

Biological Agents Database in the Armed Forces

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Abstract Rapid detection and identification of the biological agent during both, natural or deliberate outbreak is crucial for implementation of appropriate control measures and procedures in order to mitigate the spread of disease. Determination of pathogen etiology may not only support epidemiological investigation and safety of human beings, but also enhance forensic efforts in pathogen tracing, collection of evidences and correct inference. The article presents objectives of the Biological Agents Database, which was developed for the purpose of the Ministry of National Defense of the Republic of Poland under the European Defence Agency frame. The Biological Agents Database is an electronic catalogue of genetic markers of highly dangerous pathogens and biological agents of weapon of mass destruction concern, which provides full identification of biological threats emerging in Poland and in locations of activity of Polish troops. The Biological Agents Database is a supportive tool used for tracing biological agents' origin

as well as rapid identification of agent causing the disease of unknown etiology. It also provides support in diagnosis, analysis, response and exchange of information between institutions that use information contained in it. Therefore, it can be used not only for military purposes, but also in a civilian environment.

Keywords Biological agents · Government · Military · European defence agency

Introduction

Epidemiology is a study of modes of disease spreading and factors that influence this distribution in target populations and directly applies in health-related issues. One of the epidemiology objectives is identification of the etiology or cause of a disease including factors that increase a person's risk for disease, following determination of confirmed cases in the community, natural history of disease with prognosis and evaluation of new preventive and therapeutic measures. The significant contributions to the epidemiology of infectious diseases have made microbiology. Isolation and identification of specific pathogens from patients manifesting definite disease symptoms helped to prove infectious etiologies for many illnesses and supports investigation of numerous outbreaks. Moreover, isolation of microorganisms from animals, vectors and environmental sources allowed to identify reservoirs and determined likely modes of transmission. Integration of epidemiology with laboratory science also supports the choice of an effective treatment based on antimicrobial susceptibilities of bacterial pathogens.

Molecular epidemiology is a relatively new science discipline applied to explore global distribution of bacterial and viral pathogens as well as discover its evolutionary

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history. The above opportunity is arising due to advanced genotypic studies focused on detection and characterization of genetic variability of infectious agents. Standardized molecular epidemiology using genotyping methods allows to establish phylogeography of microorganisms; namely, based on bacterial diversity and molecular markers of bacterial strains in population, the relationship between geographical location and phylogenetic history of bacterium might be determined. Analysis of chromosomal DNA using standardized multilocus genotyping technique allows creating a molecular map for specific pathogens similar to numerical “fingerprint”, which is an unambiguous and unique molecular pattern. The high frequency of mutations and rapid evolutionary capabilities of RNA viral genomes explain the reason, why they are frequently “emerging pathogens” in new hosts and why they should be analyzed on molecular level. Standardized genotype results may be interpreted, repeated or consulted among other laboratories and stored in genomic libraries or databases. Furthermore, a “fingerprinted” strain or viral types can be compared with an online database of genotypes, as well.

The online access to the database of molecular markers of pathogens provides high-quality results in short time, in effect, is progressively being used in both, epidemiological and microbial forensic investigations. What is more, a significant challenge in molecular forensics of microbial diversity is the ability to differentiate the natural from the intentional or accidental biological event. In consequence, it supports the public health and law enforcement efforts to determine the source of infection and the perpetrators of bio-crime (Budowle et al. 2011).

This paper presents main goals and assumptions of the Biological Agents Database highlighting that epidemiological studies and microbial forensics technics are essential to putting laboratory findings into the context of a bioterrorist attack or natural outbreak. The paper sheds light on that rapid detection and full identification of biological agent combined with its geographical tracing enabling early event confirmation and information sharing between appropriate governmental agencies. The Biological Agents Database allows tracing the pathogens occurrence in case of biological incident on unknown etiology not only in Poland enhancing national biological security, but also in neighboring regions supporting information exchange and international assistance.

Goals

The idea to create Polish Biological Agents Database appeared in 2011 at the end of Poland’s participation in the European defence agency (EDA) Data Base of Bio Agents project. Experiences collected during this project by the

Military Institute of Hygiene and Epidemiology and the Ministry of National Defence resulted in a series of internal consultations leading to the above-mentioned idea. The Biological Agents Database will be an electronic software collecting highly pathogenic biological agents. It will enhance Polish Armed Forces capabilities in rapid biological agent detection and identification. Moreover, it will shorten the time of identification in cases of use of biological warfare agents or emergence and dangerous pathogens caused highly infectious diseases. It will be used for tracing an outbreak etiology as well as origin of unknown pathogens. Additionally, it allows for rapid verification of natural or deliberate pathogen origin based on clinical manifestation of the case.

It will serve not only for military purposes, but also for civilian sanitary inspections as a supporting tool during epidemic on both, regional and central level. Furthermore, the Database may serve in microbial forensic analyses of ancestry and last locations of causative biological agent and accelerate to early capture of crime perpetrator.

Moreover, the information deposited at the Biological Agents Database will allow for full biological agents identification and confirmation not only in situ in Poland, but also in other regions of the Polish soldiers deployment. It will support tracing occurrence of emerging infectious diseases in endemic areas providing credible information for upcoming prophylactic and preventive activities planning by the Military Sanitary Inspection. It also improves exchange of information contained herein between the national institution authorities engaged in response to biological incident at central level and military decision-makers in headquarters.

Background

Development of the Polish Biological Agents Database is possible thanks to Poland’s involvement in two EDA projects. The first one mentioned above, allowed to identify the need to increase national capability in efficient identification of various bio agents. The project worth more than 7 million EUR, thanks to participation of laboratories coming from 11 EU member states resulted in building solid frames for more enhanced cooperation between European laboratories. This cooperation should allow to create a fully operational network of European biological laboratories. This is therefore the main goal of the second phase of Data Base of Bio Agents project, the so-called European Biodefence Laboratory Network (EBLN). This project founded by ministries of defence from 10 European member states started early in 2013. It aims at further developing tools for a European Pathogen Comparison and Tracing System for microbial forensic purposes.

The Military Institute of Hygiene and Epidemiology, thanks to ministerial founding will continue its efforts within the EBLN project. The MIHE will focus on modernization of already established database and, based on its broad experiences will develop a Polish Biological Agents Database, the key initiative leading to increase of Polish Armed Forces capability in efficient identification of bio hazards.

Assumptions

In order to establish fully operational Biological Agents Database in Poland, the following objectives need to be implemented.

Firstly, preparation of facilities and modernization of IT infrastructure (software completion, adaptation of IT infrastructure to individual beneficiaries) will be done—updating of operating system and acquisition of new application modules.

Servicing and functionality expansion of BIONUMERICIS software—advanced statistical program—comparison of research results obtained with different methods will be possible. Furthermore, changes in the software servers make it possible to use real-time data without the necessity of updates.

Next step will be development of scientific identification protocols for pathogens. Efforts will be made to obtain the molecular characteristics of biological agents. The database will be extending to new pathogens and inventoried in order to determine reference strains, as well. Establishment of reference strains on unique features for different groups of pathogens allows to analyze pathogens of unknown origin. Additional studies required to achieve complete molecular characterization of the pathogens will be performed. Afterwards, systematic placing of newly generated molecular data into the database will be made. The primary sources of biological agents are collections from Data Base of Bio Agents project and ongoing European Biodefence Laboratory Network project. Having the access to molecular data of pathogens from EBLN partner countries, gradual introduction of a new molecular data of newly acquired microorganisms to the database will be possible. New strains or their molecular markers will be acquired through their purchase or exchange. Bacterial strains and viral types will be collected from research projects targeted for endemic regions or our partnerships, as well. New procedures for exchange of information between beneficiaries database will be streamlined, as well.

The genome sequence of selected bacterial pathogens and viruses will be analyzed. It allows increasing awareness and understanding the mutation processes of potential pathogens, which may impact the molecular characterization and

will pave way for unquestionable assignment of pathogens to the suitable group classification.

The microbial forensics techniques are more advanced in comparison to classical microbiology or epidemiology methods. Usage of additional molecular identification methods allows to analyze the results in more detail. It supports determination of the infectious source and possible ways of pathogens spread. Microbial forensic and molecular methods were successfully used in determining the source of anthrax spores in the US in September and October 2001.

Mass spectrometry analysis will be used to characterize the pathogens and assign them into various groups of pathogens. Based on the protein analysis they can be assigned to individual microorganisms even after its death. Moreover, the method allows to assess biological traces.

Due to the fact that each group of pathogens is characterized by a unique fatty acid composition, additionally fatty acid analysis will be done. It determines the type of fatty acids in microorganisms even after the death of the pathogen.

Analysis of environmental samples intended to acquire new strains to database collection from environment will be performed. Both, continuous geographical monitoring and reports on occurrence of emerging diseases outbreaks will be done. In case of any, environmental samples from target place (soil, water, etc.) will be collected. Suspicious pathogen will be isolated and analyzed. It expands the collection of database on new and/or not investigated strains.

Metagenomic analysis involving DNA cloning is acquired directly from natural environments and then sequencing of huge genomic libraries. The acquired sequences will be subjected to comparative analysis of the sequences within the database. This method allows for identification and categorization of microorganism's phylo-types occurring in environmental samples as well as for geographical assessment of probable pathogen's location.

Using advanced statistical analysis system BIONUMERICIS determination of the pathogen origin will be possible by establishment of comparison level between reference and unknown strains based on percentage of similarity. It allows finding a probable source of new and/or unknown strains.

Currently, there is a qualitative gap between the procedures used by individual Polish military biological laboratories. The results were obtained by the same method, but using different reference criteria may change the final outcome. The standard operating procedures will be developed for all networking laboratories to unify the diagnostic methods. Moreover, the same equipment and reagents will be recommended in order to obtain both, uniform and comparable results.

The evaluation of all analytical methods for obtaining molecular characteristics of pathogens will be carried out. The most appropriate for a specific pathogen will be selected and commonly used.

Then, work over the flow of information between the managing authority and the beneficiaries will be enhanced. Only selected laboratory personnel will be trained in terms of safe and secure handling and usage of the database.

The last step of the Biological Agents Database development will be both, official notification of the database readiness and functionality of it as well as procedures developed for approval of the information exchange between the managing authority and the beneficiaries in 2016.

The creation of verified reference collection of pathogens based on their molecular characteristics in the database as well as analysis by statistical system will be the final output coming from the Polish Biological Agents Database when finally established.

Beneficiaries

The main aim of this idea is to create a network of modern biological laboratories equipped with a database of broad molecular characteristics of pathogens. Military laboratory network with access to the Biological Agents Database will increase the ability of rapid identification of cases of unknown origin and increase human health, animal well-being and crops safety.

Primary beneficiaries will be military departments and units directly subordinate to the Inspectorate of Military Health Care and Director of Defense Against the Weapon of Mass Destruction Department including the Operational Command and Special Forces Command.

The Inspectorate of Military Health Care Service is an owner of the Biological Agents Database. The Biological Threats Identification and Countermeasure Centre of the Military Institute of Hygiene and Epidemiology in Pulawy is a managing department of the Database.

Free access to the Biological Agents Database will have the Inspectorate of Military Health Care Service, the Training Centre for Defence Against the Weapon of Mass Destruction in the Armed Forces at the National Defence Academy, the Training Centre for Defence Against the Weapon of Mass Destruction in the Armed Forces at Ministry of National Defence, the Contamination Analysis Centre, the Epidemiological Response Center of Polish Armed Forces, the National Operational Command as well as five Military Preventive Medicine Centers located at Gdynia, Modlin, Bydgoszcz, Wrocław and Kraków.

Algorithm of Data Analysis

According to rules of diagnosis of exclusion,¹ the Beneficiary will be obligated to analyze the suspicious probe based on reference level acceptable for his laboratory capabilities. He would compare his results with the Biological Agents Database information. In order to facilitate the process of biological agent confirmation or results verification, all outcomes will be collected and stored at the main server in MIHE. Moreover, in order to provide the most credible data on microorganisms, the suspicious probes will be analyzed twice, respectively, in Beneficiary and MIHE laboratories.

Only the Biological Threats Identification and Countermeasure Centre of the Military Institute of Hygiene and Epidemiology is appointed to introduce a new biological agents profiles or update the database. The transfer of sensitive data will be possibly only via secure computer links and BIONUMERICS software. The Beneficiaries will be equipped with necessary tools.

The Recipient will receive the information processed and analyzed by MIHE's specialist team.

The MIHE laboratory infrastructure fulfills the requirements of bio-safety and biosecurity issues. Analysis of highly infectious bacteria and viruses are performed, respectively, in Biosafety Laboratory Level 3 (BSL-3) and Biosafety Laboratory Level 2 (BSL-2). Only qualified laboratory personnel has access to work with highly infectious biological agents. In future, establishment of Biosafety Laboratory Level 4 (BSL-4) should be considered in order to provide full safety for laboratory personnel handling the most infectious biological agents at workplace and complex security of military infrastructure.

Conclusions

A combination of various disciplines must be exploited to analyze biological agents distribution and/or bio-crime evidences: not only biology, microbiology or medicine, but also geography, biostatistics, genetics and bioinformatics. Nevertheless, molecular epidemiology and forensic microbiology will be directly applied to the benefit of Biological Agents Database.

The Database will support Polish Armed Forces capabilities in detection and rapid identification of highly pathogenic microorganisms in Poland as well as in endemic regions, where Polish troops are deployed. Specific

¹ Diagnosis made by excluding all other known diseases, The American Heritage® Medical Dictionary Copyright © 2010 by Houghton Mifflin Harcourt Publishing Company. Published by Houghton Mifflin Harcourt Publishing Company.

analysis of strains or species type, revealing the manufacturing traits of bacteria culturing or specific growth media as well as geographic information associated with the narrow point in the map of suspects will support the crime investigation related to inadvertent release of microorganisms or bioterrorism act. Additionally, the molecular collection of biological agents in the Biological Agents Database may serve public health with bio-surveillance, monitoring of bacterial strains in endemic areas, pathogenic strains identification and tracking the etiology of outbreaks.

Both, covert and overt biological attack may mimic naturally occurring disease, intentionally delaying recognition of an attack. According to WHO, in the last decade if one disease was successfully eradicated, many new or re-emerging diseases have occurred posing a huge challenge for public health and national security. Advances of modern biotechnology and life sciences disciplines should go ahead and develop new identification methods,

precautionary and control measures and therapies, as well. On the other hand, they also increase the risk and make predicting future weapon threats more sophisticated.

In order to enhance the national detection and identification capability compared with joint laboratory networking in effective tracing of biological agents, advanced activities should be taken. The Biological Agents Database will stockpile the molecular markers of biological agents—not only pathogens classified as warfare agents, but also pathogens that are the most important from public health and national security point of view.

Reference

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