SHORT COMMUNICATION

Next Generation Sequencing and its Role in Clinical Microbiology and Molecular Epidemiology

Faiza Zeeshan[1], and Sadaf Razzak[1]

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INTRODUCTION

The microbiological diagnosis of infectious diseases and its related molecular epidemiology has come a long way owing to the advent of newer molecular diagnostic techniques. There was a time when specification of pathogens was determined by phenotypic methods only. Later on, the implementation of Polymerase Chain Reaction (PCR) based assays came in the limelight. Although these methodologies are less time consuming and highly sensitive, they require etiological hypothesis of a clinician and require presumptive predefined targeted sites[1]. Therefore, exploration of newer methods has been direly needed. The next generation sequencing (NGS) is now becoming the hallmark in detecting pathogenic and non pathogenic micro organisms from a given sample. The older sequencing techniques including Sanger sequencing and Maxam-Gilbert sequencing are indisputably gold standard techniques but their role in clinical microbiology, field and molecular epidemiology is somewhat restricted.

The first generation sequencing techniques were able to produce high output data but evolution of Genome analyzers in 2005 was a game changer, which took sequencing runs from 84 kilo base(kb) per run to 1 gigabase (Gb) per run and NGS staggeringly increased the data output from gigabites to terabites[2]. Also NGS reduced the time span, the cost and enabled parallel running of many samples. The NGS techniques primarily work upon one of the two strategies: sequencing by hybridization or sequencing by synthesis[3]. On the basis of these strategies, the sequencing platform can be broadly represented by Ion torrent, Roche/454, illumina/Solexa, and ABI/SOLid sequencing[3]. These NGS techniques have possibly made the tracking of unidentified and resistant micro organisms which are associated with hospital and community associated break outs. The NGS is now being used worldwide in tertiary care units for epidemiological studies and trailing chain of infection. The University Medical Centre Groningen (UMCG) in Netherlands is using Illumina Miseq? and Life Technologies Ion PGM™ sequencing for detection of resistant micro bugs and investigations for disease outbreaks. On average, the molecular laboratory of UMCG receives 5750 samples per year, out of which, 1500 samples are proceeded for NGS techniques and the results are commendable[4].

NGS is a breakthrough in whole genome sequencing (WGS) particularly in bacterial genomics. The NGS begins by obtaining good quality purified DNA using flourometric quantification method. Purified DNA is fragmented into short sequences and run through DNA sequencing process following the protocol of sequencing platform. The data is analyzed by utilizing bioinformatics tools[5]. Currently NGS is used in outbreak management, detection of genes in antimicrobial resistance, microbiological surveillance, transmission of zoonosis, molecular characterization of bugs and metagenomics. The NGS has high standard discrimination power between pathogenic and non pathogenic clones. The molecular characterization and phylogenetic analysis of *E. coli* (STEC) 0154:H4 and shiga toxin-producing Enteroaggregative *E. coli* (EAEC Stx2a+) O104:H4 is made possible because of NGS[5]. The polymicrobial detection from a single and multiple samples at the same time along with genomic analysis is an additional perk of NGS. The sensitivity and reduced bias is one of the factors that emphasizes the utility of NGS over other molecular and phenotypic methods[6].

CONCLUSION

The Next Generation Sequencing of a bacterial genome is a benchmark in Molecular diagnostics and

1 Lecturer, Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan

Correspondence: Dr. Faiza Zeeshan, Lecturer, Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan

Email: faiza.zeeshan@jsmu.edu.pk

Microbiology. It eases clinical diagnoses of various lethal infections, the causative agents behind them which in turn collectively aid patient's treatment and eventually a safer community buildup. The future is waiting for the new developments in the field of microbiological genomics. Therefore, clinicians and microbiologists should buckle themselves up for future challenges and new advancements.

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