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Detection of Emerging Zoonotic Pathogens: An Integrated One Health Approach

AUTHORS DETAIL

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Received: Sept 19, 2022 Accepted: Dec 8, 2022

INTRODUCTION

Now a day the management of human health is all about finding and combating new challenges imposed by emerging pathogenic as well as opportunistic microorganisms with major global concerns. Such emerging diseases are crossing the borders and transferring throughout the world in almost every kind of environment. A major concern gathering the attention of healthcare system worldwide is the transmission of many diseases from animals to humans via zoonosis (Rahman et al. 2020). Such zoonotic diseases can emerge because of intermittent disease outbreaks or new epidemic diseases. Such animals are the main cause of spreading various diseases in humans as well. The opportunistic pathogens can get an entry from animal host to human host through two ways, either interacting directly via zoonoses or by using some vectors, ultimately disturbing human health. Such interaction between humans and animals affecting the health of both comes under the umbrella of "one health". The concept of One health links all three components i.e., animal, human and environment health, together (Bird and Mazet 2018).

Therefore, understanding and working on one health approach is much important in controlling the new emerging zoonotic diseases. By applying the one health approach for zoonotic disease management we may consider the involvement of multiple disciplines, such as mammologists, entomologists, ornithologists, ecologists, physicians and epidemiologists for successful investigations and diagnostics (Humboldt-Dachroeden et al. 2020). In this chapter, we will seek to review and explain the various diagnostic methods for the detection of newly infecting agents. Different affective approaches will also be discussed to improve the disease surveillance programs by engaging the local community for the rapid discovery of new threats to human as well as animal health.

The Emerging Zoonoses Pathogen Context

According to an emerging disease hypothesis, pathogenic determinants breeding is considered in evolved disease patterns by utilizing any possible convenient biological host. One of the most commonly available biological host for resistant pathogenic determinants breeding, is animal. Almost 50% of the pathogens causing diseases in humans originate from animals which is the reason for more zoonotic threats to humans as compared to other diseases (Daszak et al. 2000; Cleaveland et al. 2001; Woolhouse and Gaunt 2007; Jones et al. 2008). According to 17th US-National Institutes of Health, almost all pathogens of Category A are zoonotic in nature which can cause severe human illnesses (Woolhouse and Gowtage-Sequeria 2005). However, these pathogens having zoonotic nature are only a minor fraction of total existing pathogenic organisms (Bebber et al. 2007; Anthony et al. 2013). The remaining unknown pathogens yet to discover, pose the need for a sustainable detection networks under the principles of one-health to prevent any pandemic situation in the world (Grange et al. 2021).

Along with biological factors, there is also a strong influence imposed by the sociology, ecology and behavioral reactions of animals and humans in the transmission of newly emerging infectious agents from animal hosts to the first human host which can result in the great dissemination as well as transmission in the human population at mass level (Wolfe et al. 2007; Morse et al. 2012; Kreuder Johnson et al. 2015).

There is an intense need for the robust and broad-based detection systems for the emerging zoonotic pathogens i.e., Ebola virus (Zaire ebolavirus), as being a relatively well-known pathogen, exposed in 2013 in Liberia, can be taken as an example for such needs (Baize et al. 2014; Dixon and Schafer 2014; Dudas et al. 2017). Of the fact that disease by Ebola virus has come into recognition from over forty years, this outbreak intensified rapidly with sixty times higher case rate as compared to the previous outbreak of Ebola (WHO 1978). An approximate increase of 11000 casualties was reported in a time of three years. Also, the virus was emerged in seven more countries where it affected individuals severely. It was reported that improved pathogen detection and disease control programs being stretched to local as well as international levels helped in the timely recognition and diagnosis of the virus (Bell 2016; Coltart et al. 2017).

Citation: Mehmood M, Faisal MN, Abdullah M, Khan AK, Nasir W, Haider U, Khan NU and Gul A, 2023. Detection of Emerging Zoonotic Pathogens: An Integrated One Health Approach. In: Khan A, Abbas RZ, Aguilar-Marcelino L, Saeed NM and Younus M (eds), One Health Triad, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. I, pp: 175-181. https://doi.org/10.47278/book.oht/2023.26

Although there was a tremendous increase in advancement in diagnostic methodologies since 1976, there is still a need for preparation of integrated and resilient laboratories along with efficient surveillance systems for both human and animal diseases. Hence, rapid identification of potential health risks via technical expertise is need of the hour. All this requires a sustainable and consistent funding, technical training sessions and involvement of partners from all over the world (Kuleš et al. 2017).

Biology and Behavior: An Interface Between Animals, Humans and Pathogens

There has always been a strong relationship between humans and animals since the beginning. Along with several benefits gained by animals, such as food, companionship, and fiber there is also a change in lifestyles, behaviors and food choices which can influence the dynamics of zoonotic disease emergence (Lloyd-Smith et al. 2009). Increasing human population has increased the demand for wild and domesticated animal products. Such increased demands result in need for land to grow animals and their feedstuffs. This whole condition can lead to the expansion of animal's production system in wildlife abundant areas resulting in high-consequence zoonotic disease emergence such as henipa viruses, Crimean-Congo hemorrhagic fever virus, Middle East respiratory syndrome-coronavirus, tick-borne bunyaviruses, thrombocytopenia syndrome virus, monkeypox and ebolaviruses (Croser and Marsh 2013; Liu et al. 2014; de Wit et al. 2016). However, direct contact of humans and wildlife for their meat consumption, hunting and slaughtering remains a key driver of above mentioned zoonoses emergence (Robertson et al. 2011; Engering et al. 2013; Suwannarong and Schuler 2016). There exists a continuous close interaction and affiliation between animals, humans, and pathogenic organisms all around the world which increases the risk for the emergence of pandemic diseases. Such pandemic conditions can threaten the lives of many humans and animals (Karesh et al. 2012; Morse et al. 2012; Morens and Fauci 2013). To overcome these challenges related to zoonotic disease exposure in future, intensive and early engagement of the community by disease diagnosis and surveillance professionals and medical anthropologists becomes imperative so that to make such leaders a part of various comprehensive and integrated disease surveillance system for zoonotic diseases (Richardson et al. 2016; Shultz et al. 2016).

The Emerging Pathogen-detection Pathway

The initial pathogen detection of zoonotic threats may begin at local levels. One can start the detection methods by simply observing the sick individuals either humans or animals for the signs and symptoms of the disease. Assistance can be taken by someone who is familiar to the common diseases and their signs, prevailing in the specific area. Such initial observation never gets reported at mass level however, some cases can lead to the involvement of local, national, and international agencies at government level. Centralized systems are developed in many countries for epidemiological as well as laboratory techniques of disease diagnosis at national level diagnostic centers which are basically distant from main area of disease emergence with a high risk of human to animal interaction (Alemnji et al. 2014). In such instances, clinical information of patient/animal, mortality number and diagnostic samples of patients and dead persons for clinical testing are transferred from local areas to such referral centers at national and international levels. Instead of several successes, failure of this centralized system can occur due to issues including poor communication and transportation facilities, less no. of trained workers and suboptimal reporting systems at national levels, which can all add up in delaying the process of recognition, disease diagnosis and emerging disease risk control (Best and Sakande 2016).

Building Effective Surveillance Networks

The main goal of any disease surveillance system should be an early, rapid, and easy detection of emerging threats by integrating the animal and human health sectors as close as possible. To achieve such goals both animal and human health workers as well as ecology testing teams of field level who are working for common scientific and health goals of common public would work together for detecting newly emerging zoonotic pathogens of animal and human population. Such integrated approaches may work ideally at local levels as compared to the centralized systems, by using the local technical training along with diagnostic laboratory systems for zoonotic ailment's detection (Bird and Mazet 2018). Although it may become difficult to handle the distributed diagnostic systems at local levels in terms of expenses and training of staff, but by choosing a closely established and highly integrated detection system at local community level, it may result in rapid detection and a smoother and easy follow up of rare health events (Land et al., 2019).

The need of the hour is to build the effective surveillance networks at local as well as regional levels under the utilization of sustainable funds, infrastructure, and lab technologies in an integrated way. In wake of pandemic situation by SARS in 2001, International Health Regulations (IHR) were adopted in 2005 by 196 countries who were member of World Health Organization (WHO). Therefore, regulations like IHR have cleared the idea of spread of diseases from animals to humans or humans to animals through movement which emphasizes the need to link human and the animal's surveillance systems closely (WHO 2016).

Many funding agencies such as World health organization (WHO), world bank, USAID, UN Food and Agriculture

Organization and government services can collaborate to augment the surveillance programs and manage the training burdens for facilitating the rapid and robust training sessions to enhance the disease surveillance and diagnostic capacities. Such hands-on personnel trainings are much necessary for developing one-health surveillance team. Trainings for surveillance programs vary country to country such as the PREDICT program engages many countries including highly advanced ones with proper medical care centers to the countries with lowest possible facilities in terms of basic infrastructure and number of trained staff to span the disease diagnosis spectrum.

Predict

A project, named PREDICT was initiated for USAID Emerging Pandemic Threats Program, in 2009. The main concern was to address the need of strengthening the capacity of pathogen discovery and detection methods for viruses having pandemic potential along with their zoonotic importance. Some most important zoonotic diseases caused by highly contagious viruses include corona virus, Nipah virus, filoviruses as Ebola virus and influenza viruses. These pathogens were highly focused in second 5-year phase of this PREDICT project. PREDICT is working with thirty countries and sixty in-country laboratories. The main purpose is to improve disease recognition and to minimize the pandemic risks by focused strategy making policy development under one health approach at global level (Carlson 2020). The main goal of this project is to identify the infectious agents of zoonotic importance at an early stage of disease spread so that the pandemic situation could be avoided. For that purpose, surveillance systems and pathogen diagnostic laboratories are being improved for their diagnostic capabilities, especially by using modern diagnostic techniques such as PCR (Anthony et al. 2015). The leader institutes of the project are trying to build some one health partnership at global level. Such cross disciplinary collaboration is critically important for the integral linkage between humans, animals and their environment for timely detection and control of zoonotic threats (Kelly et al. 2020).

The Tanzania VISHA (VIRUS-SHARING) Project

The Tanzania VISHA virus sharing is another collaborated model One-Health project of one health institute by The Sokoine University of Agriculture in Morogoro, Tanzania and The University of California, Davis. The purpose was to focus on enhancement of capacity building for increasing disease detection systems within the country to ultimately improve the health status of animals and the people coming in contact with the animals. Recently, this collaborated project has been referred as Healthy Animals and Livelihood Improvement (HALI). Many improvements are made with reference to the disease surveillance programs, trained laboratory staffs and lab detection protocols for zoonotic diseases under intense consideration of environmental and ecological influence on pathogen and population dynamics (Mazet et al. 2009).

Laboratory Testing System Integration

Over the past 40 years, there has been a tremendous advancement in the quality and variety of sample collection and the diagnostic technologies (Sridhar et al. 2015). Such vast evolution in the field of diagnostics and the use of modern technologies has led to a comprehensive understanding of emerging microbes and their potential zoonoses. Diagnostic laboratories are being equipped with all the required materials and technically trained staff. Diseases are being detected both for their acute and convalescent phases (Bird et al. 2009; Erickson et al. 2016).

Disease Diagnostic Methods

A lot of revolution has been made in the field of pathogen discovery methods. Many highly specific and sensitive molecular techniques have been discovered such as NAAT technologies including PCR for DNA amplification and RT-PCR using reverse transcriptase enzyme for RNA amplification. Such techniques based on the principle of direct detection of pathogen's genome. Other NAAT techniques having real-time quantitative PCR, consensus PCR and Sanger whole-genome sequencing have played a great role in changing the diagnostic spectrum such as for viral pathogens (Zumla et al. 2014). However, a limitation while using these techniques is the low knowledge about the new emerging pathogen's genome or variations in the new strains of the pathogens. Strategies are being made to overcome such limitations by developing further modern detection protocols and methods i.e., high-throughput sequencing or unbiased next-generation sequencing (NGS) (Radford and Bushell 2012; Radford et al. 2012; Chiu 2013; Moustafa et al. 2017). Traditional PCR techniques are also being refined as per strategies for using intensely degenerate primers for detection and amplification of genomic material across the pathogenic viral families (Linhart and Shamir 2005; Souvenir et al. 2007).

Conventional Methods for Zoonotic Disease Diagnosis

Conventional methods for zoonotic disease diagnosis can be categorized as macroscopic and microscopic pathogen identification by using different stains for morphological identification of disease-causing agents under microscope. Staining procedures include gram stain, immuno fluorescent stain and fluorochrome stain helping in narrow organism identification (Bunn and Sikarwar 2016).

Microscopic Analysis

Disease diagnosis initiates with direct and indirect demonstration of pathogenic organisms such as viruses, bacteria, fungi, and parasites in the samples of fluids, tissues, and host excreta (Bunn and Sikarwar 2016; Schwarz et al. 2017). Microscopic analysis, staining and microbial growth are serving for pathogen detection since ages (Rodrigues et al. 2010). In such techniques, phenotypic characters play most important role in pathogen detection. Such characters may include cytopathic effects in tissue cultures for detecting viral agents, bacterial fermentation profiles and fungal and parasitic morphology under the microscope. One limitation of these techniques is the time consumption. All of these procedures are time consuming and may take more time to identify the pathogenic agent that's why genome-based analysis is more common in laboratories.

Phenotypic Methods

Phenotypic methods help in discriminating between species, genera and isolates of pathogenic organisms. However, these tests are not so effective when distinguishing the differences within species. The recently emerged pathogens and the bioterrorist agents have made it much necessary for the development of new, rapid, and efficient methods for pathogen detection (Gilbert 2002).

Pathogen Isolation and Culture

Another conventional technique for pathogen detection is the isolation and culturing. Pathogenic agents including SARS-CoV, Sosuga virus, Ebola virus and Nipah virus can be isolated and analyzed by this method. It is a sensitive technique in which pathogen remains available for further analysis and is also useful for detection of unknown pathogens. However, the choice of media or cell line may limit the level of sensitivity as well as detection for unknown samples (Paton et al. 1999; Ksiazek et al. 2003; Albarino et al. 2014). Manual reading of plates by staff is a lengthy process and take much time which also interrupts the normal flow of biochemical analysis thus extending the whole procedure (Eydmann 2011).

Rapid Diagnostic Tests or Lateral Flow Assays

Rapid tests like plate agglutination tests are also being used which need no electricity, low operating skills and are thermostable. However, sensitivity is limited and can't interpret weak results. It is also not suitable for the discovery of unknown pathogens. Viruses like Influenza virus and Ebola virus can easily be detected by using this technique (Cazacu et al. 2003; Phan et al. 2016).

Conventional PCR/RT-PCR and Sequencing

One of the most common techniques that involve genome sequencing methods is PCR. It is highly sensitive and specific technique depending on the design of primer. It can broadly react and PCR amplicons are usually sequenced for detailed confirmation and characterization of the pathogen. Limitations include moderate requirements for technical and lab work (Towner et al. 2008). Also, the reagents being used in PCR require the maintenance of cold chain. Contamination may occur easily so high germ-free environment is required while dealing with the method otherwise inaccurate and difficult interpretation of the results may happen. Some examples of the pathogens being detected by this method are Bundibugyo virus, Sin Nombre virus, MERS-like CoV, Ngari virus and coronaviruses (Bowen et al. 2001; Anthony et al. 2017).

Modern Methods for Zoonotic Disease Diagnosis

When the pathogen can't grow outside the host environment and can't be detected by simple methods like microscopic analysis then some modern methods are used for diagnostic purposes. Such methods include a sensitive and specific molecular diagnosis of the pathogen (Nissen and Sloots 2002; Leland and Ginocchio 2007).

Electron Microscopy

This is a type of modern microscopic method using electron microscope. It is being used for in situ Visualization of the pathogenic organism. High technical expertise is required for slide preparation and running of microscope. Examples of pathogens being detected include SARS-CoV and expensive Ebola virus (Ksiazek et al. 2003).

Histology/Immunohistochemistry

Immunohistochemistry is being used for analyzing tissue pathology. This technique works on the antigen-antibody reaction principle, and requires cross reactive antisera. Highly technical expertise are needed for pathogen detection. Viruses like Zika virus in CNS tissue of neonates is being detected by this method (Martines 2016).

Antigen Capture and IgM ELISA

ELISA is being used to capture the antigen and it is based on antigen-antibody principle. Specifically, IgM ELISA is being used for multiple commercial as well as experimental purposes. It is adapted with high-throughput screening and proves to be excellent counterpart for molecular analysis of unknown pathogens during a newly emerged outbreak. Limitations include the requirement of cross-reactive

antisera. Some samples may also have sticky serum which can result in false positive reports (Towner et al. 2008; Broadhurst et al. 2016).

IgG ELISA Techniques

IgG ELISA is being used for the broad detection of infections which have been occurred in the past. Multiple experimental and commercial assays are analyzed by this method. Highly specific and cross-reactive antibody titers are being used for such analysis. It serves as the primary tool for the serosurveys postexposure to the infection. IgG ELISA requires specific antigen and antisera. Cross reactivity with antibody may confound the results (Hernández-González et al. 2018).

Phage Display Antibody Detection

Phage display method has a great potential for wide range detection of antibodies of diverse pathogens. However, it is an expensive and high demanding technique being used for experimental purposes (Xu et al. 2015).

Whole Genome Sequencing

Whole genome sequencing techniques include NGS or HTS being used for unbiased genome detection specially for unknown pathogens. Risk assessment is also possible by this method (Al Dahouk et al. 2013). NGS techniques are being used for tracking of virus dispersal i.e., ongoing transmissions versus reintroduction in a population. Limitations include need of highly expert lab staff specially for bioinformatics, low sensitivity, and high cost of the procedure. List of pathogens being detected include Heartland virus, Bas-Congo virus, Sosuga virus and SFTS virus (Xu et al. 2011; Yu et al. 2011; Grard et al. 2012; McMullan et al. 2012).

Quantitative Real-time PCR/RT-PCR

QRT PCR is a highly sensitive and specific method. It is an example of NAAT (Nucleic Acid Amplification Test) technology being used for rapid test of organisms like methicillin resistant *Staphylococcus aureus* (MRSA) colonization in the nasal sinuses. Mostly it is being used for known pathogens. Another example includes Marburg virus detection in bat reservoir. Use of RT-PCR technique neglects the need of traditional detection and subculture of suspected isolates being present in normal flora (Stephanie et al. 2001). Recently innovations are made to ease the use of such quick methods up to the limit of a single click and the result is generated automatically. Chances for lab contamination, false positive and false negative results are also less in these methods. There are some limitations to use this technique which include less pathogen discovery for unknown agents and PCR amplicons may not be suitable for detection and genome sequencing (Towner et al. 2008).

Conclusion

Although there is a huge advent in the field of diagnostics in the past few years, for the timely detection of disease-causing organisms, however, need for robust system development for rapid detection of emerging zoonotic pathogens remains a massive challenge. Establishment of sustainable, long term diagnostic methods at both local as well as national levels remains a key step in identification and alert to public health organizations to avoid any approaching pandemic condition and zoonotic threats. We must need to be well prepared for recognizing the prevailing threats and to work in collaboration to avoid the infection spread and consequent health issues for both animals and humans present globally. For optimal use of diagnostic methods, sensitivity, specificity, cost effectiveness and availability of relevant staff to deal with specific methods must be considered before deciding a diagnostic method for a specific infectious agent. Now a days, diagnostic techniques involving manipulation with nucleic acid of pathogenic organisms are being used and become a center to all future approaches to health care system. A continues improvement in all mentioned techniques is ever needed. In this context, nanotechnology stands as a promising approach that provides new direction to the scientific community for developing much simplified assays with advanced detection capabilities. Such methods will play a promising role in designing the detection systems in coming future.

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