboratory staff

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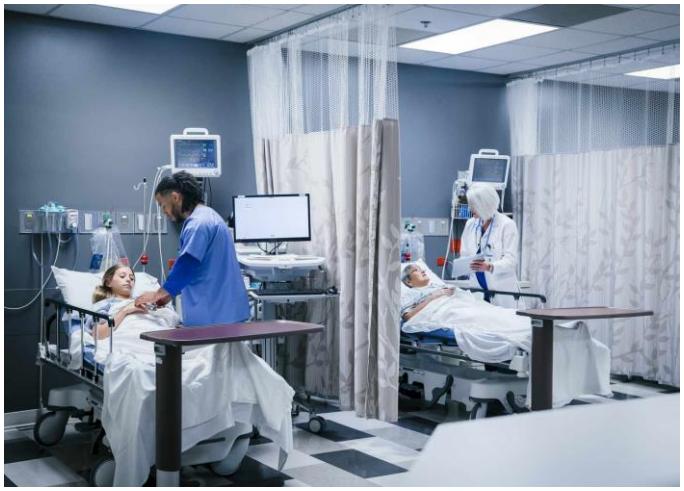
Topic 5. Hospital-Acquired Infections

Learning objectives :



5.1 Hospital-acquired (nosocomial) infections: concepts, etiology, epidemiology, classification, and prevention.

5.2 Global and national initiatives for prevention of hospital-acquired infection. The control of HAI.

5.3 Safe handling of human materials and transportation of medical waste.

5.1 Hospital-Acquired (Nosocomial) Infections: Concepts, Etiology, Epidemiology, Classification, and Prevention

Technological advances in medicine and public health can also inadvertently promote the emergence and spread of infectious disease. It has become commonplace to quip that you go to the hospital, and such infections killed around 40 times as many people as SARS did in 2003. A **hospital-acquired (nosocomial) infection** (HAI) is any clinically recognizable microbiological disease that affects a patient as a consequence of him being admitted to hospital or attending for treatment, or the hospital staff as a consequence of their work, whether or not the symptoms of the disease appear while the affected person is in the hospital. Such infections may be acquired from another person in the hospital (*cross-infection*), from an object or substance *(environmental infection),* or carried by the patient before the appearance of the hospital-acquired disease (*self-infection*). The pattern of hospital-acquired infection depends on a number of factors in the structure, organization, and activities of the hospital. The situation in a hospital differs from that in other types of institution in a number of ways of transmission. Various combinations of four main factors influence the frequency and nature of infections:

1 Low resistance of patients to infection due to the presence of the pre-existing disease, the medical or surgical treatment, age, or injury of the skin or mucous membranes, artificial contamination of the tissues or sterile areas.

2 Contact with infectious persons.

3 Contaminated environmental sites.

4 Drug resistance of endemic microbes.

The microbes responsible for hospital infection are classified into:

- **conventional** pathogens that cause disease in healthy persons in the absence of specific immunity to them;

- **conditional / opportunistic** pathogens that cause disease only in persons with reduced resistance to infection (including newborn infants) or when implanted directly into tissue or a normally sterile area of the body.

Depending on the localization of the HAIs, they are divided into the bloodstream infection, urinary tract infection, pneumonia, surgical site infection, and gastrointestinal infection.

Central line-associated bloodstream infections (CLABSIs) are serious infections that occur when germs (usually bacteria or viruses) enter the bloodstream through the central line. This infection depends on the care taken during insertion and handling of the intravascular catheter and the term of its use. Diagnosis is made by examination of blood culture and semi-quantitative culture from the catheter tip and catheter lumen.

Patients infected with CLABSI have a fever and may also have redness of the skin and soreness around the central line. According to CDC recommendation healthcare providers can take the following steps to prevent CLABSIs:

1 Follow recommended central line insertion practices to prevent infection when the central line is placed:

- Perform hand hygiene.
- Apply appropriate skin antiseptic.
- Ensure that the skin prep agent has completely dried before inserting the central line.
- Use all five maximal sterile barrier precautions: sterile gloves, sterile gown, cap, mask, and a large sterile drape.

2 Once the central line is in place:

- Follow recommended central line maintenance practices.
- Wash their hands with soap and water or an alcohol-based handrub before and after touching the line.

3 Remove a central line as soon as it is no longer needed. The sooner the catheter is removed, the less chance of infection.

Healthcare-associated pneumonia (HCAP) or ventilator-associated pneumonia (VAP) is the most important infection in patients on ventilators in intensive care units. It has a high mortality rate and is often associated with serious comorbidities. It is defined as a lower respiratory tract infection that appears during or after hospitalization of a patient who had no incubation infection on admission. Criteria for diagnosis are fever, cough with purulent sputum, a new infiltrate on a radiograph,

and Gram staining of sputum, ET aspirate, and bacteria. Healthcare-associated pneumonia is acquired by the inhalation of respiratory droplets or aerosols, or aspiration of colonized oropharyngeal and gastric secretions in conditions of low gastric acidity. Infection can also be acquired through the oropharynx during suction procedures due to inadequate hand washing and inappropriate disinfection of respiratory devices. Risk factors of healthcare-associated pneumonia are age, coronary bypass surgery, abdominal surgery, existing pulmonary, and neurological diseases, decreased clearance of respiratory secretions due to coma, sedation, etc. Invasive devices bypass natural defense mechanisms like mechanical ventilation, intubation, tracheostomy, and enteral feeding. Medications – such as antibiotics, antacids, immunosuppressive agents, and chemotherapy.

To prevent ventilator-associated pneumonia, doctors, nurses, and other healthcare providers can do the following:

- Keep the head board of the patient's bed elevated, between 30 and 45 degrees, unless other medical conditions allow it.
- Check the patient's ability to breathe on his or her own every day so that the patient can be taken off the ventilator as soon as possible.
- Clean their hands with soap and water or an alcohol-based hand rub before and after touching the patient or the ventilator.
- Clean the inside of the patient's mouth on a regular basis.
- Clean or replace equipment between use on different patients.

Urinary tract infections (UTIs) are the most common and associated with the use of an indwelling urinary catheter. UTI is an infection involving any part of the urinary system, including urethra, bladder, ureters, and kidneys. The most important risk factor for developing a catheter-associated UTI is a prolonged use of the urinary catheter. Diagnosis is based on the clinical symptoms of fever, suprapubic tenderness, frequency of urination and dysuria along with the presence of bacteria in the urine in significant quantities. The urine culture of the patient shows no more than two species of organisms identified, at least one of which is a bacterium at concentration more than 10^5 CFU/ml. The source of organisms can be the endogenous or exogenous microflora through the hands of staff or contaminated instruments. Risk factors of UTI include indwelling urinary catheter, instrumentation of the urinary tract, poor aseptic preparation during insertion of the catheter, poor catheter maintenance, advanced age, female gender, severe underlying illness. The risk of UTI can be reduced by ensuring that catheters are used only when needed and removed as soon as possible, that catheters are placed using proper aseptic technique, and that a closed sterile drainage

system is maintained. Hospitals should follow the recommendations in the 2009 CDC Guideline for Prevention of Catheter-Associated Urinary Tract Infections.

Surgical site infections (SSI) are the infection of the site of surgery, earlier called wound infection. The main risk factor of surgical site infections (SSIs) is the extent of contamination during the procedure (clean, clean contaminated, contaminated, dirty), which is largely dependent on the site of surgery, length of the operation, and the patient's general condition. SSI usually occurs within 30 days of the operative procedure. However, all deep infections related to the operative site and to the implant within 1 year of an operation should be considered a postoperative infection. The main risk factors for SSI include age and gender, overweight, wound grading by severity score, diabetes, and long duration of surgery. SSI can be superficial incisional, deep incisional and organ / space. Diagnosis is based on the clinical symptoms of pain, tenderness, localized swelling, redness or heat, pus discharge from deep incision, spontaneous dehiscence or "gaping" of wound, fever >38 °C.

To prevent SSIs, doctors, nurses and other healthcare providers should follow CDC infection prevention guidelines:

- Clean their hands and arms up to their elbows with an antiseptic agent just before the surgery.
- Clean their hands with soap and water or an alcohol-based hand rub before and after caring for each patient.
- If indicated, remove some of the patient's hair immediately before the surgery using electric clippers if the hair is in the same area where the procedure will occur.
- Wear special hair covers, masks, gowns, and gloves during surgery to keep the surgery area clean.
- When indicated, give antibiotics to the patients before the surgery starts. In most cases, antibiotics should be taken within 60 minutes before the surgery starts and the antibiotics should be stopped within 24 hours after surgery.
- Clean the skin at the surgical site with a special soap that kills germs.

Gastrointestinal infections are the most common HAI. The infection may be acquired from contaminated food or water, infected patients or staff, contact with the environment contaminated with organisms, or instruments entering the alimentary tract such as endoscopes. Healthcare-associated diarrhea often appears as an outbreak. The risk factors associated with hospital associated diarrhea are advanced age; achlorhydria; oral or systemic antibiotic therapy; disruption of normal flora;

overgrowth of resistant or sensitive pathogens; gastrointestinal procedures such as insertion of nasogastric tube, endoscopy; overcrowding of the unit; understaffing; and inadequate hand washing facilities.

Another classification of the HAI is based on the route of the transmission. **Contact** infections are transmitted via the transfer of an infectious agent through a contaminated intermediate object or person. **Droplet** infections occur when an infection is transmitted via the person coughs, sneezes or talks, or during certain procedures. Droplets are infectious particles >5 microns in size. **Airborne** transmitted infections may occur via the spread of the particles containing infectious agents that remain suspended in air over time and distance. Small-particle aerosols (<5 microns) are created during breathing, talking, coughing or sneezing and secondarily, by evaporation of larger droplets in conditions of low humidity. **Vector-borne transmission** refers to transmission of microorganisms by vectors (insects) and can be prevented by appropriate construction and maintenance of an HCF, having closed or screened windows and proper housekeeping.

The most effective way to prevent HAI is by introducing a barrier between the source of infection and the susceptible host. They include standard and transmission-based precautions.

Standard precautions are a set of activities designed to prevent the transmission of organisms between patients / staff and, in turn, prevent HCAIs. They include hand hygiene; appropriate use of PPE; following aseptic techniques, paying attention to established practices for cleaning and decontamination of soiled instruments, followed by either sterilization or high-level disinfection; appropriate disposal of biomedical waste (BMW); appropriate cleaning and disinfection of the environment, improving safety in operating rooms and other high-risk areas where the most vulnerable patients are housed and where is a high risk of exposure to infectious agents. Approaches to the HAIs prevention are dependent on the route of transmission. Prevention of the respiratory transmitted infections includes the use of a particulate respirator, e.g. a N95 mask with a proper fit; restricted entry to susceptible healthcare personnel; immunization of the susceptible persons.

When a patient is known (or suspected) to have a transmissible infectious organism / disease, **transmission-based** precautions are used in addition to standard precautions. Transmission-based precautions are additional measures focused on the particular mode of transmission. They should be applied when caring for patients with known infection, patients who are colonised with an infectious organism, asymptomatic patients who are suspected of / or under investigation for colonisation or infection.

Precautions based on transmission are classified by the route of transmission of the infectious agent:

Contact Precautions are required for patients known or suspected to be infected or colonised with microorganisms that can be transmitted by direct contact or through the patient's secretions or bodily fluids, i.e. contact which occurs when performing patient care activities that require touching the patient's skin, secretions or bodily fluids; or indirect contact, i.e. touching potentially contaminated environmental surfaces or equipment in the patient's environment. Examples include *Staphylococcus aureus* (MSSA or MRSA), Vancomycin resistant *Enterococci* (VRE), *Clostridium difficile* infection (CDI) and scabies. Contact Precautions should include hands of HCWs, clothing after caring / for a patient colonised or infected with an infectious agent, the use of patient care devices that are shared with patients without cleaning and disinfecting, and insufficiently disinfected surrounding surface.

Droplet Precautions are required for patients known or suspected of being infected with microorganisms transmitted by droplets. Droplets can be generated by coughing, sneezing, talking or during the performance of procedures (e.g. nebulisation). Examples include pertussis, influenza, rubella and mumps. Droplet Precautions include placing the patient (1–2 m inter-bed distance), observing cough etiquette, and wearing a three–layer surgical mask 1–2 m away from the patient.

Airborne Precautions are required for patients known or suspected to be infected with microorganisms that can be transmitted to other patients / staff by the airborne droplets, such as in dust. Examples include tuberculosis, chickenpox, and measles.

A combination of Contact, Droplet and Airborne Precautions may be applied for diseases that have multiple routes of transmission or in case of epidemiologically important organisms, risk group 4 organisms, or when transmission routes are unknown. Combined precautions are recommended in cases of Ebola and Nipah virus diseases. They are always to be used in addition to standard precautions and should be applied to all suspect, probable and confirmed cases.

5.2 Global and National Initiatives for Prevention of Hospital-Acquired Infection and the control of HAI

Most HAIs are preventable and can be reduced by up to 70 % through effective infection prevention and control (IPC) measures. In 2016, the World Health Organization has published recommendations on effective IPC strategies. The CDC has determined the main areas of IPC measures in all healthcare facilities: administrative

measures, physical distancing, hand hygiene, and the appropriate use of personal protective equipment.

The World Health Organization and the Centers for Disease Control and Prevention have recently published the guidelines for the prevention of surgical site infections (SSIs). There are 7 strategies to prevent HAI after surgery. These strategies could be applied in other types of healthcare departments. They include:

Hand hygiene is the most important, simplest, and the least expensive means of reducing the prevalence of HAIs and the spread of antimicrobial resistance (AMR). The WHO defines the key moments when healthcare workers should perform hand hygiene:

1) before touching a patient,
2) before clean / aseptic procedures,
3) after body fluid exposure / risk,
4) after touching a patient, and
5) after touching the patient's surroundings.

Basic rules of the hands washing:

Hand washing should be done with alcohol gel or soap and water. Whatever you use to wash your hands, you must follow the six principles demonstrated below. The relative duration of the procedure is 30-60 seconds (Fig. 8).

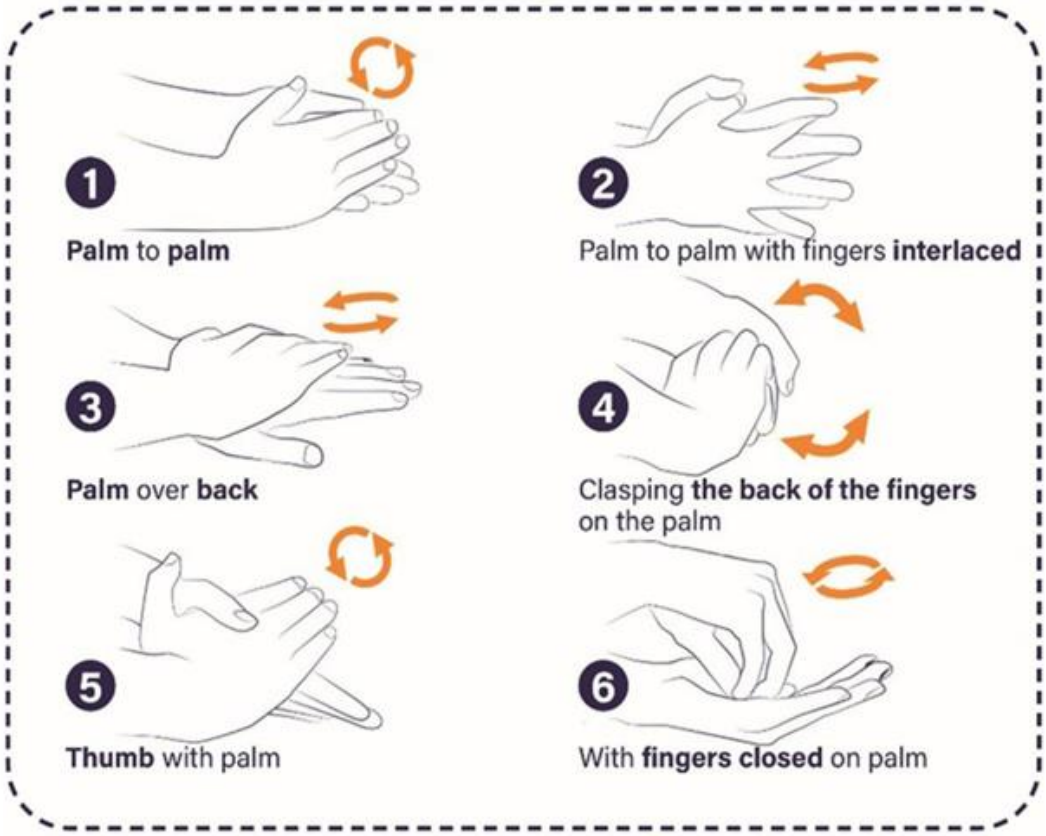


Figure 8 – Handwashing chart

Environmental hygiene is a fundamental principle of infection prevention in healthcare settings. Contaminated hospital surfaces play an important role in the transmission of microorganisms. Microbial contamination can result from the patients, their visitors and healthcare workers. Contamination can be reduced by general cleaning and disinfection.

Cleaning with warm water and detergent is a process that removes visual dirt and contamination, and in most cases is effective for decontaminating both equipment and the environment. Routine cleaning of the environment should be undertaken at least daily. Thorough cleaning with neutral detergent and water is commonly used. If soiling (with blood and / or bodily fluids) is evident, then general cleaning should be followed with a disinfectant clean, using a chlorine releasing product / sodium hypochlorite or a chlorine dioxide solution. If using a hypochlorite solution, the area should then be rinsed and dried although this is not required with some chlorine dioxide solutions.

During an outbreak of infection or an unusual increase in the incidence of a particular organism, **enhanced routine cleaning** (minimum twice daily) is recommended. This will entail cleaning / disinfecting the environment including frequently touched surfaces such as bed tables, bed rails, the arms of chairs, sinks, call bells, door handles and push plates, and any area / piece of equipment that may potentially be contaminated. Depending on the type of outbreak in the healthcare facility, certain areas will require more frequent cleaning and disinfection, e.g. sanitary areas during an outbreak of gastrointestinal infection.

Terminal cleaning is the thorough cleaning / disinfection of all surfaces including floors and re-useable equipment either within the whole healthcare facility or within an individual ward / department / unit. This may be required in the following circumstances:

- Following an outbreak or increased incidence of infection.
- Following discharge, transfer or death of a patient who has had a known infection.
- Following isolation / contact precaution nursing of a patient.

Disinfection is a process that reduces the number of microorganisms to a level at which they do not present a risk to patients or clients. It is only effective if surfaces and equipment have been cleaned thoroughly with detergent and water beforehand. Warm water and detergent should be used to clean hard surfaces followed by disinfection with 0.1% chlorine releasing agent/hypochlorite solution or chlorine dioxide solution. The hypochlorite or chlorine dioxide solution will kill both bacteria and viruses. Hypochlorite solutions are corrosive and it is recommended that the solution is rinsed off commodes, mattresses and stainless steel surfaces with warm

water at the end of the process. Some chlorine dioxide solutions do not need to be rinsed off.

Every healthcare facility should have written protocols to guide cleaning and disinfection ensure that all areas of the environment are regularly cleaned and disinfected to a satisfactory standard. Staff undertaking cleaning should follow agreed protocols and have access to adequate resources and equipment to achieve the required standard of cleaning.

Screening and cohorting patients are effective in groups of the immunocompromised patients and patients with planed invasive manipulations or surgical interventions. Isolation or grouping of colonized / infected patients helps to prevent transmission of microorganisms from infected or colonized patients to other patients, hospital visitors, and healthcare workers. Early detection of multidrug-resistant organisms is an important component of any infection control program. The active screening of preoperative patients for MRSA, with decolonization of carriers, results in reductions in postoperative infections caused by MRSA. Isolating a patient with highly resistant bacteria is beneficial in stopping patient-to-patient spread.

Surveillance systems allow the evaluation of the local burden of HAIs and AMR and contribute to the early detection of HAIs including the identification of clusters and outbreaks. It comprises national and local (facility) infection prevention and control programs. National surveillance systems should be an integral part of the public health system.

Antibiotic stewardship programs (ASPs) can facilitate protection against the horizontal spread of infection by rational reducing antibiotic exposure and preventing the emergence of antibiotic resistance. ASPs prevent surgical site infections via optimized use of surgical antibiotic prophylaxis.

Adherence to the guidelines is also important for infection control and reducing microbial transmission in hospitals.

Patient safety is the lack of preventive harm to the patient in the process of healthcare and reducing the risk of unnecessary harm associated with healthcare to an acceptable minimum.

5.3 Safe Handling of Human Materials and Transportation of Medical Waste

Healthcare activities generate huge amounts of waste. The most of it is non-hazardous, but approximately 15 % is considered hazardous material that may be infectious, chemical, or radioactive. To avoid adverse health outcomes associated with waste handling, the WHO introduced the safety standard of the work with human-

derived materials and transportation of medical waste. Depending on the type of potential threat, medical waste is divided into several groups:

- **Infectious waste:** waste contaminated with blood and other bodily fluids, cultures and stocks of infectious agents from laboratory work, or waste from patients with infections.
- **Pathological waste:** human tissues, organs or fluids, body parts and contaminated animal carcasses.
- **Sharps waste:** syringes, needles, disposable scalpels and blades, etc. (Fig. 9).

Figure 9 – Biohazard waist and sharps disposal container

- **Chemical waste:** chemicals used in laboratory, disinfectants, sterilant and heavy metals contained in medical devices and batteries.
- **Pharmaceutical waste:** expired, unused and contaminated drugs and vaccines.
- **Cytotoxic waste:** waste containing substances with genotoxic properties such as cytotoxic drugs used in cancer treatment and their metabolites.
- **Radioactive waste:** products contaminated by radionuclides including radioactive diagnostic material or radiotherapeutic materials.

The following general principles of waste segregation, storage and transportation relate to the control of waste flow from generation to disposal:

- Healthcare waste is generated in a medical area and must be segregated into different fractions, based on their potential hazard and disposal route, by the person who produces each type of waste.
- Separate containers should be available in each medical area for each segregated waste fraction.
- Waste containers should be labelled to help managers control waste production.

- Closed local storage inside or near to a medical area may be needed if waste is not collected frequently.
- Hazardous and non-hazardous wastes should not be mixed during collection, transportation, or storage.
- Collected waste is often taken to central storage sites before onsite or offsite treatment and disposal.
- Staff should understand the risks and safety procedures for the wastes they are handling.

Highly infectious waste should be collected separately and autoclaved at the point of generation. Once disinfected, the waste leaves the medical area in a container for infectious medical waste. Waste containers can come in many shapes and sizes and be made from different materials (Fig. 10). They should be sturdy and leak-proof, and (except for sharps containers) should be lined with a strong plastic bag. Containers should have well-fitting lids, either removable by hand or preferably operated by a foot pedal. Hazardous waste should be stored in utility rooms, which are designated for cleaning equipment, dirty linen, and waste. From here, the waste can be kept away from patients before removal, then collected conveniently and transported to a central storage facility. Hazardous and non-hazardous waste should always be transported separately. In general, there are three different transport systems:

Figure 10 – Biohazard waste container

- Waste transportation trolleys for general waste should be painted black, only be used for non-hazardous waste types, and labelled clearly “General waste” or “Non-hazardous waste”.
- Infectious waste can be transported together with used sharps waste. Infectious waste should not be transported together with other hazardous waste to prevent the possible spread of infectious agents. Trolleys should be colored in the appropriate color code for infectious waste (yellow) and should be labelled with an “Infectious waste” sign. Central storage area(s) are fenced, lockable and isolated from patients and the public. Maximum storage time before treatment or disposal of infectious waste is no longer than – moderate climate: 72 hours in winter and 48 hours in summer – hot climate: 48 hours in the cool season and 24 hours in the hot season.
- Other hazardous waste, such as chemical and pharmaceutical waste, should be transported separately in boxes. Off-site transporting hazardous healthcare

waste should comply with national regulations, and with international agreements if waste is shipped across an international frontier for treatment (Secretariat of the Basel Convention, 1992) or recommendations on the transport of dangerous goods published by the United Nations. Vehicles must transport waste in a closed or covered container, and the driver must know what to do in case of an accident or incident during transportation on public roads.

- Staff receives instructions on three-bin waste segregation and safe handling and storage of healthcare waste. Staff know how to protect themselves from injuries and waste infection. Transport staff should be vaccinated against at least hepatitis A and B, polio and tetanus.

References:

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Topic 6. Biosafety and Biosecurity of the Environment

Learning objectives :

6.1 Environmental biosafety.
Environmental infectious disease.
Climate impact on environmental biosafety.
6.2 Plant related biosafety.
6.3 Food biosafety.
6.4 Export control.

6.1 Environmental Biosafety. Environmental Infectious Diseases. Climate Impact on Environmental Biosafety

Biosafety and biosecurity have direct relevance to food safety, the conservation of the environment (including biodiversity), and sustainability of agriculture. Biosecurity constitutes three sectors (namely food safety, plant life and health, and animal life and health). Microbes are everywhere in the biosphere, and their effects on the environment can be beneficial or harmful or inapparent. All these organisms together form a pulsating, living community in soil, water, plant, and animals.

The soil microbiome is the community of microorganisms found in soil. One gram of healthy soil usually contains a microbiome comprising many millions of microbes, including archaea, bacteria, and fungi. Some microbes colonize the area around plant roots, known as the rhizosphere, forming mutually beneficial associations (symbioses) with plants. These symbiotic microbes and other free-living soil microbes contribute to crop growth and soil health by cycling nutrients, improving soil structure, and increasing organic matter content, conferring disease resistance to crops by out-competing pathogenic microbes and stimulating complex biochemical plant defenses, improving the resilience of plants to environmental stresses, and enhancing root growth and nutrient uptake. The microbial populations in soil vary depending on the type of soil. Intensive agriculture leads to soil degradation, erosion and changing of the soil microbiome diversity and function. Apart that a variety of classic and emerging soil-related pathogens cause serious human disease. Infection may occur by direct inoculation or ingestion, ingestion of contaminated food, or inhalation. In addition to classical soil-related diseases such as tetanus, anthrax and botulism, soil bacteria may cause gastrointestinal, wound, skin and respiratory tract diseases (fungal infections).

Aquatic microbiology is devoted to advancing the study of microbes in aqueous environments, with a focus on fresh water, estuarine and oceanic ecosystems. Water can support the growth of many types of microorganisms. Some of them can cause

microbial diseases. Bacteria that live in the intestinal tracts of humans and other warm-blooded animals, such as *Escherichia coli, Salmonella, Shigella,* and *Vibrio,* can contaminate water if feces enters the water. The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, enteroviruses, and coxsackievirus. Another group of concern in water microbiology are protozoa such as *Giardia* and *Cryptosporidium*. They can cause debilitating and prolonged diarrhea in humans.

Many microorganisms in fresh and salt water can be important in the food chain that forms the basis of life in the water. Another microorganism found in salt water can cause many fish to die. Humans can become ill by eating contaminated fish. Water can also be an ideal means of transporting microorganisms from one place to another. One of these organisms, a bacterium called *Vibrio cholerae,* causes life threatening diarrhea in humans.

With environmental change and pressure on a global scale (i.e. climate, deforestation, increasing population), infectious diseases are finding new ways to re-emerge. New contacts between wild fauna and humans and their livestock increase the risk of cross-species infection. Rodents continue to be sources of re-emerging infections. Intensified production of meat and meat products has led to new infections. For instance, BSE, or "mad cow disease", emerged in British cattle in 1986 because of industrialized cannibalism, whereby rendered neural tissue and bone meal from slaughtered cattle were recycled into cattle feed, as well as into pies and hamburgers for human consumption. The increasing predilection for meat of exotic species has exacerbated the risk of exposure to infections not previously encountered, and this situation probably triggered the SARS epidemic. In Africa, bushmeat also poses a serious problem for emerging infectious diseases, as well as for nature conservation. For example, 21 human deaths owing to Ebola virus infection ensued from the butchering of a single chimpanzee. HIV has crossed from chimpanzees to humans on at least three occasions, and a higher number of zoonotic events from sooty mangabeys are indicated for HIV-2.

The urban environment has only recently become the dominant human habitat. Rapid urbanization boosts certain established infectious diseases, such as childhood pneumonia, diarrhea, tuberculosis and dengue, and facilitates dissemination of various "emerging" diseases (SARS). Crowded and dilapidated public housing can potentiate infectious disease transmission through drug abuse and sexually transmitted infections.

Climate variability and climate change are affecting infectious-disease transmission patterns in multiple ways. For example, diseases traditionally associated with tropical and subtropical regions are reaching new areas of the world. Rising temperatures and precipitation are making temperate, northern, or mountainous

countries more susceptible to outbreaks of "southern" or “low land” diseases like malaria. Nepal, previously too cool for dengue fever, suffered its first outbreak in 2006, with a handful of cases. Since then, the incidence of dengue has increased significantly. Before 1970, dengue fever caused severe outbreaks in only nine countries. Now it is endemic in more than 100 countries, according to the World Health Organization.

A loss of wildlife habitat is linked both to climate change and to disease outbreaks. An estimated 75 % of new infectious diseases are zoonotic, meaning they transmit from animals to humans, for instance the Ebola, Zika and Nipah viruses.

Air pollution also could help viruses become airborne and more deadly. An increase in fine particulate pollution of just one microgram per cubic meter corresponded to a 15% increase in COVID-19 mortality.

Melting of ice and permafrost could lead to the reemergence of ancient diseases. Permafrost is a very good preserver of microbes and viruses because it is cold, devoid of oxygen, and dark. Fragments of ribonucleic acid (RNA) from the 1918 Spanish flu virus were found in corpses buried in mass graves in Alaska's tundra. In 2016, a 12-year-old child died and 20 people were infected by anthrax in a remote part of Siberia where a heat wave had thawed permafrost soil, exposing the corpse of a reindeer that had died 75 years earlier from the disease. Global warming could cause viral mutations that resist our defenses for fighting illness. A changing climate could also unlock new infectious diseases as pathogens mutate and evolve to adapt to warmer temperatures in much of the world.

Another area in which environmental microorganisms can have detrimental effects is that of biodeterioration, whereby economically important materials such as wood, paper, textiles, petroleum and even metals and concrete may be subject to damage by a range of microorganisms, mainly fungi and bacteria. The microbes accomplish biodegradation and carbon cycling from everything organic, which includes foods and grains stored in granaries, supermarket or refrigerator, as well as natural structural materials and textiles used for our shelters and clothing.

In many countries environmental microbiology is the subject of legislation. These tendencies are demonstrated in the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement), the Convention on Biological Diversity (CBD) and its Cartagena Protocol on Biosafety. It is further addressed in the FAO/WHO Codex Alimentarius, the International Plant Protection Convention (IPPC) and the FAO Code of Conduct for Responsible Fisheries. The IPPC makes a key contribution to biosecurity by reducing the risks of introduction of plant pests that may affect agriculture and the environment.

6.2 Plant Related Biosafety

Due to climate change and growth of human population increasing agricultural productivity per unit area is critical to reducing land conversion and biodiversity erosion. Agriculture is facing the challenge due to a combination of factors. Microbial diseases of the plants, as one of such factors, may lead to serious economic or social consequences. Microbial plant diseases can reduce the crop yields and it can result in inadequate food to humans or lead to starvation and death. For example, late blight disease of potato, which is caused by *Phytophthora infestans*, destroyed potatoes which were the main crop in Ireland during 1845–1850. This resulted in the Great Famine (or Great Hunger), where about one million people died and another million emigrated to Canada, the USA and other countries. Plant microbial diseases continue to reduce the quality and nutritional value of fruits.

One of the most common ways in which plant diseases can affect humans is through the release of toxic metabolites "mycotoxins" by fungi infecting plant products. These mycotoxins can directly affect humans and animals, resulting in diseases and deaths. Mycotoxins can be found in several products, especially peanuts, pistachios and maize. Infection of these products by mycotoxin-producing fungi can occur in the field or during storage. In addition, mycotoxins can be consumed indirectly by humans through the consumption of meat from animals fed on food contaminated with mycotoxins.

Aflatoxins are one of the most common and serious toxins produced by *Aspergillus* species. Aflatoxin B_1 is lethal at high doses and is carcinogenic to humans at low doses, and it can result in reduced liver function, vomiting and abdominal pain. In some parts of Africa 250,000 people die because of aflatoxin per year. *Fusarium graminearum* secretes a specific mycotoxin, deoxynivalenol (also known as vomitoxin), that can kill pigs, cows and poultry. Ergot (Fig.11) is also a disease of some grains including wheat. It is caused by some fungi belonging to the *Claviceps* genus. Consumption of bread produced from contaminated flour can result in ergotism disease in humans.

Figure 11 – A detail of the painting by Mathias Grunewald showing a man suffering from St. Anthony's fire (ergotism)

Ergotism has been reported to result in death, loss of peripheral sensation or hallucinations.

Although most plant pathogens do not infect humans, consumption of rotten or moldy fruits and vegetables or food contaminated with toxin-producing fungi should be avoided. Removing diseased parts of the fruits can help reduce inoculation of pathogens and rotten parts of the fruits. However, it may not ensure that all contamination has been excluded as some fungi and their toxins can diffuse into symptomless parts of fruits. Although cooking may result in the decomposition of some mycotoxins, there are some mycotoxins that are not degraded by intense heat. The action of some mycotoxins can be reduced by adding some mycotoxin-binding agents or by deactivation.

Several bacterial plant pathogens have been shown to act also as pathogens for animals. For example, some *Agrobacterium* and *Erwinia* (Pectobacterium) species, well-known phytopathogens with wide plant host ranges, were found to cause opportunistic infections in animal hosts. In humans they can cause bacteremia in immunocompromised patients. *Erwinia species* were isolated from an individual who presented an urinary tract infection, cutaneous infection, and bacteremia. Fungi *Alternaria infectoria* cause phaeohyphomycosis after renal transplant and was linked to keratitis in post-traumatic infections.

Plant-associated viruses are not considered to present any potential pathogenic threats to humans or other vertebrates, although some plant viruses may replicate within the bodies of insect hosts that are acting as transmission vectors. For example, Tobacco mosaic virus (TMV) and Pepper mild mottle virus are extremely stable plant viruses that have been detected in animal and human faecal samples. TMV is highly stable in tobacco products and was detected in the lungs of active or passive smokers and the bronchoalveolar lavage of intubated patients.

Invasive species are a major cause of crop loss and can adversely affect food security. Invasive species present significant threats to global agriculture. The biggest agricultural producers (China and the United States) could experience the greatest absolute cost from further species invasions. For instance, Cogongrass is an Asian plant that arrived in the United States as seeds in packing material. It is now spreading through the Southeast, displacing native plants. It provides no food value for native wildlife and increases the threat of wildfire as it burns hotter and faster than native grasses.

6.3 Food biosafety

Food safety is a scientific discipline describing handling, preparation and storage of food to prevent food-induced illnesses, such as infections, intoxication, and allergies. Food safety refers to all hazards, whether chronic or acute, that may cause problems ranging from flu-like symptoms to serious illness – even death.

Food originated hazards can be:

1 Biological: harmful bacteria, viruses, and parasites.

2 Chemical: food additives, pesticides / agricultural products, veterinary drugs, mycotoxins, natural toxins, environmental contaminants, marine toxins, processing induced chemicals, furan.

3 Physical: jewelry, hair and fingernails, insects, plasters, broken glass, string, bits of equipment, bits of shell or bone, pest droppings, dust, and dirt.

Biosafety represents all measures taken to reduce or eliminate potential risks that may arise as a consequence of using spoiled, infested, or genetically modified food, which could have adverse effects on human health. These effects may be direct or indirect, immediate, or delayed. Microbiological hazards constitute the greatest risk for human health.

There are several factors which are likely to contribute to outbreaks of foodborne illness, including a raw food supply that is frequently contaminated, a lack of awareness among the general public, mistakes in food handling and food preparation at home and the deliberate consumption of raw and undercooked foods of animal origin, often described as “risky eating behavior”. Food can provide ideal conditions for bacteria to multiply and produce toxins.

Raw foods, including meat and poultry, raw eggs, fish and shellfish, and fruits and vegetables, should all be considered as potential entry sources of foodborne pathogens into the home. The human and animal occupants of the home can also serve as sources of foodborne pathogens. Pathogens can be transferred from various sources to inanimate contact surfaces in the home or directly to other foods or human occupants via transient carriage on the hands.

To assess and manage risks associated with the intake of microbiological hazards through food the several regulations were launched. A set of food manufacturing practices is used to minimize biological food hazards through safe and clean operations to protect public health from foodborne diseases. Food hygiene is a crucial aspect of ensuring food safety and preventing foodborne illnesses.

Food safety spans all phases from sourcing raw materials, through processing, packaging, transportation, and finally, to the product’s readiness for sale. During processing, food is vulnerable to biological, physical, chemical, and allergic

contamination. Food hygiene standards mainly cover conditions, rules, and procedures for preventing biological contamination of food that lead to foodborne illnesses. The measures range from proper food handling, thorough cleaning activities, preventing cross-contamination, etc. By comparison, traceability and proper labeling are part of food safety management, but are not necessarily in part of food hygiene standards.

The key food hygiene behavior, principles, and guidelines comprise cleaning, cooking, chilling, cross-contamination, safe transport, personal hygiene, proper waste management, safe water and pre- and post-operation sanitation. Effective **cleaning** is vital to ensure that all equipment and surfaces are free from contamination. This principle applies to kitchen utensils, working areas, and even your raw materials. Cleaning also pertains to the sanitation process before working. Thorough **cooking** is necessary to kill harmful bacteria that potentially cause food poisoning. Each food item requires a different amount of cooking time and a safe and adequate temperature up to the center of your product. This principle of food hygiene also includes reheating your food when left for a while in ambient temperatures to ensure that any potential contaminations are controlled. Proper **chilling** is crucial to stop bacterial growth and keep food safe, particularly for perishable food such as ready-to-eat salad, cooked meat, etc. Storing your food products before use or even after use prevents the multiplication of harmful microorganisms. Chilling means storing your food in conditions with temperatures around or equal to at least 35°F to 40°F (2°C to 4°C). Food leftovers should be kept in an airtight container within 2 hours of cooking. Any pieces that have been standing at room temperature for an extended period should be discarded.

Cross-contamination or bacteria spread among food, equipment, and work areas occurs as a result of improper segregation of materials, using similar utensils for raw and cooked food, improper wiping of working area, and others. **Proper segregation** is one of the best ways to avoid cross-contamination. Raw ingredients must always be separated from cooked ones to avoid contamination of already processed food. Because there is no other processing after the food is cooked, the contamination cannot be controlled. Proper segregation also includes setting a space for dried and wet products. Because wet products have more tendency to have a higher microbial count, lumping them with dry ingredients can rehydrate the latter materials and contaminate them. This situation also contaminates the dry ingredients in terms of quality.

Figure 12 – The proper food storage

Cross-contamination can also be prevented by implementing a good workflow and by proper cleaning. Improper handling during food transportation may lead to contamination and spoilage. The containers used for transportation should provide adequate protection from potential contamination, keep the appropriate temperatures for chilled or frozen products (refrigerated vans, cool bags) and separate raw products from ready-to-eat ones. This principle also includes regular cleaning of the vehicle. Food waste includes biodegradable and non-biodegradable materials, and they must be kept far from the work area to prevent possible contamination. Safe drinking water is one of the most widely used ingredients in all businesses. is used not only for formulating and cooking such as in juices or in cooking for a restaurant, but also for washing both raw materials and utensils. Water quality in all areas must be ensured by clean potable sources. Before starting work the work area must be cleaned and sanitized properly to make sure that no contaminants are present. It is equally important to sanitize your work area after work.

Foodborne diseases represent serious threats to the health of thousands of millions of people. There are three main types of foodborne illnesses. The first type is generally referred to **foodborne infections** and it is realized after multiplication of microbes. The second type is **food poisoning**: it occurs when a person ingests food containing a foodborne pathogen, and the illness results from the production of toxins. The third type of food borne illness commonly refers to as "**foodborne intoxications**" and it occurs when a pre-formed toxin, made by a microorganism, is ingested by the

person, resulting in illness. The most widely spread foodborne infection is dysentery. Common examples of food poisoning organisms include *Listeria monocytogenes*, *Salmonella enterica* and *Campylobacter* spp. Examples of food intoxication include *Staphylococcus aureus* and *Bacillus cereus* intoxications, as well as the seafood toxins such as paralytic shellfish toxin / poisoning and domoic acid.

Staphylococcus aureus, one of the leading causes of foodborne illnesses, is commonly transferred through cross-contamination. This bacterium is a commensal, which means it lives in some parts of our body, including our skin, ears, hands, and nose. Therefore, touching your skin and then touching the food you are handling can cause cross-contamination.

6.4 Export Control

Export Control Regulations constitute a body of law enacted by the federal government to protect national and economic security and advance foreign policy goals by prohibiting the unlicensed transfer of items that are subject to trade restrictions or have proprietary, military, or economic applications to foreign nationals. There are two sets of export controls that govern dual-use items, technologies, software with commercial and military applications, and materials, technologies and software specially designed for military applications. The export control regulations provide the institution and the individual rules. Note that these regulations apply to you in all circumstances, regardless of whether the activity is conducted. For health care professionals researchers in the health sciences or researchers working with biological materials, there are several items that require export licenses prior to being shipped outside. These include attenuated forms of bacteria, toxins, viruses, and fungi as well as animals or materials containing those agents. There are many items that are not isolated agents but do require export licenses for even minuscule quantities or for snippets of genomic elements.

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Topic 7. Biological Warfare, Weapons, and Bioterrorism

Learning objectives :

7.1 Biological warfare and weapons.

7.2 History of biological warfare. The Biological Weapons Convention.

7.3 Bioterrorism.

7.4 Defense against biological weapons (Biodefense).

7.1 Biological Warfare and Weapons

Biological weapons disseminate disease-causing organisms or toxins to harm or kill humans, animals or plants. They generally consist of two parts – a weaponized agent and a delivery mechanism. In addition to strategic or tactical military applications, biological weapons can be used for political assassinations, the infection of livestock or agricultural produce to cause food shortages and economic loss, the creation of environmental catastrophes, and the introduction of widespread illness, fear and mistrust among the public. Almost any disease-causing organism (such as bacteria, viruses, fungi, prions or rickettsia) or toxin (poisons derived from animals, plants or microorganisms, or similar substances produced synthetically) can be used in biological weapons. The agents can be enhanced from their natural state to make them more suitable for mass production, storage, and dissemination as weapons. Biological weapons can be directed against crops and livestock, in addition to humans. Biological agents can be delivered as an aerosol, by food or water, by a vector, by injection.

Biological weapons, unlike other types of weapon referred to as weapons of mass destruction, are incapable of mass destruction of infrastructure, buildings, or equipment. Biological weapons are difficult to detect, economical and easy to use. The cost of biological weapons is estimated at about 0.05 % of the cost of conventional weapons while producing the same amount of mass casualties per square kilometer. Moreover, their production is very simple, since the technology can be used for the production of biological weapons, vaccines, food, spraying devices, beverages, and antibiotics. Ideal characteristics of a biological agent to be used as a weapon against humans are high infectivity, high virulence, non-availability of vaccines and availability of an effective and efficient delivery system. Stability of the weaponized agent (the ability of the agent to retain its infectivity and virulence after a prolonged period of storage) may also be desirable, particularly for military applications, and the ease of creating one is often considered. Control of the spread of the agent may be another desirable characteristic.

Entomological warfare (EW) is a type of biological warfare that uses insects to attack the enemy. The concept has existed for centuries, and research and development have continued into the modern era. EW was used in combat by Japan, and several other nations have developed and been accused of using the Entomological Warfare program. EW may employ insects in a direct attack or as vectors to deliver a biological agent, such as plague. Essentially, EW exists in three varieties. One type of EW involves infecting insects with a pathogen and then dispersing the insects over target areas. The insects then act as vector of infection, infecting any person or animal they may bite. Another type of EW is a direct attack of insects on crops; the insects may not be infected with any pathogen but instead represent a threat to agriculture. The latter method uses uninfected insects, such as bees or wasps, to attack the enemy directly.

7.2 History of Biological Warfare Use. The Biological Weapons Convention

The use of infectious agents and poisons against enemy personnel is an ancient practice in warfare. Rudimentary forms of biological warfare have been practiced since antiquity. The earliest documented incident of the use of biological weapons is recorded in Hittite texts of 1500–1200 BCE, in which victims of tularemia were driven into enemy lands, causing an epidemic. The Assyrians poisoned enemy wells with the ergot fungus, though with unknown results. Scythian archers dipped their arrows and Roman soldiers their swords into excrements and cadavers – as a result, the victims contracted tetanus.

One of the first recorded uses of biological warfare occurred in 1347, when Mongol forces are reported to have catapulted plague-infested bodies over the walls into the Black Sea port of Caffa (now Feodosiya, Ukraine), at that time a Genoese trade center in the Crimean Peninsula. Some historians believe that ships from the besieged city returned to Italy with the plague, starting the Black Death pandemic that swept through Europe over the next four years and killed some 25 million people (about one-third of the population).

Biologicals were extensively used in many parts of Africa from the sixteenth century AD, most of the time in the form of poisoned arrows, or powder spread on the war front as well as poisoning horses and water supply for enemy forces. The creation of biologicals was handled by a special professional class of physicians.

In 1710 a Russian army fighting Swedish forces barricaded in Revel (now Tallinn, Estonia) also hurled plague-infested corpses over the city's walls. In 1763 British troops besieged at Fort Pitt (now Pittsburgh) during Pontiac's Rebellion passed blankets infected with smallpox virus to the Indians, causing a devastating epidemic among their ranks.

During World War I (1914–1918) Germany initiated a clandestine program to infect horses and cattle owned by Allied armies on both the Western and Eastern fronts. The infectious agent for glanders was used. For example, German agents infiltrated the United States and surreptitiously infected animals prior to their shipment across the Atlantic in support of Allied forces. In addition, there reportedly was a German attempt in 1915 to spread plague in St. Petersburg in order to weaken Russian resistance. One of these German itself became a victim of similar attacks – horses bound for Germany were infected with Burkholderia by French operatives in Switzerland. The horrors of World War I caused most countries to sign the 1925 Geneva Protocol banning the use of biological and chemical weapons in war. Geneva Protocol prohibits the use but not the possession or development of biological and chemical weapons.

With the onset of World War II, the Ministry of Supply in the United Kingdom established a biological warfare program at Porton Down, headed by the microbiologist Paul Fildes. The research was championed by Winston Churchill and soon tularemia, anthrax, brucellosis, and botulism toxins had been effectively weaponized. In particular, Gruinard Island in Scotland was contaminated with anthrax during a series of extensive tests for the next 56 years. Although the UK never offensively used the biological weapons it developed, its program was the first to successfully weaponize a variety of deadly pathogens and bring them into industrial production.

Japan also began their own biological weapons programs and engaged in a massive and clandestine research, development, production, and testing program in biological warfare. It used biological weapons against Allied forces in China between 1937 and 1945. The Japanese also experimented on and killed more than 3,000 human subjects (including Allied prisoners of war) in tests of biological warfare agents and various biological weapons delivery mechanisms. They experimented with the infectious agents for bubonic plague, anthrax, typhus, smallpox, yellow fever, tularemia, hepatitis, cholera, gas gangrene, and glanders. In 1940, the Japanese Army Air Force bombed Ningbo with ceramic bombs full of fleas carrying the bubonic plague. Many of these operations were ineffective due to inefficient delivery systems, although up to 400,000 people may have died. During the Zhejiang-Jiangxi Campaign in 1942, around 1,700 Japanese troops died out of a total 10,000 Japanese soldiers who fell ill with disease when their own biological weapons attack rebounded on their own forces.

The most horrifying war crimes ever committed were conducted by Japan scientists in the Unit 731. They did experiment on hypothermia, investigated the effects of disease and injury on the fighting ability of an armed force, rape and forced pregnancy and tested biological weapons on Chinese civilians.

Figure 13 – Unit 731 personnel conduct a bacteriological trial upon a test subject in Nong'an County of northeast China's Jilin Province. November 1940

The Japanese use of biological warfare agents against the Chinese led to an American decision to undertake biological warfare research. The U.S. established a large research program and industrial complex at Fort Detrick, Maryland in 1942 under the direction of George W. Merck. The biological and chemical weapons developed during that period were tested at the Dugway Proving Grounds in Utah. Soon there were facilities for the mass production of anthrax spores, brucellosis, and botulism toxins, although the war was over before these weapons could be of much operational use. The United Kingdom, Germany, and the Soviet Union had similar programs during World War II, but only Japan has been proved to have used such weapons in the war.

In the Cold War era, which followed World War II, both the Soviet Union and the United States, as well as their respective allies, embarked on large-scale biological warfare research and production programs. United States developed an anti-crop capability during the Cold War that used plant diseases (bioherbicides, or mycoherbicides) for destroying enemy agriculture. Biological weapons also target fisheries as well as water-based vegetation. It was believed that the destruction of enemy agriculture on a strategic scale could thwart Sino-Soviet aggression in a general war. Diseases such as wheat blast and rice blast were weaponized in aerial spray tanks and cluster bombs for delivery to enemy watersheds in agricultural regions to initiate

epiphytotic (epidemics among plants). On the other hand, some sources report that these agents were stockpiled but never weaponized. When the United States renounced its offensive biological warfare program in 1969 and 1970, the vast majority of its biological arsenal was composed of these plant diseases. Enterotoxins and Mycotoxins were not affected by Nixon's order. The United States and Britain discovered plant growth regulators (i.e., herbicides) during the Second World War, which were then used by the U.K. in the counterinsurgency operations of the Malayan Emergency. Inspired by the use in Malaysia, the U.S. military effort in the Vietnam War included a mass dispersal of a variety of herbicides, famously Agent Orange, with the aim of destroying farmland and defoliating forests used as cover by the Viet Cong. Sri Lanka deployed military defoliants in its prosecution of the Eelam War against Tamil insurgents.

The Soviet biological weapons (BW) program was the largest and most sophisticated program ever undertaken by any nation. It was initiated in 1928. It continued during WW 2 and directly afterwards. After 1945, the Soviet program benefited from information obtained from the wartime Japanese and U.S. BW programs. In the pre-1972 Soviet BW program used classical genetic selection techniques for enhancing the virulence and hardiness of pathogens that could incapacitate or kill men, animals or plants. In addition, an effort was made to obtain antibiotic-resistant strains of these organisms. The latter ability would permit the pathogen to overcome the opponents' defenses, both vaccines and antibiotics, as well as evade detection and identification systems. The post-1972 Soviet BW program was composed of four major components. The main elements were major facilities in the Ministries of Defense, Agriculture and Health and the newly created nominally civilian Biopreparat organization. Between them they comprised 40–50 research, development and production facilities plus the large military testing site on Vozrozhdeniye Island in the Aral Sea. These were cumulatively capable of initiating production of thousands of tons of BW agents within a year of being ordered to in anticipation of a major war with the U.S. By 1990 the USSR had proof-tested 13 agents as well as delivery systems for them. However, its existing BW stockpiles at that time were still composed of classical not-genetically modified bacterial and viral strains. The BW delivery systems produced by the Soviet military were spray systems for medium bombers and bomblet multiple munitions to be contained in air-delivered munitions.

In the 1980s Soviet Ministry of Agriculture had successfully developed variants of foot-and-mouth disease, and rinderpest against cows, African swine fever for pigs, and psittacosis to kill the chicken. These agents were prepared to spray them down

from tanks attached to airplanes over hundreds of miles. The secret program was code-named "Ecology".

In the largest biological weapons accident known the anthrax outbreak in Sverdlovsk (now Yekaterinburg) in the Soviet Union in 1979 sheep became ill with anthrax as far as 200 kilometers from the release point of the organism from a military facility in the southeastern part of the city and still off-limits to visitors today. The Soviet Union attracted international suspicion after the 1979 Sverdlovsk anthrax leak killed approximately 65 to 100 people.

To stop the biological weapons spread, the United States government announced the end of its offensive biological weapons (BW) program on November 25, 1969. U.S. BW stockpiles were destroyed in 1971-1972, and facilities converted. Great Britain, by then also divested of its BW program, proposed a treaty banning BW, which had been until that time always combined with chemical weapons in arms-control negotiations. The Soviet Union approved a massive expansion of the Soviet offensive BW program at the end of 1971. From 1975 on the Soviet BW program existed in violation of the international treaty.

After being discussed and negotiated in the United Nations' disarmament forum starting in 1969, the Biological Weapons Convention (BWC) opened for signature on April 10, 1972, and entered into force on March 26, 1975. This program prohibits the development, production, acquisition, transfer, stockpiling and use of biological weapons. As of March 2021, 183 states have become party to the treaty, including Palestine, and four signatories (Egypt, Haiti, Somalia, Syria, and Tanzania). Ten states have neither signed nor ratified the BWC (Chad, Comoros, Djibouti, Eritrea, Israel, Kiribati, Micronesia, Namibia, South Sudan, and Tuvalu). The BWC established a strong global norm against biological weapons, but its effectiveness has been limited due to insufficient institutional support and the absence of any formal verification regime to monitor compliance.

Moreover in 1985, the Australia Group was established, a multilateral export control regime of 43 countries aiming to prevent the proliferation of chemical and biological weapons. In 2004, the United Nations Security Council adopted Resolution 1540, which obliges all UN member states to develop and enforce appropriate legal and regulatory measures against the proliferation of chemical, biological, radiological and nuclear weapons and their means of delivery, in particular to prevent the spread of weapons of mass destruction to non-state actors.

Nowadays, according to an unclassified U.S. Department of State report in 2005, nations suspected of continued offensive biological warfare programs in violation of the BWC include China, Iran, North Korea, Russia, Syria, and possibly Cuba.

7.3 Bioterrorism

Terrorists consider biological agents an attractive alternative to conventional weapons because of their relatively low cost, accessibility, and easy production, and delivery. Their use or even the threat of their use has the potential to cause widespread social disruption. The CDC separates potential bioterrorist agents that cause infections in humans into three categories, designated as A, B, and C. Category A agents carry the highest priority because they can be easily disseminated or spread person-to-person, can be highly lethal, have the potential for serious public health impact, can potentially cause public panic and lead to social disruption. Category B agents carry the second highest priority because they are moderately easy to disseminate, usually result in moderate morbidity, and are generally less lethal. Category C agents carry the third-highest priority. They include emerging pathogens that can potentially be engineered for future mass dissemination.

Table 4 – Examples of potential bioterrorist agents / diseases by CDC category

Category A	**Category B**	**Category C**
Botulism	Cholera	Hendra
Hantavirus	E. coli O157:H7	Tick-borne encephalitis
Lassa	Hepatitis A	Nipah
Marburg	Ricin toxin	SARS
Plague	Salmonella	Rabies
Antrax	Typhus fever	N1H1 influenza
Tularemia	Yellow fever	HIV

Over the last fifty years, there have been three the most famous terrorist the attacks using biological weapons. The first and the largest bioterrorist attack in the United States history was in 1984 when 751 people suffered food poisoning in The Dalles, Oregon, U.S. due to the deliberate contamination of salad bars with *Salmonella* in ten local restaurants. A group of prominent followers of Rajneesh (later known as

Osho) led by Ma Anand Sheela, hoped to incapacitate the city so that their own candidates would win the 1984 Wasco County elections.

Having previously gained political control of Antelope Oregon, Rajneesh's followers, who were based in nearby Rajneeshpuram, sought election to two of the three seats on the Wasco County Circuit Court that were up for election in November 1984. Fearing they will not gain enough votes, some Rajneeshpuram officials decided to incapacitate voters in The Dalles, the largest population center in Wasco County. The chosen biological agent was *Salmonella enterica* serovar Typhimurium, which was first delivered through glasses of water to the two county commissioners and then, on a larger scale, in salad bars and in salad dressing.

A sample of bacteria matching the contaminant that had sickened the town residents was found in a Rajneeshpuram medical laboratory. Two leading Rajneeshpuram officials were convicted on charges of attempted murder and served 29 months of 20-year sentences in a minimum-security federal prison.

The other attack happened in the Tokyo underground on 20th March, 1995. Twelve people were assassinated and more than one thousand were injured and required medical treatment. The sarin was used in the series of attempts by the Aum sect to use biological weapons. Earlier Aum scientists tried to culture *Clostridium botulinum*, but all attempts to produce and use biological weapons failed. Shortly three vehicles were equipped with aerosol generators and driven through Tokyo to the Narita International Airport and to the U.S. naval bases at Yokohama and Yokosaka. However, no effect of these actions could be observed. Similar conclusions can be drawn about Aum's attempt to cultivate and spread *Bacillus anthracis*. Aum was only able to get hold of a non-virulent strain of the pathogen, used for the production of vaccine. Reports about this event can only lead to the conclusion that the production of aerosol from anthrax bacteria solution completely failed – nobody was infected with the pathogen. Two further attempts to spread the anthrax pathogen using the same spraying vehicles used previously used for botulinum toxin also failed.

Last attack occurred between October 4 and November 20, 2001. An unknown actor mailed a powder containing infectious anthrax spores to two U.S. senators and several media outlets. A total of 22 cases of infection with anthrax happened in New York and in Washington, D.C. Half of the total were of the inhaled form, while the other eleven cases were of a skin infection with anthrax. Five of the patients suffering from the inhaled form of anthrax died. Post office staff who contacted with the letters or simply worked at the sites where the letters were sorted or forwarded were affected by anthrax. Lately it has been revealed that all anthrax spores belonged to a strain, which was first isolated in a laboratory in Ames, Iowa. Law enforcement investigators

reached the conclusion that a U.S. bio-defense researcher who worked for a military laboratory at Fort Detrick conducted the attacks. The researcher, Bruce Ivins, killed himself in 2008 during the investigation. Ivins, however, was never formally charged with a crime, and no direct evidence links him to the attacks.

Agroterrorism is an act of terrorism targeting the agricultural or agribusiness sector. Four biological agroterrorism cases are described: (1) in 1952, Mau Mau poisoned cattle in Kenya by using a plant toxin from the African milk bush plant; (2) in 1985, the USDA claimed that Mexican contract workers were involved in the deliberate spread of the screwworm (*Cochliomyia hominivorax*) among livestock; (3) in 2000, Palestinian media reported that Israeli settlers released sewer water into Palestinian agricultural fields; and (4) in 2011, a person was sentenced to prison after threatening the U.S. and U.K. livestock with the deliberate spread of foot-and-mouth disease virus. All four cases can be assigned to political groups.

7.4 Defense Against Biological Weapons

The best way to combat biological warfare and terrorism is to be prepared to respond to a biological weapons attack. A successful civil defense against major biological attacks requires effective sensors, warning systems, vaccines, medicines, responce training, and public education, as well as contingency planning procedures. A variety of state and local agencies are involved in public health emergency preparedness and response. CDC and National Biological Threat Characterization Center (NBTCC) conduct studies and experiments on current and future biological threats, assesses vulnerabilities and conducts risk assessments, and determines potential impacts to guide the development of countermeasures such as detectors, drugs, vaccines, and decontamination technologies. State and local health departments, as well as public and private hospitals and local law enforcement agencies, would also be involved in responding to a bio-weapon public health emergency. Their roles are outlined in national response plans and are addressed in detail by organization-specific plans.

An effective policy to limit the risks of exposure is to vaccinate military personnel against various biological threats and provide them with detection gear, protective masks, and suits. The entire population is not vaccinated. Special vaccines have been created, tested, and approved to deal with the most lethal biological agents that can also be most easily weaponized: anthrax, smallpox, plague and cholera. Currently there are no effective vaccines to prevent infection with glanders, brucellosis, staphylococcal enterotoxin B, ricin, or T-2 mycotoxin. Special training for military medical personnel and the medical community at large are provided to recognize and

treat victims of a biological weapon attack. Early detection capability is an essential tool in cases of suspected uses of biological weapons. The sooner a bioterrorist attack is detected, the faster the medical community can respond to prevent additional exposure and to begin treatment of those who have been exposed. The network of regional labs provides rapid analysis and identification of selected biological agents and provides a defense against biological warfare.

Most lethal biological agents used as weapons are intended to be delivered as aerosols, which can cause infections when inhaled by targeted personnel. For this reason, the most-effective defense against biological weapons is a good protective mask equipped with filters capable to block bacteria, viruses, and spores larger than one micron from entering the user's nasal passages and lungs. Protective overgarments, including boots and gloves, are useful to prevent biological agents from contacting open wounds or scratches on the skin. Also, decontaminants can neutralize biological agents in infected areas after a biological attack.

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Topic 8. Advances in Biotechnology and Biosafety Issues

Learning objectives :

8.1 History of synthetic biology.

8.2 Potential application of synthetic biology.

8.3 Potential risks of synthetic biology.

8.4 Laboratory-level precautions biosafety and biosecurity issues related for synthetic biology.

8.5 Regulatory policy related to synthetic biology.

8.1 History of Synthetic Biology

Synthetic biology is a multidisciplinary research area that combines biology with chemistry, mathematics, computer science, and engineering and focuses on engineering of biological systems by modifying, designing, and *de novo* constructing biological components with new functions.

Synthetic biology emerged at the beginning of the 21st century when the first DNA regulatory elements were constructed based on simple mathematical models. From 2004 to 2007, electrical or mechanical engineering principles were introduced into the study of biological systems. In 2013, a revolutionary genome-editing method, CRISPR-Cas (clustered regularly interspaced short palindromic repeats with associated proteins), was invented. This technology improves the accuracy and efficiency of editing pathogens, animals, plants, and human genomes and speed up traceless modification of genomes. The technology can help enhance the pathogenicity, virulence, or transmission of toxins or bacteria or disrupt essential genes in humans, animals, and plants.

The first synthetic genome of the poliovirus was constructed in 2002. Full-length poliovirus cDNA, which was transcribed into RNA by ordinary RNA polymerase, was synthesized using chemical methods. Then transcribed RNA was incubated on Hela cells, and the infectious virus was successfully obtained. Later, in 2005, the Spanish influenza virus was synthesized on the basis of the common influenza virus. The virus particles containing eight gene fragments were cultivated on human kidney cells. In 2008, a minimal prokaryotic genome of *Mycoplasma genitalium* was chemically synthesized and assembled. Then, in 2009, the first synthetic yeast genome project was launched to redesign and chemically synthesize the entire *Saccharomyces cerevisiae* genome. In 2010, an artificial *M. mycoides* cell “Synthia,” with the expected phenotype

and self-replication capability, was created. In 2015, scientists inserted the bat coronavirus SHC014 spike protein into mouse adaptive SARS-CoV skeleton. This resulted in the ability of the virus skeleton to recognize the ACE2 receptor via the SHC014 spike protein and to replicate successfully in human respiratory cells. In 2008, virologist David Evans synthesized horsepox virus, which was produced by introducing DNA into cells using recombinase. Horsepox virus is closely evolutionary related to variola virus. Scientists from Switzerland, Germany, and Russia reconstructed the novel coronavirus in yeast using the publicly available SARS-CoV-2 sequences. In 2019, a four-mega base *E. coli* genome was created and then transformed into a bacterium called "Syn61" that uses only 61 codons. The synthetic bacteria exhibited a complete viability.

Theoretically, synthetic biology could be used in the future to design new types of biological warfare agents. Most of the biosecurity concerns in synthetic biology are focused on the role of DNA synthesis and the risk of producing genetic material of lethal viruses (e.g. 1918 Spanish flu, polio) in the lab.

8.2 Potential Application of Synthetic Biology

Synthetic biology has been used in wide range of fields. In the chemical industry microbes are used for production and manufacture of enzymes, biofuels, and creation of bio-based specialty products. Engineered bacteria that live on cornstarch have been engineered to produce high-tech fabrics. DDT pollutants can be degraded by engineered bacterial strains to stable 4-chlorobenzoic acid metabolites.

Synthetic biologists are trying to create microbial biosensors for pollution monitors. Engineered bacteria are used to monitor the concentration of naphthalene in water and gas phases, organophosphorus pesticides. The rapid, inexpensive detection of Ebola and Zika viruses has been achieved by implementing biosensor gene networks.

Agricultural biotechnology is a major beneficiary of synthetic biology. The synthetic biology principles facilitate improvements and developments in sustainable farming practices, animal health, disease-resistant crops and their yields, and speciality foods, reducing our dependence on traditional crops. Genetic modification is a special set of synthetic biology that alters the genetic machinery of such living organisms as animals, plants, or microorganisms. Combining genes from different organisms is known as recombinant DNA technology and the resulting organism is said to be "Genetically modified (GM)", "Genetically engineered" or "Transgenic". Genetic engineering technology has great potential to contribute increasing that productivity and reduce deforestation and biodiversity loss in forests.

Biotechnology products have been shown to increase the yield and reduce the use of insecticide in agriculture. The main transgenic crops grown commercially in the field are herbicide- and insecticide-resistant soybeans, maize, cotton, and canola. There are fruit and nut trees that produce years earlier, and plants that produce new plastics with unique properties. Despite several advantages of GM crops they possess potential negative environmental impact such as 1) gene flow between the transgenic plants and their sexually compatible relatives, 2) changes in weediness or invasiveness of GM crops or their wild relatives, 3) horizontal transfer of engineered traits to other species, 4) non-target effects, and 5) development of pest resistance or new secondary pests. The main concerns about adverse effects of GM foods on health are the transfer of antibiotic resistance, toxicity, immune suppression, cancer, and allergenicity.

In healthcare research synthetic biology facilitates rational drug design research, immunotherapy for cancer research, and sustainability to medical practices. Microbes were used to produce chemicals for decades. For instance, *Saccharomyces cerevisiae* is widely used in industry for the production of artemisinin acid, opioids and some rare medicinal extracts. Synthetic biology has also suggested using microbes (*Yersinia pseudotuberculosis* and some viruses) to invade tumour cells. The invading bacteria are programmed to trigger drug expression to repress tumour growth. Synthetic bacteriophage expresses enzymes capable of lysing biofilms and drug-resistant bacteria.

Another direction of synthetic biology is vaccine development. There are bananas that produce human vaccines against infectious diseases such as hepatitis B. Significant progress has been made in the development of the messenger RNA **(**mRNA**)** vaccine for SARS Cov2 infection. This type of vaccine uses genetically engineered mRNA to induce production of the S protein of the COVID-19 virus. After vaccination, host cells produce S protein and display it on cell surfaces. This stimulates the formation of specific immunity against Covid 19.

8.3 Potential Risks of Synthetic Biology

Development and application of synthetic biology raise biosafety and biosecurity conserns that they may expose public health and the environment to unknown hazards. The problems are allergies, antibiotic resistance, carcinogenicity, and pathogenicity or toxicity as risks to human health and environmental risks of alteration or depletion of the environment; competition with native species, horizontal gene transfer and pathogenicity or toxicity.

Discrete risks in synthetic biology can be categorised into three types of risk:

- Synthetic biology does not always produce the desired results. A typical example is gene editing in human babies.
- Synthesis of the pathogen is much easier than before. The genetic sequences of highly pathogenic bacteria and viruses are available on websites, such as GenBank, EMBL, and DDBJ. There are many technical service companies that can provide support from experimental design to products through network orders. Methods for improving the pathogenicity and transmission of dangerous viruses or bacteria have been published available in many academic journals.
- Formation of antibiotic-resistant superbugs after artificial application of the plasmids.

8.4 Laboratory-Level Precautions for Biosafety and Biosecurity Issues Related to Synthetic Biology

To address the biosafety issues associated with synthetic microbes technical efforts have been made to develop precautionary measures at the laboratory level to limit the release and survival of synthetic microbes in the environment.

Genetic safeguards are effective biocontainment strategies against the accidental release of genetically modified microbes into the environment. This strategy involves the early design of biocontainment systems using toxin gene expression cassettes. It is a conditional suicide system for bacteria that use a toxin gene that kills various bacteria when expressed. A combination of condition-regulated promoters and toxin gene cassettes will stop the growth of cells when released from the designed environment. Another toxin-controlled system is based on toxin / antitoxin pairs of bacteria, where the main mechanism involves neutralization of the toxin by the antitoxin at either the transcriptional or translational level. In addition, genetic safeguards can be built based on auxotrophic mechanisms. For example, a biological isolation system based on the expression of an essential gene was developed in *Salmonella enterica* which was initially designed for arabinose-dependent growth. The transcription of arabinose-regulated genes is shut down in this microbe due to a lack of arabinose when the bacteria enter host cells. Moreover, the down regulation of these genes will activate the synthesis of antisense mRNA of targeted essential genes and this action eventually will cause cell lysis of bacteria.

Genetic firewall or xenobiology is an important branch of synthetic biology that aims to construct and synthesise xenonucleic acids or engineer proteins with non-canonical amino acids. Xenonucleotides or non-canonical amino acids do not exist in nature; therefore, synthetic organisms dependent on such artificial molecules will not survive outside their designed environment. The strategy can effectively eliminate the

risks related to genetic information exchange and preclude horizontal gene transfer between synthetic and existing natural organisms. Moreover, genetic materials released by dead synthetic cells cannot be incorporated into a natural organism because they cannot be recognized by a natural DNA polymerase.

DNA watermarks or barcodes are based on detection and identification of contaminating synthetic DNA or organisms. In this regard, unique synthetic DNA sequences, embedded in multiple loci of synthetic genomes, provide valuable means for isolating or identifying and tracking synthetic organisms. Several DNA watermarks have been independently devised for DNA coding regions, regulatory sequences, and non-coding DNA sequences to encrypt information by the DNA-Crypt algorithm. Watermarks or barcodes not only help to trace and identify synthetic organisms, but can also provide proprietary protection for engineered strains.

8.5 Regulatory Policy Related to Synthetic Biology

Legislation of synthetic biology in different countries is issued according to the areas of research and applications of synthetic biology. Government regulations on synthetic biology are similar in the European Union and United States. Current synthetic biology continues to use techniques subject to Directives 2009/41/EC and 2001/18/EC, the governance of which includes the use and deliberate release of genetically modified organisms into the environment. European Union legislation on the use and regulation of GMOs is mainly based on Directive 90/219/EC, which regulates genetic modification of microorganisms as well as their cultivation, storage, transport, destruction and disposal.

The production of medicines in the United States are regulated by the Federal Food, Drug, and Cosmetic Act (FDCA). Toxic substances are controlled with Plant Insect Law, Public Health Security and Bioterrorism Preparedness Response Act of 2002, the Bioshield Act, the Biological Defense and Pandemic Vaccine and Drug Development Act, the National Bioengineered Food Information Disclosure Standard, the U.S. Government's Regulatory Policy for Life Sciences Dual-Use Research, and other laws and regulations.

China has promulgated laws and regulations on the biosafety governance of synthetic biology in laboratory practice to ensure biosafety and biosecurity. The Law of the People's Republic of China on Biosafety in 2020, the Regulation on the Safety Management of Biotechnology Research and Development (Biotechnology Research Regulations), and a series of policy acts have been issued regarding the prevention and control of infectious diseases and laboratory management.

In the EU three separate pieces of legislation govern the use of GMOs in foods : Council Directive 90/220/EEC, the EC Novel Foods Regulation (EC № 258/97) and Council Regulation (EC № 1139/98) (labelling of certain foodstuffs). They focus on the protection of human health and the environment. They focus on the protection of human health and the environment. They cover the environmental risk assessment and approval release of all GMOs in the research and development phase, as well as the placing on the market of products containing or consisting of GMOs.

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