Review article

Utility of Pathogen Genomics in Clinical Care: A Review

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Abstract

Objectives: The primary objective of this review was to describe the impact of genomic and molecular diagnostic tools in diagnosis of infectious disease in clinical settings and to enumerate existing shortfalls in infectious disease diagnostics in Kenya looking at the scope and implementation of use of newer and robust technologies.

Data source: Data was obtained from peer reviewed publications containing surveys of the most common methods used in infectious disease diagnostics clinically in Kenya. Few data sources appear in regards to implementation of much newer methods of infectious disease diagnostics in tuberculosis patients and mixed pathogen species from cerebral spinal fluid.

Conclusions: Current laboratory techniques can be laborious and expensive when it comes to identifying pathogens responsible for many infectious disorders. Faster methods of diagnosis could expedite the administration of the appropriate treatment, reduce healthcare expenditures, and improve infection control and harnessing preparedness of future pandemics. In a clinical setting, when quick and robust choices regarding patient management are required to be made for the purpose of achieving the best possible results and preventing the spread of infectious disease, precision is very crucial. The technologies that are available today can test for a wide variety of pathogens using a variety of clinical samples, and they can produce results in under an hour while requiring less than a minute of manual intervention. The power of pathogen genomics diagnostic tools presents prospects that would take a more anticipatory approach to accurate clinical diagnosis, prevention and control of outbreaks.

Key words: Pathogen genomics, Molecular diagnostics

Introduction

Within the past ten years, advancements in genetics have led to numerous improvements across the board in healthcare systems all around the world. It is encouraging to see that Kenya, despite having a low GDP per capita, has become one of the pioneers in Eastern and Sub-Saharan Africa, in the spread of the application of genomic technology in molecular diagnostics. In this article, we provide a spectrum of pathogen genetic diagnostic tools that are both presently accessible for use, as well as those that, if implemented, would enhance the quality of healthcare provided to a number of patients at all stages of the medical process, from the laboratory to the bedside. We address their skills in terms of supporting clinicians in obtaining a higher diagnostic yield of attributed infectious agent, as well as some of the limitations they confront, such as cost. Technologies such as Polymerase Chain Reaction (PCR) and next-generation sequencing are able to perform simultaneous and complete detection of a wide variety of infectious pathogens directly from patient samples. Use of this genomic technologies also present a potential in infectious disease surveillance and ability to equip healthcare systems with future pandemic preparedness. Even though genomic tests for infectious diseases hold an amazing amount of potential in changing public health in Kenya, it is still unclear whether we will be able to surmount the substantial obstacles that stand in the way of molecular diagnostics becoming applicable on a more widespread scale.

Infectious disease genomics

To enhance healthcare outcomes and provide timely patient intervention, the area of molecular genomics has been considered a multidisciplinary one that sees an interplay of genomic techniques, data analytics,

and clinical history (1). The current fight against the Covid-19 pandemic has made it necessary to use genomic technologies like whole genome sequencing and PCR to monitor the spread and evolution of the SARS-CoV-2 virus in Kenya. Understanding and controlling infectious diseases at the patient and population level has benefited greatly from this genomic approach with significantly improved knowledge of the pathogen and the host population.

One of the most innovative new scientific breakthroughs to emerge and completely alter the field of molecular biology is Polymerase Chain Reaction (PCR). This procedure needs a template molecule, which can be either DNA or RNA, a DNA polymerase enzyme, a primer, which is a brief, precise sequence of nucleotides that aids in the beginning of the DNA copying process, and a chain of nucleotides (which comprises the four bases A, T, C, and G)(2). These components are combined in a tube and put in a device known as a thermocycler, which allows repeated cycles of DNA amplification to happen in three steps: denaturation, which causes the double helix strand of DNA to separate; annealing, which occurs when the temperature is lowered to enable the specific primers to bind to the target DNA if their sequences are complementary; and extension, which occurs when the temperature is raised again to extend the primers through the target DNA (3). The procedure produces billions of clones of a particular DNA fragment, giving biologists the chance to discover and recognize gene sequences as well as visually gauge its size.

With changes to the conventional PCR technology throughout the years, significant advancements have been made; in this case, DNA amplification is monitored while it is taking place. Real-Time PCR is a technique whose primary goal is to quantify and differentiate nucleic acids in a sample (4). Quenchers and reporters are two types of specialized fluorescently labeled oligonucleotides that are used in this procedure. These oligonucleotides give signals during amplification and provide data output that is depicted in graphical format (4). Fluorescent signals are measured at each PCR cycle and a cycle threshold value is determined and this value is inversely proportional to the viral or bacterial copy number in a specimen (1).

Within the clinical context and HIV burden, the Kenyan Government through the National AIDS and STIs Control Program (NASCOP) has been able to partner with UNAIDS seeking to achieve the goal of ending HIV by 2030 (5). In order to accomplish this goal, they have collaborated with a variety of partners to deliver point-of-care tests that monitor patient outcomes and progress while on receiving antiretroviral medication. In order to provide information on the levels of viremia in HIV-1 positive individuals, the viral load assay has been developed for use by local healthcare providers (6). Real-Time Polymerase Chain Reaction (rPCR) is used in the current technologies that are involved. The HIV-1 virus is extracted from blood plasma using this technology, and quantification is performed. Not only has this system been utilized in the delivery of point-of-care services to HIV/AIDS patients, but it has also been utilized in the fight against Covid-19 in recent years.

In March 2020, at the beginning of the pandemic, the World Health Organization (WHO) provided interim guidance on laboratory testing for coronavirus disease in suspected human cases. These techniques included serological testing, viral sequencing, and screening by real time reverse transcriptase-PCR (rRT-PCR). In addition, the WHO also recommended that all suspected human cases be tested (7). Because of this, it was possible to implement the use of this technology at a variety of testing facilities across Kenya. As a consequence, the government was able to arrive at well-informed decisions concerning the tracking of contacts and the management of transmission inside the country.

An expanding number of potentially dangerous variants of the SARS-CoV-2 virus, which were caused by mutations in the virus itself, led to the development of new insights into methods of surveillance and tracking based on molecular sequencing. This idea was initially conceived in the early 1970s in an effort to determine the sequences in DNA via primed synthesis with DNA polymerase. This was the first time this concept had been presented. Then, in the early 1990s, automated fluorescent sequencing equipment was developed, which made a significant contribution to mammalian genomics research as well as the famous Human Genome Project. The Human Genome Project's research effort was aimed at deciphering the chemical make-up of the entire human genome. Automated fluorescent sequencing equipment played a significant role in both of these endeavors (8).

As a result of the high prevalence of HIV-1 disease in Kenya, additional points of care besides the measurement of viral load have been established. These points of care monitor drug susceptibility in order to determine whether or not a patient with HIV has a form of the virus that is resistant to antiretroviral treatment. The testing is carried out to provide clinicians with assistance in the selection of active medications to use when altering their ART regimens. Therefore, Sanger sequencing has been utilized in order to pinpoint with accuracy locations within the pol region, which is a gene within the HIV virus that codes for proteins that are ART medication targets (9). Using this approach provides more accuracy in determining resistance and provides detailed and comprehensive reports from the Stanford HIV Database regarding drug regimens that the patient has developed resistance to over the PCR viral load assay which determines the levels of viremia.

In just a few short decades, the scientific profession has become increasingly dependent on computers in regard to medical informatics, medical imaging, telemedicine, and medical research. In addition, advancements in computing capacity led to the creation of Next-Generation Sequencing Technology, which brought together improved sensitivity, higher capacity, shorter turnaround time, and the ability to sequence hundreds of genes simultaneously (10). The scope has been relatively novel in Kenya, with various stakeholders developing comprehensive workshops that have highlighted the importance of access to Next Generation Sequencing and bioinformatics in a few research laboratories. These research laboratories include the Kenya Medical Research Institute (KEMRI), the International Livestock Research Institute (ILRI), and the Centre for Molecular Biosciences and Genomics (CMB). These research laboratories have been involved in the surveillance of variants and emerging variants of concern in Kenya (11). However, the use of this platform has only been described in infectious disease research and species identification in plant pathology strategies.

Tuberculous molecular diagnostics

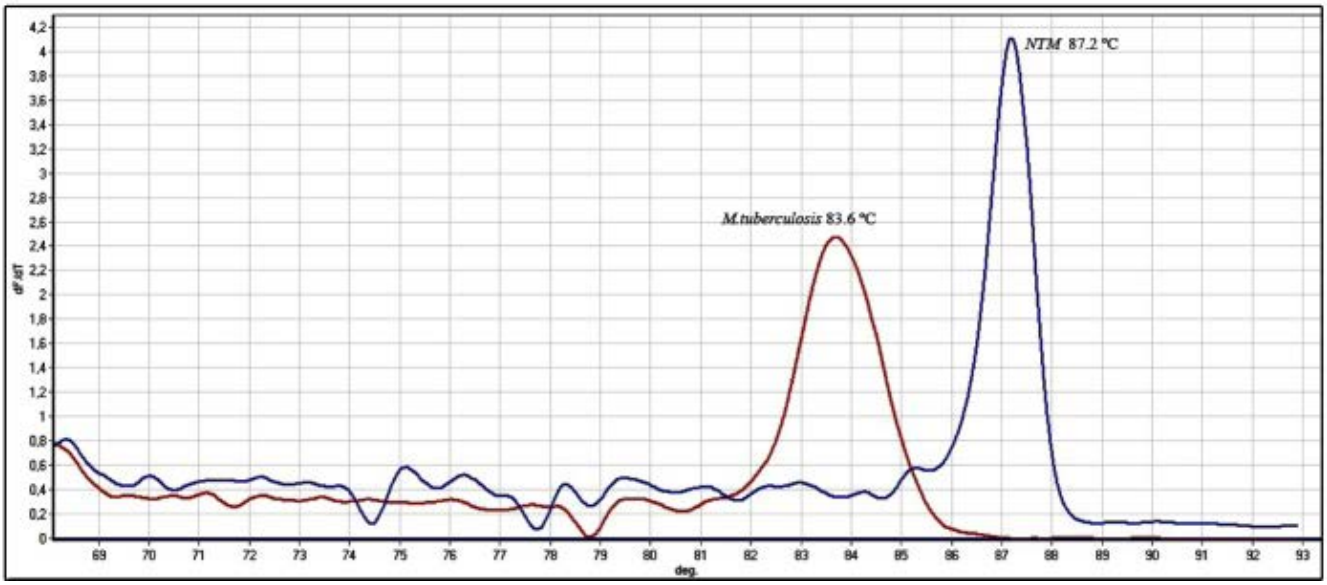
The World Health Organization has identified Kenya as one of the 30 states with the highest rate of tuberculosis (TB), and the Kenyan Ministry of Health has made it an extremely high priority to locate people who are afflicted with TB disease and provide them with the most effective treatment possible (12). Non-molecular TB diagnostic have been extensively used due to low costs and require direct visualization of acid-fast bacilli making it the most common method of diagnostics globallyheBACTEC™ mycobacterial growth indicator tube (MGIT™) platform from BD (USA) is a culture method also being used in Kenya and takes up to four weeks to generate a test result and although there remains a large turnaround time, the method is highly sensitive and can be used for phenotypic determination of drug resistance.

Molecular TB diagnostics offer the advantage of higher sensitivity, faster turnaround time of 3 days and relatively low cost. There has been the development of Nucleic Acid Amplification based diagnostics that are accurate in diagnostic testing and employ PCR techniques to amplify a specified region of genomic DNA; nevertheless, in the context of tuberculosis diagnostics, it has a number of problems, including but not limited to specimen type such as sputum, which produces a low number of pathogens, and difficulty in lysing the cell wall to liberate nucleic acids. Since it takes months to diagnose TB using culture-based procedures (which are the gold standard for TB diagnostics), the use of these nucleic acid amplification techniques to identify and diagnose TB is more efficient and accurate. It is still quite evident that culture-based methods cannot be discarded entirely; however, it is recommended that clinicians and laboratory personnel collaborate in order to conflate the use of nucleic acid amplification techniques in conjunction with liquid culture-based methods in order to improve the accuracy.

Non Tuberculous Mycobacteria (NTM) is currently an increasing opportunistic infection within the Kenyan population and is phenotypically indistinguishable from tuberculosis (TB), which remains a challenge in poor nations where several clinical and phenotypic aspects of NTM species are comparable to those of MTB (13). Because of this, non-tuberculous mycobacterium displays comparable traits such as morphology, and as a result, they can often be confused for MTB when it comes to methods such as microscopy and liquid culture.

Specialized techniques such as Sanger sequencing and multiplex real-time PCR-High Resolution Melting (PCR-HRM) techniques can rapidly identify non-tuberculous mycobacteria with high throughput to aid in the therapy of patients despite their limited sensitivity and lengthy turnaround time (14). The benefits of obtaining precise results with a short response time exceed the aspect of cost implications, owing the fact that the service is exorbitant.

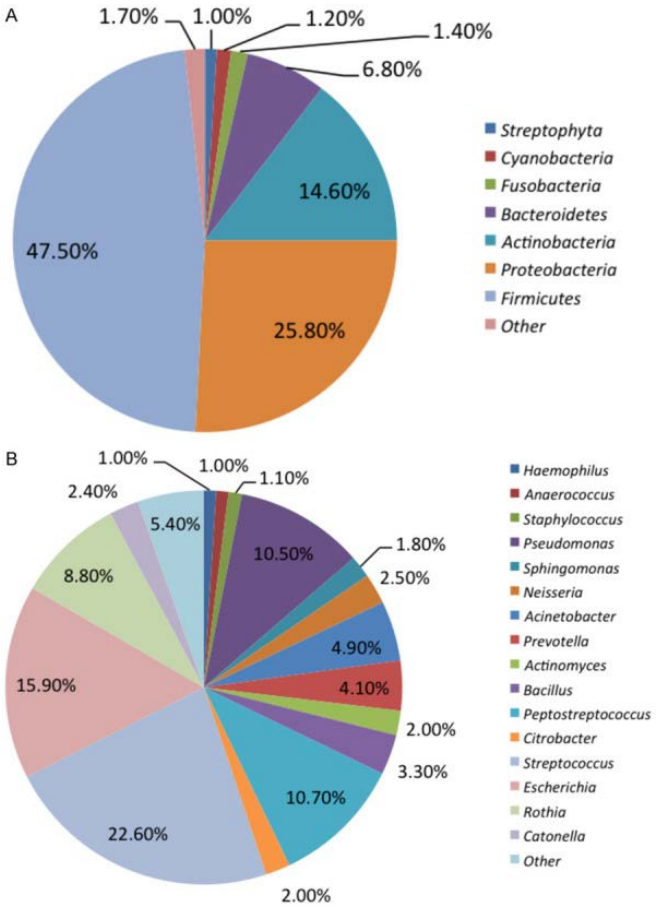
Figure 1: Standardization of melting temperatures in multiplex real-time PCR-HRM showing differences in nontuberculous mycobacteria and mycobacteria tuberculosis in two patient samples (14).



Utility of molecular diagnostics in isolating pathogens in cerebrospinal fluid

The bacteria *Streptococcus pneumoniae, Neisseria meningitidis,* and *Haemophilus influenzae* are the most prevalent in the development of endemic illnesses such as meningococcal meningitis. In most cases, the diagnosis of bacterial meningitis is made based mostly on the results of a positive cerebrospinal fluid (CSF) culture or a positive CSF latex agglutination test (15). The majority of the diagnostic labs in Kenya are equipped to carry out tests such as the Bacterial Antigen Testing (BAT) of cerebrospinal fluid (CSF) by latex agglutination test with a turnaround time for this test estimated to be two hours and the typical cost is approximately four thousand Kenyan shillings (US$35).One of the shortcomings of the culture-based approaches however is the presence of many different types of culture media as well as the possibility of a high sample size and contamination. With this in mind, a recently published study by Men *et al* (15) where routine clinical laboratory diagnostics was performed through the use of microscopy and culture-based procedures on thirty-one patients, showed that thirty patients (97%) exhibited negative culture results. In order to circumvent false positives, genomic DNA was isolated, and subsequent next-generation sequencing as well as 16S ribosomal DNA analysis were carried out.

Figure 2: Distribution of predominant microflora in 31 CSF samples (15)



According to the results obtained by Men *et al* (15), it is abundantly clear that the majority of the bacteria were not identifiable through the use of standard culture-based approaches, which end up producing false negative cultures. Therefore, sequencing approaches have shown that they are capable of identifying bacterial species from culture-negative samples, which is a capability that current clinical detection methods lack with benefits of sequencing approaches perform with high accuracy and in a relatively short amount of time of three days. This context is also applicable to many other areas such as vaginal, respiratory and wound specimens.

Current laboratory techniques can be laborious and expensive when it comes to identifying pathogens responsible for many infectious disorders. Faster methods of diagnosis could expedite the administration of the appropriate treatment, reduce healthcare expenditures, and improve infection control and harnessing preparedness of future pandemics. In a clinical setting, when quick and robust choices regarding patient management are required to be made for the purpose of achieving the best possible results and preventing the spread of infectious disease, precision is very crucial. The technologies that are available today can test for a wide variety of pathogens using a variety of clinical samples, and they can produce results in under an hour while requiring less than a minute of manual intervention.

Conclusions

Current laboratory techniques can be laborious and expensive when it comes to identifying pathogens responsible for many infectious disorders. Faster methods of diagnosis could expedite the administration of the appropriate treatment, reduce healthcare expenditures, and improve infection control and harnessing preparedness of future pandemics. In a clinical setting, when quick and robust choices regarding patient management are required to be made for the purpose of achieving the best possible results and preventing the spread of infectious disease, precision is very crucial. The technologies that are available today can test for a wide variety of pathogens using a variety of clinical samples, and they can produce results in under an hour while requiring less than a minute of manual intervention. The power of pathogen genomics diagnostic tools presents prospects that would take a more anticipatory approach to accurate clinical diagnosis, prevention and control of outbreaks.

Recommendation

We recommend that clinical specialists and laboratory scientists begin to wade deeper into the subject of the utility of pathogen genomics for clinical intervention and possible implementation.

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