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Next Generation Sequencing: A Critical Review of Its Applications in Clinical Microbiology

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Abstract

Next-generation sequencing (NGS) technology is fast supplementing and improving the current conventional sequencing. This is as a result of its ability to sequence pathogen genomes and interpret the information in near real time. The aim of this paper is to review the applications of next-generation sequencing in clinical microbiology. With the speedy advances in NGS innovations, clinical and public health microbiology labs are progressively accepting NGS innovation in their workflows into their diagnostic procedures. In this review, it has been found that the applications of NGS in the clinical and public health microbiology settings is not disposable and have the potential to guide clinicians in tailoring treatment to dynamic genomic changes of microbes. Next-generation sequencing has opened a broad new area of research with the potential to revolutionize personalized cancer medicine. Advances in NGS have demonstrated a distinct advantage in diagnostic microbiology, fundamentally lessening the time from diagnosis to clinical treatment.

Keywords: Fastidious; Non culturable; Next-generation sequencing

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Introduction

Clinical microbiology and public health laboratories have begun to utilize next-generation sequencing (NGS) for a range of applications, including whole genome sequencing, microbiome analysis/ metagenomics, transcriptome profiling, infectious disease diagnosis, pathogen discovery, and public health surveillance (1, 2). The term 'next-generation sequencing' refers to new sequencing technology which supplemented and improved on the current conventional Sanger deoxy chain termination sequencing method (3). What attracts the most attention is the ability to sequence pathogen genomes and interpret the information in near 'real time'. There are potentially vast applications of this technology, which will bring benefits in terms of clinical management and public health aspects. Interestingly, there are already several platforms available commercially despite its presumed infancy in terms of application (4-6). In view of the high amount of data generation, at relatively low cost, this technology has the potential to produce a true revolution in the way things are done in the clinical microbiology laboratories and in public health diseases or outbreaks prevention strategies (7, 8).

Molecular methods have begun to revolutionize the workflow and changed the paradigm in clinical microbiology as shown in Figure1. Polymerase chain reaction (PCR) was a very popular technique at the initial

phase and provided qualitative detection of pathogens. Variants of the technique were then seen, e.g. multiplex PCR, real time PCR, etc. Molecular detection of pathogens has evolved from a mere qualitative detection of the genetic material, to an array of quantitation of the DNAs or RNAs, sequencing of the genes and now mass sequencing of all genes (9). This has created a lot of interest on its clinical applicability on patient management, understanding diseases pathogenesis and the control of infectious diseases. To adopt this technology into the routine clinical microbiology workflow requires a paradigm shift. A significant shift from more than 100 years of knowledge 'perfecting' on skills of detection, identification and susceptibility testing to a computer-driven genome processing.

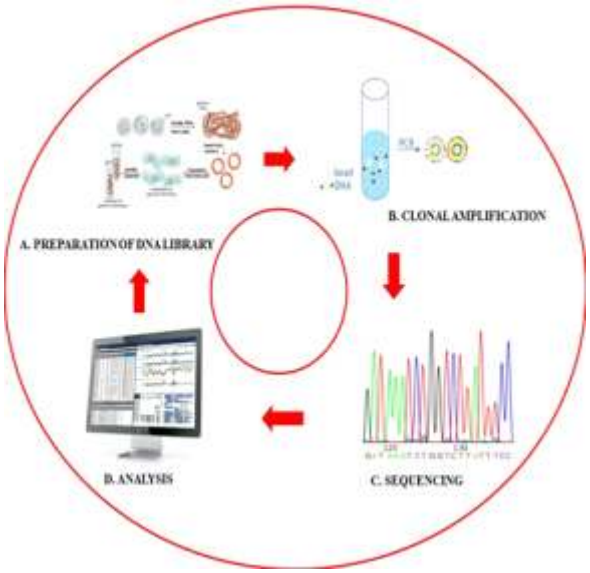

Figure 1: Schematic representation NGS workflow paradigm in clinical microbiology

Clinical microbiology is generally divided into diagnostic microbiology and public health microbiology. In diagnostic microbiology, it involves management of patients, infection control and surveillance of infectious diseases. Public health microbiology works on a larger scale encompassing detecting and monitoring outbreaks and identifying emergence of new infectious diseases. The current workflow of diagnostic laboratories involved detection of pathogen, identification and susceptibility testing (10). All involve rigorous 'trial and error' of series of biochemical tests and perfected through time and now became the gold standard of patient management support in diagnostic laboratories globally (11). A reference laboratory may have a slightly advanced technique for epidemiology characterization or pathogen typing. This will help the public health to outline strategies on how to contain the spread of the infections or outbreaks. For detection and identification of viral infections, serology still accounts for the most common tests done in the laboratories. In reference laboratories, a virus culture facility may be available and is very useful (12). It is obvious a lot of evidence-based studies need to be done to justify this shift. An increasing number of studies are done worldwide on how this technology can be applied in clinical microbiology (13-15). Therefore, the aim of this paper is to critically review these applications.

Epidemiology and Typing

In terms of epidemiology and typing, NGS plays an important role in defining transmission pathways of pathogens and supporting outbreak investigations (16). It is without doubt that sequencing the entire genome will provide excellent resolution for epidemiology studies. NGS has been successfully used in numerous instances. NGS was used in an investigation of prolonged outbreak of Methicillin resistant Staphylococcus auerus (MRSA) in a neonatal intensive care unit (NICU) (17). NGS was also used to investigate the Haiti 2010 cholera outbreak. This study revealed that Haitian strains were closely related to South Asian strains, notably from Bangladesh. The investigators concluded that the Haiti outbreak had been caused by imported Vibrio cholera strains from South Asia (18,19). Another study in the southern region of USA, NGS was used to solve mysterious cases among native-born Americans. In the same region, it is discovered that the Wild 9-banded armadillos were infected with Mycobacterium leprae. A unique M. leprae genotype (3I-2-v1) was found in 28 of 33 wild armadillos and on 25 of 39 US patients (20). To adapt NGS as a routine method to replace the current conventional tests, require vigorous testing and evidence on its superiority over the current conventional methods. What matters most is whether NGS can provide greater clinical benefit in terms of management and treatment of patients. To the managing physician, speed and reliability of the result are important. To the laboratory technicians and pathologists, the technical requirement ought to be simplified.

The challenge of using NGS in detection and identification of pathogens is making sure the test turnaround time is better than culture-based methods. The software must be user-friendly, and the results are

clinically meaningful in terms of clinical management. There are other issues such as quality control procedures that need to be developed, including software validation and proficiency testing (21,22). There is already a move to seriously look at the issues of establishing a formal mechanism for inter-laboratory test performance to ensure harmonization and standardization in NGS and data analysis (23).

Currently, in general, a comprehensive set of genetic determinants of antimicrobial resistance would need to be identified for each species. There should also be an international effort to have a uniform database with the sequence details. Most importantly, prediction on resistance and susceptibility should ideally be accurate. Therefore, the reliability of NGS as a means of predicting antimicrobial susceptibility is critically dependent upon the availability of a current and curated database of reference sequences (24,25). It is a huge challenge to develop a database that include all known virulence determinants and can update seamlessly with the latest determinants. Good and reliable software is needed to analyze genome sequences for the presence and absence of known virulence determinants as well as conducting association studies. With data collected through NGS, it is essential to share nationally and internationally, so that it is more meaningful and useful for many other users. There is also a potential to advocate paradigm shift to replace many species-specific typing methods as a single technology for typing all pathogens. Adopting this technology requires a truly paradigm shift. It requires major changes in the organization, skill mix and infrastructure of diagnostic laboratories (26, 27). The area of focus will be strengthening competence in bioinformatics and software development at global level, in terms of surveillance of infectious diseases required an agreed standards and political will (28).

Detection and Identification

Applying NGS in clinical microbiology has to, in a way overcome some of the inherent characteristics of current conventional methods, namely cheaper cost, and relatively rapid and efficient methods. In the detection and identification of pathogens, by clinical laboratories, NGS faces with severe disadvantage with its high cost (29). The current methods of using the century-proven culture-based coupled with series of biochemical tests, had been time-tested and over the years had proven to provide good and reliable results. Over time microbiologists had mastered the skills of identifying pathogens through these methods. To justify the high costs, NGS may probably play a role in certain important related issues such as accurate detection of organisms which cannot be cultured or difficult to culture, especially fastidious bacteria and anaerobes, and the discovery of novel pathogens or variant of known pathogens (30). In other words, this technology can provide rapid results without the need to isolate and culture the pathogens as well as prior knowledge on pathogen sequence (31,32). Faced with problems with laboratory diagnosis of culture and PCR, a group had described the role of NGS in detecting Francisella spp in clinical specimen in a patient who presented with a right thumb ulcer and right axillary lymphadenopathy (33, 34). A new arenavirus, named Lujo virus, was discovered in South Africa after an outbreak of hemorrhagic fever with nosocomial transmission (35, 36). Within 72 hours of sample receipt, the virus was identified and detailed phylogenetic was characterized. Not only a new virus has been discovered, its genetic composition and relation to other viruses were also made available from the technology within a short period of time. Another novel arenavirus was also discovered in transplant-associated disease clusters (37, 38). The detection of human papillomavirus that has not been cultured, had a good application potential where NGS can provide useful and meaningful results for clinicians (39, 40). The study of intestinal microbiome is even made much easier with NGS. Study in mice showed some role of monitoring vancomycin resistance enterococci in at-risk patients and offer appropriate treatment intervention (41).

Detecting Virulence Determinants

It is an established fact that certain bacterial sequences encode for virulence factors. To apply this fact into clinical practice, the NGS technology can assist in identifying the virulence genes rapidly and this can give the managing doctors an inference of potential virulence of a particular pathogen (42, 43) which may affect or alter clinical decisions. In addition, it also provides the opportunity to discover new virulence factor through association studies.

In an investigation of increased pathogenic potential of meningococci and its genetic basis, whole genome comparisons of a large collection of the pathogen were performed (44, 45). The authors discovered the presence of a meningococcal prophage in the bacteria. The phage

is secreted from the bacteria via the type VI pilin secretin. Another interesting study looked at the evolutionary dynamic of Staphylococcus aureus during the progression from carriage to disease in a patient over a period of time (46). The conclusion of the study revealed both carriage and invasive bacteria formed distinct clades within the host. It was also noted that invasive bloodstream bacteria emerged from a nasal population of methicillin sensitive Staphylococcus aureus.

Drug Susceptibility Testing

In the crucial area of antibiotic susceptibility testing, it is possible to predict resistance phenotypes simply by linking it to genetic determinants of antibiotic resistance (47). Currently, the role of NGS is limited due to incomplete data associating genotype and phenotype (48). Moreover, the current established phenotypic testing is cheaper. Nevertheless, NGS could be used to rule in resistance for certain antibiotics. In certain circumstances, such as a complete or near-complete congruence between genotype and phenotype as well as when phenotypic testing is very slow (due to slow growing bacteria), NGS could be regarded as the best method to detect resistance. A good example is Mycobacterium tuberculosis, which is an ideal organism for genotypic testing. This is owing to its slow growth rate and resistance arises through point mutations or small insertion or deletion. Though currently, probe-based hybridization assay and the Cepheid Xpert MTB/RIF assay are available, NGS can provide added value. Precise Mycobacterium species can be identified, and susceptibility testing can be done for remaining antibiotics. At public health level, epidemiological typing can also be done and provide useful information for interventions (49, 50). Compare to the current conventional Sanger sequencing technology, NGS can provide faster and cheaper phenotypic tests (51). Minor variants can be detected easily; HIV is a good example where there is a good congruency between genotype and phenotype in terms of anti-viral resistance (52). Therefore, NGS can provide better alternatives than existing tests.

Conclusion

This review provides distinguishing proof as well as expectation of the antimicrobial resistance in clinical materials exclusively by molecular methods in the diagnostic microbiology research center isn't novel. Nevertheless, capacity to sequence huge numbers of bacterial genomes as well as delivering and interpreting the resultant sequence information in real time is the basis of NGS technologies with numerous applications and success in clinical microbiology and public health field.

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