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# *MOLECULAR DETECTION AND CHARACTERIZATION OF THE fbpA GENE IN MULTI DRUG RESISTANT (MDR) Mycobacterium tuberculosis ISOLATES FROM MAKASSAR*

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***Abstract***

***Antibiotics have been widely used to treat tuberculosis (TB), yet the emergence of drug-resistant strains remains a serious global concern, highlighting the need for new molecular biomarkers to assess treatment success more accurately. Mycobacterium tuberculosis adheres to host tissues through the fbpA gene, which plays a vital role in bacterial virulence and persistence. The novelty of this study lies in exploring the fbpA gene as a potential biomarker for evaluating TB treatment response, differing from previous research that mainly targets drug-resistance genes such as rpoB, katG, or inhA. This study offers a new perspective by focusing on a virulence-related gene that may reflect bacterial survival and therapy outcomes, particularly in Indonesian clinical samples where such data remain scarce. The study aimed to assess the potential of the fbpA gene as a treatment biomarker by detecting a 900 bp fragment using molecular techniques. Ten sputum samples were collected from the Tuberculosis Unit of HUM-RC, Hasanuddin University Hospital, Makassar, and underwent sputum decontamination, Ziehl-Neelsen staining, acid-fast bacilli examination, DNA isolation, PCR amplification, and gel electrophoresis. The results showed the presence of DNA bands in all samples, with variations in thickness and intensity corresponding to DNA concentration. These findings suggest that the fbpA gene could serve as a rapid and reliable molecular biomarker for monitoring bacterial load and evaluating treatment efficacy, supporting the development of more precise diagnostic tools for multidrug-resistant TB management.***
***Keywords: Electroforesis; fbpA gene; MDR-TB; PCR.***

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## 1. INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the deadliest infectious diseases in the world (1)(2). Prior to the COVID-19 pandemic, TB had overtaken HIV/AIDS as the leading cause of death from a single infectious agent. In 2021, TB caused approximately 1.6 million deaths an increase from the previous year with Indonesia ranking second globally in TB incidence after India. According to the World Health Organization (WHO), an estimated 10.6 million people were infected with TB in 2021, representing a 4.5% increase from 2020. Without treatment, TB has a mortality rate of around 50% within the first year. Indonesia is among the 30 countries classified by the WHO as having a high burden of TB cases. According to the Ministry of Health of the Republic of Indonesia, the Southeast Asia region accounts for nearly half of the global TB burden, with 4.82 million cases or 45.4% of the total. Eight countries are responsible for approximately 66% of global TB cases, with Indonesia (9.2%) ranking second after India. Furthermore, the 2022 Global TB Report stated that there were 969,000 TB cases in Indonesia in 2021 (3)(4). Based on data from the Makassar City Health Office, there were 3,250 TB cases reported in 2020 with a treatment success rate of 85%, and the number increased to 3,911 cases in 2021.

The use of antibiotics is a common and widely accepted treatment for TB. However, in some cases, antibiotic resistance develops in *M. tuberculosis* due to the accumulation of mutations in the genes targeted by antibiotics or due to altered drug titration (5). TB patients who are non-compliant with their treatment regimen are particularly vulnerable to developing antibiotic resistance, which may lead to MDR-TB (Multi-Drug-Resistant Tuberculosis). MDR-TB occurs when the bacteria become resistant to rifampicin and isoniazid, the two most powerful first-line anti-TB drugs. In more severe cases, TB may progress to XDR-TB (Extensively Drug-Resistant Tuberculosis), a condition in which the bacteria are resistant not only to rifampicin and isoniazid, but also to fluoroquinolones and at least one of the three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) (6).

Treatment biomarkers utilizing specific techniques in MDR-TB patients are used to determine whether the patient has fully recovered. Identifying treatment biomarkers in MDR-TB patients is crucial for evaluating therapeutic effectiveness. One gene with potential as a treatment biomarker is the *fbpA* gene, which plays a role in cell wall synthesis and the adhesion of *Mycobacterium tuberculosis* to alveolar macrophages. This gene encodes the Ag85A protein (Antigen 85 complex A), which has acyltransferase activity and contributes to the formation of mycolic acid, a key component in bacterial virulence and resistance (7)(8). In addition, Ag85A acts as a fibronectin-binding adhesin that facilitates the

attachment of M. tuberculosis to host tissues, which is critical for infection and persistence (9). These dual roles in in cell-wall biosynthesis and host-cell adhesion provide a strong rationale for selecting the fbpA gene as a potential treatment biomarker. Several studies have also reported the immunodominance of Ag85A and its application in vaccine research, further supporting its relevance for diagnosis and treatment monitoring. Overall, the *fbpA* gene, which encodes Antigen 85A (Ag85A), is not only immunodominant but also directly involved in the pathogenicity of *Mycobacterium tuberculosis* (10). This study aims to isolate and identify the *fbpA* gene from MDR-TB patients in Makassar City using PCR techniques. The process involves amplification of *Mycobacterium tuberculosis* DNA and visualization of the results via electrophoresis. The presence of the *fbpA* gene is expected to serve as a molecular indicator (treatment biomarker) for evaluating the success of TB treatment (whether the patient has recovered or not).

## 2. METHODS

This study was a descriptive laboratory research employing a molecular approach. Sputum samples were collected from 10 patients diagnosed with Multidrug-Resistant Tuberculosis (MDR-TB) at the Tuberculosis Unit, Hasanuddin University Medical Research Center (HUM-RC), Universitas Hasanuddin Hospital, Makassar, along with one negative control sample from a healthy individual. Ethical approval was not required, as anonymized specimens from routine diagnostic procedures were used without any patient-identifiable information. All procedures were conducted under appropriate biosafety protocols, and patient selection followed the Indonesian Ministry of Health (2022) guidelines.

Sputum samples were collected in the early morning using sterile containers, then decontaminated with 4% NaOH and 2.9% Na-citrate (1:1) inside a Biological Safety Cabinet (BSC). Samples were homogenized, incubated, centrifuged, and washed with Phosphate Buffer Saline (PBS), followed by Ziehl-Neelsen (ZN) staining and Acid-Fast Bacilli (AFB) examination in accordance with IUATLD and WHO standards.

DNA isolation was performed using a Geneaid Biotech Ltd. kit, including heating, addition of proteinase K and Gel Sample Buffer (GSB), and purification through a Genomic DNA (GD) column. The extracted DNA was stored at −20°C. Amplification of the fbpA gene was carried out using the PCR technique with specific primers (forward 5’-GATCGCTAGCATGGGTGAATTACGGTTG-3’ and reverse 5’-TATCTCGAGCGGCACGCTATCCCA-3’), both validated using BLAST analysis. PCR was conducted for 30 cycles with an annealing temperature of 56.8°C.

PCR products were analyzed by 2% agarose gel electrophoresis in Tris-Borate-

EDTA (TBE) buffer with ethidium bromide (EtBr) staining and visualized under a UV transilluminator. The appearance of a ~900 bp DNA band confirmed successful amplification of the fbpA gene.

## 3. RESULTS AND DISCUSSION

### Results

Sample Characteristics

This study utilized sputum samples from MDR-TB patients, categorized based on Acid-Fast Bacilli (AFB) test results. These characteristics are crucial to ensure that the samples used truly originate from cases with high drug resistance and are appropriate for evaluating the presence of the *fbpA* gene. Additionally, one negative control sample from a healthy individual was included to confirm the specificity of the results.

Table 1 presents the list of sputum samples used in this study, consisting of 10 samples from MDR-TB patients and 1 negative control. The samples were obtained from the Tuberculosis Unit of HUM-RC, Universitas Hasanuddin Hospital, and categorized according to the results of AFB examination.

Table 1. List of putum samples obtained from the Tuberculosis Unit of HUM-RC, Universitas Hasanuddin Hospital.

| No. | Sample code | category |
|---|---|---|
| 1 | MDR 1308 | 3+ |
| 2 | MDR 1283 | 2+ |
| 3 | MDR 1276 | 2+ |
| 4 | MDR 1274 | 2+ |
| 5 | MDR 1269 | 3+ |
| 6 | MDR 1218 | 2+ |
| 7 | MDR 1211 | 3+ |
| 8 | MDR 1198 | 3+ |
| 9 | MDR 1196 | 3+ |
| 10 | MDR 1113 | 3+ |

*Sources: Primary Data, 2025*

ZN Staining and Initial Identification

Ziehl-Neelsen (ZN) staining of the 10 sputum samples from MDR-TB patients revealed characteristic pink-colored acid-fast bacilli (AFB) in the 2+ and 3+ categories, while the single negative control sample showed no bacilli (Figure 1). These findings confirm that all patient samples contained active *Mycobacterium tuberculosis*.

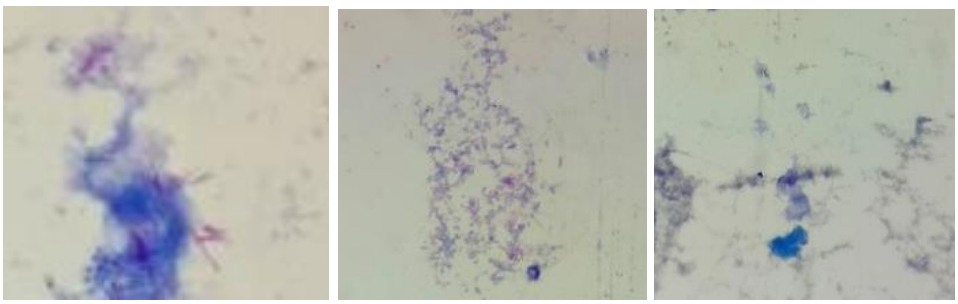

Figure 1. Results of Ziehl-Neelsen (ZN) staining of Mycobacterium tuberculosis from sputum samples and AFB examination showing a sample with 3+ category (a), a sample with 2+ category (b), and a negative control sample (c).

**Amplification of the *fbpA* Gene by PCR**

**Visualization of Electrophoresis Results**

The gel electrophoresis visualization shown in Figure 4 demonstrates that all sputum samples from MDR-TB patients produced a DNA band of approximately 900 bp, which corresponds to the size of the *fbpA* gene. The bands appeared at the same position as the 900 bp DNA marker band. In contrast, no band was observed in the negative control sample, indicating the specificity of detection exclusively for *Mycobacterium tuberculosis*.

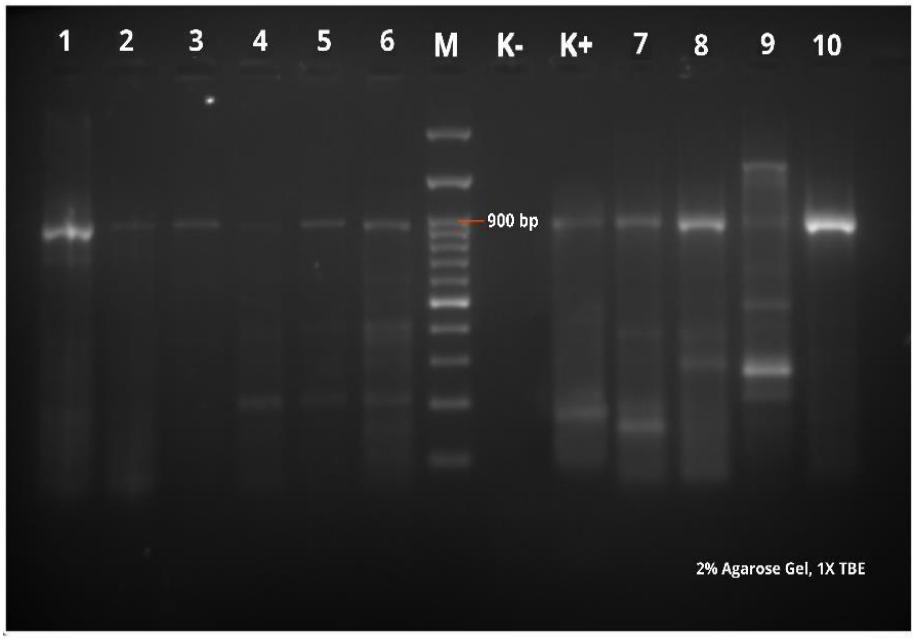


Figure 2. Gel electrophoresis visualization results

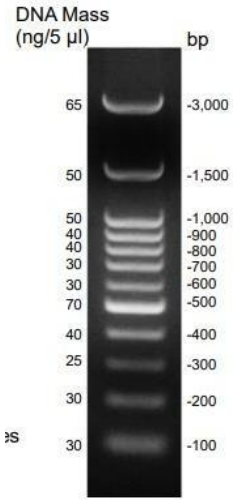


Figure 3. Size reference of DNA marker from Geneaid (Geneaid, 2023)

The DNA bands observed in the samples showed variations in intensity; samples 1, 8, and 10 exhibited thick and sharp bands, indicating a high concentration of isolated DNA and relatively intact DNA quality. In contrast, the bands in samples 2, 3, 4, 5, 6, 7, and 9 appeared fainter, suggesting lower DNA concentration or possible degradation during the extraction process. Some samples also showed smearing, which may indicate the presence of contaminants such as RNA or DNA damage due to physical or chemical factors during isolation. A smile effect was also observed in certain bands, likely caused by technical issues such as uneven electric field distribution, non-uniform gel composition, or suboptimal ethidium bromide (EtBr) concentration.

The 100 bp DNA marker used as a reference consists of 12 bands and serves to visually determine the size of the target DNA fragment. The third band from the top indicates a size of approximately 900 bp, which corresponds to the patient sample bands. The use of ethidium bromide (EtBr) as an intercalating dye and loading dye as a sample migration indicator enabled the visualization of DNA bands under UV light at a wavelength of 254 nm. These results further confirm that the amplification of the *fbpA* gene was successful and clearly detectable through 2% agarose gel electrophoresis

**Primer Validation Using BLAST**

The forward and reverse primers used for the amplification of the *fbpA* gene were validated using BLAST analysis. Based on the results of the BLAST (Basic Local Alignment Search Tool) program available on the NCBI website (10), as shown in Figures 4 and 5, it was found that the *fbpA* gene sequence had 100% similarity with the designed forward and reverse primers. This indicates that the primers have high specificity and are capable of accurately recognizing and binding to the desired target gene, making them suitable for the amplification process. Through the *fbpA* coding

gene, the genotypic characteristics of *Mycobacterium tuberculosis* can be identified.

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 36.2 bits(18) | 0.002 | 18/18(100%) | 0/18(0%) | Plus/Minus |

```
Query  11       TTTTCCCGGCCGGGCTTG  28
                 ||||||||||||||||||
Sbjct  4266707  TTTTCCCGGCCGGGCTTG  4266690
```

Figure 4. BLAST result of the forward primer for the fbpA gene (NCBI, 2023).

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 50.1 bits(25) | 2e-07 | 25/25(100%) | 0/25(0%) | Plus/Plus |

```
Query  6        TCTGTTCGGAGCTAGGCGCCCTGGG  30
                 |||||||||||||||||||||||||
Sbjct  4265011  TCTGTTCGGAGCTAGGCGCCCTGGG  4265035
```

Figure 5. BLAST result of the reverse primer for the fbpA gene (NCBI, 2023)

**Discussion**

The successful amplification of the *fbpA* gene from all MDR-TB patient samples and its absence in the negative control indicate the high sensitivity and specificity of the PCR method in detecting *Mycobacterium tuberculosis*. These findings are consistent with previous studies that have identified *fbpA* as a promising molecular detection target due to its role in adhesion and cell wall synthesis (11). The presence of the *fbpA* gene suggests an interaction between fibronectin and *Mycobacterium tuberculosis*, implying that the bacteria produce the *fbpA* gene to synthesize fibronectin—a major extracellular protein found in human tissues and organs. This protein functions as an extracellular adhesion molecule, facilitating the attachment and invasion of *Mycobacterium tuberculosis* into human tissues, particularly the lungs in TB patients. Overall, the fbpA gene, which encodes Antigen 85A (Ag85A), is not only immunodominant but also directly involved in the pathogenicity of Mycobacterium tuberculosis; Ag85A's functions as a mycoloyl-transferase essential for mycolic acid synthesis and as a fibronectin-binding adhesin underpin its central role in bacterial virulence and persistence (12). This interpretation is supported by recent reviews and experimental studies showing that the Ag85 antigens (including Ag85A encoded by *fbpA*) are highly immunogenic and has been developed as a therapeutic and vaccine target: ag85a/b immunotherapy has been reported to reduce bacterial load and lung lesions in animal models, and antigen-specific antibody responses have been investigated for treatment monitoring (10)(13). Ag85 antigen detection in sputum and antigen-specific antibody changes have been investigated as potential markers of treatment response (14)(15).

Variations in DNA band intensity can be influenced by several factors, including the concentration of the template DNA, isolation efficiency, and DNA integrity. Intact and non-fragmented DNA generally produces sharp and thick bands. Conversely, bands that appear faint

or smeared may indicate DNA degradation, pipetting errors, or RNA contamination. This aligns with findings by Pandey et al. (2024), which state that the level of DNA fragmentation due to degradation is directly proportional to the appearance of smear patterns in electrophoresis, where DNA with high integrity shows a single, clear, and focused band. In addition, Sowersby et al. (2023) explain that technical factors such as electrical imbalances, uneven gel distribution, and suboptimal buffer concentrations can cause variations in band intensity or even a smile effect in electrophoresis results (16)(17).

The presence of *fbpA* as part of the Ag85 complex also has implications for therapeutic strategies. The Ag85 protein is one of the dominant antigens considered in TB vaccine development and therapeutic targeting (18). Therefore, molecular detection of this gene is not only valuable for diagnosis but also contributes to mapping bacterial virulence. These results reinforce the potential of the *fbpA* gene as a treatment biomarker for TB, especially in MDR-TB cases. Clinical applications of this genetic detection include therapy effectiveness monitoring, early relapse detection, and serving as a tool for screening high-risk populations. However, this study did not analyze patients' treatment histories or clinical characteristics such as disease severity or therapeutic response; therefore, correlations between *fbpA* detection and clinical outcomes could not be assessed. Future studies should incorporate treatment history and relevant clinical data to better establish the clinical relevance of *fbpA* as a treatment biomarker.

For future development, this research can be expanded using a quantitative approach such as qPCR, along with Ag85A protein expression assays to establish a correlation between gene expression and patients' clinical phenotypes, thereby strengthening the clinical applicability of fbpA as a molecular biomarker. With this approach, *fbpA* holds strong potential to be developed as a molecular biomarker in more targeted and specific treatment strategies for TB patients. As an initial step in utilizing molecular biomarkers, *fbpA* gene detection via PCR can support laboratory-based clinical decision-making. Furthermore, these results have implications for enhancing laboratory-based health information systems for TB control. Such genetic molecular detection can be integrated into national TB surveillance systems to accelerate the identification of MDR-TB cases, improve referral efficiency, and support more effective TB control efforts across all levels of healthcare services.

## 4. CONCLUSION

The fbpA gene was successfully identified from sputum samples of MDR-TB patients using PCR and gel electrophoresis techniques, with DNA bands confirming the presence of the gene in all samples. Variations in band intensity indicated differences in DNA concentration. These findings highlight the potential of the fbpA gene as a molecular biomarker for the detection of *Mycobacterium tuberculosis* in drug-resistant

patients and for evaluating infection status. Further studies are recommended to strengthen the validity of this gene as a DNA-based marker and to support the development of more accurate molecular diagnostics and targeted TB therapy strategies that are both specific and efficient.

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