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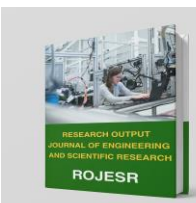

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Genomic Epidemiology of Infectious Diseases

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ABSTRACT

Genomic epidemiology is a transformative field that integrates microbial genomics with epidemiological data to enhance the understanding, prevention, and control of infectious diseases. The advent of next-generation sequencing (NGS) technologies has made it possible to rapidly sequence microbial genomes, providing detailed insights into the transmission dynamics, evolution, and resistance patterns of pathogens. This paper explores the methodologies and applications of genomic epidemiology in outbreak investigations, transmission dynamics, and antimicrobial resistance monitoring. It also addresses the challenges of data privacy, ethical considerations, and the integration of genomic data with public health systems. By leveraging genomic data, researchers and public health officials can develop more effective strategies for detecting, monitoring, and controlling infectious disease outbreaks.

Keywords: Genomic Epidemiology, Next-Generation Sequencing (NGS), Infectious Diseases, Outbreak Investigations, Transmission Dynamics.

INTRODUCTION

Genomic Epidemiology: Genomic epidemiology is a newly developed field that emerged with the improvement of second and third-generation nucleic acid sequencing technologies and third and future biochemical technologies. Due to the decreasing cost of deep sequencing of nucleic acids, microbial genomes can be obtained at a rate of more than 10,000 bases per second. These data provide the scientific community with the opportunity to describe how a particular infectious strain was disseminated, as it is now possible to trace with high accuracy isolated strains available from infected people or other immunocompromised individuals [1]. Infectious diseases are derived from various sources. The causal agents of infectious diseases are bacteria, viruses, fungi, and parasites. Bacteria, viruses, fungi, and parasites can cause a wide range of serious illnesses. Infectious agents are generally related to the subcategory above. The diagnosis of an infectious disease is initially based on an accurate and influential medical history. Access to medical history prevents unnecessary expenses and reduces the duration of laboratory diagnoses. The identification of the causal agents of infectious diseases at the molecular level greatly shortens the pathogenesis of infectious diseases. Virus isolation is generally performed by three groups of methods: classical virology methods, immunological methods, and molecular biology tests [2]. Genomic epidemiology: Genomic epidemiology is a newly developed field that emerged with the improvement of second and third-generation nucleic acid sequencing technologies and third and future biochemical technologies. Due to the decreasing cost of deep sequencing of nucleic acids, microbial genomes can be obtained at a rate of more than 10,000 bases per second. These data provide the scientific community with the opportunity to describe how a particular infectious strain was disseminated, as it is now possible to trace with high accuracy isolated strains available from infected people or other immunocompromised individuals. The incredible expansion of genomic and epidemiological data has the potential to improve our knowledge of infectious diseases by facilitating the establishment of control methods suitable for treating, detecting, and eradicating diseases. Epidemiologists and microbiologists became more enthusiastic about the analysis of mutations and spread of resistant bacterial strains since the development of WGS is made accessible for routine experimental analysis. Also, it has revealed that drug resistance and other adaptive traits are an important factor that may be influential. WGS is particularly useful for the epidemiology of infectious diseases and also a tool to study transmission events

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at the molecular level. Subsequently, this is a considerable advantage for the determination of public health information in detection and monitoring studies. Genomic epidemiology includes infectious disease-causing microorganisms, treatment and resistance scenarios, and is the basis for the multimodal detection of infectious disease outbreaks that need to be monitored [3].

DEFINITION AND SCOPE

Genomic epidemiology integrates microbial genomic data and epidemiological data to identify and characterize the transmission of infectious diseases in an epidemiological context, and to inform the prevention and control of such diseases. The big, growing and diverse body of genomic data requires smart mechanisms for data dissemination and data handling. These mechanisms must meet global standards, provide strong bioinformatics and data interpretation services, and assure full FAIRness (i.e., the data are findable, accessible, interoperable and reusable). This section provides exhaustive details concerning the requirements for the scope, data types, methodology and protocols for infectious disease genomic epidemiology, population and functional genomics [4]. The pathogen genomes, also referred to as 'isolate data', kept in public databases, such as GISAID, NCBI GenBank and the European Nucleotide Archive ENA, are one important source for genomic epidemiology. In addition, we must consider the variation among genomic data of various inclusive and exclusive large and small outbreak sizes (e.g., local, national and international) in terms of setting and disease. Moreover, epidemiologists must consider divergence from the reference pathogen genomes to obtain meaningful results. Considering all of these issues, the key focus of this section addresses the data types, methodology for analysis, and protocols for generation. Data types describe the data collection as 'clinical' and 'epidemiological' (a composite term that includes pre-clinical and underlying comorbidity). Methodology for analysis describes how to identify and confirm possible clones/transmission routes/aetiologies. Also, disease transmission and causal effects change in different settings and disease aetiology [5].

SIGNIFICANCE IN INFECTIOUS DISEASE RESEARCH

In the infectious disease research community, the innovative field of genomic epidemiology has provided valuable contributions to enhancing our understanding of disease etiology, identification of nosocomial pathogen transmission, as well as insights into epidemic control. Since deciphering the complete genome of Neisseria meningitidis in 2000, researchers have focused on deciphering the sequences of other pathogens expansive to humans and agriculture. This genomic data is highly informative in providing fundamental knowledge and improving diagnostics, epidemiology, and the session. Francis Collins, who was NHGRI director at the time, recognized that this would be the first field to benefit primarily from the Human Genome Project (HGP) [6]. Currently, high-throughput technologies for clinical microbiology, pathology, and public health laboratories were introduced in two essays in Science in 2012. Optimizing data analysis, integration, and sharing to support public health response being one of the most important fields, the use of whole-genome sequencing in public health microorganisms has erupted. WGS had become an important aspect of microbiology. Further reduction in genome-sequencing prices and increasing throughput has attracted many additional researchers to infectious disease research, increasing the field's relevance. The applications of WGS for pathogen testing are the crux of massive programs recently launched by countries such as Nigeria, as well as The Gambia-focused Ebola Preparedness Program (EPP), which include implementing this strategy to monitor infectious diseases [7].

GENOMIC TECHNOLOGIES IN EPIDEMIOLOGY

The progress over recent decades in the understanding of the genetic basis of life and disease is a testimony to the widespread appeal and value of genetics. However, most of this knowledge is confined to the domain of human genetics. With the advent of new high-throughput tools facilitating nucleotide sequencing, various other fields are beginning to take their steps into genetic technologies. This also holds for the field of epidemiology [8]. The genetic revolution in epidemiology goes by the name of 'genomic epidemiology', which is the title of this special issue edited by Marion Koopmans and Bas Oude Munnink. The special issue came about as a result of workshops on disease detectives organized in the frame of the Valletta (Malta) conference series on virus evolution (preserved in the papers in this special issue) and several invited members of the EFSA Working Group on Whole Genome Sequencing (WG-WGS) of public health microbial pathogens. It stands as an example of the meeting point of diverse disciplines and research interests: the role of genomics (defined as the totality of DNA sequences) in typically epidemiological studies, in other studies regarding human and veterinary infectious disease diagnostics, particularly the elucidation of outbreak origins, and more generally in studies of invasion, genetic diversity and micro-evolution in pathogen populations. The special issue binds together epidemiological questions and methods in the analysis of variation in nucleotide sequences generated by current sequencing methods, in interpretation of the billions of DNA base pairs thus generated, and in

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forward genetic studies (qualitative changes in infection dynamics at the population scale resulting from particular sequence alterations). Several selected papers show that nucleotide variation between genomes may be used to identify chains of transmission in classical epidemiological studies of disease outbreaks, an application that is founded in the basic principles of forensic genetics. Another group of selected papers addresses the computational challenges associated with analysis of such data [9].

NEXT-GENERATION SEQUENCING

Next-generation sequencing (NGS) has revolutionized the study of infectious disease epidemiology. Analogous to the way pulsed-field gel electrophoresis (PFGE) coincided with the field evolution from DNA fingerprinting to molecular epidemiology, NGS has changed the epidemiological landscape of infectious diseases and shifted it from pulsotype or restriction enzyme analysis to genomic-level sequences. Over the past decade, NGS has been applied to many aspects of the study of infectious diseases, such as pathogen discovery, investigation of foodborne pathogens and environmental samples, studies of antimicrobial resistance, the field of vaccinology, and phylogenomic analysis. NGS platforms highly vary in their sequencing scope and depth, read length, and collection time. These platforms include the dominant short-read sequencing technologies, the third-generation long-read sequencing technologies, and the soon-to-be-deleted works-on-alternative-samples sequencing methods [1].

The rapid advancement of NGS has also precipitated a series of ethical and logistical concerns, which have prompted professional societies, programs, and organizations to develop guidelines and best practices for investigators and public health laboratories that specifically address the analysis of genome sequencing data in the context of public health surveillance and infectious disease epidemics. These concerns include the misuse of infectious disease surveillance data in bioterrorism and warfare, the exposure of the ancestry and genealogy of participants from deeply conserved genomes, and the potential for data breaches. In addition, infectious disease surveillance using genomic data collection has raised issues regarding informed consent to collect and use the data obtained [10].

BIOINFORMATICS TOOLS

Biological samples must be sequenced to generate pathogen genomic data. However, this output contains unresolved "biological variation," poor sequences, and distinct epidemiological and evolutionary processes. Therefore, bioinformatics tools are needed to interpret and and determine the "genotype," which is a description of the DNA sequence of the pathogen isolate. Many bioinformatics tools have been developed for a wide range of applications such as assembling and annotating whole pathogen genomes, genotyping, investigating genomic variation, phylogenetic estimating, identifying antimicrobial resistance, forward predicting the functions of proteins, and so on. Additionally, many public tools and databases have been developed to provide more powerful support for genotyping analysis and genomic data interpretation. Here, we do not describe all of the bioinformatics tools but focus on the main steps of genomic epidemiological data analysis and interpretation increasingly reported in the field [11]. Bioinformatics tools can currently be used to identify polymorphic markers or whole genome sequences, adjust the data cell of the raw and assembled contigs. They can also be employed to align and model data to determine variation caused by both random and systematic false negative and error rates, generate the phylogenetic tree, and interpret the results. We should take precautions as many of the tools have different default settings and priorities. The tools differ in the amounts of epidemiological and genomic uncertainties considered, refill appropriate evolutionary and epidemiological models, and incorporate independent information of the organism being analyzed. These online databases and underpinning data can improve the level of interpretation of analysis results at each step. They can contribute to identifying possible transmission links, antimicrobial resistance markers, new variants, and typing on the outbreak or strain results [12].

APPLICATIONS IN INFECTIOUS DISEASE CONTROL

1. Outbreak Investigations

Genomic epidemiology became relevant to infectious disease outbreak detection, investigation, and management. Genomics was demonstrated to support epidemiological investigations in tracing infections and outbreaks, confirming and challenging epidemiological hypotheses, and reporting transmission patterns across countries, regions, and globally. By delivering genotype information beyond subtyping, genomics has also helped in separating highly genetically related isolates in minor, non-matching subtypes that would have been impossible with subtyping for tracking potential outbreak sources [13].

2. Transmission Dynamics

Genomic epidemiology can also provide insights into the transmission dynamics of specific infections. For many infectious diseases, long-range and hierarchical transmission patterns have been observed. Whole

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genome sequencing and phylodynamic analysis have further been able to identify and quantify the impact of demography and geography on the transmission of EIDs, allowing, for example, the identification of genetic diversity that traces drivers in the animal reservoir and human behavior that correlates with pathogen transmission. The scale and source of data required for implementation into case or outbreak investigations or AMR or surveillance studies vary across different applications as well as between different settings and systems [14].

OUTBREAK INVESTIGATION

During an outbreak, genomic epidemiology offers rapid analysis and tools for tracking the transmission of an infectious disease. Once the pathogen has been identified, the genome sequences of most pathogens can be determined in hours or days. This process used to be time-consuming, expensive, and confined to readily cultivable microorganisms or HIV. But as next-generation sequencing (NGS) has become faster and less expensive, this technique is increasingly being used. For this reason, an increasing number of genome sequences of bacteria, viruses, fungi, and other groups can be found in public databases. Depending on the pathogenic agent, the genome sequences themselves or special sequence polymorphisms and other "readouts" of these sequences can be evaluated to understand the outbreak dynamics [15]. "Genomic epidemiology is crucial for outbreak investigations" and can be used when traditional methods are not sufficient, e.g., during low-prevalence outbreaks. In the course of outbreak investigation, the disease under study and its symptoms will be examined. Affected individuals, as well as the people surrounding them, will be interviewed. Further data are collected at the series of the occurrence, i.e., at the district and national levels. A key question is: How is the infectious disease transmitted between individuals or through other modes (e.g., food, water, wildlife, domestic animals) and how does the virus/genome sequence relate to the transmission route? The large amount of genomic data available on, amongst others, bacteria can be used to track an outbreak, to find sources of an outbreak, and to disprove or confirm a link of some cases with an outbreak [16].

TRANSMISSION DYNAMICS ANALYSIS

Transmission is a fundamental process in epidemiology. The identification of the patterns and mechanisms of disease transmission can effectively help us to control infectious diseases. A well-known epidemiological curve, which represents the number of new cases over time at a population level, can already provide insights into the patterns of disease transmission. The genomic epidemiology research group aims to employ a posteriori collected genetic data to shed light on patterns and mechanisms of pathogen transmission. Contrary to environmental microbiology, less focus is given in medical microbiology to the generation and analysis of metadata, which are essential for epidemiological investigations. This includes how the transmission is building and establishing new infections, and how the pathogen is spreading in a host population. We aim to analyze this process [17]. In epidemiology, the study of transmission chains (orderly successions of transmission between cases) augments insights gained from isolated cases of disease. Transmission chains can be traced back to one or several sources in different settings. Some studies focus on the potential likelihood of individual students. Other studies collect experimental strains and try to infer transmission chains and clusters of infector groups and infectee groups. Along with disease categories, this study views each case and the strain isolated from that case as essentially identical. This study seeks to establish the possibility of determining the time that has elapsed between a source and transmission [18].

CHALLENGES AND FUTURE DIRECTIONS

Compared to the few studies which present practical applications of genomic epidemiology, many more define specific strategies for analyzing datasets and imputing missing data. These utilized tools or methodologies share similar objectives diluted throughout the literature: being able to handle missing data, reduce potential bias, and mitigate the potential for false associations generated by incomplete data. While tackling these objectives is important, all studies used a priori operational definitions within their analyses, signifying their findings may not necessarily reflect the actual incidence of infectious diseases or prevailing resistance profiles [19]. Multiple challenges emerge when using genomics in an epidemiological context, especially for infectious diseases. De-identification and data privacy issues must be taken into account when gathering genomic information (which would also be linked to patient medical records). In areas of mid- to low-income countries, public genomic databases are not ubiquitously available, with personal genomic testing not widely available. Furthermore, consortiums or collaborations may be difficult to establish due to cost or conflict of interest (a result of national security implications, fear of misuse, and competitive industry implications). Large-scale data sharing is essential for international collaborations, but this poses ethical issues. In the context of infectious disease, there must be very rapid feedback from genomic epidemiology into public health systems. The extent of this feedback

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might reside at multiple levels, including the patient through to patients and practitioners. Therefore, better incorporation of genomic analyses into standard epidemiological analyses is required, particularly in the understanding of varying effects attributed to specific determinants (i.e., attributable fraction). These may then be best mitigated through intervention and are an essential component of public health strategies [20].

DATA PRIVACY AND ETHICS

As discussed above, a great deal of data is often required for genomic epidemiological studies, and these are often collected directly from patients. In consequence, the use and storage of personal data is a fundamental part of research in genomic epidemiology, raising a host of ethical considerations that must be addressed. Some types of data collected from, or generated by, patients already have stringent data protection and ethical requirements linked to them, such as clinical data in Europe. Others, such as genomic data, are not necessarily covered by these rules. For instance, when patient data were required to be shared with international collaborators in a study conducted by the Network for Genomic Surveillance in South Africa, the research team had to navigate ethical and legal obstacles in order to comply with local laws and regulations. For this reason, it is important that research teams establish relationships with clinical and regulatory bodies to ensure that they work within a safe and ethical framework [21]. One of the foundations of ethical practices in science is that every effort should be made to protect the privacy of the individuals who volunteer their data. When DNA or RNA sequences are determined for bacterial or viral isolates from patients, identifying information about those individuals is often retained alongside the isolate's genomic data. Therefore, it is possible that a set of isolates could be used to infer the genomic sequences of the patients from whom those isolates were obtained. To avoid this, it is common practice to 'de-identify' the genomic and associated data, in other words, to remove or code the information that could identify individual patients. The most efficient way to de-identify a bacterial isolate is to remove any information about the patient from whom it was isolated, which means that the sequences can be used to infer aspects about the nature of the pathogens themselves without compromising individual privacy. The processes and protections required to de-identify data must be supported by legal, ethical, and regulatory frameworks, for both the original de-identification process and the subsequent sharing of these data [22].

INTEGRATION WITH PUBLIC HEALTH SYSTEMS

Integration of genomic epidemiology with local, national, and international public health systems is anticipated to result in several practical advantages. These include setting the long-term research agenda for genome-based molecular epidemiology efforts, providing data for situational awareness during an outbreak, improving outbreak response (for example, through rapid species and strain identification), helping to identify and manage the impacts of super-spreaders and chains of transmission, identifying possible sources of infection, classifying levels of colonization, and cross-border validation of test results (e.g., proving freedom from disease), as well as linking outbreaks together [16]. However, there are also a range of practical and theoretical challenges that will need to be addressed for genomic epidemiology to become widely integrated with public health systems. For example, there is a lack of harmonized international regulatory standards, political fragmentation, and uncertainty in terms of the accountability and legal responsibilities of those involved in the interpretation of phylogenetic, bioinformatics, and surveillance information, and subsequent public health interventions. Other challenges include equity of access, cost, breach of privacy and security, the meaningful interpretation of studies, and diversity in the practical utility of genomic information between different types of pathogens. Many of these challenges are, however, gradually being resolved through a series of initiatives, outlined in section 1.6 [8]. Over the next few decades, it is likely that laboratory-based whole-genome sequencing will rapidly replace many of the current standard clinical microbiology tests. While small-scale genotyping could remain, it is anticipated that it will become inconsequential and unnecessary to disease diagnosis and routine outbreak detection and response. Full genome sequences are the ultimate biological identifier and are unambiguous and comprehensive for capturing within and between taxonomic group variation in pathogen biology. In particular, "prohibitive-scale analysis" of the changes within complete genomes or a comprehensive subset (including point mutations, changes within regulatory or coding areas, changes in genome organization and mobility, as well as changes due to selective forces, genome reduction, and adaptation) will make high-quality large-scale databases more informative than to date, and particularly relevant to the study of new pathogens and drug resistance. The fact that genomic information can be gathered with little bias and characterizes all biologically relevant phenomena of an organism means that we can begin replacing signals of transmission and causality with direct evidence for the public health and clinical relevance of any pathogen(s) in a given sample [23].

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CONCLUSION

Genomic epidemiology has revolutionized the field of infectious disease research and public health by providing high-resolution insights into pathogen transmission and evolution. The integration of genomic data with epidemiological studies allows for more precise outbreak investigations and effective control measures. However, the field must address significant challenges, including data privacy, ethical concerns, and the need for robust bioinformatics tools. As genomic technologies continue to advance, the incorporation of genomic epidemiology into public health systems will likely become more widespread, ultimately improving global health outcomes through enhanced disease surveillance and control strategies.

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