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Review Article

Molecular Epidemiology and Surveillance Program in Iran: Present Status and Future Prospect

Masoud Hajia*

Professor of Medical Microbiology, Department of Molecular Biology, Health Reference Laboratories Research Center, Ministry of Health and Medical Education, Tehran, Iran

Abstract

Background and aims: The food-borne pathogens appear to be a reemerging and endless problem in the human community all over the world. Hence, all the outbreaks should be constantly monitored for pandemic strains and new mutant genotypes. The main purpose behind the molecular typing methods is the comparison of bacterial isolates to obtain genomic relatedness regarding epidemiological aspects. One of these methods that have been recently reported in several Iranian studies is pulse field gel electrophoresis (PFGE). The aim of this study was to review and discuss the current situation and difficulties, and also the necessity of planning for tracking new and re-emerging food-borne pathogens investigating PFGE future status in Iran as a molecular epidemiology tool.

Results: According to the results, it was found that this technique requires high investments in both fields of required equipment and software some of which are now available in many research centers. In addition, investigations on various medical search engines revealed that hundreds of studies have been published after 2010 in Iran. These reports indicated that most of these studies were not able to provide an efficient epidemiological analysis of the outbreaks and prevention of future events, except for a few exceptions.

Conclusion: A review of the capabilities available in the country in this respect led the researchers to infer that it may be the best time to make a plan on the existence of a general network of collecting and analyzing the results, as well as integrating them into the international databases. It is expected that these responsible institutions to make the required design in this field.

Keywords: Molecular Typing Methods, Surveillance Program, Pulse Field Gel Electrophoresis

***Corresponding Author:**
Masoud Hajia, No. 48, Shahrokh Alley, Zartoshtian St., Hafez Av., Tehran, Iran, P.O. Box. 1131636111.
Fax: +982166728121,
Email: massoudhajia@yahoo.com

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Introduction

After more than 30 years of the development of pulse field gel electrophoresis (PFGE) and its first use as a typing method, it has effectively been used as a third-generation molecular sub-typing tool in outbreak investigations and surveillance. Researchers are trying to revolutionize their ability to differentiate the relatedness of isolates use of new molecular methods. The value of any data-generating process ultimately resides in the degree to which the produced information to be meaningfully understood and analyzed whether simple or specialized.[1-3] This method is a powerful tool for genome characterization that has led to the construction of the physical map of bacterial chromosomes specifically food-borne pathogens. It has been used for over a decade in epidemiologic studies and has proved to be a robust typing method for investigations of food-borne outbreaks and hospital epidemiology.[4,5] Nevertheless, PFGE along with a variety of other microbiology-related assays are moving toward the analysis which is based on the DNA sequence. Nonetheless, it is used as a gold standard typing method in the enteric pathogens. In recent years, many reports have been released on the great advances of bacterial total genome sequencing and bioinformatics analysis as a fourth-generation typing method. However, at present, no other reliable method exists to answer the questions regarding the molecular epidemiology. The PFGE technique has been applied to numerous bacterial pathogens for surveillance and outbreak investigations.[6,7] This method is one of the most reproducible and highly discriminatory typing methods available which is generally used for many epidemiological evaluations. Besides, this technique has been established as a powerful device owing to its ability to differentiate and provide standardization ability for universal molecular sub-typing network and food-borne-disease surveillance.

The present study aimed to review and discuss the current situation and difficulties, as well as the required designed steps in order to be successful in tracking the reemerging food-borne pathogens. Eventually, it is the future status of PFGE as a molecular epidemiology tool was also dealt with in this study.

Influential Variables

Unfortunately, almost all the Iranian released reports did not complete the required steps for having a suitable influential. The PFGE needs to be carried out with the combination of perfect laboratory performance and use of accurate software to analyze the results.[8] This combination enables the researchers to accurately interpret the provided results. Interpretation of the result is the second required parameter that needs to have an internationally accepted definition of specific criteria.[9] Finally, epidemiological analysis of the incidents requires integrated information of laboratory networks throughout the country.[10]

A Brief Description of the PFGE Method

Intact chromosomes are digested using the restriction enzymes in this method to generate a series of varied-sized DNA fragments that form different patterns. The resulting DNA fragments are too large to be separated by conventional agarose gel electrophoresis. Through periodically changing the direction of the electrical field in which the DNA is separated, the PFGE allows the separation of DNA molecules of over 1000 kbp in length. Contour-clamped homogeneous electric field (CHEF) uses a more complex electrophoresis chamber with multiple electrodes to achieve highly efficient electric field conditions for separation. Typically, the electrophoresis apparatus reorients the DNA molecules by switching the electric fields at 120° angles. The finding of isolates that have identical or related restriction endonuclease patterns suggest spread from the single strains.[11-13]

PFGE Interpretation Criteria

Tenover et al proposed a guideline for interpretation of the PFGE.[14] According to this guideline, a banding pattern difference of three fragments could have occurred due to a single genetic event. Therefore, these isolates were classified as highly related. The differences of four to six restriction fragments were likely happened due to two genetic events. Moreover, differences greater than seven restriction fragments were found to occur owing to three or more genetic events. Isolates that differed just by three fragments in the PFGE analysis probably represent epidemiologically-related subtypes of the same strain.

Methods of Result Integration into a Framework

Lack of comparability has greatly reduced the power of molecular subtyping methods. Thus, analysis of PFGE patterns is recommended using the same software program. The World Health Organization (WHO) has suggested the application of BioNumerics for uniformity of the results. Standardized PFGE protocols have been developed by the PulseNet national food-borne disease surveillance network.[15-19] A quality assurance program has also been instituted for PulseNet to ensure the integrity of the results obtained by the standardized PFGE techniques. This program requires strict adherence to standardized PFGE protocols. This network has been successful in engaging many laboratory centers in the field of food safety on a global scale. It has been able to create a platform for data sharing, as well as the comparison of clinical, veterinary, and food isolates. Moreover, it has been proved to be a good performance as a laboratory network for molecular epidemiology more than 20 years ago.

PFGE Method Limitations

Although PFGE method is widely used, it has some pitfalls and limitations which need to be acknowledged. This method requires at least 3–4 days completing a test and also strict adherence to standardized protocols for inter-laboratory comparison. Therefore, it is technically time-consuming. It may also lack the resolution power to distinguish the bands of nearly identical sizes. Moreover, the probability of degrading DNA can occur during PFGE process making those strains untypeable. The PFGE method is laborious and has an influence on litter performance errors and quality variations. Hence, it warrants the need for identifying more robust and reproducible methodologies for the molecular typing-based surveillance in the future. Another point is high discriminatory power of the technique to investigate clonal relationships that have been changed over the years.[10]

PulseNet in the World

Public health laboratories are responsible for the verification of bacterial isolates identity and also determining their serotypes and PFGE profiles all over the world.[20] More than a hundred laboratories in 86 countries have set up the PulseNet model in seven regional networks till now. These networks include the United States, Europe, Canada, Asia Pacific, Latin America and the Caribbean, Middle East, and Africa. They work together for disease surveillance and outbreak response using standardized analysis methods. Each region has a coordinating laboratory that is in charge of training, quality control, and quality assurance programs. Coordinator laboratory organizes regular conference calls, meetings, and communication of epidemiological information.

Adoption of a standardized PulseNet PFGE protocol has efficiently enhanced the inter-laboratory comparison. Consequently, tracking of the enteric pathogen has been improved among the endemic countries in the region. These laboratories use the same molecular sub-typing protocols which were developed to ensure that laboratories compare the same data in a consistent manner and that the information can be easily shared within and between the regions.[21-24]

Whole-Genome Sequencing Implementation

PulseNet International is recently trying to improve its worldwide surveillance of food-borne bacterial pathogens through transitioning to Whole-Genome Sequencing (WGS) as a detection tool. The development and implementation of WGS methods and analytic processes in PulseNet USA is influenced by the needs, ideas, and practical strategies arising from PulseNet laboratories and vice versa.[25] Recently, recommendations have been published to guide both developed and developing countries

in determining their readiness for implementing WGS. The required readiness is depended on a wide variety of political, technical, economic, and political factors including infrastructure, equipment, training, operating funds, and policies [M. Hajia, unpublished material].[26]

The Status of Iran at the Global Epidemiological Surveillance of the Infectious Disease

None of the laboratories and research centers is able to cover all the regions of Iran alone for all outbreaks. A referral laboratory needs to corporate with a group of centers. This net-labs all together can provide the desired goal of PulseNet as is it carrying out in other countries. Recently, the PFGE system has been set up in many research centers. Looking through the released reports reveals that all the published papers have been carried on specific limited fields. In addition, it can be observed that the health system in any academic field of Iran does not use the results of its own research centers. Most of the reported results cannot be potentially used for the health system. Furthermore, they cannot improve the health criteria or prediction of any epidemic disease.[27-31] Another problem is the main goal of the molecular epidemiology. As previously discussed, the aim of the type identification is to find out the source of infection either in the food reservoirs or any other sources. Besides, the purpose of research is not just to report the classification. A suitable study should compare the human types with other isolated pathogens in the animal or food fields as reservoir sources. However, the lack of a standard analysis and a general network to collect and analyze the macro-restriction mapping has limited the effective application of typing by the PFGE. This limitation has also led to the lack of Iranian reports integration into the global databases for epidemiological surveillance of infectious diseases.

A summary of the main challenges for setting up the PulseNet laboratories in Iran are as follows:

1. The most pressing challenge is probably the insufficient funding allocated in this regard. The primary source of funding for food-borne disease surveillance and PulseNet activities must be under the responsibility of the Center for Disease Control of the Ministry of Health and Medical Education. However, accurate and complete details of a practical proposal are first required to start with.
2. Establishment of national laboratory centers that are equipped with the required facilities in various regions of Iran is another problem the national PulseNet members are encountered with.
3. Having expert and qualified personnel in reference laboratories is another issue of concern. Throughout the country, members of these PulseNet laboratory centers are in charge of collecting the specimens from the outbreaks and analyzing them. They should transfer the analyzed samples and report the data to the coordinator laboratory.
4. Such coordinator laboratory is responsible for supervising the reference laboratories. Personnel of the coordinator laboratory must have the necessary qualifications for training and quality control. As a result, quality assurance programs must be regularly performed by the coordinator center in all of the involved laboratories.[32]
5. Tracking the causative agent of the food-borne pathogen in all the fields including human infection, as well as animal and food sources should also be taken into account. The greatest challenge to the food-borne system is possibly setting up a comprehensive plan for collecting the specimens from all sources.

Conclusion

According to the results of this study, it can be claimed that it may be the best time for the responsible institutions to make a plan on the existence of a general network to collect and analyze the macro-restriction mapping results and integrate them into the international databases. Therefore, these institutions are recommended to make the necessary design in this field.

Ethical Approval

Not applicable.

Conflict of Interest Disclosures

None.

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