NEXT GENERATION SEQUENCING: APPLICATIONS AND LIMITATIONS IN CLINICAL MICROBIOLOGY

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Infections caused by multidrug resistant (MDR) pathogens are recognized as the biggest health challenge. In order to reduce the burden of bacterial disease it is imperative that infections be diagnosed and characterized in a timely manner. In this backdrop since last two decades clinical microbiological laboratories have witnessed an escalating trend in molecular diagnostic methods [1]. High throughput DNA sequencing or the Next Generation Sequencing (NGS) is becoming a common place and has changed the landscape of clinical microbiology [2]. NGS has not only reduced the cost of total sequencing but has introduced versatile applications under one platform. Outbreak investigations, microbial identification, source detection, metagenomics, transcriptome profiling, identification of antibiotic resistance genes and virulence factor, food microbiology, forensic microbiology, public health surveillance and microbial taxonomy; all can be done after single sequencing [3]. That is why there is an increasing interest in microbial identification (advanced molecular detection) through NGS. NGS may reduce sequencing cost to $2 to $300 per bacteria [4].

Conventional methods (PFGE, MLST) to investigate outbreaks though rely on genomic differences but are not sophisticated enough [5]. Nonetheless, NGS can provide information about whole genome at high speed and high precision level. It can be positively expected that NGS will replace other conventional molecular typing techniques in near future as it provides larger data in shorter time and in less cost [5].

There are many unculturable bacteria and they are overlooked by tradition at methods. Likewise, if the clinical specimen is complex and contain multiple species then traditional methods cannot be used. Whereas the NGS allows sequencing of the whole genome of numerous pathogens in one sequence run, either from bacterial isolates of (different) patients, or from multiple species present in patient material from one individual (metagenomics) [1]. The greatest advantage of using NGS is that a single protocol can be used for all pathogens for almost all applications [1]. There is no need for target specific primers rather in NGS the genome is fragmented (100-1000 bases). Fragments are then fused to adapters and barcoded. Nextera XT Library preparation kit helps to do fragmentation and adapter fusion in one step. Next are the amplification, normalization and sequencing steps. After sequencing the main challenge is data analysis and handling. Many commercial and free bioinformatics software are available. During data analysis each signals is assigned a quality score then it is further processed to trim the adapters, filtered and assembled. From assembled genome all relevant information can be mined [6].

There are several hurdles to mark the effective clinical utilization of NGS; high running cost, complex procedure and lack of expertise [7]. Though the technique is available since one decade yet no FDA approved test for pathogen identification and resistance detection has been introduced [6]. It is therefore required that clinical laboratories must establish their own test and validate them through their own quality standards by using standard reference materials. To make a transition of technique from research labs to diagnostic clinical labs sufficient training, user friendly bioinformatics tools, standard reference materials are prerequisite and it definitely need a joint and collaborative effort of all stakeholders [6].

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