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Review Article

Current Understanding of *Mycobacterium tuberculosis* Drug Resistances and Diagnostics in Indonesia: A Review

Ayu Rahayu[1*], Oktaviani Naulita Turnip[2], and Anwar Rovik[3]

[1] *Department of Microbiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. Jl Farmako Utara, Sendowo, Sleman 55281, Yogyakarta, Indonesia*
[2] *Department of Microbiology, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya 78474, Kalimantan Tengah, Indonesia*
[3] *Master Program in Biotechnology, The Graduate School of Universitas Gadjah Mada. Jl Teknika Utara, Caturtunggal, Sleman 55281, Yogyakarta, Indonesia*

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***Corresponding Author:**
Ayu Rahayu,
Department of Microbiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. Jl Farmako Utara, Sendowo, Sleman 55281, Yogyakarta, Indonesia
E-mail addresses: ayurahayu@ugm.ac.id

ABSTRACT

Tuberculosis is a significant global infectious disease cause of mortality, with 25% of the world's population caused by *Mycobacterium tuberculosis* (Mtb). The highest case recorded was 821,200 cases in 2023 over one decade. Drug resistance significantly impacts tuberculosis treatment and has been observed since the first drug discovery, streptomycin. In Indonesia, drug-resistant tuberculosis (DR-TB) is a significant public health issue, with an estimated 24,666 cases recorded in 2022. A comparative literature search was conducted using various articles, including research studies, guidelines, narrative reviews, reports, and meta-analyses, with the inclusion criteria for reference sources including ten years of published articles. Drug resistance in TB can reduce treatment success rate and increase therapy duration. In the result, several general mechanisms proposed for drug resistance in Mtb include efflux pump, enzyme inactivation, permeability barrier, mutations in drug-target genes, and epigenetics. Currently, TB treatment in Indonesia involves combination therapy using three or more types of drugs, such as rifampicin and isoniazid. The International Standard for TB Care (ISTC) serves as the basis for TB diagnosis in Indonesia, which involves a combination of clinical and laboratory tests. Early diagnosis and universal access to the Drug Susceptibility Test (DST) are crucial for TB resistance. In Indonesia, several diagnostic methods, such as sequencing, PCR testing, Gene Xpert, and culture, can be used to identify TB resistance. Understanding the mechanisms of drug resistance and developing effective diagnosis strategies for TB resistance is crucial for managing global infection.

Keywords: *Mycobacterium tuberculosis*; drug-resistant; diagnostic; Indonesia

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INTRODUCTION

The global infection caused by *Mycobacterium tuberculosis* (Mtb) is one of the most significant worldwide infectious disease causes of mortality (Papakonstantinou et al. 2021). Tuberculosis is transmitted through the inhalation process by droplets from the aerosols. The global spread of Mtb is mainly driven by human movement, such as exploration, migration, and trade. Every minute, another three people in the world die of tuberculosis. With more than 8 million new cases of active disease and nearly 1.5 million deaths annually, tuberculosis is a global health emergency of overwhelming proportions (Galagan 2014). According to the WHO, 25% of the world's population is latently infected with TB, providing a large reservoir for future cases of active TB, and it is caused by the emergence of drug resistance, especially multi-drug resistance (Oudghiri et al. 2018; World Health Organization 2018).

Drug resistance has a significant impact on tuberculosis treatment. Several kinds of drug resistance in tuberculosis are single drug resistant (SDR), multidrug-resistant (MDR), and extensively drug-resistant (XDR). In addition, some countries such as India, Italy, South Africa, and Iran have reported that they are resistant to all drugs, known as totally drug-resistant (TDR) (Galagan 2014). In 2012, there were approximately 450,000 new cases of MDR-TB and 170,000 deaths—the highest MDR rates found in Eastern Europe and Central Asia. China and former Soviet Union countries have particularly a high burden of MDR-TB. In 2011, data showed in Belarus that 35% of new patients had MDR-TB, while 75% of patients had been treated for TB (Fenner et al. 2012; Seung, Keshavjee, and Rich 2015).

Drug resistance is a biological condition observed in TB since the first drug discovery, streptomycin (Seung, Keshavjee, and Rich 2015). Drug resistance occurs at the gene level when a mutation arises and impacts the efficacy of antituberculosis treatment (Seifert et al. 2015). The mutation of the Mycobacterium genome can evade the drugs commonly used to inhibit them. Mutation occurs by decreasing the accumulation or inactivation of the drug. In addition, virulence factors, genetic factors of the host, immunity, and incomplete treatment of the patient contribute to the emergence of drug-resistant TB (Khawbung, Nath, and Chakraborty 2021). The accurate and timely diagnosis of multidrug-resistant tuberculosis (MDR-TB) is crucial for effective patient management and public health. Misdiagnosis or delayed diagnosis can lead to treatment failure, the development of extensively drug-resistant TB (XDR-TB), increased mortality, and the spread of drug-resistant strains within communities. For this reason, a better understanding of how biological factors might contribute to drug resistance and effectively diagnose MTb is needed.

METHODS

A comparative literature search was conducted using PubMed, Scopus, Web of Science, and Google Scholar databases. The keyword terms included "*Mycobacterium tuberculosis*," "Cases," "Diagnosis," and "Resistance" with the following combination: *Mycobacterium tuberculosis* AND cases; *Mycobacterium tuberculosis* AND diagnosis; *Mycobacterium tuberculosis* AND resistance. Various articles include research studies, guidelines, narrative reviews, reports, and meta-analyses. Inclusion criteria for reference sources include ten years of published articles in English and Indonesia, and available free-accessed data. Conducting a literature review and compiling the results into this review is the responsibility of all authors.

RESULTS AND DISCUSSION

TB Cases in Indonesia Over a Decade

Tuberculosis continues to be a significant public health issue in Indonesia. According to recent data, Indonesia has one of the highest tuberculosis burdens in the world. It is estimated that there are approximately 845,000 new cases of tuberculosis in Indonesia each year. According to World Health Organization (2022), Indonesia consistently ranks among the top 20 countries with the highest TB cases globally. In 2010, Indonesia reported 343,000 new cases of TB, which increased to 433,000 cases in 2019, indicating a steady rise in the disease cases (Figure 1). An estimated 969,000 cases of tuberculosis (TB) are reported in Indonesia, or one case every 33 seconds. It represents an increase of 17% from 2020, or 824,000 cases. In Indonesia, there are 354 cases of tuberculosis (TB) for every 100,000 persons or 354 cases of TB for every 100,000 people (KNCV, 2022). Considering that 969,000 TB cases occur annually, 724,309 instances (75%), or 25% of cases, have been reported; the remaining 25% remain unreported, undiscovered, or unreached. The anticipated number of MDR/RR TB cases in 2021 is 28,000, or 10 cases per 100,000 people; this represents a 17% increase from 24,000 cases in 2020 and a 15% rate per 100,000 people. The number of TB-DR cases discovered was 12,531, with 51% coverage (Ministry of Health RI 2023)

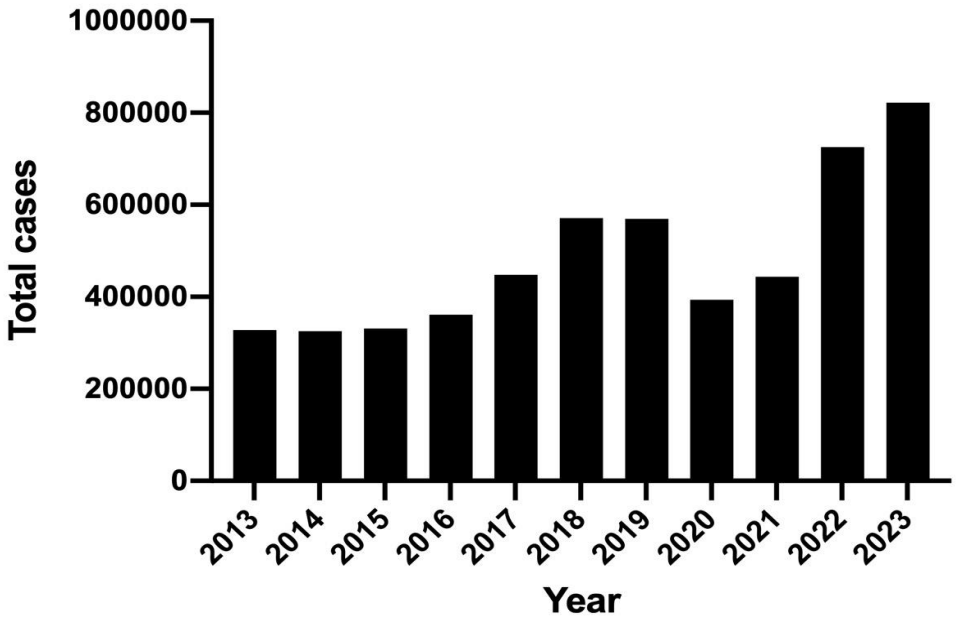

Figure 1. TB cases in one decade (2013-2023). Based on available data, 2023 recorded the highest number of tuberculosis cases (821,200) found over the decade (Ministry of Health RI 2023).

The province with the highest number of TB cases in Indonesia is West Java, with 43,000 cases reported in 2019. It is followed by East Java, with 34,000 cases, and Central Java, with 28,000 cases. The provinces with the lowest TB cases are Papua, with 1,000 cases, and West Papua, with 1,500 cases (World Health Organization 2022). The high incidence of TB in West Java is attributed to several factors, including poverty, malnutrition, and limited access to healthcare services. The province has a large population and a high level of urbanization, which can contribute to spreading the disease. Indonesia's population in 2022 is 274,859,094 people, consisting of 137,890,954 men and 136,968,140 women. The provinces with the largest population are West Java (18.4%), East Java (14.7%), and Central Java (12.8%), while the province with the lowest population is in the provinces of West Papua (0.18%), South Papua (0.19%), and Southwest Papua (0.2%) (Ministry of Health RI 2023). Additionally, the province has a high prevalence of HIV/AIDS, which can increase the risk of TB infection (Widyastuti, Riyanto, and Fauzi 2018). In contrast, the provinces with the lowest number of TB cases, such as Papua and West Papua, have a lower population density and a higher level of ruralization. These provinces also have limited access to healthcare services, making diagnosing and treating TB cases more complicated.

The disease affects people of all ages and genders, but the incidence of TB varies across different age groups and sexes. According to the World Health Organization (2018), the adult population is more exposed to cases of illness due to TB, with the highest cases occurring in the population aged 5-14 years. It could be because the population under 20 is more likely to get sick when infected (Suryawan. S and Siagian 2021). In terms of gender, more TB cases occur in men

than women. A study conducted in Bandar Lampung City found that 56.74% of TB patients were male, while 43.26% were female. It is in line with the 2021 Indonesia Health Profile data, which shows that the number of male TB patients was 57.75% and the number of female TB patients was 42.25% (Tuntun, Aminah, and Yusrizal Ch 2022).

The Ministry of Health has used an intensive tracking methodology called IK (Contact Investigation) since 2018. This model is designed for individuals who have close contact with TB patients. Each community health center uses the involvement of local community organizations and health cadres to carry out IK activities. In terms of the Case Index aim that IK has implemented, Nusa West Southeast (69%), Bali (69%), Central Java (62%), East Java (50%), and Bangka Belitung (48%) are the provinces that have scored the highest. Maluku (13%), DKI Jakarta (17%), and North Maluku (11%) were the provinces that attained the lowest IK Case Index targets (Ministry of Health RI 2023). The investigation of tuberculosis (TB) cases in Indonesia has been hindered by several barriers. One of the significant challenges is the need for more awareness among the general public regarding the importance of seeking medical attention for TB symptoms. It is evident in the fact that many patients with TB do not seek medical care until they have experienced severe symptoms, which can lead to a delay in diagnosis and treatment. Another significant barrier is the limited access to healthcare services, particularly in rural areas. A study conducted in Bandar Lampung City found that most TB patients were from urban areas, indicating that rural areas have limited access to healthcare services (Tuntun, Aminah, and Yusrizal Ch 2022).

Drug Resistance in MTb

In Indonesia, drug-resistant tuberculosis (DR-TB) is very high; in 2022, there were approximately 24,666 cases recorded (Ministry of Health RI 2023). The leading causes of DR-TB in Indonesia include inadequate treatment, poor adherence to treatment, and the lack of effective treatment options. A study reported that the leading cause of DR-TB was insufficient treatment, with 96.2% of patients experiencing inadequate treatment. Moreover, the prevalence of DR-TB was higher among patients who had a history of TB treatment failure (Nugrahaeni, 2015).

Mycobacterium tuberculosis, the bacteria that causes tuberculosis (TB), can develop resistance to the drugs used to treat it. This drug resistance is a significant public health threat. Increased MTB resistance can reduce treatment success rate and increase therapy duration (Falzon et al. 2013). Several factors influence the emergence of MTB resistance, including duration of therapy, compliance with drug consumption by patients, access to treatment, and economic conditions (Khawbung, Nath, and Chakraborty 2021). Therefore, understanding resistance mechanisms is essential for developing antibiotic use strategies.

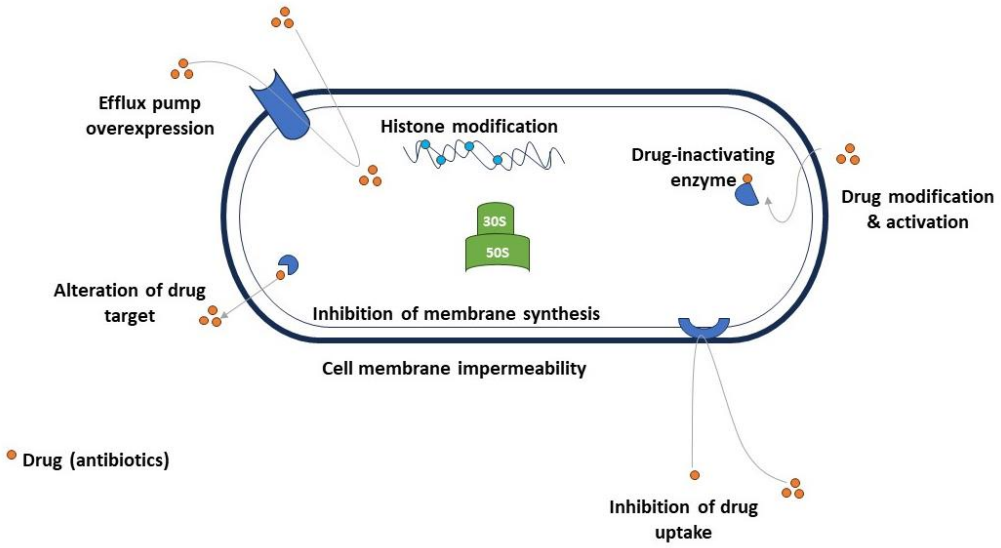

Figure 2. General proposed mechanisms of drug resistance in MTb (designed from various sources)

The development of drug resistance in MTb is a complex process involving multiple mechanisms (Cohen, Bishai, and Pym 2014; Khawbung, Nath, and Chakraborty 2021). There are several general mechanisms proposed for drug resistance in MTb (Figure 2), as follows:

Efflux Pump

This protein channel plays an essential role in bacterial cells' metabolism and physiological conditions, such as signaling molecules and facilitating the transport of nutrients to toxic compounds. Efflux pumps pass through the inner and outer membranes to pump drugs out of bacterial cells (Abraham 2020). Increased expression of efflux pumps may make it difficult for drugs to accumulate within the bacteria to levels high enough to be effective. Long-term exposure to low doses of drugs can activate transporter proteins that play a role in efflux, the process of removing drugs that accumulate in cells (Miotto, Cirillo, and Migliori 2015). The study reported the outer membrane channel protein CpnT in MTb, which has a dual role in nutrient absorption and selective susceptibility to antibiotics (Danilchanka et al. 2015).

Enzyme Inactivation

Some drugs work by inhibiting enzymes that are important for bacteria's survival. MTb can develop resistance by producing enzymes that modify antibiotics, rendering them inactive. The β-lactamase enzyme produced by MTb can inhibit β-lactam antibiotics, resulting in resistance (Flores, Parsons, and Pavelka, 2005).

Permeability Barrier

The MTb cell membrane envelope comprises three components: the plasma membrane, the wall, and the capsule. Cell walls can act as a barrier to drug penetration. Cell wall MTb consists of peptidoglycan bound to arabinogalactan, which is covalently bound to mycolic acid (Sharma et al. 2016). The arabinogalactan layer and mycolic acid complex ensure low permeability for the absorption of drug components (Miotto, Cirillo, and Migliori 2015). Antibiotics accumulate around cells, so MTb can slowly detoxify antibiotic components. An in silico study showed that the content of Rv1973 and Rv1698 in the MTb membrane plays a role in the drug resistance mechanism (Singh et al. 2008). It may increase the risk of phenotypic resistance, leading to multidrug resistance.

Mutations in Drug-Target Genes

Mutations can change the structure of the drug's target protein and the antibiotic's target location or interfere with the drug's activation pathway. For example, mutations in the rpoB gene can inhibit the binding of the drug rifampicin. Likewise, mutations in the katG gene can prevent the conversion of the drug isoniazid to its active form (Khawbung, Nath, and Chakraborty 2021).

Epigenetics

Although less explored, epigenetic modifications such as DNA methylation and histone modifications may play a role in the development of antibiotic resistance. MTb was reported to modify DNA-binding protein (histone), thereby downregulating the transcription of the katG gene (Niki et al. 2015).

Currently, TB treatment is carried out with combination therapy, using three or more types of drugs. Active TB patients are generally given rifampicin, isoniazid, pyrazinamide, and ethambutol for the first two months and then continued with rifampicin and isoniazid for four months (Muttaqin, Z. et al. 2023). Here, we highlight the most common drugs in Indonesia: rifampicin and isoniazid. Rifampicin (RIF) is considered the most effective first-line TB treatment. MTb resistance to rifampicin is a significant problem, especially during long-term treatment. Resistance to rifampicin occurs mainly due to mutations in the rpoB gene. Mutations in the ropB gene can cause changes in RNA polymerase structure, decreased drug binding affinity, increased efflux pump activity, and changes in drug metabolism (Goossens, Sampson, and Van Rie 2020; Singh et al. 2017). Rifampicin attaches to the β subunit of RNA polymerase, inhibiting the RNA chain polymerization process. The four polypeptides in RNA polymerase, namely the α, β, β', and σ subunits, are encoded by the rpoA, rpoB, and rpoC genes (Khawbung, Nath, and Chakraborty 2021). These genes are conserved in MTb bacteria. (Miotto et al. 2018) reported mutations at codons 426-452 in rpoB were associated with MTb resistance to rifampicin. (Muttaqin, Z. et al. 2023) reported that rpoB gene

mutations at codon 531 cause high levels of resistance in MDR Tuberculosis, followed by codons 526 and 516. The ripA and ripB genes encode peptidoglycan in the MTb cell wall. (Martinelli and Pavelka 2016) reported that ripA gene mutations can increase antibiotic susceptibility. However, the ripA gene mutation in A701G causes localization of peptidoglycan hydrolase, thereby causing sensitivity to β-lactam antibiotics.

Isoniazid (isonicotinyl hydrazine, INH) has high sensitivity in the treatment of TB. The drug isoniazid enters MTb cells as a prodrug, so it requires activation by the enzyme. The drug isoniazid is stimulated by the catalase or peroxidase enzyme encoded by the katG gene (Rawat, Whitty, and Tonge 2003). MTb resistance to the drug isoniazid is associated with mutations in the katG, kasA, ahpC, and inhA genes. The katG gene mutation in S315T increases MTb resistance to isoniazid (Fenner et al. 2012). The study reported point mutations in the katG gene at G1388T, G2161A, C1061T, and G1261A in isoniazid-resistant isolates. In silico studies show that mutations in the katG gene at S315T or S315N are involved in forming hydrogen bonds between isoniazid and amino acid residues (Purkan et al. 2018). In the wild-type katG gene, this hydrogen bond is not formed, so it can encourage the formation of toxic free radicals in MTb cells (Jena et al. 2014).

Activated isoniazid produces nicotinoyl NAD, then binds to enoyl-ACP-dependent NADH reductase encoded by the inhA gene (Khawbung, Nath, and Chakraborty 2021). This condition disrupts the process of mycolic acid synthesis in bacterial cell walls so that MTb cell growth is disrupted. However, mutation of the inhA gene reduces the binding between inhA and NADH, resulting in overexpression of the inhA gene (Machado et al. 2013). InhA protein is an enzyme that catalyzes the reduction of long-chain trans-2-enoyl-ACP in synthesizing type II fatty acids for the meromicolic acid pathway. Meromicolic acid is the primary material for mycolic acid formation, the main component of MTb cell walls (Joshi, Kandari, and Bhatnagar 2021).

Overexpression of genes encoding efflux proteins also has an essential role in resistance to isoniazid. The study conducted knockout experiments on the inhA gene on isoniazid-resistant MTb bacteria (Colangeli et al. 2020). The results showed that the inhA gene plays a role in efflux pump activity. The overexpressing efflux genes in isoniazid-resistant isolates. However, there are no known mutations related to isoniazid resistance. This suggests a role for efflux pumps (Rv0194 and Rv0507) in isoniazid resistance (Narang et al. 2017).

Multidrug resistance is defined by MTb resistance to the most common drugs, i.e., rifampicin and isoniazid. Following this condition, bedaquiline is highly recommended by the FDA for the long-term treatment of patients with RR/MDR TB. Bedaquiline has higher inhibitory power among several antibiotics, with a lower minimal inhibitory concentration (MIC). Bedaquiline inhibits ATP synthesis in MTb cells (Andries et al. 2014; Li, Sun, and Zhang 2019). Bedaquiline resistance is associated with mutations in the atpE gene (Setyawan et al. 2023). In

addition, bedaquiline resistance is also associated with linezolid resistance encoded by the rplC and rrl genes, as well as cross-resistance to clofazimine in the pepQ, Rv0678, and Rv1979c genes (Andries et al. 2014; Li, Sun, and Zhang 2019).

A study by Rukmana, Gozali, and Erlina (2024), found that the Rv1979c gene mutation was the most frequently detected in MTb isolates. This mutation results in amino acid changes in Asp286Gly and Leu393His. The mutations in the atpE gene cause changes in various amino acids, including amino acids at sequences 28, 61, 63, and 66 (Andries et al. 2014). These changes shorten the amino acid sequence and can affect the structure of the rotor ring of APTase, which plays a role in ATP production (Giraud-Gatineau et al. 2020; Zhang et al. 2019). Activation of the efflux pump system is considered the first step towards broader resistance levels. The study from Setyawan et al. (2023), concluded that mutations in Rv0678 alter the efflux of the MmpS5-MmpL5 repressor pump, which promotes primary resistance to bedaquiline. The unique mutations of amino acid insertions and deletions in the transmembrane transport protein MmpL5 (Rv0676) among drug-resistant Mtb (Farnia et al. 2024). Thus, using efflux pump inhibitors may increase the activity of the drug bedaquiline against MTb cells (Martin et al. 2020).

Diagnosis of MTb in Indonesia

The International Standard for TB Care (ISTC), created by a global professional organization, serves as the basis for TB diagnosis in Indonesia and is approved by the Indonesia Doctors Association and the National Tuberculosis Control Programme. The diagnosis of TB in Indonesia involves a combination of clinical and laboratory tests (Safithri 2011).

Table 1. Study of drug resistance in TB patients in Indonesia

Study Area	Resistant to	Detection Method	Reference
Banten	Rifampicin	GeneXpert	(Christopher, Cucunawangsih, and Widysanto 2019)
Java	Rifampicin, isoniazid, streptomycin, and ethambutol	WGS	(Tania et al. 2020)
West Java	Rifampicin, isoniazid, bedaquiline, clofazimine, and linezolid	WGS, GeneXpert	(Lidya Chaidir et al. 2019; Rukmana, Gozali, and Erlina 2024; Soeroto et al. 2019)
Papua	Rifampicin, isoniazid, ethambutol, streptomycin, and pyrazinamide	WGS	(L. Chaidir et al. 2015; Maladan et al. 2021)
South Halmahera	Rifampicin	GeneXpert	(Dewi 2020)
Makassar	Rifampicin and streptomycin	PCR, Multiple Allele Specific Polymerase Chain Reaction (MAS-PCR)	(Muttaqin, Z. et al. 2023; Umar et al. 2020)
Surabaya	Rifampicin and bedaquiline	PCR, Sanger sequencing, GeneXpert	(Koentjoro et al. 2021; Mertaniasih et al. 2021; Setyawan et al. 2023)

In Indonesia, laboratory tests used for TB diagnosis include sputum smear microscopy, culture, Interferon-gamma release assay (IGRA), serologic test, Gene Xpert MTB/RIF assay, Loop-mediated isothermal amplification (LAMP), polymerase chain reaction (PCR), and next generation sequencing (NGS). The effectiveness of the subsequent stage of TB therapy implementation depends significantly on the diagnosis's accuracy.

Microscopy method

Diagnosing Tuberculosis (TB) in Indonesia using the microscopic method is crucial in identifying the disease. The microscopic method involves examining sputum samples under a microscope to detect the presence of Mtb bacteria. This method is widely used in Indonesia due to its simplicity, low cost, and high sensitivity (Safithri 2011). A study conducted in Indonesia found that the microscopic method effectively diagnosed TB in rural areas with limited access to laboratory facilities. The microscopic method involves staining sputum samples with Ziehl-Neelsen stain, which allows for the visualization of MTB bacteria under a microscope. The stain combines acid-fast dyes that bind to the bacterial cell wall, making it visible under a microscope (Noviyani 2020).

Culture

Culture is a gold standard for diagnosing mycobacterial infections. Mtb bacteria can be identified in sputum samples by cultivating it in controlled conditions. It is commonly used in Indonesia due to its high sensitivity and specificity in identifying tuberculosis (Susilawati and Larasati 2019). In Indonesia, the culture method is used to confirm the diagnosis of tuberculosis (TB) in conjunction with other diagnostic techniques like microscopy and genetic testing. A study conducted in Indonesia found that combining culture and microscopy was more effective in diagnosing TB (Noviyani 2020).

Lowenstein-Jensen (LJ) solid medium is a widely used method for culturing MTb. The LJ medium is composed of a complex of organic substances, including egg, potato starch, and other substances with varying compositions, as well as mineral salts, asparagine, glycerol, and malachite green. The cultures are incubated at 37°C, and growth is observed. Colonies of Mtb are identified by their rough, crumbly, waxy, buff-colored appearance, which develops 2-3 weeks after inoculation. The colony with doubtful morphology was confirmed by ZN staining (Kumari et al. 2020). It is recommended that the tubes of LJ medium that have been inoculated with bacteria be positioned at an angle for at least 24 hours to distribute the specimen evenly across the surface of the medium.

The MGIT medium is a liquid medium that supports the growth of MTB, allowing for the detection of bacteria in sputum samples. MGIT consists of a liquid broth medium, which gives better and faster bacterial growth. It contains 7 mL of modified Middlebrook 7H9 broth base. A growth supplement MGIT OADC (Oleic acid, Albumin, Dextrose, and Catalase), essential for the growth of many mycobacteria, is added to make the medium complete. Each tube was inoculated with 0.5 mL of the processed specimen. Then, the tubes were kept in the MGIT 960 instrument at 37°C and were monitored automatically after every hour for the increase in fluorescence for a maximum of six weeks. Any sample that shows a growth signal is taken out of the instrument (Kumari et al. 2020). From the positive tube, a smear preparation is done for microscopic examination of AFB.

Interferon-gamma release assays (IGRAs)

IGRAs measures the level of interferon-gamma (IFN-γ) released by T-cells in response to the presence of Mtb antigens. IGRAs are used in Indonesia to diagnose latent TB infection (LTBI), which is a condition where the patient has been infected with MTB but does not show symptoms of active TB. IGRAs are not used to diagnose active TB, as they do not detect the presence of MTB bacteria in the body. In Indonesia, IGRAs are used in conjunction with other diagnostic tests, such as the tuberculin skin test (TST), to confirm the diagnosis of TB. IGRAs are considered a more specific and sensitive test than TST, especially in populations vaccinated with BCG (Adilistya 2016).

Serologic Test

Tuberculosis (TB) diagnosis in Indonesia using serologic tests, specifically the tuberculin skin test (TST). TST is a widely used diagnostic method that involves injecting a small amount of tuberculin, a protein derived from Mtb, into the patient's skin. The TST method works by measuring the reaction of the patient's immune system to the tuberculin protein. If the patient is infected with MTB, their immune system will react to the protein by producing a skin reaction, typically within 48-72 hours after injection. TST is used to diagnose latent TB infection (LTBI), a condition where the patient is infected with MTB but does not show symptoms of active TB (Adilistya 2016; Farlina et al. 2016).

A study conducted in Indonesia found that TST was effective in diagnosing LTBI in children, with a sensitivity of 83.3% and a specificity of 92.3%. Another study found that TST was less effective in diagnosing active TB in adults, with a sensitivity of 50% and a specificity of 80%. The limitations of TST include its low sensitivity and specificity, particularly in populations vaccinated with BCG. Additionally, TST can produce false positive results in individuals exposed to non-TB mycobacteria (Tspot, 2024).

Gene Xpert MTB/RIF assay

GeneXpert MTB/RIF is a molecular diagnostic test that detects Mtb complex and resistance to rifampicin (RIF) in a single test. The GeneXpert MTB/RIF test is a rapid diagnostic test that can provide results within 2 hours, making it a valuable tool for early diagnosis and treatment of TB. A study conducted in Indonesia found that the GeneXpert MTB/RIF test was practical in diagnosing TB in patients with a sensitivity of 92.3% and a specificity of 98.3%. Another study found that the test was also practical in diagnosing TB in patients with a history of TB exposure, with a sensitivity of 95.5% and a specificity of 97.5% (Novianti, Simarmata, and Lolong 2020; Putri and Hartono 2024). Expert MTB/RIF can identify borderline rifampicin resistance, typically linked to minor levels of rifampicin resistance. However, when deficient bacteria levels are present, specific sequences on the MTB/RIF probes may not amplify adequately, leading to probe attachment failure and inaccurate identification of rifampicin resistance. Moreover, the absence or delay in detecting specific probes like B or E can produce false-positive results for rifampicin resistance by Expert MTB/RIF (Karuniawati et al. 2023).

Loop-mediated isothermal amplification (LAMP)

LAMP is a molecular diagnostic test that amplifies DNA targets in a single reaction. It allows for rapid and sensitive detection of TB, with a sensitivity of 87.5% and a specificity of 95.6%. The LAMP assay provides a reliable and accurate choice for TB diagnostic testing in resource-limited settings or where advanced PCR or cultural methods are unavailable (Juliasih 2020).

Polymerase Chain Reaction (PCR)

PCR is a susceptible and specific method for detecting MTB DNA in samples including real-time PCR and Line Probe Assay (LPA) method. It is used in Indonesia because it detects TB quickly, typically within 2 hours. The PCR method involves amplifying specific DNA sequences of MTB using primers and probes. This process allows for detecting MTB DNA in samples, even in the presence of other microorganisms (Kurniati, Suameitra Dewi, and Purwani 2019).

Sequencing

Sequencing is a recent development that has shown promising results in improving the accuracy and speed of TB diagnosis. It is a high-throughput technology that allows for the simultaneous analysis of multiple genetic regions, enabling the detection of multiple genetic mutations and variations in a single test. Sequencing technology has demonstrated exceptional potential for reliable and comprehensive resistance prediction. Moreover, the most complete method of molecular-based DST is provided by whole genome sequencing (WGS), which enables analysis of all mutations that might provide medication resistance to the infecting organism (Dookie et al. 2022). Otherwise, high-quality sequence data enables the precise identification of mutations linked to first-line and second-line drug resistance. Mutations in genes associated with drug resistance in Mtb were identified from the entire genome, utilizing TB Profiler. TB Profiler exhibited complete consistency (100%) with phenotypic DST results for INH, RIF, STR, ETB ETH, and fluoroquinolones (Maladan et al. 2021). Based on the above explanation regarding the types of diagnostic methods for drug-resistant TB, we summarized the diagnostic tools recommended for detecting TB resistance in Indonesia.

Table 2. Recommendation Diagnostic for Detecting DR-TB in Indonesia

Assay	Material	Advantages	Disadvantages
Culture Susceptibility Testing: LJ and MGIT	Sputum, bronchoalveolar lavage, tissue biopsy	Able to provide quantitative data on bacterial growth and test a wide range of drugs, suitable for low-resource settings	Requiring biosafety level 3 (BSL-3) laboratory facilities and consuming a lot of time
Gene Xpert	Sputum and bronchoalveolar lavage	Rapid (results in about 2 hours), high sensitivity and specificity, Its closed amplification system minimizes the potential for cross-reactions.	Expensive equipment and cartridges, limited to types of resistance
PCR Assay: Real time PCR and Line Probe Assay (LPA)	Sputum, bronchoalveolar lavage, tissue biopsy	Rapid (results in 1-2 days) and able to detect resistance to multiple drugs	Requires skilled personnel and expensive equipment, Limited to known mutations
Sequencing: WGS and NGS	Cultured isolates, sputum (with high bacterial load)	WGS: Comprehensive detection of all resistance-related mutations and Provide detailed genetic information. NGS: Focuses on specific resistance genes, reducing cost and complexity, faster than WGS	Requires specialized equipment and expertise, Expensive and requires advanced bioinformatics support, Longer turnaround time compared to rapid tests

Early diagnosis and universal access to DST are required by the WHO End TB Strategy. Lack of funding, delays in enhancing and implementing TB diagnostic policies, a lack of qualified human resources and a high turnover rate, substandard facilities in laboratories and health systems, and inadequate protocols for biosafety and equipment maintenance represent some of the factors contributing to the slow progress of technology development for DR-TB detection (World Health Organization 2018). Stronger political commitment along with coordinated measures to develop the health system that are specific to the needs of each nation are necessary to overcome all of these challenges.

The most comprehensive are new instruments that make use of molecular technology, including tests for nucleic acid amplification. Tests that identify TB, drug resistance, or both TB and drug resistance are among the technologies now under development. These include next-generation sequencing for the simultaneous identification of many resistance-conferring mutations and microarray-based multiplexing diagnostic tools (Gilpin, Korobitsyn, and Weyer

2016). National TB programs are encouraged to make the most of the tests that are now available for identifying drug resistance and to strengthen laboratory networks at the national level immediately as feasible, including efficient systems for referring patients and specimens.

CONCLUSIONS

The recent development of drug resistance in Mtb and the significant increase in TB incidence worldwide present severe challenges to the treatment of TB and global public health. The combinations of intrinsic and acquired drug resistance mechanisms, including efflux pump, enzyme inactivation, permeability barrier, mutations in drug-target genes, and epigenetics, render the *Mtb* cells resistant to most antibiotics.

The only diagnostic test suitable for use at lower health system levels that the WHO still recommends is Xpert MTB/RIF, which can detect TB and rifampicin resistance simultaneously. Since new technologies appropriate for use at the point of care are unlikely to be developed shortly, National TB programs are urged to make the most of the tests currently available to detect drug resistance and to strengthen national laboratory networks—which include efficient mechanisms for referring patients and specimens—as quickly as possible.

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