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Next Generation Sequencing in Clinical Microbiology

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ABSTRACT

Some Molecular diagnostics of human pathogen does not give sufficient information for study. Next generation sequencing gives rapid and accurate diagnostic for known as well as unknown pathogen. Next generation sequencing is able to sequence 1K to 1M of DNA in a single sequence run and the obtained sequence can be further used for investigation. In this a basic introduction of NGSs is given which consist of laboratory preparation , machines and common types of NGSs .Some applications were described including pathogen identification, outbreak detection and intervention, rapid identification of bacteria and zoonotic micro-organism. Here we described the use of NGSs in clinical microbiology in future with some certain requirements.

Keywords: NGS, Zoonotic Micro-Organism, Sanger Sequencing, Virulence finder and Resfinder

1. INTRODUCTION

Identification of microbes that give rise to infections in humans are important for treatment of patients but some bacterial species cannot be identify by currently present molecular tests because they are not able to detect the genetic features in unknown pathogen which is spread in humans, animals and the environment. Unidentified pathogens can easily cause hospital breakout by putting patient's life at risk. Currently molecular diagnostic methods have met a rapid development growth and increasingly take part in clinical microbiology. These methods have reduced the timeof test and made it possible to detect pathogens which are not known. . One of the most popular molecular methods used in clinical microbiology is the sequencing of genes of the pathogens. Sequencing of genes can be used to describe the genetic relationship of bacteria or viruses and detect mutations in bacterial genomes leading to resistance against antibiotics. There are two main types of sequencing the old method is Sanger method which is also called chain termination method and the new method is Next Generation Sequencing method. But clinical material is more complex so the results obtained by Sanger sequencing are not reliable and hard to identify the specific pathogens also the cost of Sanger sequencing for these tasks is high, and time taking but NGS can process a large number of DNA sequence quickly and the results are more accurate than in sanger sequencing. Because sangers sequencing is preceded by amplification of each gene by using certain primers. This can also be applied for the identification of pathogens in clinical material. But clinical material contains several types of sample for example faecal sample, urine sample so this method becomes very troublesome and cannot be applied to clinical materials.

2. SOFTWARE DATA ANALYSIS

The biggest challenge in the introduction of NGS in the clinical microbiology is the data analyses. We can perform NGS data analyses for disease diagnostic purposes , even if we have little knowledge of bioinformatics, with the help of some software package that are available. For more in-detail analysis scientific knowledge is required for the identification of genomic features and the biological background of micro-organism. Usually large fragments have easier and more accurate genome assembly. Software packagesn, such as CLC Genomic Workbench, SPAdes and Velvet, are used in laboratory for the assembly of genome. Many more software packages, such as SeqSphere (Ridom)

and BioNumerics , or online tools, such as Entero Base and BIGSdb (Bacterial Isolate Genome Sequence Database) can be used to find the genetic relationship between isolates, which can also investigated by using a gene-by-gene comparison with the help of multi-locus sequencing typing (MLST), either by the conserved core genome (cgMLST), or the whole genome (wgMLST), which consist of set of variable accessory genes. cgMLST allows the introduction of a common nomenclature for genetically related strains. .it is not clear that to call a gene identical how many alles should be differ. The same problem arises when comparing two genomes by using single-nucleotide polymorphisms. Many methods to analyse NGS data are found on the website of the Center for Genomic Epidemiology. For identification of virulence- and resistance genes, Virulence Finder and ResFinder, can be used. Similarly, the Comprehensive Antibiotic Resistance Database (CARD) and the Virulence Factor Database (VFDB) can be used for the detection of resistance and virulence genes. In these online tools assembled or non assembled genome can uploaded. But the results obtained through these websites needs confirmation by using other methods. To decrease the sequencing costs, data storage and data analyses cost have increased because of the large amounts of data, and its complexity.

3. NGS IN CLINICAL MICROBIOLOGY

NGS has been already applied in many clinical microbiology labs. It is used for outbreak management, molecular case finding, identification of pathogens etc. The advantages of using whole genome sequencing (WGS) - based typing is it promotes the NGS implementation for public health investigations. WGS has the potential to make an adequate contribution to determine the presence of virulence factors by using different online tools. It is very important and helpful in outbreak detection and monitoring the evolution and dynamics of multi-drug resistant pathogens. Apart from multidrug-resistant bacteria, WGS is applicable to characterize highly-virulent bacteria, such as shiga toxin-producing Escherichia coli, this bacteria has been responsible for large outbreak. The importance of NGS is that it is a high power tool to differentiate between clones with specific properties and to obtain knowledge for patient management, infection prevention and evolutionary studies. In addition to outbreak management, the use of WGS also allows the control measures to avoid the spreading of resistant bacteria clones. NGS databases can search the cases in complex and comprehensive outbreaks. This helps in detection of cases that would not have been found by traditional epidemiological investigation. Many other examples of molecular case finding in NGS databases have been reported after the discovery of the plasmid resistance gene. Novel resistance genes in bacteria can also be identified by NGS, both in current as well as in historical strain collections. The current procedure for characterization of pathogens is based on a large variety of bacteriological, biochemical and molecular methods, this procedure are laborious, time-consuming, and expensive. NGS serve as a perfect tool to study a wide range of pathogen characteristics and identification and is applicable on a broad range of pathogens. NGS allows culture-free detection of number of pathogens**.** Analysis of large data requires a high knowledge of bioinformatics and computational resources that is currently absent in diagnostic medical microbiology laboratories. Other met genomics approaches are time consuming. However NGS is an excellent approach to detect and identify bacterial species. When compared to met genomics, NGS is faster, less complicated and cheaper. Apart from identifying pathogens, a met genomics approach can also be used to study the resist me. The gut is also known as a reservoir for antibiotic resistance genes (ARG), and antibiotics treatment has an impact on the intestinal resistome, which can result in horizontal gene transfer and the selection of resistant bacteria. NGS can also be applied directly to the clinical specimens. Not only by using a targeted NGS approach, but also by sequencing the DNA or RNA from patient samples by met genomics sequencing by using this method, it is possible to identify the presence of pathogens and the presence of virulence and resistance genes in one sequence run. It also reveals more about zoonotic transmission of micro-organisms.

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