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Next-generation sequencing technology in relation to our understanding and tackling of Antimicrobial Resistance

[Tecnología de secuenciación de próxima generación en relación con nuestra comprensión y lucha contra la resistencia a los antimicrobianos]

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Resumen

Las tecnologías de secuenciación han sufrido mejoras en su desempeño en los últimos años. La secuenciación de próxima generación se está utilizando con más frecuencia para controlar enfermedades infecciosas, para conocer y anticipar la resistencia antimicrobiana (AMR) y en los controles de vigilancia contra posibles brotes infecciosos. Los ensayos moleculares utilizados para detectar agentes patógenos o resistentes a los antibióticos requieren mucho tiempo y esfuerzo, y a menudo no se recopila suficiente información para tomar decisiones. La secuenciación de próxima generación parece dilucidar en el menor tiempo posible toda la secuencia de ADN y nos proporciona datos suficientes para conocer la resistencia, la virulencia y la tipificación que se pueden analizar y una gran ayuda en la investigación y la toma de decisiones. NGS es una tecnología muy prometedora, para que se use ampliamente, requiere el desarrollo de plataformas de análisis de datos y la reducción de los costos de prueba que aún es muy alta para un uso masivo.

Palabras clave: Tecnologia de secuenciación, Resistencia antimicrobiana, comprensión.

Abstract

Sequencing technologies have suffered over the last few years improvements in its performance, Next-generation Sequencing is being used more frequently to control infectious diseases, to know and anticipate antimicrobial resistance (AMR) and in surveillance controls against possible infectious outbreaks. Molecular assays used to detect pathogenic or antibiotic resistant agents take a lot of time and effort, and often enough information is not collected to make decisions. Next- generation sequencing appears to elucidate in the least time possible the whole DNA sequence and provide us with enough data to know resistance, virulence and typing that can be analyzed and a great help in research and decision making. NGS is a very promising technology, in order for it to be used extensively, requires the development of data analysis platforms and reduction of trials costs that still is very high for a massive use.

Keywords: Sequencing technology, Antimicrobial Resistance, comprehension

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1. Introduction

Development of Antimicrobial Resistance (AMR)

Antimicrobial Resistance is one of the greatest threats to public health worldwide, current data indicate that AMR produces close to 700 000 deaths per year, and it is estimated that by the year 2050 this figure will reach 10 million if measurements and strategies are not taken to face it (Otto, 2017).

Antibiotics have been used since the twentieth century and it caused a great impact in the battle against diseases, without them millions of lives would have been lost and humanity as we know it today would not exist. The antibiotics have the property of killing or inhibiting bacterial growth without causing greater damage to the cells that are their receptors or to the tissues that surround them. Antibiotics are the most prescribed drugs by doctors with 70 million doses administered by 2010, increasing by 36% in consumption compared to 2000 (Crofts, et al., 2017). Antibiotics have been produced in nature since ancient times, they can be found in the environment, and therefore, resistance to these antibiotics have also been produced naturally by microorganisms from the environment. Even genes resistant to some antibiotics currently used have been found in permafrost preserved for thousands of years out of the reach of humans. There is evidence that the increase of genes resistant to antibiotics in soil samples have been caused by indiscriminate use in health, agriculture and industry. Worldwide, AMRs with a high death rate are those related to malaria, tuberculosis and HIV, while in the developed countries hospital infections related to methicillin resistant Staphylococcus Aureus (MRSA) and Enterobacteriaceae beta- lactam are the most recurrent problems (Otto, 2017). Advances in the DNA sequence have brought the possibility of monitoring AMR epidemiology with much detail that was previously impossible to perform.

Next Generation Technologies

NGS is a tool that in the future will replace the traditional microbiological methods that are used for the analysis of pathogens. The development of faster tools for the DNA sequence came with the Human Genome Project in 1990, in which more advanced technologies were needed to comply with the genome analysis.

There are three kinds of sequencers, the ¨first- generation¨ was Sanger Method. The Sanger sequencing produces long (500-1000 bp) high quality DNA sequences (Besser et al., 2018) , but it takes a long time to get the results. In 2005 appeared the NGS that allowed the DNA sequence with millions sequences read in a single run, this was called second-generation sequencing. Different sequencers have been developed and allow the analysis of short (500-400 bp) or long (1-100 kb) reads, among them those who dominate the market are Illumina and ThermoFisher sequencers. There is a third- generation of sequencers that can generate large fragments (>200 kb), which offers fast sequence runs and long reads. Among them is Sequel (Pacific Biosciences) that can provide the identity and the whole genome sequence of a pathogen, but it has low accuracy. MinION (Oxford Nanopore), is a portable device with little consumption of instruments and consumables, which makes cost-efficient. The third generation of sequencers is not yet used in laboratories due to their low throughput and high cost (Deuremberg et al., 2017; Quainoo et al., 2017).

Although NGS has different technologies, the steps to be taken to obtain the whole genome of an organism are shown in Figure 1.

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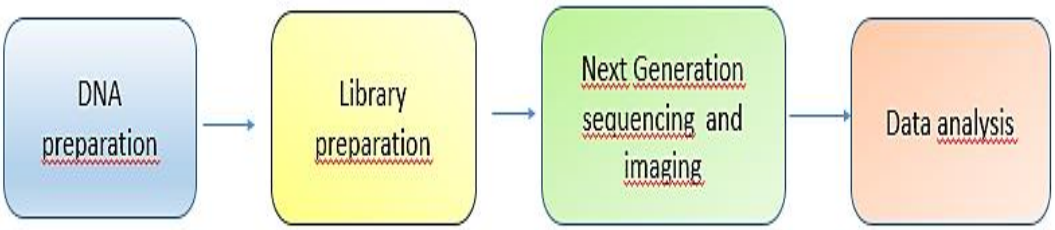

Fig.1. Next generation sequencing workflow

NGS to control the antibiotic resistance emerging in clinics

NGS plays an important role in outbreaks infections in hospitals or clinics, helps to elucidate the pathogen that causes the infection and how it is transmitted between patients. This technology was useful in University Medical Center Groningen, Netherlands, where CTX-M-15 producing Klebsiella pneumoniae were transmitted between patients from a center and after to the whole institution, using NGS was detected K.pneumoniae, high-risk-clone (HiRic) in the shortest possible time and control measures were taken to avoid the dissemination (Zhou et al., 2016).

Harris et al. (2013) made an investigation of an MRSA outbreak occurred in a children's unit, where a patient was infected with the same outbreak strain of an MRSA-positive patient who left the unit 64 days earlier, using WGS was confirmed that MRSA strain was the same as the previous outbreak. The staff members were screened and a member was identified as a carrier of MRSA, a quick analysis with NGS identified that MRSA strain was the same as the infected patient. Consequently, the member was relieved of his work in the hospital and received treatment. In these cases, it has been determined that the use of NGS is very beneficial to control possible infectious outbreaks and take corrective actions on a clinical level.

Next-generation sequencing to detect resistance genes in infectious diseases.

NGS still does not have a massive use in hospitals or clinics worldwide due mainly to the high cost of this technology, however, it offers many advantages compared to the current microbiological analysis specially with the turnaround time and the amount of information about the pathogen that you can get it in a single trial. Definitely NGS provides a more complete genome data about the pathogens compared with the purely microbiological methods. Two examples of highly transmissible diseases with a high incidence in less developed countries, these diseases are being studied with the help of NGS, giving very encouraging results that would help to reduce their spread and find more suitable treatments for the patient.

Mycobacterium Tuberculosis

TB is a disease that produces about 1.7 million deaths annually, identifying and deciphering the MTBC genotype is imperative to understand human-to-human transmission. Currently the MIRU-VNR method is used to have a deeper understanding in MTBC sequencing, but it is not very useful in regions where this disease is endemic and there is a high rate of conserved genotypes (Allix-Beguec, 2008). Nikolayevskyy et al. (2016) analyzed 12 publications and determined that NGS performs better discrimination and allows to subdivide clusters, also showed that cut-off value of < 6 SNPs between isolates allows to predict a recent TB transmission, additionally, the data obtained gives us information about mutant genes that are related to drug resistance and that would be very useful in TB diagnosis laboratories.

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Another study was achieved by Daum et al. (2014) regarded to Pyrazinamide (PZA), this antibiotic is part of multidrug antituberculosis therapy and the increase in resistance to this drug has been spreading worldwide even though there is a standard drug testing for this compound, when the concentrations of resistant organisms are low, the phenotypic testing can give false results that are observed after the treatment is started. It was screening 26 isolates multidrug resistance and 9 of them were PZA resistance. Next generation sequencing was applied to pncA gene and it was found that all 9 isolates have an amino acid mutation that could provide PZA resistance. NGS appears as the most promising tool to show the gene mutations regarded drug-resistant MTB cases.

HIV-1

It has been determined that HIV-1 positive patients provide evidence that the incidence of resistant mutant genes can make treatment with antiretrovirals not very effective. Several studies have compared NGS with Sanger sequencing method with the ability to capture minor resistant variants in the HIV virus, resulting in NGS identifying more drug-resistant mutations (DRMs), almost 50% more compared with the amount of variants found by Sanger Sequencing (Lefterova et al., 2015; Simen et al., 2009). Therefore, this fact is important since the existing resistant mutations can determine the treatment failure. In many researches carried out it has been shown that the quality of analysis with NGS is much higher than Sanger sequencing, which has led to the implementation of HIV-1 resistance tests using NGS.

NGS a relevant tool in the development of antibiotics

It is necessary to take strategies that face AMR, past experiences have indicated that every time a novel antibiotic is launched, soon after resistance mechanisms will appear and spread worldwide. Functional metagenomics is being used to screen different soil samples from different origins and has found novel resistant genes to several antibiotics. The use of metagenomics of human microbiome as saliva or gut has also given interesting results to be taken into account in the development and testing of novel antibiotics (Crofts et al., 2017). The development of antibiotics should not only consider the mechanisms of resistance existing in clinics, but also those that exist in the environment. Novel antibiotics in development should be compared against a library metagenomics to predict a possible emergence of resistance before it is released to the market.

A study by Diaz-Torres et al. (2006) discovered that in the oral microbiome contained genes resistant to three antibiotics: tetracycline, gentamycin and amoxicillin. Andries et al., (2005) published a research about the use of second generation sequencing technology to find F0 subunit of the ATP synthase as the target of bedaquiline, which later became the first antibiotic approved for TB after 40 years. A resistome study by Cox et al. (2017) screened a small number of molecules to find compounds that improve the activity of gentamicin. Of 27 selected compounds that presented this activity, two were chosen for a later validation, it was confirmed that both of them inhibit aminoglycoside-2 "-O-nucleotidyltransferase (ANT (2 ")-Ia -mediated resistance, but if they acted alone they exhibited poor antibiotic activity. Then a screening of the combination of the two drugs was carried out and it was possible to find the resistant inhibitor genes.

An approach that needs to be investigated in more detail is drug repurposing, this would mean giving use to existing antibiotics and directing them in combination to prevent resistance. Researchers studied the effect of three β-lactam antibiotics: meropenem, piperacillin, tazobactam that, used in combination eliminates the bacteria methicillin-resistant S.aureus (MRSA) N315 in vitro and in mouse infection models. In this case, the combined use of these three antibiotics totally kills the resistant bacteria without the possibility of a future emergence due to the elimination of its resistant gene.

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NGS for surveillance of antimicrobial resistance

Current procedures to identify AMR include microbiological analyzes, biochemical methods, that consume a lot of time, thus delaying the time to give a quick response to the problem. The spread of resistance is a global problem that affects all countries, therefore the monitoring of their evolution and dissemination is of vital importance to prevent pandemic diseases. NGS gives us a light in the battle against antibiotic resistance genes, performs a rapid analysis of the genome of pathogens, providing its characteristics, genotype, antibiotic resistance profiles and other important data that help researches to elucidate the risk that could present to global health.
Willman et al. (2015) carried out an investigation at the University of Tubingen, where two patients with ciprofloxacin treatment for six days, presented antibiotic resistance genes (ARGs) in their gut, the researchers used metagenomics in order to identify the appearance of these resistance genes. These results are useful for clinicians make more precise decisions on antibiotic prescriptions that do not have an effect on bacteria in the gut's patients, and therefore reduce the presence of resistant genes.

In the 90s, multidrug-resistance Salmonella Typhimurium DT104 unleashed an epidemic with high death rates worldwide. Initially, it was thought that local animals were infecting humans, but a WGS analysis of human and livestock samples from Scotland showed that pathogen strains were not related, which suggested that imported meat could have been the source of the disease (Mather et al., 2013; Koser et al., 2014).

Kluytmans-van den Bergh et al. (2016) performed a study in the University Medical Center Groningen (UMCG), highlighting on AMR bacteria. Through the WGS they found the mcr-1 gene in three E.Coli strains obtained from chicken meat, although this gene is not present in humans, two of these E.Coli strains belonged to a common clone in humans and chickens, called ST117.
In China there is a monitoring plan that monitors antimicrobial resistance in animals that are bred for human consumption, due to this surveillance program was detected the plasmid-borne colistin resistance gene, mcr1. This resistant gene endangers the use of colistin in humans, however, its detection in an initial phase helped to take action to minimize its diffusion (Du et al., 2016; Crofts et al., 2017).

The monitoring of AMR genes in different areas using Next generation sequencing opens a more reliable and faster approach to study and detect at an early stage a possible outbreak of resistant bacteria.

2. Conclusions

It has reviewed some points related to clinical microbiology and the application of NGS as a tool to identify and analyse a pathogen. The great advantage offered by NGS is that we can get a lot of data from genome in a single run, being one of the most important feature: antimicrobial resistance. It is a promising tool that will revolutionize a future information obtained from pathogens that allows to take action in a real-time to avoid infectious outbreaks. However, it is necessary to develop at the same time Bioinformatics pipelines (genome assembly, comparative genome studies) for the analysis of data, and a metagenomics software that is easy to use so that people do not need an exhaustive knowledge of informatics to be able to handle it.

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