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MODERN TECHNOLOGIES FOR STUDYING THE GENOME OF MYCOBACTERIA

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Molecular technologies play a leading role in the laboratory diagnosis of tuberculosis and mycobacteriosis. The successes in studying the genome of Mycobacterium have contributed to significant progress in understanding the evolution, variability, and genetic diversity of pathogens, as well as the development of diagnostic technologies, including research into resistance to anti-tuberculosis drugs.

***The aim of this research** is to conduct a comparative study of the spectrum of modern technologies for studying the genomes of mycobacteria and their impact on the efficiency of the laboratory diagnosis of tuberculosis.*

***Materials and methods**: a search for sources of information was carried out in the PubMed, Medline, Web of Science, and Google Scholar databases. Materials related to the technology of molecular diagnosis of tuberculosis and mycobacteriosis and for determining the susceptibility of pathogens to anti-tuberculosis drugs were selected.*

***Results:** it was determined that the modern methods for studying the genome of mycobacteria include amplification technologies (PCR analysis), hybridization, restriction, spoligotyping, sequencing, and their various combinations. The main methods are standard and modified protocols of PCR (RAPD-PCR, AP-PCR, rep-PCR, Real-time PCR, Inverse PCR, TB-LAMP, HIP, LM-PCR). Genomic Restriction Analysis can be used in studies of MTBC and NTM strains (RFLP, AFLP analysis, MIRU-VNTR genotyping). The most effective method for genome analysis is WGS. Complex methods that utilize a combination of molecular technologies allow for the direct detection of mycobacteria in clinical samples.*

***Conclusions**: the widespread application of genomic technologies in the study of mycobacteria will contribute to the effective implementation of the global WHO strategy for the prevention, treatment, and control of tuberculosis and mycobacteriosis*

***Keywords:** Amplification, Genome, Hybridization, Molecular Diagnosis, Mycobacterium, Restriction, Sequencing, Spoligotyping, Tuberculosis*

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1. Introduction

At the current stage of development in the field of laboratory diagnosis of infectious diseases, molecular technologies play a leading role. Their advantages lie in the high sensitivity, specificity, and reproducibility of results, the quality of which is not dependent on sample collection, transportation conditions, and the state of the biomaterial.

In the context of tuberculosis infection, which remains one of the leading causes of global mortality with 3 million deaths annually, molecular diagnostic technologies have confidently taken the forefront among laboratory diagnostic procedures [1]. According to WHO estimates, in 2021 compared to 2020, the incidence of tuberculosis increased by 3.6 %. There were more new cases (by 0.4 million people) and more deaths (by 0.1 million people, out of which 187,000 were HIV-positive). Amid the COVID-19 pandemic, tuberculosis daily claimed twice as many lives as COVID-19 [2].

In the current circumstances, timely and effective tuberculosis diagnosis is the cornerstone of the global strategy to combat this dangerous infection. It involves microscopic, cultural, immunological, and molecular genetic methods. Since the biological basis of the pathogenicity of the pathogen is the structure of its genome, studying its composition is essential for the successful control and prevention of the disease. Therefore, starting from 1998, when the genome of the tuberculosis-causing agent Mycobacterium tuberculosis strain H37Rv was sequenced [3], there has been significant progress in studying the evolution, variability, and genetic diversity of the species and the development of diagnostic technologies, including investigating mechanisms of resistance to anti-tuberculosis drugs. Whole-genome sequencing of other species within the Mycobacterium tuberculosis complex (MTBC) and non-tuberculous mycobacteria (NTM) has contributed to the creation of highly effective in vitro tests for diagnosing tuberculosis,

including its latent and multidrug-resistant forms. Given that, according to the World Health Organization's (WHO) statement, there is no "gold" standard for tuberculosis diagnosis, molecular technologies justifiably hold a leading position in the complex diagnostic procedures for both children and adults in medical practice [4, 5].

The above rationale has justified the aim of this research - to conduct a comparative study of the spectrum of modern technologies for studying the genomes of mycobacteria and their impact on the efficiency of the laboratory diagnosis of tuberculosis.

2. Materials and methods

A literature search was conducted in the PubMed, Medline, Web of Science, and Google Scholar databases using the keywords: Genome, Molecular Diagnosis, Mycobacterium, Tuberculosis. Materials related to the technology of molecular diagnosis of tuberculosis and mycobacteriosis, as well as methods for determining the susceptibility of pathogens to anti-tuberculosis drugs, were selected.

3. Results

Analysis of available literature sources has shown that the modern arsenal of methods for studying the genomes of tuberculosis pathogens and mycobacterial infections includes amplification technologies (PCR analysis), hybridization, genome restriction marker analysis, spoligotyping, sequencing, and their various combinations.

Currently, one of the main methods for molecular-genetic typing, identification, and molecular epidemiological research of mycobacteria is genome analysis using PCR. It allows for distinguishing closely related strains and identifying their individual genotype features, even in cases of the absence of phenotypic differences. Additionally, it enables the conducting of epidemiological studies of challenging-to-culture and non-culturable microorganisms. The advantages of PCR analysis include its relative speed, simplicity, and cost-effectiveness compared to other methods of molecular research [6].

PCR protocols have been standardized and successfully employed in contemporary research, both in the classical format and in numerous modifications [7].

Various clinical specimens are utilized for the detection of mycobacteria using the PCR method, including sputum, bronchial secretions, pleural exudate, cerebrospinal fluid, and other biological fluids, as well as biopsies. Samples from environmental objects are also taken. Prior to analysis, DNA must be extracted from the diagnostic material. In the case of studying oligobacillary samples, preliminary cultivation of mycobacteria is employed to obtain the required DNA quantity [8, 9].

PCR is commonly used in conjunction with other laboratory methods for differential diagnosis of tuberculosis, detection of extrapulmonary forms of the disease, strain and species typing of MTBC and NTM, and identification of drug resistance markers, including multidrug resistance (MDR). However, it is not recommended to conduct screening studies to identify tuberculosis patients. The PCR method is employed for analyzing genes such as 16S rRNA, hsp65, IS, ITS sequences, and other targets for differentiating mycobacteria [10–12].

The specificity level of PCR depends on the choice of molecular target. For genotyping to obtain a unique set of DNA fragments of different sizes for each microorganism, various PCR analysis modifications are commonly employed, such as RAPD-PCR, AP-PCR, rep-PCR, and many others [13].

To simultaneously detect multiple molecular targets in a single sample, multiplex PCR can be employed. This technique utilizes a set of primer pairs to amplify different regions of target sequences and/or multiple genetic markers simultaneously [6].

Currently, there are numerous commercially available kits and reagents for conducting PCR analysis of mycobacteria: Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test system (AMTD; Gen-Probe Inc., USA) and INNO-LiPA Mycobacteria (Innogenetics, Belgium), which are based on rRNA sequence amplification; AMPLICOR MTB assay (Roche Molecular Systems, USA), Xpert MTB/RIF assay (Cepheid, USA); GenoType MTBDRplus and GenoType MTBDRsl (HainLifescience, Germany), among others [14].

PCR analysis exhibits high sensitivity, up to 98 %, detecting as few as 10 to 100 bacterial cells in a sample. Its specificity reaches 98.8 %. Factors limiting the efficiency of PCR in mycobacterial research directly within biomaterial include the low quantity of mycobacteria, intracellular parasitism, peculiarities of cell wall structure, and the presence of reaction inhibitors. Due to these challenges, DNA amplification from pure mycobacterial cultures is considered more reliable. A known limitation of PCR is the risk of intra-laboratory contamination with nonspecific DNA fragments, potentially distorting analysis results. To address this, specific rules for conducting amplification reactions have been developed, involving strict zoning of laboratory premises for various stages of research, as outlined in relevant regulatory documents [15–17].

Real-time PCR technology is technically simpler to execute, less costly, and less susceptible to contamination by extraneous DNA fragments. This is because in real-time PCR, amplification and detection of results occur within the same tube [6]. Real-time PCR serves as a tool for quantitative analysis of genetic markers and can be performed in a multiplex format. This method is commonly used for distinguishing between MTBC and NTM species, offering high sensitivity, rapid execution, and feasibility in field conditions. The specificity of real-time PCR can be enhanced by combining it with other molecular and chemotaxonomic methods for mycobacterial research.

The foundation of popular test systems like Xpert MTB/RIF (Cepheid, USA) and COBAS® (Roche Diagnostics, Switzerland) lies in the real-time PCR technology. These systems are employed for identification and detection of drug resistance in MTBC and the M. avium-complex (MAC). The portable test system Truenat MTB (Molbio Diagnostics, India), developed in 2013, is designed for quantitative determination of *M. tuberculosis* DNA in sputum samples within one hour. It utilizes a microchip format and runs on battery power throughout the entire process of DNA extraction, amplification, and result detection. Its modification, Truenat MTB-Rif Dx, is intended for determining resistance to rifampicin.

According to the latest assessments by WHO experts, the sensitivity and specificity of these test systems, as alternatives to sputum smear microscopy, are comparable to Xpert MTB/RIF in drug resistance testing as well as TB-LAMP [13].

Inverse PCR is particularly effective for studying flanking regions of nucleotide sequences [7, 18].

Loop-mediated isothermal amplification (LAMP) is a relatively new, simple, rapid, cost-effective, sensitive, and highly specific method of molecular diagnostics. It is performed without the need for a thermocycler at a constant temperature (60–65) °C, eliminating the need for temperature cycling and ensuring efficient amplification. In terms of the number of amplicons produced, LAMP significantly surpasses PCR amplification [19–21].

A detailed description of the LAMP reaction mechanism is available on the website of the molecular diagnostics test system manufacturer, Eiken Chemical Co., Ltd. [22]. The currently available diagnostic test system TB LAMP (Eiken Chemical Co Ltd, Japan) is designed for the detection of tuberculosis pathogens in sputum, urine, and blood samples, as well as for the identification of MTBC and MAC species in cultures. This investigation does not require expensive equipment, as results are assessed visually. The procedure can be completed in 15 to 40 minutes and boasts a sensitivity of 88 % and specificity of 94 %.

PURE-LAMP is a second-generation TB-LAMP technology with accelerated DNA extraction, offering heightened sensitivity and specificity. Overall, LAMP methods are intended for analyzing various genome sequences of mycobacteria, such as gyrB, IS6110, and sdaA [23, 24].

A modified LAMP technique that combines PCR and dUTP-UNG strategies stands out for its enhanced specificity and adaptability for diagnosing both pulmonary and extrapulmonary tuberculosis. In this method, the occurrence of false-positive amplification results is prevented by reducing sample contamination with non-specific nucleotides. This is achieved through the utilization of uracil-DNA glycosylase (UNG) in the reaction mixture.

The Hybridization technology with DNA probes belongs to rapid methods for nucleic acid analysis. As these methods require a substantial amount of target DNA, they necessitate prior cultivation of mycobacteria or conducting an amplification reaction to accumulate the required amount of DNA target. For the identification of cultured MTBC, MAC, *M. gordonae*, and *M. kansasii* species, the AccuProbe Culture Identification technology (Hologic, USA) has been developed, along with corresponding test systems featuring genus- and species-specific DNA probes labelled with chemiluminescent reagents. These probes hybridize with complementary rRNA sequences. The capabilities of DNA hybridization extend beyond identification. DNA probe technology has also become a reliable tool for drug resistance analysis in mycobacteria [25–27].

Complex technologies that utilize a combination of amplification and hybridization methods allow for the direct detection of bacterial DNA in clinical samples. These techniques are rapid, taking 2 to 8 hours, and are effective for studying low-bacillary clinical specimens. They enable the detection of both DNA and RNA of mycobacteria. Depending on the type of target nucleic acid, various amplification methods are employed – classical PCR, real-time PCR, Nucleic Acid Sequence-based Amplification, and Strand Displacement Amplification. Target sequences can be either whole PCR products or fragments cleaved by restriction enzymes. DNA probes have been developed for differentiating a wide range of mycobacterial species, including MTBC and NTM, and commercially available tests are accessible for most clinically significant species.

An exemplary blend of amplification and hybridization techniques is the Line Probe Assay (LPA) – a fast, highly sensitive, and specific method for detecting mycobacterial DNA and drug resistance in a matter of hours when analyzing sputum or pure cultures. The fundamental principle behind the LPA technology is hybridization between two complementary DNA sequences: target regions of the bacterial genome and DNA probes. LPA enables the detection of drug resistance in tuberculosis agents to first- and second-line anti-tuberculosis drugs, as well as broad drug resistance (XDR-TB) to fluoroquinolones, aminoglycosides, and ethambutol, by detecting mutations in drug resistance-associated genes – rpoB, katG, inhA, among others.

In 2008, the World Health Organization (WHO) approved the use of the first-generation LPA-based GenoType MTBDRplusV1 (Hain Lifescience, Germany) test systems for rapid detection of tuberculosis-causing bacteria and resistance to rifampicin and isoniazid in sputum and cultures. Subsequently, new highly sensitive technologies such as GenoType MTBDRplusV2 were developed. Diagnostic kits like NTM+MDRTB2 (Nipro, Japan) and strip technology INNO-LiPA® MYCOBACTERIA (Innogenetics (Fujirebio), Belgium) were introduced, offering extended capabilities to detect MTBC and drug resistance in sputum, bronchoalveolar lavage, pleural and cerebrospinal fluid from patients with both pulmonary and extrapulmonary tuberculosis forms. The GenoType MTBDRslV1 test system became the first commercially available tool for detecting resistance to second-line anti-tuberculosis drugs. Its updated version, GenoType MTBDRslV2 (2016), additionally detects mutations associated with resistance to certain fluoroquinolones (ofloxacin and levofloxacin), all second-line injectable drugs (kanamycin, amikacin, and capreomycin), and ethambutol. Despite the described merits of the method, the use of LPA does not replace the need for confirming results through traditional drug susceptibility testing [28].

For species identification of cultures of atypical mycobacteria, DNA hybridization tests with genus- and species-specific probes immobilized on nitrocellulose membranes have been developed. Using the GenoType Mycobacterium CM test system, it is possible to differentiate between 14 species of NTM (*M. avium ssp., M. chelonae, M. abscessus, M. fortuitum, M. gordonae, M. intracellulare, M. scrofulaceum, M. interjectum, M. kansasii, M. malmoense, M. peregrinum, M. marinum/M. ulcerans, and M. xenopi),* while the GenoType Mycobacterium AS system can additionally differentiate 17 species *(M. simiae, M. mucogenicum,*

M. goodii, M. celatum, M. smegmatis, M. genavense, M. lentiflavum, M. heckeshornense, M. szulgai, M. intermedium, M. phlei, M. haemophilum, M. kansasii, M. ulcerans, M. gastri, M. asiaticum and *M. shimoidei*).

The Reverse Blot Hybridization (REBA) technology from YD Diagnostics (Thailand) employs 25 DNA probes capable of detecting mutations in drug resistance-related genes and species-specific probes complementary to amplicons of the rpoB gene sequences. This method facilitates the rapid identification of MTBC cultures and over 21 NTM species. The advantage of this manufacturer's test systems is the ability to differentiate between *M. abscessus* and *M. massiliense* species.

The principle of hybridization underlies promising fast, miniaturized analytical technologies known as DNA microarrays, which enable simultaneous analysis of a large number of molecular targets for identification, spoligotyping, and detection of drug resistance in mycobacteria [29, 30].

Among the most well-known manufacturers of biochips and diagnostic test systems of this type are Veredus Laboratories (Singapore, VereMTB detection kit, https://vereduslabs.com/products/molecular-testing-loc/), Akonni Biosystems (USA, TruArray MDR-TB Assay, https://akonni.com/products/trudiagnosis/truarray-mdr-tb-assay/), Beijing Capital Biotechnology Ltd (China, GeneChip, http://www.capitalbiotech.com/en/products-content.html?id=67) [31–33].

Genomic Restriction Analysis currently serves as the foundation for a variety of complex typing and identification technologies for mycobacteria. In this regard, restriction fragments can be used either directly as targets or as molecular probes in studies of MTBC and NTM strains. The initial methods of genomic typing for various *M. tuberculosis* strains were based on the analysis of restriction fragment length polymorphism (RFLP), a class of molecular targets generated through endonuclease restriction. The detection method of IS6110, one of the most well-known and studied specific insertion sequences in MTBC species, is based on RFLP analysis. IS6110 was initially identified in the *M. tuberculosis* H37Rv strain. It has been established that the half-life of changes in the IS6110-RFLP pattern is approximately 3–4 years, and the stability of IS6110-RFLP depends on the frequency of transposition events [34].

Differences in copy number and their genomic localization, which contribute to the high degree of IS6110 polymorphism, determine the utility of these sequences as specific molecular markers for genotyping *M. tuberculosis* strains. Currently, IS6110-RFLP genotyping remains the most widely used method employed in molecular epidemiological studies of tuberculosis pathogens. The addition of an amplification procedure has increased the sensitivity of the IS6110-RFLP method [35].

The modern IS6110-RFLP technology has been standardized and adapted for numerous studies, allowing results obtained from various laboratories to be comparable and catalogued. The method is highly selective and reproducible, enabling the differentiation of epidemiologically related and distinct strains. The primary limitations of IS6110-RFLP typing are associated with the requirement for a substantial amount of high-quality DNA for enzyme work (2 μg), necessitating the prior cultivation of the studied bacteria for several weeks, as well as the utilization of complex software. The discriminatory power of the method is insufficient for strains with ≤ 6 copies of IS6110 (which are found among *M. bovis* isolates from cattle and Asian-origin *M. tuberculosis* isolates). To compare IS6110-RFLP results with data available in the international IS6110 template database (RIVM-Bionumerics), additional results from spoligotyping and MIRU-VNTR typing of the material are required. Furthermore, some NTM possess multiple copies of IS6110-homologous sequences capable of hybridizing with the IS6110 probe, leading to false-positive results.

The Heminested Inverse PCR (HIP) method, based on the amplification of the 5' fragment of IS6110 and its flanking sequence up to the proximal BsrFI restriction site, is equivalent to standard IS6110-RFLP analysis in terms of reproducibility and resolving power but is faster and simpler to execute.

Currently, despite its limitations, the IS6110-RFLP method remains one of the most frequently used techniques for genotyping *M. tuberculosis* and has long been considered the "gold standard" among methods for typing MTBC representatives. However, since 2006, the role of the gold standard has shifted to the genotyping of the MIRU-VNTR loci [36].

The molecular target of the multilocus VNTR (variable number of tandem repeats) analysis method is the repeated loci with variable numbers of tandem repeats of unique coding and spacer sequences found in specific regions of the bacterial chromosome. The initial VNTR descriptions included major polymorphic tandem repeats (MPTR) and exact tandem repeats (ETR). MPTR were identified not only in MTBC species but also in *M. gordonae, M. kansasii,* and *M. szulgai*. RFLP analysis with MPTR as probes enabled the detection of multiple subtypes within the *M. kansasii species*, indicating varying degrees of pathogenicity for humans. MPTR are part of the 3'-end of PPE protein-encoding genes, and it is believed that polymorphism in these repetitive elements contributes to antigenic variability in mycobacteria. ETR elements, exclusively found in MTBC strains, consist of repeats ranging from (53–79) bp. Unlike MPTR, all known ETR loci are variable.

During the *M. tuberculosis* H37Rv genome sequencing project, new VNTR-type repeated loci were identified and termed MIRU (mycobacterial interspersed repetitive units). These tandem repeats range from 46 to 101 bp and were subsequently found in the genomes of *M. tuberculosis* CDC155 and *M. bovis* AF2122/97 strains. The most polymorphic VNTR/MIRU loci are analyzed in the MIRU-VNTR typing method, allowing strains to be differentiated based on the number and length of tandem repeats in each locus of an isolate. The variability of specific loci can serve as a marker for the natural diversity of a strain and its epidemiological parameters.

Currently, the optimized 24-locus MIRU-VNTR typing scheme is considered the "gold standard" for *M. tuberculosis* research, providing information on phylogenetic relationships and transmission pathways of the tuberculosis-causing agent. It has been established that 96 % of all detected genomic polymorphisms in

M. tuberculosis strains can be attributed to 15 out of these 24 loci. Individual sets of 12 primers are used to differentiate strains of certain genotypes.

MIRU-VNTR genotyping of *M. tuberculosis* stands out for its speed, simplicity, high sensitivity, reproducibility, and resolving power. The method is suitable for large-scale genetic or evolutionary studies, as well as tracking key epidemiological events. The simple digital format of results, indicating the number of MIRUs in a specific locus, allows for result comparison across different laboratories, contributing to publicly available international databases [37].

The detection of MIRU-VNTR loci in NTM genomes is applied for genotyping strains of *M. avium, M. avium subsp. paratuberculosis, M. intracellulare, M. ulcerans, M. marinum*, and other species.

Another group of genotyping methods, alternative to traditional IS6110-RFLP genotyping, includes methods designed to study the variability of IS6110, based on PCR with ligation-mediated amplification (LM-PCR) [36].

They distinguish themselves by reducing the testing time and not requiring bacterial culture, as only 1 ng of chromosomal DNA is sufficient for restriction. Several variations of LM-PCR have been developed for the differentiation of MTBC strains.

Through the optimization of the working procedure, a rapid ligation-mediated PCR (FLiP, fast ligation-mediated PCR) was developed, which is much simpler and faster than the original LM-PCR method, taking only 6.5 hours compared to the two-day process of the original protocol.

Later, a new variant of LM-PCR was proposed - the fast ligation amplification polymorphism method (FLAP). It was demonstrated that the resolution capabilities of FLiP and FLAP methods are comparable to or exceed those of IS6110-RFLP or the 15-locus MIRU-VNTR typing method [36].

Despite their rapid setup, LM-PCR methods are less applicable for isolates with a low number of IS6110 copies and have relatively low reproducibility, which hinders the creation of informative databases. Therefore, LM-PCR-based methods are not used independently but serve as additional tools for epidemiological investigations.

Amplified Fragment Length Polymorphism (AFLP) is a comprehensive method that also combines restriction and PCR analysis procedures. Its improved version, Fluorescent amplified fragment length polymorphism (fAFLP), has higher resolution capabilities comparable to IS6110-RFLP. The AFLP method has been successfully applied to various NTM species, including representatives of MAC and *M. haemophilum.* Additionally, AFLP analysis enables differentiation between *M. marinum* and *M. ulcerans,* which are challenging to distinguish using phenotypic methods [37, 38].

It has been established that the genomes of 40 % of known bacteria across different systematic groups contain a family of distinct DNA repetitive elements known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). CRISPR loci usually consist of a non-coding, A/T-rich leader sequence and a varying number of identical direct repeats (DR) of 36 base pairs, alternating with unique short variable segments called spacers [39].

CRISPRs are often associated with Cas genes (CRISPR-associated), forming CRISPR-Cas loci, which serve as an adaptive immune system in prokaryotes, providing resistance against phages.

Among mycobacteria, CRISPR loci were initially identified in the vaccine strain *M. bovis* BCG P3 and a few other species, such as *M. tuberculosis, M. bovis,* and *M. avium*. However, integrated CRISPR-Cas loci are only found in *M. tuberculosis* and *M. bovis,* suggesting horizontal gene transfer from other bacteria to explain their presence in NTM.

The analysis of DR polymorphism in mycobacteria is the basis of SPOLIGOTYPING – a method introduced to laboratory practice in the late 1990s. In the classical version of spoligotyping for MTBC strain differentiation, PCR technology is used with two inversely oriented primers complementary to DR sequences. The biotinylated PCR products are then hybridized with a set of 43 synthetic oligonucleotides representing unique spacers of *M. tuberculosis* H37Rv and *M. bovis* BCG P3, covalently bound to a nitrocellulose membrane or microspheres. Different strains can be differentiated based on the presence or absence of individual spacers from the probe set. Spoligotyping results obtained in different laboratories can be easily compared, interpreted, and digitized. Since 2006, online databases of spoligotyping patterns have been available, with the main databases – SpolDB4 and SITVIT [40, 41].

Modern spoligotyping technologies are faster, simpler to use, economically efficient, highly reproducible, fully automated, and capable of simultaneously analyzing up to 96 samples. They employ multiplex PCR, as well as fluorescent dyes or MALDI-TOF MS, for the detection of target products. Spoligotyping results can be obtained within 2 days, and their reliability is determined by the high stability of the DR locus. An important advantage of spoligotyping is its high sensitivity, estimated at 10 fg of chromosomal DNA, which is equivalent to the DNA from 2-3 bacterial cells. This allows the typing of MTBC isolates directly from clinical samples without prior cultivation, enabling the detection of fragmented DNA measuring 75 base pairs in smears, histopathological specimens, paraffin tissue sections, and mummified [36].

Spoligotyping allows for the identification of MTBC representatives at the species or subspecies level and is currently one of the most commonly used PCR-based approaches for studying the phylogeography of MTBC strains. For instance, it has been established that *M. tuberculosis* spoligotypes are characterized by the absence of spacers 33–36, while clinical *M. bovis* isolates typically lack spacers 39–43, and *M. bovis* BCG carries spacers 3, 9, and 16. Furthermore, spoligotyping enables the identification of genotypes of clinically and epidemiologically significant isolates. For example, the majority of Beijing genotype strains are characterized by spacers 35-43. In order to enhance the resolution power of the spoligotyping method for MTBC, new sequence sets consisting of 51 and 68 spacers have been proposed.

Limitations of spoligotyping include the use of complex equipment and lower resolution capacity when typing strains with a high copy number of IS6110 compared to IS6110-RFLP. This method is recommended to be used in conjunction with other MTBC genotyping

technologies as well as independently for screening large collections of mycobacterial isolates. Given that the *M. tuberculosis* genome has an exceptionally high content of GC pairs (>65.5 %), genotyping methods have been developed that target GC-rich sequences. The most common type of these sequences in MTBC is polymorphic GC-rich repetitive sequences (PGRS). It is known that PGRS, present in multiple chromosomal loci of studied MTBC species, consist of multiple repeats of a 9-bp consensus sequence (5'-CGGCGGCAA-3'), tandemly arranged in segments up to 1.5 kb in length. Although PGRS were initially identified in *M. tuberculosis* strains, they are now known to be present in NTM species as well, such as *M. kansasii*, *M. gastri*, and *M. szulgai.*

PGRS share important similarities with the aforementioned MPTR sequences, including host range, structure, genetic stability, and copy number in the mycobacterial genome. Like MPTR sequences, PGRS loci are part of the PE gene family. It is suggested that PE proteins play a role in the antigenic variability of mycobacteria, similar to PPE-MPTR proteins.

As the number and distribution of PGRS elements vary among different strains, these sequences have been proposed as genetic markers for *M. tuberculosis* genotyping. The PGRS-RFLP typing method is widely used, with a procedure similar to IS6110-RFLP but with some differences.

It has been shown that PGRS-RFLP analysis or pTBN12-RFLP fingerprinting has relatively high resolution power, especially for strains with a low number of IS6110 copies. While PGRS are not typically used for typing NTM strains, RFLP analysis with PGRS isolated from *M. tuberculosis* as a probe has been successfully used for differentiating isolates of species like *M. kansasii* and *M. ulcerans*.

Trinucleotide repeat sequences (TRS), characteristic of the PE and PPE gene families, are also targeted for genotyping *M. tuberculosis* strains with few or no copies of IS6110, as well as for differentiating isolates of *M. kansasii, M. ulcerans, M. marinum, M. szulgai*. TRS sequences such as (CGG)5 and the plasmid pTBN12 are used as probes. PCR primers containing sequences (CAC)4 and (CGG)4 have also been developed, as well as a PCR method for amplifying two repetitive elements, IS6110 and PGRS (DRE-PCR, double-repetitive-element), allowing the detection of polymorphism and the number of these sequences in the genome, as well as the distance between them. A similar methodology is used in the amplityping method for analyzing variability in the distance between IS6110 and MPTR elements in M. tuberculosis. A promising technology involves the use of synthetic oligonucleotides (GTG)5 as primers or probes for recognizing specific DNA fragments between IS6110 and (GTG)5 repeats in strains of *M. tuberculosis* with a low number of IS6110 copies, as well as in species like *M. gordonae*, *M. scrofulaceum*, *M. diernhoferi*, and representatives of MAC. As a tool for the epidemiological control of tuberculosis infection, a high-resolution multiplex IS6110-Mtb1-Mtb2 PCR-typing method has been proposed, utilizing primers recognizing TIR and flanking sequences of IS6110, as well as short 16-bp sequences of Mtb1 and Mtb2 with a high G-C content in the *M. tuberculosis* genome.

In the genomes of certain mycobacteria, palindrome sequences of 126 bp in length known as ERIC (Enterobacterial repetitive intergenic consensus) have also been identified. The variability of these sequences forms the basis of the ERIC-PCR typing method for assessing the genetic diversity of *M. tuberculosis, M. gordonae, M. intracellulare, M. szulgai, M. fortuitum, M. chelonae, M. abscessus strains* [8].

Genotyping based on single nucleotide polymorphisms (SNPs) allows for SNP-typing, which reveals both main types of SNPs: synonymous SNPs (s-SNPs) and non-synonymous SNPs (ns-SNPs). It has been established that ns-SNPs, along with missense point mutations, small deletions, and duplications in specific chromosomal loci, determine the resistance of *M. tuberculosis* to anti-tuberculosis drugs. Therefore, ns-SNP screening provides important information about the molecular mechanisms and dynamics of drug resistance development. s-SNP screening is utilized in population genetics and for studying phylogenetic relationships between mycobacterial strains.

Based on the analysis of a combination of s-SNP and ns-SNP markers in the structure of specific genes, a phylogenetic structure scheme of the MTBC population has been constructed, comprising three genetic groups (PGG1-PGG3) containing nine major clusters (I –VIII and II.A). Additionally, a diagnostic algorithm based on SNPs has been developed, allowing the identification of 17 MTBC phylogenetic lineages with high specificity. The SNaPshot method (Thermo Fisher Scientific, USA), utilizing multiplex PCR, enables the simultaneous detection of up to 10 SNPs. A conceptually similar multistage multiplex technology, iPLEX Gold, has been tested for the identification of MTBC species, in which the amplified products are detected using mass-modified terminators and the MALDI-TOF mass spectrometry method [42].

Methods for detecting single nucleotide polymorphisms (SNPs) underlie commercial test systems for detecting drug-resistant strains of MTBC, including the most commonly used ones today: GenoType MTBDR, GenoType MTBDRplus (Hain Lifescience GmbH, Germany), and Xpert MTB/RIF (Cepheid, USA).

The cartridge-based technology Xpert MTB/RIF was introduced into healthcare practice in 2010 following WHO recommendations. It is a fully automated, highly sensitive, and specific diagnostic system based on PCR. This system is designed for identifying *M. tuberculosis* and simultaneously testing the organism's susceptibility to rifampicin. Linear probes detecting mutations in the rpoB gene are used for this purpose. Currently, this test system is widely used for the primary diagnosis of both pulmonary and extrapulmonary tuberculosis in adults, children, and HIV-infected patients. It is recommended by WHO as a screening test in countries with a high burden of disease. The advantage of this method includes the capability to test clinical sputum samples without prior sample preparation, full automation of the process, autonomous cartridges, eliminating cross-contamination issues, and the use of software synchronized with bioinformatics databases, facilitating analysis of large volumes of generated data. The sensitivity of Xpert MTB/RIF reaches 99.8 % for positive results from microscopy and culture, and 90.2 % for cases with negative

smear analysis but positive culture results. Its enhanced version, Xpert MTB/RIF Ultra, exhibits increased sensitivity comparable to the culture method. The hardware and software of this platform are continually improved.

Sequencing, which determines the primary nucleotide sequence of nucleic acids, is the most effective method for analyzing individual genes and entire genomes to identify bacterial species and subspecies, even in the absence of known species-specific molecular targets. The "gold standard" for determining the DNA nucleotide sequence in sequences up to 1000 bp is the enzymatic dideoxynucleotide method involving DNA polymerase or the "chain termination" method based on the procedure developed by F. Sanger in 1977. After comparing the established sequences of obtained reads with sequences from genomic libraries (Microseq library (AB); Lab-specific custom library; Genbank BLAST (NCBI), and others), it is possible to determine the sequence and localization of nucleotides in the genome and reconstruct the entire genome sequence. This principle forms the basis of whole-genome sequencing [43].

Whole-genome sequencing (WGS) provides valuable information for studying mycobacteria, serving both identification and typing purposes, as well as enhancing the understanding of their genomic functioning. The complete decoding of the *M. tuberculosis* H37Rv genome in 1998 marked a significant milestone, fostering progress in the study of genetic diversity among clinical mycobacterial isolates and expanding diagnostic methods in the field of phthisiology. Today, bioinformatics databases house results from the whole-genome sequencing of over 5000 strains of *M. tuberculosis* exhibiting various phenotypes and genotypes, along with reference strains of all officially recognized mycobacterial species [44].

Presently, there are five major technological platforms for mycobacterial WGS: 454 pyrosequencing (Roche), HiSeq/MiSeq (Illumina), SOLiD (Thermo Fisher Scientific), Ion Torrent/Ion Proton (Thermo Fisher Scientific), and PacBio (Pacific Biosystems). These genome sequencing technologies employ a common protocol, involving DNA fragmentation to create libraries of nucleotide sequences, followed by amplification with real-time quantitative signal measurement. For characterizing new bacterial species, platforms that enable the analysis of longer DNA fragments (reads), such as PacBio RS (Pacific Biosciences, USA), are recommended. Shorter reads, as offered by Illumina MiSeq (Illumina, USA) or Ion Torrent PGM (Thermo Fisher Scientific, USA), are suitable for drug resistance profiling.

The advent of next-generation sequencing (NGS) platforms significantly augmented the informativeness of fundamental and applied research in diagnosing and epidemiology of infectious diseases, including tuberculosis and mycobacterioses [45].

This technology enables the simultaneous sequencing of numerous different DNA sequences using methods involving restriction, amplification, ligation with fluorescent, chemiluminescent, or chemical signal detection. These procedures are cost-effective, fully automated, miniaturized, high-throughput, require no prior cultivation of the investigated biomaterial samples or DNA cloning, are relatively fast, within a few hours or days, and provide information on both individual isolates and conduct metagenomic studies of macroorganism microbiomes and environmental objects.

Third-generation nanopore sequencing technology, MinION (Oxford Nanopore Technologies), achieves a throughput of up to 30 billion nucleotides per cycle. It allows sequencing of single molecules spanning several thousand bases without the need for amplification, boasting cost-effectiveness and equipment mobility.

There are several different NGS platforms available that can generate long or short sequences. For mycobacterial research, the PacBio platform is widely used [46, 47].

In general, whole-genome sequencing (WGS) methods of individual strains and metagenomic sequencing of bacterial associations within specific biotopes provide information about the entire microbial genome. They allow the identification of practically all types of molecular markers detectable by various genotyping methods. Consequently, they are the most accurate approaches for detecting strain variability and origins. WGS methods offer extensive and in-depth insights at every level and stage of the epidemic process, ranging from the global to the local scale. Additionally, these methods can be used to investigate genetic relationships among different mycobacterial species, the evolution of mechanisms shaping mycobacterial populations, drug resistance, virulence, and immunogenicity of isolates. This applies to both community-acquired and hospital-acquired cases [35, 48, 49].

Furthermore, numerous studies have revealed that from a genetic perspective, the genus Mycobacterium displays high diversity, with the most significant distinctions emerging when comparing genotypes of the Mycobacterium tuberculosis complex (MTBC) and non-tuberculous mycobacteria (NTM). In contrast to many NTM species, MTBC has smaller genome sizes and lacks extrachromosomal genetic elements. Within MTBC, there exists a genetically remarkably homogeneous group, with individual species sharing over 99.7 % genome identity. It is believed that evolutionarily, all species within this group share a common ancestor. Among all pathogenic mycobacteria, the first complete genome sequencing was achieved for Mycobacterium tuberculosis strain H37Rv [3, 50]. The genome size of *M. tuberculosis* is approximately 4.4 million base pairs (around 4000 genes) and is characterized by a high G+C content (up to 65 %). Currently, GenBank contains information on the complete genomes of over 6500 strains of M. tuberculosis, as well as reference strains for all species within the MTBC. It has been established that the human tuberculosis pathogen, due to the unique structure of its genome, exhibits a population structure consisting of distinct genetic lineages. Some of the genetic lineages of *M. tuberculosis* are confined to specific geographic areas, while others, possessing higher virulence and transmissibility, are widespread globally (for example, the Asian Beijing lineage and the Latin American-Mediterranean LAM lineage). However, influenced by various external factors, including pressure from anti-tuberculosis drugs, the pathogen's genotype is continually evolving. Effective infection control demands reliable and specific molecular investigations of the most informative regions of the pathogen's genome [51, 52].

In-depth analysis of the genome of virulent *M. tuberculosis* strains has revealed its high conservation. Approximately 3 % of the genome consists of repetitive insertion sequences (IS), which contribute to DNA polymorphism. Additionally, variable numbers of tandem repeats (VNTR), direct repeats (DR), major polymorphic tandem repeats (MPTR), polymorphic GC-rich repeat sequences (PGRS), and phages (phiRv1, phiRv2) are significant elements associated with pathogenicity. Among the 56 IS loci of different families, the IS6110 sequence is the most commonly encountered. The genomes of other MTBC species also contain various types of short nucleotide repeat sequences (PGRS and MPTR) and direct DR repeats interspersed with variable spacer sequences [47, 53, 54].

Study limitations. The limitation of the study is the narrowing of the consideration of the problem mainly within the limits of the description of molecular technologies for the study of the genome of pathogenic mycobacteria and specifically the causative agent of tuberculosis M. tuberculosis.

The prospects for further research lie in exploring the significance of molecular genome research technologies for the identification of new species of non-tuberculous mycobacteria and for the differential diagnosis of mycobacteriosis.

5. Conclusions

Genomic research technologies form the foundation for the advancement of modern laboratory methods for diagnosing tuberculosis and mycobacterial infections. Currently, the most widely used standardized molecular genetic typing methods for *M. tuberculosis* are based on the detection of mobile or repetitive elements within the bacterial chromosome, such as IS6110 and MIRU/VNTR. These methods provide reliable differentiation of strains, identification of the most epidemiologically significant isolates, detection of laboratory contamination, cases of mixed infections, and differentiation between endogenous and superinfection during disease relapses. For conducting comprehensive global and local analysis of circulating strains and monitoring drug resistance, the use of unrelated genetic markers, such as mutations, deletions, or polymorphisms in the variable spacers of the DR locus in spoligotyping, is also effective. However, the most informative method for molecular analysis remains whole-genome sequencing. Currently, regularly updated public databases of MTBC genotypes are available, and their analysis reveals the presence of more significant genetic variability even among closely related isolates than previously believed.

The widespread application of genomic technologies in the study of mycobacteria will contribute to the effective implementation of the global WHO strategy for the prevention, treatment, and control of tuberculosis, aimed at the complete elimination of this dangerous disease.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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Data availability

The manuscript has no associated data.

References

1. Global tuberculosis report 2020 (2020). World Health Organization, 208. Available at: https://apps.who.int/iris/handle/10665/336069

2. Global tuberculosis report 2022 (2022). World Health Organization, 51. Available at: https://apps.who.int/iris/handle/10665/363752

3. Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D. et al. (1998). Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature, 393 (6685), 537–544. doi: https://doi.org/10.1038/31159

4. Global tuberculosis report 2019 (2019). World Health Organization, 283. Available at: https://apps.who.int/iris/handle/10665/329368

5. Barbova, A. I., Zhurilo, O. A., Trofimova, P. S., Mironchenko, S. V. (2019). Experience of selection, indication and identication of non-tuberculous mycobacteria, got during I National research on study of distribution of drugresistant of tuberculosis in Ukraine. Tuberculosis, Lung Diseases, HIV Infection, 2 (37), 63–71. doi: https://doi.org/10.30978/tb2019-2-63

6. Yang, S., Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. The Lancet Infectious Diseases, 4 (6), 337–348. doi: https://doi.org/10.1016/s1473-3099(04)01044-8

7. Clark, D. P., Pazdernik, N. J. (2016). DNA Synthesis In Vivo and In Vitro. Biotechnology. Applying the Genetic Revolution, 97–130. doi: https://doi.org/10.1016/b978-0-12-385015-7.00004-1

8. Nurwidya, F., Handayani, D., Burhan, E., Yunus, F. (2018). Molecular Diagnosis of Tuberculosis. Chonnam Medical Journal, 54 (1). doi: https://doi.org/10.4068/cmj.2018.54.1.1

9. Chia, J.-H., Wu, T.-L., Su, L.-H., Kuo, A.-J., Lai, H.-C. (2012). Direct identification of mycobacteria from smear-positive sputum samples using an improved multiplex polymerase chain reaction assay. Diagnostic Microbiology and Infectious Disease, 72 (4), 340–349. doi: https://doi.org/10.1016/j.diagmicrobio.2011.12.008

10. Van Crevel, R., Hill, P. C. (2017). Tuberculosis. SECTION 2 Syndromes by Body System: The Respiratory System. Infectious Diseases, Vol. 1, 271–284. doi: https://doi.org/10.1016/b978-0-7020-6285-8.00031-9

11. Percival, S. L., Williams, D. W. (2014). Mycobacterium. Microbiology of Waterborne Diseases. Microbiological Aspects and Risks, 177–207. doi: https://doi.org/10.1016/B978-0-12-415846-7.00009-3

12. Shapovalova, O. V., Pozmogova, S. A., Zavgorodniy, A. I. (2022). Molecular technologies of mycobacterial research. Annals of Mechnikov Institute, 1, 9–20. doi: https://doi.org/10.5281/zenodo.7721843

13. Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children: rapid communication (2020). World Health Organization, 8. Available at: https://apps.who.int/iris/handle/10665/330395

14. Kohli, M., Schiller, I., Dendukuri, N., Yao, M., Dheda, K., Denkinger, C. M., Schumacher, S. G., Steingart, K. R. (2021). Xpert MTB/RIF Ultra and Xpert MTB/RIF assays for extrapulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database of Systematic Reviews, 2021 (1). doi: https://doi.org/10.1002/14651858.cd012768.pub3

15. Tuberculosis laboratory biosafety manual (2012). World Health Organization, 50. doi: https://apps.who.int/iris/handle/10665/77949

16. Pro zatverdzhennia derzhavnykh sanitarnykh norm i pravyl "Orhanizatsiia roboty laboratorii pry doslidzhenni materialu, shcho mistyt biolohichni patohenni ahenty I-IV hrup patohennosti molekuliarno-henetychnymy metodamy" (2008). Hakaz MOZU No. 26. 24.01.2008. Available at: https://zakon.rada.gov.ua/laws/show/z0088-08#Text

17. Gerilovich, A. P., Stegnii, B. T., Zavgorodnii, A. I., Vlizlo, V. V. et al. (2014). Molekuliarno-genetichni metodi diagnostiki u veterinarnii meditcini ta biotekhnologii. Kyiv: ST-Druk, 286.

18. Chauhan, T. (2019). Inverse PCR: Principle, Procedure, Protocol and Applications. PCR Technology. Genetic Education. Available at: https://geneticeducation.co.in/inverse-pcr-principle-procedure-protocol-and-applications/#google_vignette

19. Notomi, T., Mori, Y., Tomita, N., Kanda, H. (2015). Loop-mediated isothermal amplification (LAMP): principle, features, and future prospects. Journal of Microbiology, 53 (1), 1–5. doi: https://doi.org/10.1007/s12275-015-4656-9

20. Notomi, H., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T. (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Research, 28 (12), 63e–663. doi: https://doi.org/10.1093/nar/28.12.e63

21. Kim, J., Park, B. G., Lim, D. H., Jang, W. S., Nam, J., Mihn, D.-C., Lim, C. S. (2021). Development and evaluation of a multiplex loop-mediated isothermal amplification (LAMP) assay for differentiation of Mycobacterium tuberculosis and non-tuberculosis mycobacterium in clinical samples. PLOS ONE, 16 (1), e0244753. doi: https://doi.org/10.1371/journal.pone.0244753

22. Molecular Test "LAMP". Available at: https://www.eiken.co.jp/en/products/lamp/

23. Innovative Tool "TB LAMP". Available at: https://www.eiken.co.jp/en/ourfields/infection/tb_diagnosis/

24. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance (2016). World Health Organization. Available at: https://apps.who.int/iris/handle/10665/249154

25. Caulfield, A. J., Wengenack, N. L. (2016). Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. Journal of Clinical Tuberculosis and Other Mycobacterial Diseases, 4, 33–43. doi: https://doi.org/10.1016/j.jctube.2016.05.005

26. Line probe assays for detection of drug-resistant tuberculosis: interpretation and reporting manual for laboratory staff and clinicians (2022). World Health Organization, 31. https://apps.who.int/iris/handle/10665/354240

27. Freeman, W. M., Robertson, D. J., Vrana, K. E. (2000). Fundamentals of DNA Hybridization Arrays for Gene Expression Analysis. BioTechniques, 29 (5), 1042–1055. doi: https://doi.org/10.2144/00295rv01

28. Manual for selection of molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis (2022). World Health Organization, 29. https://apps.who.int/iris/handle/10665/353596

29. Talbot, E. A., Raffa, B. J. (2015). Mycobacterium tuberculosis. Molecular Medical Microbiology, Vol. 3, 1637–1653. doi: https://doi.org/10.1016/B978-0-12-397169-2.00092-5

30. Adam, M. A. M., Hamdan Ali, H. M., Khalil, E. A. G. (2019). Diagnostic predictive values of the hain genotype MTBDRsl assay in mycobacterial strains isolated from Sudan. Pan African Medical Journal, 32. doi: https://doi.org/10.11604/pamj.2019.32.124.12762

31. Ou, X., Li, Q., Su, D., Xia, H., Wang, S., Zhao, B., Zhao, Y. (2020). A pilot study: VereMTB detection kit for rapid detection of multidrug-resistant mycobcterium tuberculosis in clinical sputum samples. PLOS ONE, 15 (3), e0228312. doi: https://doi.org/10.1371/journal.pone.0228312

32. Algorithm for laboratory diagnosis and treatment-monitoring of pulmonary tuberculosis and drug-resistant tuberculosis using state-of-the-art rapid molecular diagnostic technologies: expert opinion of the European Tuberculosis Laboratory Initiative core group members for the WHO European Region (2017). World Health Organization. Regional Office for Europe. Available at: https://apps.who.int/iris/handle/10665/344108

33. The use of molecular line probe assay for the detection of resistance to isoniazid and rifampicin: policy update (2016). World Health Organization, 56. https://apps.who.int/iris/handle/10665/250586

34. Roychowdhury, T., Mandal, S., Bhattacharya, A. (2015). Analysis of IS6110 insertion sites provide a glimpse into genome evolution of Mycobacterium tuberculosis. Scientific Reports, 5 (1). doi: https://doi.org/10.1038/srep12567

35. Jagielski, T., van Ingen, J., Rastogi, N., Dziadek, J., Mazur, P. K., Bielecki, J. (2014). Current Methods in the Molecular Typing of Mycobacterium tuberculosisand Other Mycobacteria. BioMed Research International, 2014, 1–21. doi: https://doi.org/10.1155/2014/645802

36. Jagielski, T., Minias, A., van Ingen, J., Rastogi, N., Brzostek, A., Żaczek, A., Dziadek, J. (2016). Methodological and Clinical Aspects of the Molecular Epidemiology of Mycobacterium tuberculosis and Other Mycobacteria. Clinical Microbiology Reviews, 29 (2), 239–290. doi: https://doi.org/10.1128/cmr.00055-15

37. Goulding, J. N., Stanley, J., Saunders, N., Arnold, C. (2000). Genome-Sequence-Based Fluorescent Amplified-Fragment Length Polymorphism Analysis of Mycobacterium tuberculosis. Journal of Clinical Microbiology, 38 (3), 1121–1126. doi: https://doi.org/10.1128/jcm.38.3.1121-1126.2000

38. Kassama, Y., Shemko, M., Shetty, N., Fang, Z., MacIntire, G., Gant, V. et al. (2006). An Improved Fluorescent Amplified Fragment Length Polymorphism Method for Typing Mycobacterium tuberculosis. Journal of Clinical Microbiology, 44 (1), 288–289. doi: https://doi.org/10.1128/jcm.44.1.288-289.2006

39. Varun, C. N. (2014). Clustered Regularly Interspaced Short Palindromic Repeats – Crispr. MICROBOIDS. Available at: https://varuncnmicro.blogspot.com/2014/02/clustered-regularly-interspaced-short.html

40. Demay, C., Liens, B., Burguière, T., Hill, V., Couvin, D., Millet, J. et al. (2012). SITVITWEB – A publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology. Infection, Genetics and Evolution, 12 (4), 755–766. doi: https://doi.org/10.1016/j.meegid.2012.02.004

41. Brudey, K., Driscoll, J. R., Rigouts, L., Prodinger, W. M., Gori, A., Al-Hajoj, S. A. et al. (2006). Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiology, 6 (1). doi: https://doi.org/10.1186/1471-2180-6-23

42. Bouakaze, C., Keyser, C., Gonzalez, A., Sougakoff, W., Veziris, N., Dabernat, H. et al. (2011). Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry-Based Single Nucleotide Polymorphism Genotyping Assay Using iPLEX

Gold Technology for Identification of Mycobacterium tuberculosis Complex Species and Lineages. Journal of Clinical Microbiology, 49 (9), 3292–3299. doi: https://doi.org/10.1128/jcm.00744-11

43. Burian, A. N., Zhao, W., Lo, T., Thurtle-Schmidt, D. M. (2021). Genome sequencing guide: An introductory toolbox to whole-genome analysis methods. Biochemistry and Molecular Biology Education, 49 (5), 815–825. doi: https://doi.org/10.1002/bmb.21561

44. Bacterial Diversity Metadatabase BacDive. Available at: https://bacdive.dsmz.de/about

45. The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex: technical guide (2018). World Health Organization, 112. Available at: https://apps.who.int/iris/handle/10665/274443

46. Zhou, X., Wu, H., Ruan, Q., Jiang, N., Chen, X., Shen, Y. et al. (2019). Clinical Evaluation of Diagnosis Efficacy of Active Mycobacterium tuberculosis Complex Infection via Metagenomic Next-Generation Sequencing of Direct Clinical Samples. Frontiers in Cellular and Infection Microbiology, 9. doi: https://doi.org/10.3389/fcimb.2019.00351

47. Anochie, P. I., Onyeneke, E. C., Ogu, A. C., Onyeozirila, A. C., Aluru, S., Onyejepu, N. et al. (2012). Recent advances in the diagnosis of Mycobacterium tuberculosis. GERMS, 2 (3), 110–120. doi: https://doi.org/10.11599/germs.2012.1021

48. Leão, S. C., Martin, A., Meija M, G. I., Palomino, J. C., Robledo, J., da Silva Telles, M. A., Portaels, F. (2004). Practical handbook for the phenotypic and genotypic identification of mycobacteria. Available at: http://hdl.handle.net/1854/LU-7188307

49. Schlossberg, D. (Ed.) (2017). Tuberculosis and nontuberculous mycobacterial infections. Washington: ASM Press, 800. doi: https://doi.org/10.1128/9781555819866

50. Schaaf, H.S., Zumla, A. (Eds.) (2009). Tuberculosis: A Comprehensive Clinical Reference. Philadelphia: Saunders Elsevier, 1046.

51. Balasingham, S. V., Davidsen, T., Szpinda, I., Frye, S. A., Tønjum, T. (2009). Molecular diagnostics in tuberculosis: basis and implications for therapy. Molecular Diagnosis & Therapy, 13 (3), 137–151. doi: https://doi.org/10.1007/bf03256322

52. Scarparo, C., Piccoli, P., Rigon, A., Ruggiero, G., Scagnelli, M., Piersimoni, C. (2000). Comparison of enhanced Mycobacterium tuberculosis amplified direct test with COBAS AMPLICOR Mycobacterium tuberculosis assay for direct detection of Mycobacterium tuberculosis complex in respiratory and extrapulmonary specimens. Journal of Clinical Microbiology, 38 (4), 1559–1562. doi: https://doi.org/10.1128/jcm.38.4.1559-1562.2000

53. da Silva, D.A., de Pina, L.C., Rêgo, A.M., Ferreira, N.V., Redner, P., Caetano, L., Antunes, M.; Tang, Y.-W., Stratton, C. W. (Eds.) (2018). Advances in the Diagnosis of Mycobacterium tuberculosis Infection. Advanced Techniques in Diagnostic Microbiology. Vol. 2: Applications. Springer Nature Switzerland AG. 101–136. doi: https://doi.org/10.1007/978-3-319-95111-9_4

54. European Centre for Disease Prevention and Control. Handbook on tuberculosis laboratory diagnostic methods in the European Union – Updated 2018 (2018). Stockholm: ECDC. Available at: https://www.ecdc.europa.eu/en/publications-data/handbook-tuberculosis-laboratory-diagnostic-methods-european-union-updated-2018

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