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MYCOBACTERIUM TUBERCULOSIS AND DNA FINGERPRINTING: A VITAL TOOL FOR TRACKING OUTBREAKS

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Abstract: Genetic fingerprinting has been incorporated into epidemiological research to investigate TB micro-epidemics. TB control aims to pinpoint areas of transmission to halt the spread of the disease. accurate diagnosis of TB infection is essential to prevent disease progression. Traditional epidemiological studies, which relied on identifying plausible opportunities for transmission based on shared environments, have been greatly enhanced by the introduction of genetic techniques. The ability to characterize infecting organisms through DNA fingerprints has provided invaluable insights into the transmission dynamics of tuberculosis. By integrating these genetic data with traditional clinical, epidemiological, and public health information, we have gained a more comprehensive understanding of how tuberculosis spreads within populations. Moving forward, the continued use and refinement of tuberculosis DNA fingerprinting techniques will be crucial in identifying transmission links, guiding targeted interventions, and ultimately, reducing the burden of tuberculosis worldwide..This powerful tool holds the potential to revolutionize tuberculosis control strategies and bring us closer to achieving the goal of eliminating this disease as a global health threat.

Keywords: DNA Fingerprinting, Multi Drug Resistance, Genes, Strains,

1. Introduction

Traditionally, the study of infectious disease epidemiology has depended on observed occurrence patterns to deduce transmission dynamics. When dealing with diseases like tuberculosis (TB) that have a variable incubation period, a continuous notification rate at the population level can obscure a series of concurrent micro-epidemics. Recently, genetic fingerprinting has been incorporated into epidemiological research to investigate these TB micro-epidemics[1,2]. An essential component of any TB control initiative is the capability to pinpoint areas of transmission to halt the spread of infection. This involves identifying newly infected individuals and offering them preventive therapy to prevent the progression to active disease[2,3,].

Our ability to observe patterns of transmission within populations has been greatly improved by the use of genetic fingerprinting of Mycobacterium tuberculosis. It has made it easier to pinpoint the

points at which related people have contracted unrelated strains of the virus and brought attention to such connections[4].

There are two more intermediate clinical stages of tuberculosis (TB) apart from the infection and disease state: incipient TB and subclinical TB. This is because the spectrum of TB is defined by a dynamic equilibrium between the host immune system and Mtb[30,31]. Although there are no clinical, radiological, or microbiological symptoms of the disease, early tuberculosis (TB) is defined by the reproduction of bacteria in the affected tissues and a partial failure of the host immune response. If therapy is not received, it is believed that this stage may progress to tuberculosis disease. The absence of symptoms is the hallmark of subclinical tuberculosis (TB), despite a rise in Mtb replication that can be measured by microbiologic assays and in tissue damage that may be seen by radiological imaging. People with compromised immune systems, such as those living with HIV infection, diabetes, receiving immunosuppressive medication, or co-infected with SARS-CoV-2, have been shown to have an increased risk of developing tuberculosis (TB)[31,32].

Standardized diagnostic instruments for identifying individuals with incipient tuberculosis are still lacking. With 1.7 billion people expected to have traumatic brain injury (TBI) , an accurate diagnosis of TB infection is essential as a tool to avoid disease progression and provide preventive therapy, which is a vital component for worldwide TB control [3,4,6,33,234,35] We present the research tests for TBI diagnosis in this review, with an emphasis on recently developed technologies[36,37,38].In this paper, we outline the procedures and examine the developments, benefits, and constraints of a potent molecular biology application to the study of a long-standing human illness[5,6].

1.1 Epidemiology at the molecular level

Based on realistic chances of transmission, traditional epidemiologic research deduced tuberculosis transmission. If it was determined that there was adequate environmental sharing for transmission to have taken place, two or more cases were deemed related. Early in the 1990s, laboratory methods formerly only used in genetic research were accessible for broader epidemiologic and public health studies. Through the use of "DNA fingerprints," which can be paired with conventional clinical, epidemiologic, and public health data, these techniques enable the characterization of infectious organisms[7,8].To create genetic fingerprints of tuberculosis isolates, restriction fragment length polymorphism (RFLP) analysis is the most often employed molecular approach. The repeating base pair sequences known as "insertion sequences" found in the chromosomal DNA of M. tuberculosis are unevenly dispersed across the organism's genome.5 Laboratory techniques generate a fingerprint for every isolate based on the quantity and arrangement of these components. IS6110 is the most often used insertion sequence in M. tuberculosis research.[6,7]

A restriction endonuclease, which consistently recognises and cuts at a certain sequence of base pairs (the restriction site) is used to cut the organism's DNA in order to form the fingerprint. Thousands of DNA fragments of various lengths are the end product, which are further divided by size using gel electrophoresis. These divided fragments are then hybridised with a probe that detects the DNA sequence of IS6110 on a membrane (a process known as Southern blotting)[7,8]. The majority of M. tuberculosis isolates will generate a DNA fingerprint consisting of one to twenty-five bands that hybridise with IS6110. A distinct banding pattern unique to that isolate is produced by the combination of fragment size and quantity; the number of bands is equal to the number of IS6110 elements and the distance they move over the gel, reflecting the portion carrying IS6110's molecular weight. The intricacy of various patterns overwhelms one after studying several hundred isolates, making visual pattern comparisons impractical when studying DNA fingerprints across a population. Rather, a computer scan of the DNA fingerprints is done beforehand to enable visual comparisons of the banding patterns with computer assistance[8,9].

The same isolate's genetic fingerprints generated before and after multiple laboratory passages have been demonstrated to be similar, suggesting that an isolate's DNA fingerprint is a persistent characteristic. Additionally, it has been discovered that the DNA fingerprints of patients with active tuberculosis, from whom several samples have been collected over time, are largely constant.7. After analysing every repeat isolate from the Netherlands, De Boer and colleagues have calculated that an

IS6110-based DNA fingerprint has a half-life of roughly three years[8] .These data imply that changes in DNA fingerprints occur at a slightly slower rate than secondary cases appear, which means that most cases in an outbreak should present with very similar, if not identical, DNA fingerprints. This is because it is estimated that approximately 50% of new cases of active TB occur within 2 years of infection[8].

While the molecular techniques for DNA fingerprinting are well established and standardized, there is still considerable uncertainty regarding how these patterns should be applied to epidemiologic study. The best way to evaluate results and their eventual use outside of the study setting are among the unresolved challenges. If the fingerprints from two cases are entirely distinct, we can conclude that there hasn't been any transmission.[9,10] However, we cannot always assume a direct transmission link when isolates from two patients happen to have the same fingerprints. This is especially true if there is just a small diversity of DNA fingerprints in the community or if other members of the community also share the same fingerprint. There is strong biological support for the theory that identical fingerprints indicate infection by an organism belonging to the same lineage. Nevertheless, whether or not that commonality really represents recent transfer will determine how this information is applied to understanding transmission. Although molecular techniques have significantly advanced our knowledge of tuberculosis, epidemiologic data must always be taken into account when interpreting the results. The main tactics for decreasing the spread of tuberculosis (TB) are contact tracking, early diagnosis1, and efficient treatment of infection and illness[11,12,13]. Beyond the primary interest of the clinician in providing the patient with successful care, there is a shared obligation to stop additional infection and disease transmission. An organized effort to quickly and precisely trace patient relationships is a crucial first step towards limiting tuberculosis transmission. TB can have a protracted incubation period between infection and disease onset, which increases the difficulty of epidemiologic studies meant to pinpoint the points of infection and transmission. Molecular epidemiology and study of genes related to Mtb is an improved cooperative approach to understanding the dynamics of disease spread that emerged from the combination of traditional epidemiological approaches and contemporary molecular protocols for strain identification at the DNA level. Genetic fingerprinting(Table 1) is one of the components of this innovative approach that has already been utilized to detect many suspected and unsuspected transmission linkages that would have gone unnoticed a mere ten years ago[14,15,16,17,18].

Table 1: Table of different genes related to Tuberculosis

Gene Name	Function	References
rpoB	Codes for RNA polymerase subunit beta, associated with resistance to rifampicin, a first-line TB drug.	**55,56**
katG	Codes for catalase-peroxidase enzyme, associated with resistance to isoniazid, a first-line TB drug.	**56,57**
inhA	Involved in mycolic acid synthesis, associated with resistance to isoniazid.	**58**
embB	Codes for arabinosyltransferase enzyme, associated with resistance to ethambutol, a first-line TB drug.	**58**
pncA	Codes for pyrazinamidase/nicotinamidase enzyme, associated with resistance to pyrazinamide, a first-line TB drug.	**56,57,58**
gyrA	Codes for DNA gyrase subunit A, associated with resistance to fluoroquinolones, a second-line TB drug.	**59,60,61**
gyrB	Codes for DNA gyrase subunit B, associated with resistance to fluoroquinolones.	**61,62**
rrs	Codes for 16S ribosomal RNA, associated with resistance to aminoglycosides, a second-line TB drug.	**60,61,2**
eis	Enhances expression of aminoglycoside resistance genes, associated with resistance to aminoglycosides.	**62**

2. Standard examinations and their latest advancements

A traumatic brain injury (TBI) is a condition when there are no radiological, microbiological, or clinical symptoms of the illness, but rather an immunological response to Mtb. Since there are no standardised technologies to detect Mtb bacilli, we use the tuberculin skin test (TST) and interferon (IFN)-γ release assays to determine the Mtb-specific immune response as an indirect marker of infection. Mtb is most likely in a dormant or low-replicative state. There isn't yet a gold standard for TBI diagnosis. The basis of TST involves injecting pure protein derivative (PPD) subcutaneously, which causes a delayed-type hypersensitivity reaction. 48–72 hours after PPD injection, the diameter of induration is measured to assess the TST response [30]. TST has insufficient specificity to identify Mtb infection because PPD is a combination of antigens, many of which are shared by M. bovis, Mtb, bacille Calmette – Guérin (BCG), and other species of environmental mycobacteria [18,19]. Furthermore, in immunocompromised patients, it is characterised by limited sensitivity [Citation20]. There have been proposals for updated skin test versions to increase TST accuracy. They work by injecting the 10-kDa culture filtrate protein (CFP-10) and the Mtb-specific antigens early secretory antigenic target (ESAT-6) intradermally. These antigens are encoded in the Mtb-specific region of difference (RD)-1 and are not present in BCG or the majority of environmental mycobacteria [21] which makes them more specific than TST. These assays, which are ESAT-6/CFP-10 (EC)-based skin tests, are the c-Tb test (Serum Institute of India, Pune, India), the EC-skin test (Anhui Zhifei Longcom Biopharmaceutical, Anhui, China), and the Diaskintest (Generium, Moscow, Russian Federation) [22,34]. A recent report on their accuracy was made [25]

IGRA tests are performed using either whole blood or peripheral blood mononuclear cells (PBMCs). By measuring the release of IFN-γ following in vitro stimulation with Mtb-specific antigens, such as ESAT-6 and CFP-10, they assess the cellular immune response. The QuantiFERON-TB Gold In-Tube (QFT-GIT) (QIAGEN, Venlo, The Netherlands) and the T-SPOT.TB (Oxford Immunotec) were the first IGRAs to be made commercially available[33,34,35]. [36,37,38,39].

2.1 Genetic markers utilized for strain identification

It used to be challenging to distinguish between different strains of the Mycobacterium tuberculosis complex. Epidemiological data in determining whether an infection was a reactivation or an exogenous reinfection, or whether an unexpectedly positive culture result was actually a false positive, possibly because of cross-contamination in the laboratory, could only be supported by unique strain characteristics (biochemical reactions or characteristic resistance patterns). At most, strain discrimination based on varying susceptibility to mycobacteriophage invasiveness has produced a small number of strain types due to its technological difficulties. The ability to accurately distinguish between M. tuberculosis strains at the DNA level has been made possible by advances in molecular biology techniques, the publication of the entire DNA sequence of M. tuberculosis, and a better understanding of the molecular genetics of mycobacteria[39,40,41].A certain level of genetic variability is required for strains of M. tuberculosis to function in any DNA-based system. By connecting the outcomes of various fingerprinting techniques, it is possible to take advantage of the varying rates of change among distinct fingerprinting markers. A sizable family of repetitive DNA sequences is another characteristic of the M. tuberculosis genome. It is now known that distinct forms of stable, repetitive DNA sequences can be found on chromosomes at different places and in varied copy quantities. One of several mycobacterial mobile genetic elements, insertion sequence 6110 (IS6110), has been the subject of most TB fingerprinting research. Transposable genetic element IS6110 occurs spontaneously and appears to be present exclusively in organisms that belong to the M. tuberculosis complex[42,43]. Relatively impartially, IS6110 can replicate itself and transpose to various locations on the M. tuberculosis chromosome. As a result, there is considerable variation in the amount of IS6110 element copies and their chromosomal positions, which serves as the foundation for each individual genetic fingerprint. The variables influencing the frequency of IS6110 transposition are not well understood. Although there seems to be a significant background variability in strains that are not connected epidemiologically, IS6110 remains a durable molecular marker of strain identity throughout the majority of epidemiologic studies[44,45,46].

The features of IS6110 have been integrated into an internationally standardised molecular technique especially designed for the DNA typing of M. tuberculosis complex strains, using technology based on M. tuberculosis restriction fragment length polymorphism (RFLP).For 8 M. tuberculosis strain differentiation by RFLP to occur, a cell mass that is big enough to produce enough DNA for molecular manipulation—ideally luxuriant development on a solid culture medium—must be available[47,48] After cells have been extracted, chromosomal DNA is separated and digested by enzymes. A restriction enzyme (PvuII) derived from Proteus vulgaris and its matching restriction site on the DNA molecule play a crucial part in the RFLP technique. PvuII cleaves DNA at each instance of a certain six-nucleotide palindromic sequence it detects in the molecule. (The enzyme does not cleave the DNA at these locations because of particular DNA changes that prevent cleavage in the cell that produces the enzyme.) Following agarose gel electrophoresis, the DNA fragments are moved to a nylon membrane and probed with a segment of IS6110, producing distinct patterns for unrelated strains. In order to facilitate collaborative efforts between laboratories, RFLP patterns are regularly stored into a computer database for both intragel and intergel pattern comparison[49,50,51]. Priority cases can be fingerprinted in as little as one to two working days because to a novel TB DNA fingerprinting technique called spacer oligonucleotide type analysis, or spoligotyping, which has been used since 1997. Spoligotyping is a test that uses polymerase chain reaction (PCR) and doesn't require a lot of cells. Cells from a developing culture at an early stage, an old nonviable culture, or a culture of M. TB combined with nontuberculous mycobacteria can all be used for the spoligotyping PCR reaction. Compared to RFLP analysis, this assay's speed and reduced cost have made fingerprinting much more common[52,53,54].

The M. tuberculosis complex comprises all members that possess the direct repeat region. Multiple copies of a conserved 36-base-pair DNA sequence make up the area, which is divided into "spacer" sequences that range in length from 34 to 41 base pairs[53.] Another epidemiological indication is the presence or lack of these spacer sequences. Similar to RFLP, internationally standardised protocols are used for performing the spoligotyping assay and nomenclature[51,52]. PCR is used to first amplify the direct repeat region from the strain of interest in order to perform spoligotyping[54]. After that, 43 synthetic DNA oligonucleotides that have been attached to a nylon membrane are hybridised with the labelled PCR products. In order to speed the results of DNA fingerprinting and to rank the samples for RFLP, a spoligotyping assay is typically run first.Six Often, spoligotyping offers enough details to fulfil the requirement without requiring the more time-consuming confirmatory RFLP[48,49].As of right now, no single DNA fingerprinting technique can accurately discriminate between all strains of M. tuberculosis. Excellent findings are obtained when analysing recent transmissions and doing cross-contamination examinations in laboratories when spoligotype and RFLP analyses are combined[49,50,51]

3. Application of DNA Fingerprinting

3.1 Analysis of outbreak characteristics

When doctors or public health officials suspected tuberculosis (TB) transmission, they used RFLP techniques for DNA fingerprinting to confirm the case. RFLP results have been utilised in hospital-based research to validate the epidemiologic evidence of nosocomial transmission among AIDS patients receiving medical care.[11,18,19] Although there have been reports of HIV-positive individuals progressing more quickly from TB infection to active disease, RFLP techniques have been utilised to confirm the route of transmission and the pace of development.[10] Furthermore, it has been consistently shown by DNA fingerprinting that tuberculosis can spread even in the absence of "close personal contact." An outbreak that was discovered to be concentrated in a local pub serves as an excellent illustration.[52,53,54]

3.2 Detection of laboratory cross-contamination

When two patients are reported to have TB but the bogus "transmission" was actually caused by organisms from an active TB patient's sample contaminating one patient's sample, this is known as a pseudo-outbreak. RFLP analysis has shown instances of false-positive cultures caused by spills that

occur during sample processing in the lab[13] or by organisms moving between culture vials via needles used to track growth [48,49].

3.3 Assessment of treatment ineffectiveness

Exogenous re-infection during or after successful treatment of active tuberculosis in patients with AIDS has been documented by the use of DNA fingerprinting.[15] After being declared entirely healed of drug-sensitive active tuberculosis, several patients developed drug-resistant illnesses. Such apparent relapses were previously linked to poor treatment compliance and the development of medication resistance. Some of these "relapses" were actually the consequence of re-acquired infection with a different, drug-resistant strain, according to RFLP research. Such exogenous reinfection has also been documented in patients who are not HIV positive in more recent times.[16] Comparably, two M. tuberculosis strains with different fingerprints have been linked to an instance of active tuberculosis, as shown by RFLP analysis.[46,47,48]

3.4 Identification of recent transmission

According to RFLP surveys, the percentage of persons with active TB who believe their condition was caused by recent transmission may be significantly greater in some communities than previously thought[18,19,20,21]. The high percentage of shared fingerprints in cities like San Francisco, New York, Bern, and Los Angeles suggested that 25%–50% of active TB cases in those areas were the result of recent transmission, despite the fact that recent transmission was previously thought to account for only 10% of all active TB cases. Post hoc evidence of epidemiologic relatedness based on questionnaires and other conventional approaches validated these findings.

3.5 Recognition of distinct transmission patterns

Using DNA fingerprinting, researchers in San Francisco have deduced that the majority of tuberculosis cases among overseas-born individuals were caused by the reactivation of a distant infection.[22,33,34,35,45] Rarely did individuals who were born outside of the United States belong to "clusters" as defined by the RFLP, where transmission was confirmed by epidemiology. The inability of contact investigation to identify infected contacts was determined by the same authors to be the most significant vulnerability of the TB control programme. The final secondary cases were not listed as contacts in the source case in about 70% of the clusters.[22,23,24] In the San Francisco region, increased control efforts have led to a decline in secondary TB cases, as shown by RFLP analysis.[23,45]

3.6 Analysis of communicability and pathogenicity characteristics

DNA fingerprinting has shown that infection can spread among individuals with TB who are culture-positive but smear-negative.[24] Smear-negative patients were thought to be responsible for at least 17% of secondary active cases in San Francisco, despite the fact that these people are often thought to pose a negligible risk of contagion and that their condition is not identified in the majority of the world.

In certain outbreaks, it seems that TB spreads quickly, whereas in others, it spreads more slowly. It has been proposed that host features and environmental factors influence the rates of transmission. Using RFLP techniques, an outbreak was documented in which the infecting M. tuberculosis strain appeared to exhibit certain traits reflected in its abnormally high transmission rates.[25] DNA fingerprinting revealed a mean gap of 21 weeks between the relevant contact and the onset of active disease among HIV-seronegative individuals with active TB within 4 years of contact with an infectious index case.[26]

The microbe that produce multidrug-resistant tuberculosis (MDR TB)(Table 2) are resistant to at least isoniazid and rifampin, the two most effective TB medications. All patients with tuberculosis are treated with these medications.Treatment for MDR TB should involve consultation with TB specialists.As supplemental drugs, fluoroquinolones (FQs) are used to treat tuberculosis (TB). By blocking mycobacterial DNA gyrase activity, which stops bacterial DNA from unwinding and duplicating, these broad-spectrum antibacterial drugs with bactericidal efficacy against Mycobacterium TB produce their bactericidal effects (1,–3).The World Health Organisation (WHO)

recommends doxifloxacin (MFX), a "fourth-generation" fluoroquinolone (FQ), for the treatment of multidrug-resistant tuberculosis (MDR-TB), which is defined as resistance to at least two of the most effective antituberculosis drugs, isoniazid and rifampin. Studies have shown that MFX has better activity against M. tuberculosis than OFX.

Table 2: Outlining different drugs used to treat tuberculosis (TB) and their association with drug-resistant TB

Drug	Mechanism of Action	Common Resistance Mechanisms	References
Isoniazid (INH)	Inhibits mycolic acid synthesis	katG mutation, inhA mutation	62,63
Rifampicin (RIF)	Inhibits RNA polymerase	rpoB mutation	64,65
Ethambutol (EMB)	Inhibits arabinogalactan synthesis	embB mutation	63,64,65
Pyrazinamide (PZA)	Unknown, believed to disrupt membrane	pncA mutation	64,65
Streptomycin (SM)	Inhibits protein synthesis	rrs mutation	65
Fluoroquinolones	Inhibit DNA gyrase	gyrA, gyrB mutations	64,66
Second-line injectables (e.g., Kanamycin, Amikacin, Capreomycin)	Inhibit protein synthesis	rrs mutation, eis promoter mutation	65,66

4. Conclusion

In conclusion, the advent of tuberculosis DNA fingerprinting has revolutionized our approach to understanding and combating the spread of this infectious disease. Traditional epidemiological studies, which relied on identifying plausible opportunities for transmission based on shared environments, have been greatly enhanced by the introduction of genetic techniques. The ability to characterize infecting organisms through DNA fingerprints has provided invaluable insights into the transmission dynamics of tuberculosis. By integrating these genetic data with traditional clinical, epidemiological, and public health information, we have gained a more comprehensive understanding of how tuberculosis spreads within populations. Moving forward, the continued use and refinement of tuberculosis DNA fingerprinting techniques will be crucial in identifying transmission links, guiding targeted interventions, and ultimately, reducing the burden of tuberculosis worldwide. This powerful tool holds the potential to revolutionize tuberculosis control strategies and bring us closer to achieving the goal of eliminating this disease as a global health threat.

Conflict of Interest: None

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