DNA SEQUENCING TO TRACE DRUG RESISTANCE - AN OVERVIEW

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ABSTRACT

Drug resistance is the reduction in efficacy of a drug such as an antimicrobial, anthelmintic and antineoplastic in curing a disease or condition. The various causes for antimicrobial drug resistance includes selective pressure, mutation, gene transfer, societal pressures, inappropriate drug use, inadequate diagnostics, hospital use and agricultural use of drugs. The diagnosis of drug resistance has been performed by lab tests that challenge the isolated microbes to grow and survive in the presence of the drug. In this review we discussed about some DNA sequencing methods to identify drug resistance.

KEYWORDS: Drug resistance, antimicrobial, anthelmintic, antineoplastic and DNA sequencing test.

INTRODUCTION

The acquisition of drug resistance by pathogenic microorganisms found to be the most significant public health threats facing humanity in the 21st century. Mutation in target enzymes will cause or negate its destructive effect, resulting in antibiotic resistance. Bacteria are capable of not only altering the enzyme targeted by antibiotics, but also by the use of enzymes to modify the antibiotic itself and thus neutralize it. Resistance to chemicals is only one aspect of the problem, another being resistance to physical factors such as temperature, pressure, sound, radiation and magnetism, found at physical factors affecting microbial life. In the domestic environment, drug-resistant strains of organism may arise from activities such as the use of bleach, tooth-brushing and mouth washing, the use of antibiotics, disinfectants and detergents, shampoos and soaps, particularly antibacterial soaps, hand-washing, surface sprays, application of deodorants, sun blocks and any cosmetic or healthcare product, insecticides, and dips. The chemicals contained in these preparations, besides
harming beneficial organisms, may intentionally or inadvertently target organisms that have the potential to develop resistance.[1, 2]

DNA sequencing is a technique used to determine the nucleotide sequence of DNA (deoxyribonucleic acid). The nucleotide sequence is the most fundamental level of knowledge of a gene or genome. It is the blueprint that contains the instructions for building an organism, and no understanding of genetic function or evolution could be complete without obtaining this information.[3]

Early efforts at sequencing genes were painstaking, time consuming, and labor intensive, such as when Gilbert and Maxam reported the sequence of 24 base pairs using a method known as wandering-spot analysis. Frederick Sanger developed several faster, more efficient techniques to sequence DNA. Indeed, Sanger's work in this area was so groundbreaking that it led to his receipt of the Nobel Prize in Chemistry in 1980. Over the next several decades, technical advances automated, dramatically sped up and further refined the Sanger sequencing process. Also called the chain-termination or di-deoxy method, Sanger sequencing involves using a purified DNA polymerase enzyme to synthesize DNA chains of varying lengths.[4, 5]

In this paper some identification methods of drug resistance where discussed.

**Mechanisms of drug resistance**

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are drug inactivation, e.g., enzymatic deactivation of Penicillin G in some penicillin-resistant bacteria through the production of β-lactamases, alteration of target site, e.g., alteration of PBP— the binding target site of penicillin’s — in MRSA and other penicillin-resistant bacteria, alteration of metabolic pathway: e.g., some sulfonamide-resistant bacteria do not require para-amino benzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides. Instead, like mammalian cells, they turn to utilizing preformed folic acid and reduced drug accumulation, by decreasing drug permeability and increasing active pumping of the drugs across the cell surface.[6]

**Management of drug resistance**

The chances of drug resistance can sometimes be minimized by using multiple drugs treatment. Hence individual mutations can be independent and may tackle only one drug at a
time. Beta-lactam bacterial resistance can also be overcome by administering beta-lactam antibiotics with drugs that block beta-lactamases such as clavulanic acid so that the antibiotics can work without getting destroyed by the bacteria first. Researchers have recognized the need for new drugs that inhibit bacterial efflux pumps, which cause resistance to multiple antibiotics such as beta-lactams, quinolones, chloramphenicol, and trimethoprim by sending molecules of those antibiotics out of the bacterial cell. Destruction of the resistant bacteria can also be achieved by phage therapy, in which a specific bacteriophage (virus that kills bacteria) is used. In the future, there is a possibility that antimicrobial peptides might replace novel antibiotics.\[^7\]

**New and improved diagnostics for detection of drug resistant diseases**

Pulmonary Tuberculosis, Second-line drug susceptibility testing (DST) is complex and expensive. Automated liquid culture systems and molecular line probe assays are recommended by the WHO as the current 'gold standard' for first-line DST. Liquid culture DST for aminoglycosides, polypeptides and fluoro quinolones has been shown to have relatively good reliability and reproducibility for diagnosis of extensively drug-resistant TB.\[^8\]

Hepatitis C, A rapid, sensitive and accurate method to detect drug resistant hepatitis C virus (HCV) mutants has been developed. Researchers at Hiroshima University established a system to rapidly and accurately measure the presence of HCV Y93H drug resistant mutant strains and evaluate the proportion of patients harboring this mutation prior to treatment. Even in serum samples with low HCV titers, Y93H drug resistant mutation could be successfully detected in more than half of the samples. This new system for detecting mutant strains may provide important pre-treatment information valuable not only for treatment decisions but also for prediction of disease progression in HCV genotype 1b patients.\[^9\]

DNA sequencing to trace the total spread of multidrug resistant tuberculosis, Scientist have for the first time used DNA sequencing to trace the total spread of multidrug resistant tuberculosis in patients in the UK.\[^3\] The Study includes, genetic analysis of the TB bacteria revealed how a 44-year-old man who died of the disease in 2012 caught the drug-resistant infection from a healthcare worker who had worked in South Africa, when both were admitted on the same medical ward four years earlier. TB is spread by inhaling tiny airborne droplets from an infected person. The bacteria can survive in the lungs for long periods without causing symptoms - known as latent infection. In an article published in emerging
infectious diseases today, researchers report using this genetic information to trace the source of infection in a British patient who had never travelled abroad. The DNA profile of the bacteria sample was matched to that of a patient who died in 2008. The second patient had worked as a health care worker at Tugela Ferry Hospital in South Africa, the location of a serious outbreak of drug resistant TB in 2005, but was healthy upon moving to the UK to work. Admission records established that both patients were admitted on the same medical ward in a UK hospital for 8 days in 2008. As is typical with TB, the infection did not manifest itself the second patient until four years later. When he was admitted to hospital and ultimately succumbed to the infection. “Genetic sequencing enabled us to establish beyond reasonable doubt that a patient who died of multi-drug resistant TB caught the infection from another patient at a hospital in the UK,” said Dr. Crooke. Genome sequencing of pathogens is becoming part of routine practice for establishing transmission patterns for TB and other infectious diseases. This sort of analysis will help to improve our understanding of how diseases spread and identify more effective ways to stop them.\textsuperscript{[10]}

CONCLUSIONS
This review clearly shows the need for continuous phenotypic and genotypic characterization of drug resistance at the national level in order to determine the most suitable molecular marker for drug resistance in our setting. Further analysis is needed in identifying resistance mechanism, out of which DNA sequencing test is a most promising method.

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REFERENCE