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Original Article

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Current Profile of Vivax Malaria in Isolated Area of Kualuh Leidong

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Abstract

The Indonesian Ministry of Health targets to eliminate malaria by 2030. Vivax malaria, a challenging variant to eradicate, is prevalent in areas near elimination, including North Sumatra, which ranks fourth in malaria cases in Indonesia. Labura district, a part of North Sumatra, had a low-endemic status until 2020. However, an increase in cases occurred in 2021 within the Kualuh Leidong sub-district, the primary contributor to Labura's malaria cases. This shifted the endemicity status from low to moderate. The objective of the study is to assess the malaria case profile in this region. A descriptive approach was used, employing a total sampling method at Tanjung Leidong Health Center between September 2022 and July 2023. This observational study identified 494 vivax malaria cases. Predominantly affecting males (60.9%), cases peaked in adults (>18 years) with 314 cases (63.6%). Microscopic examination was the leading diagnostic tool, used in 463 cases (93.7%). The health center primarily administered national regimen therapies dihydroartemisinin-piperaquine (DHP) + primaquine in 204 cases (62.4%), whereas others received alternate therapies. All patients recovered without referrals. Over 11 months, seven recurrence cases emerged, with five receiving quinine+primaquine. Vivax malaria cases in Kualuh Leidong have seen a significant increase compared to previous years. The attention and collaboration of all parties, both from the health center and the community, are necessary to achieve malaria elimination by 2030.

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INTRODUCTION

Malaria elimination has made significant progress over the past two decades since the year 2000. The success of malaria management in the first two decades of the 21st century is even being called the golden era. Globally, the incidence of malaria cases (per 1000 at-risk population) has decreased from 80 cases in 2000 to 58 cases in 2015 and further decreased to 57 cases in 2019. Despite the significant reduction in malaria incidence cases from 2000 to 2015, malaria cases and mortality rates remained relatively stagnant in 2019, with 229 million cases and 409,000 deaths reported by the World Health Organization (WHO). In the Southeast Asia region, the reduction in malaria mortality and morbidity cases remains on target. Countries in Southeast Asia have shown a decrease in malaria cases and deaths of more than 40%, with the exception of Indonesia, which exhibited a reduction between 25% and 40% in 2020 compared to 2015.[1]

In Indonesia, the malaria elimination program is targeted for the year 2030. Over the past decade, there has been a decrease from 465,764 cases (with an Annual Parasite Incidence, or API, of 1.96%) in 2010 to 222,085 cases (with an API of 0.86%) in 2018, which accounts for more than a 50% reduction. However, the reduction in malaria cases has stagnated since 2014, indicating that interventions in the elimination program still need further development to significantly reduce the number of cases.[2]

Although malaria has seen a global decrease in the past decade, malaria cases have been negatively impacted by the COVID-19 pandemic, reversing the progress made and returning global incidence and malaria death rates to 2015 levels. The total number of malaria cases increased to 241 million cases in 2020, up from 229 million cases in 2019. This is attributed to disruptions in malaria service delivery and management during the pandemic.[3]

The pandemic's impact on malaria cases has also been experienced in Indonesia. This is evident from the rise in malaria cases in 2021 and 2022. Malaria cases in 2020 initially stood at 254,055 cases, but they increased to 304,607 cases in 2021 and rose again to 443,530 cases in 2022.[4]

The eastern portion of Indonesia, more especially the provinces of Papua, West Papua, and East Nusa Tenggara (NTT), is the majority of the country's malaria-endemic areas. Only one province outside the eastern region of Indonesia still has districts with high endemicity, namely East Kalimantan Province, especially in Penajam Paser Utara District. In North Sumatra, 11 out of 33 districts have not yet achieved malaria elimination, with 8 of them having low endemicity and the remaining 3 having moderate endemicity. Labuhanbatu Utara District is one of the districts with moderate endemicity in North Sumatra.[4]

Tanjung Leidong Health Center is a primary health center located in Kualuh Leidong Sub-District, Labuhanbatu Utara District. Tanjung Leidong Health Center serves as the primary health center for the Kualuh Leidong Sub-District, which has high malaria cases in Labuhanbatu Utara District, including recurrence cases of vivax malaria. This study aims to provide an overview of malaria cases in the work area of Tanjung Leidong Health Center.

MATERIALS AND METHODS

Materials

Data was collected retrospectively using the medical record of the Health Center from September 2022 to July 2023 and the Indonesian national malaria surveillance system from 2015 to 2021.

Methods

A descriptive analytic observational study was undertaken in the operational domain of Tanjung Leidong Health Center, the principal healthcare facility in Kualuh Leidong Sub-District. The study spanned 11 months, commencing from September 2022 to July 2023. Passive surveillance was conducted over the 11-month period for malaria patients diagnosed using microscopic examination or rapid diagnostic test (RDT) at the healthcare facility. Patients availing treatment at the center were derived from all seven villages within the Kualuh Leidong Sub-District. Employing a total sampling methodology, the data compilation encompassed variables such as gender, age, occupation, village of residence, diagnostic assessments, and malaria treatment modalities.

To provide broader context, data on malaria cases from 2015 to 2021 were obtained from the Indonesian national malaria surveillance system, covering the entire Labuhanbatu Utara District. While these data sets originate from different geographic areas and time frames, they offer a general perspective on malaria trends. Notably, Kualuh Leidong Sub-District has historically been the highest malaria cases in the district, highlighting its relevance in regional malaria epidemiology. Data was then analyzed using SPSS version 27, with the results subsequently elucidated through mean values and percentages.

Study Area

The current study offers valuable insights into the current profile of vivax malaria in the isolated area of Kualuh Leidong, emphasizing the unique challenges and characteristics of malaria cases in this specific region. The geographical isolation of Kualuh Leidong presents a noteworthy observation in this study, as the majority of vivax malaria cases were found to originate from within the area itself.

Kualuh Leidong is one of the sub-districts in Labuhanbatu Utara District (Figure 1), which is located in the North Sumatra Province. The area of Kualuh Leidong District is 340.32 km^2, and is further divided into 7 villages. It has a tropical climate, which has dry season and rainy season. The population in the district reached 34,677 people. The village with the largest population is Tanjung Leidong Village, where 9,605 people live. The Tanjung Leidong Village is also the main village of the Kualuh Leidong, as the main Health Center and many government offices are located in this village.[5]

Figure 1. The location of Kualuh Leidong, one of the sub-districts in Labuhanbatu Utara District

Kualuh Leidong Sub-District is located around 50 km from the nearest

Hospital, taking 1.5 to 2 hours of travel via land route due to the bumpy and muddy road conditions (Figure 2). The access to and from this area will be limited when it is rainy season, as the roads can become very muddy and difficult to pass. This area is located in a coastal region with a poor level of environmental cleanliness, creating numerous water puddles. The majority of the community live in wooden houses built over the water. These conditions create an optimal environment for mosquitoes to breed and spread disease.

Figure 2. Kualuh Leidong road access and environment

RESULTS AND DISCUSSION

Patient's Characteristics

Based on the data collected over the 11-month period from September 2022 to July 2023 (Table 1), there were a total of 494 malaria cases. The characteristics of malaria patients based on gender were predominantly male, with a total of 301 cases (60.9%), and adults aged 18-60 years were most affected, accounting for 63.6% of the total cases.

Based on Table 2, the data reveal a concentrated prevalence of vivax malaria in Tanjung Leidong and Pangkalan Lunang villages, both of which are in close proximity to Tanjung Leidong Health Center. This spatial association suggests that geographical factors may contribute to the higher incidence of malaria in these areas. The challenging terrain and difficult access to healthcare facilities may impact the timely diagnosis and treatment of malaria cases, emphasizing the need for targeted interventions in these high-prevalence villages. The presence of geographical challenges in an area influences the community's treatment-seeking behavior. Individuals residing farther from health facilities are more inclined to seek traditional medicine treatment without undergoing proper diagnosis.[6]

Table 1. Basic characteristics of patients from passive surveillance since September 2022 to July 2023

Patient Characteristics	Cases	Percentage
Gender		
Male	301	60.9%
Female	193	39.1%
Age Group		
Children (0-18 years)	173	35.0%
Adult (>18 years)	314	63.6%
Data missing	7	1.4%

Table 2. Origin village of patients from passive surveillance since September 2022 to July 2023

Village	Cases	Percentage
Air Hitam	17	3.4%
Kelapa Sebatang	16	3.2%
Pangkalan Lunang	131	26.5%
Simandulang	20	4.0%
Tanjung Leidong	228	46.2%
Teluk Pulai Dalam	42	8.5%
Teluk Pulai Luar	23	4.7%
Outside of Kualuh Leidong	12	2.4%
No data	5	1%

The gender tendency to experience vivax malaria varies in each region, influenced by various factors such as economic status, culture, occupation, and others. Notably, in the context of this study, there is an observed male predilection for experiencing vivax malaria. This observation aligns with the outcomes reported by Tafesse et al., where males were more impacted by malaria

parasites than females over the last 10 years.[7] However, this finding diverges from other studies that have identified a higher prevalence of vivax malaria in females compared to males.[8,9]

The gender-specific distribution of vivax malaria appears to be contingent upon diverse contextual elements, emphasizing the intricate interplay of various contributing factors across different regions. The multifaceted factors influencing the gender-specific distribution of vivax malaria in Kualuh Leidong are also applicable to age groups. Vivax malaria poses a risk across all age groups.[10] From this study, it is observed that the highest incidence of vivax malaria in Kualuh Leidong occurs in the adult age group. This contrasts with a study conducted in Ethiopia, which revealed that the age group of children under 5 years exhibited a higher prevalence of vivax malaria.[11] This difference might be because one of the main jobs in Kualuh Leidong is fishermen, which requires them to be at sea during nighttime hours. Additionally, the community members also tend to gather for social gatherings at the night, further increasing their risk of mosquito bites.

Malaria Diagnosis

Microscopic examination stands out as the most recommended diagnostic method for confirming malaria cases in Indonesia.[12] In the specific context of Kualuh Leidong, the microscopic examination diagnostic approach remains predominant, being employed in 93.7% of malaria cases (Table 3). The usage of RDT was in specific circumstances. RDT was used for patients living far from the health center and who can not easily access the health center, hence, the examination was conducted by local midwives. For cases where both diagnostic methods are used, these typically involve patients who visit the health center's emergency unit outside of working hours. An initial RDT was performed as the microscopist was not present, and microscopic examination was done to confirm the diagnosis on the next day when the microscopist was available. This observation underscores that despite the geographic isolation of the area, the diagnostic tool utilized for malaria diagnosis is not impeded, provided that health facilities possess appropriate tools and personnel receive adequate training.

Table 3. Diagnostic methods used for malaria diagnostic from passive surveillance since September 2022 to July 2023

Methods	Cases	Percentage
Microscopic Examination	463	93.7%
Rapid Diagnostic Test (RDT)	25	5.1%
RDT & Microscopic Examination	6	1.2%

Malaria Treatment

Out of the 494 malaria cases in Kualuh Leidong Sub-District, Tanjung Leidong Health Center managed 327 cases (66.2%), while the remaining 167 cases (33.8%) were handled by private clinics (Table 4).

Table 4. Malaria treatment in Kualuh Leidong from passive surveillance since September 2022 to July 2023

Treatment	Cases	Percentage
Treated in Private Clinics	167	33.8%
Treated in Health Center	327	66.2%
DHP + Primaquine	: 204 cases (62.4%)*	
Quinine + Primaquine	: 90 cases (27.5%)**	
DHP	: 6 cases (1.8%)	
Quinine	: 4 cases (1.2%)	
Not coming back for treatment	: 23 cases (7%)	

* 2 cases of recurrent malaria
** 5 cases of recurrent malaria

The primary treatment at the health center was the first-line therapy of DHP + Primaquine. Quinine was used as an alternative when DHP was stock out. Detailed treatment data for cases handled by

private clinics were not available because only the microscopic slides of patients suspected of having malaria were sent to the health center for examination. Treatment at the private clinics likely involves quinine rather than DHP, as DHP is less accessible in private settings.

From the 327 patients who sought treatment at the health center, 7 patients (2.1%) experienced a recurrence of malaria within the 11-month observation period. Throughout the 11-month observation, none of the malaria patients experienced complications. Hence, all malaria management was conducted at the basic health facility level and did not require referral.

Although severe cases of malaria are not prevalent in Kualuh Leidong, the persistence of recurrent cases, albeit at a modest percentage (2.1%), poses a formidable challenge to the complete eradication of malaria. This recurrence rate aligns with findings from a study showing that the burden of P. vivax relapse varies widely across Indian regions, with reported proportions ranging from 1.47% to 6%.[13] It is noteworthy that the nature of malaria recurrence can encompass relapse, recurrence, or reinfection, yet there currently exist no established modalities for distinguishing between these possibilities in Indonesia, especially in an isolated area such as Kualuh Leidong.[14]

Increase in Malaria Cases in the Last Few Years

The historical data on malaria cases in preceding years were extracted from the national malaria case reporting system in Indonesia. Figure 3 provides an overview of malaria cases in the Labuhanbatu Utara District annually. When examining the trend of malaria cases per year, the number of cases tends to decrease from 2015 to 2020. The declining case of malaria in the second decade of this century was also observed in another country targeting the elimination of malaria.[15-17] Since 2019, it has been observed that only *Plasmodium vivax* has been identified in Labuhanbatu Utara District, with no detection of any other type of Plasmodium. This situation mirrors the global trends in which attempts to lessen the burden of *Plasmodium vivax* have not produced a satisfactory outcome. *Plasmodium vivax* has been a challenge in malaria elimination due to the ability to be asymptomatic but still be transmissible and the ability to hide dormant in the liver stages, which can lead to relapse.[18]

In Figure 3, a noteworthy deviation occurred in 2021, where there was a surge in malaria cases, totaling 435 cases. This figure represents an increase of nearly twofold compared to 2020. Consequently, this upswing of cases in 2021 prompted a transition in the endemic status, shifting from a low-endemic area to a moderately endemic area. The interruptions brought on by the COVID-19 pandemic, which affected malaria control systems across the country, were expected to be the cause of the observed increase in malaria cases in 2021.

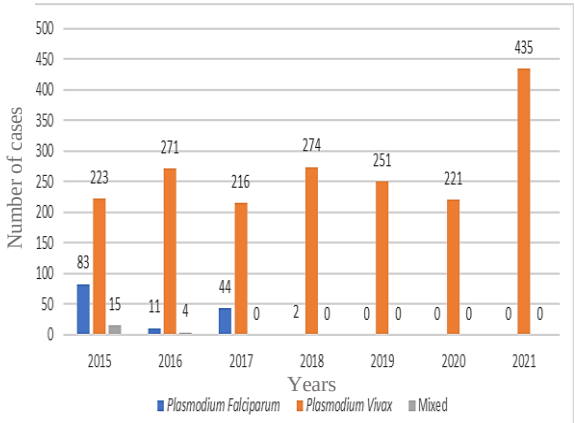

Figure 3. Number of malaria cases in Labuhanbatu Utara District from 2015 to 2021

The COVID-19 pandemic has had profound consequences on the national malaria program in Indonesia, contributing to a surge in malaria cases in 2021.[4] The Kualuh Leidong area, in particular, has not

been exempt from this impact. As illustrated in Figure 3, malaria cases doubled in 2021. This escalation is not reported only in Indonesia but also in several other countiers.[19-21] The World Health Organization (WHO) reports that the incidence of malaria rose by 5% in 2020.[22]

The pandemic of COVID-19 give a major disruption to health service delivery, including malaria management.[23] The global occurrence of hindered mobilization, isolation, and lockdown extended to China, the major provider of medical equipment, antibiotics, active pharmaceutical ingredients (API), and personal protective equipment (PPE) worldwide. The direct impact of the pandemic on malaria treatment manifested in disruptions to the drug supply chain. Consequently, this led to numerous stockouts of medications, including dihydroartemisinin + piperaquine (DHP).[24-26] In our study area, these drug shortages had even more severe consequences due to the region's optimal environment for mosquito breeding, resulting in a high mosquito population. The use of quinine requires a longer duration and higher dosing frequency (7 days, thrice daily) compared to DHP (3 days, once daily), increasing the risk of transmission by prolonging the period during which Plasmodium remained in the blood stage.

STRENGTH AND LIMITATION

The strength of this study was its ability to gather data from an isolated location, thus giving information on the malaria situation in a remote location. However, the limitation of this study was the passive data collection and the collected data did not interfere with healthcare service flow, which leads to the restriction of collected information.

CONCLUSIONS

The data collected over an 11-month period from September 2022 to July 2023 highlights a significant increase in vivax malaria cases in Kualuh Leidong compared to previous years. The discernible increase is attributed not only to local factors but also to the broader impact of the COVID-19 pandemic, which has disrupted the national malaria control program, contributing to a surge in cases globally and specifically within Kualuh Leidong. Addressing these challenges and striving towards the ambitious goal of malaria elimination by 2030 necessitates collaborative efforts from all stakeholders, encompassing both health centers and the community.

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ETHICAL CLEARANCE

Ethics approval had been obtained from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, before the study was conducted (No. 1135/KEP/USU/2021).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

We certify that each and every author has contributed to this work. Every author has contributed to the content's drafting and critical revision. Each author has approved this work and commits to take responsibility for it.

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Article Review

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Update on The Current Management of Drug-Resistant Tuberculosis (DR-TB)

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Abstract

Drug-Resistant tuberculosis (DR-TB) is a global public health threat that requires a comprehensive response from all parties. DR-TB cases are often overlooked and tend to increase every year. Efforts to overcome DR-TB cases began in 2009 with the use of a molecular test, Xpert MTB/Rif, as a diagnostic tool. This has now been developed with the procurement of a molecular test with Xpert MTB/XDR. This diagnostic update also formed the basis of the latest DR-TB classification terminology by not categorizing polyresistance into the DR-TB group. This step is still not in accordance with the low success rate of DR-TB treatment in Indonesia, ranging from 45-50%. The latest DR-TB management recommendations by WHO in 2022 have implemented a 6-month treatment regimen to minimize the occurrence of treatment dropout or patient treatment non-compliance. The BPaLM/BPaL regimen is a shorter-duration oral regimen that is expected to help achieve the End TB 2015-2030 targets. Previously used short-term regimens have now been modified with Ethionamide and Linezolid variants as alternatives for DR-TB management if the BPaLM/BPaL regimen does not meet the criteria for use.

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INTRODUCTION

Mycobacterium tuberculosis Complex infection is the causative agent of Pulmonary Tuberculosis (PTB), an infectious lung disease. After COVID-19, PTB cases ranked as the second most common cause of infection-related mortality globally. In 2022, PTB cases will result in twice as many deaths as cases of HIV/AIDS combined with other viruses. PTB continues to be a significant global concern, with an annual rise in cases.[1,2] To eliminate PTB cases by 2030, the World Health Organization (WHO) and United Nations (UN) prefer to improve the incidence rate to 65/100.000 people and the mortality rate to 6/100.000 people.[2,3]

As per the 2023 Global Tuberculosis Report, Indonesia currently maintains the second place globally in terms of PTB cases, with an estimated total of 1.060.000 cases, after India. The amount of mortality of PTB is also strongly associated with an increase in new cases, which exceeded 134.000 cases.[1] Data from the Indonesian Ministry of Health also explains that there was an 809.644 case increase in PTB cases in 2023, above the previous year.[1,4] Indonesia has noticed an increase in PTB cases, mainly because of the unsuccessful outcome of treatment. The amount of Drug Resistant Tuberculosis (DR-TB) cases in Indonesia has also increased in alongside this condition.[5,6]

Globally, by 2022, resistance testing will be conducted on 73% of new PTB cases, with 4,4% having developed DR-TB.[1,6] Incidence of DR-TB is estimated to be 10/100.000 in 2021, with a mortality rate of 52/100.000 people.[7,8] Compared to 7.876 cases in 2021, the incidence of patients with confirmed Multi Drug Resistant (MDR)/Rifampicin Resistant (RR) Tuberculosis until the beginning of 2024 was 12.531 cases.[4,8] Indonesia is one of the nations with the highest incidence of DR-TB cases globally, accounting for 28.000 cases since 2021. In that time, 45–50% of cases continue to end successfully. Along with mortality rates of 15-20% of cases, this condition is caused by a high rate of dropping out of 20-30% of cases.[1,9]

WHO recommended an oral treatment regimen in 2020 with the aim of decreasing the duration of treatment to 9-11 months for cases of MDR-TB. Through the use of injectable Anti Tuberculosis Drugs (ATD), the previous regimen failed to be as assist of prompt treatment accomplishment for each patient as it had been for others.[10,11] The use of medication is affected by reports of patients who, mainly as an outcome of drug side effects, are unable to complete treatment on time.[11] There are additional reasons to search for advanced DR-TB regimens with a shorter duration and fewer side effects, including the logistical challenges of DR-TB regimens, the high expense of current drugs, and an increasing number of treatment failures.[12,13]

The strategy for DR-TB diagnosis requirements, which experienced several changes in accordance with WHO guidelines in 2022, is closely related to tasks that aim at increasing the treatment's success rate. The development of Molecular Rapid Test exams, which can currently detect Isoniazide (INH) and Fluoroquinolone (FQ) resistance in the same test, shows changes in the procedure for the diagnosis of DR-TB. Furthermore, beginning in 2022, clinical trials into the use of ATD without injection and a shorter duration of treatment would be carried out, as the WHO.[14,15]

The Global Tuberculosis Programme of the World Health Organization (GTB-WHO) has incorporated all the latest clinical trial recommendations into one set of integrated guidelines for the global

management of DR-TB cases.[16] The newest regimens recommended by WHO are Bedaquiline, Pretomanid, Linezolid, Moxifloxacin (BPaLM), and Bedaquiline, Pretomanid, Linezolid (BPaL). Already in 2022, 40 nations began using the 6-month BPaL/M regimen for RR/MDR-TB and Pre-Extensively Drug Resistant (Pre-XDR)-TB patients. [16,17]

The National Tuberculosis Programme in Indonesia has started providing INH Monoresistance regimens and BPaLM/BPaL regimens by 2023. This effort is to close the gap between DR-TB case finding and patients who have received DR-TB regimens previously.[16,18] The WHO's decision to implement its most current DR-TB regimen in 2022 indicates a significant advancement in the revision of the Technical Guidelines for DR-TB Management in Indonesia. The success of End Tuberculosis 2030 is expected to be improved by the introduction of the BPaLM/BPaL regimen.[16,18]

DEFINITION

The common term for Tuberculosis is an infectious disease caused by bacilli of *Mycobacterium tuberculosis*. Bacilli of *Mycobacterium tuberculosis* are intracellular pathogens that can reproduce a mycolic acid layer, are immobile, and are capable of cell division every 18-24 hours.[19] The WHO 2022 summary of multiple investigations has led to an upgrade in the nomenclature used to describe the cause of PTB. The Genus of Mycobacterium, which includes *M. tuberculosis*, *M. bovis*, *M. caprae*, *M. africanum*, *M. microti*, *M. canneti*, *M. orygis*, and *M. pinnipedii*, is the source of PTB. This group of species is known as the *Mycobacterium tuberculosis* Complex.[20]

CLASSIFICATION

According to the WHO's 2022 recommended terminology, PTB can be classified according to anatomical location, previous PTB treatment history, HIV status, and drug test sensitivity.[16,19] Drug-sensitive TB (DS-TB) and DR-TB are the two categories of PTB that are based on drug sensitivity tests. The most recent DR-TB treatment is directly related to the updated DR-TB classification approach that takes *Mycobacterium tuberculosis* microbe resistance patterns into view. As shown in Figure 1, there are five groups in the DR-TB classification.[19,21]

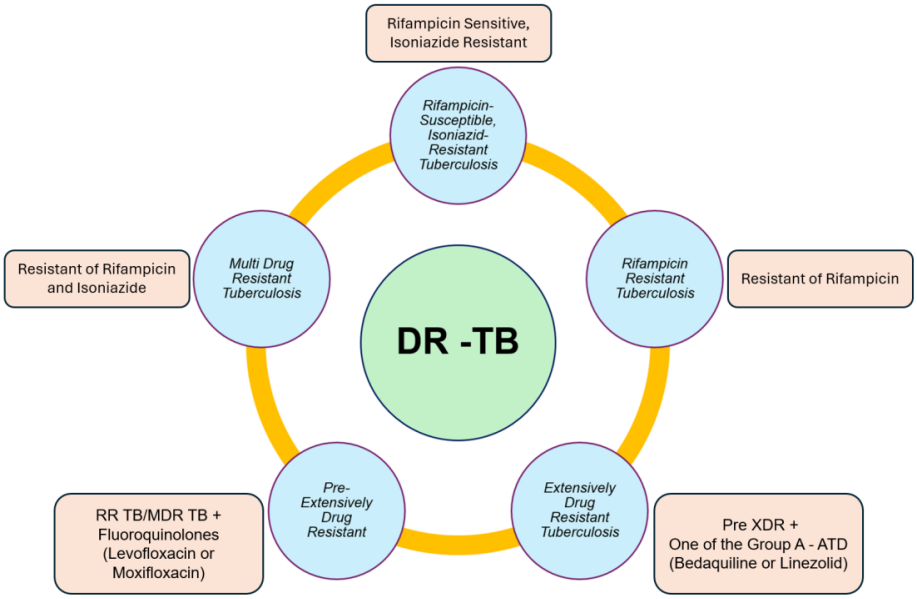


Figure 1. Classification of DR-TB[19]

CRITERIA OF DR-TB SUSPECTED

Suspected DR-TB was reduced to two criteria in accordance with WHO recommendations in 2022. For paediatric or adult DS-TB patients with or without HIV that worsened clinical (symptomatic or radiological) or bacteriological improvement by the end of the 2nd month, one month before the end of treatment, and the end of treatment, despite receiving an appropriate mix and dose and following ATD, the initial criterion is to be used. The second criteria is relevant to patients that belong to paediatric or adult with symptoms of PTB and had one of the following histories: (1) history of close contact with DR-TB patients; (2) a history of close contact with DS-TB patients that died about PTB, failed treatment, and were not committed during treatment; and (3) had history of DS-TB or DR-TB treatment.[16,18,22]

DIAGNOSIS APPROACH

The Molecular Rapid Test examination is the first approach used to diagnose PTB, employing Xpert MTB/Rif cartridges. The Molecular Rapid Test examination has been able that detect the *Mycobacterium tuberculosis* complex and measure resistance to Rifampicin, Isoniazide, and Fluoroquinolones since 2006, as evidenced by the Xpert MTB/XDR cartridge.[23] Xpert MTB/XDR was created to improve the rate of finding cases for DS-TB and DR-TB, which will decrease morbidity and mortality. The development of this Molecular Rapid Test examination also improves the diagnosis of patients who meet the criteria for suspected DR-TB. The MRT Xpert MTB/XDR examination serves as a substitute for first and second-line Probe Assay (LPA) and culture examinations, showing quicker test results.[16,23]

The algorithm for identifying PTB was also altered to prioritise the results of the Molecular Rapid Test of Xpert MTB/XDR test (Figure 2).[24] The new algorithm also enables a more targeted approach to the diagnosis of DR-TB, which in then accelerates the treatment process. The interpretation of Molecular Rapid Test examination results assists in the detection of XDR-TB, RR-TB/MDR-TB, Pre-XDR-TB, and Monoresistance TB.[24,25]

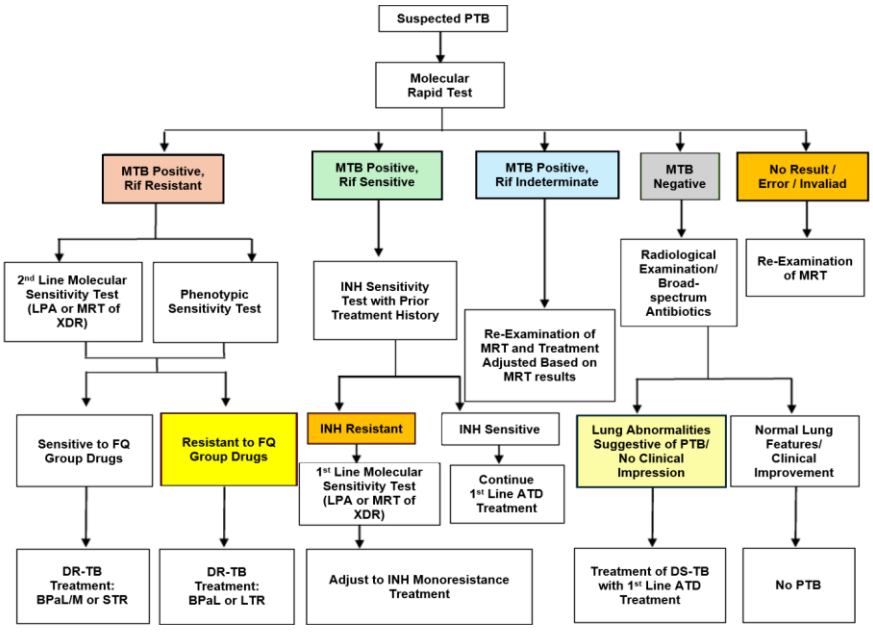


Figure 2. Update of the Diagnosis Algorithm of PTB in Indonesia with a Treatment Approach in Cases of INH Monoresistance and DR-TB based on Current Molecular Rapid Test Interpretation[18,24]

ALGORITHM OF CURRENT MANAGEMENT FOR DR-TB

So as to achieve the goal End TB 2030, WHO demonstrates that each of the bacteriologically confirmed PTB patients experiences early diagnosis of PTB and increased sensitivity tests. People who show classic symptoms of PTB, have a history of contact with PTB patients, and have comorbid or co-infected with HIV are prioritized for molecular tests. The sensitivity test is also implemented to evaluate the patient's resistance to Second Line ATD through genotypic and phenotypic analysis.[16,26]

Based on Figure 3, the WHO published the current management strategy for DR-TB in 2022. The most recent update on the management of DR-TB suggests that the BPaLM/BPaL regimen be administered and that the Short Term Regimen (STR), which variant of the Ethionamide and Linezolid options, get revised.[16,22,27]

There are three general treatment regimens that are currently used to manage DR-TB (Figure 3). The first treatment regimen consists of BPaLM, BPaL, and Monoresistance (Hr-TB) regimens and will be given for six months.[16,18,25] The second regimen, which was previously identified as the STR, refers to the nine-month treatment regimen. The STR treatment is updated in accordance with the criteria of DR-TB patients and is divided into Ethionamid and Linezolid variants. The Long Term Regimen (LTR) is the last regimen, extending for 18-24 months.[16,26,27]

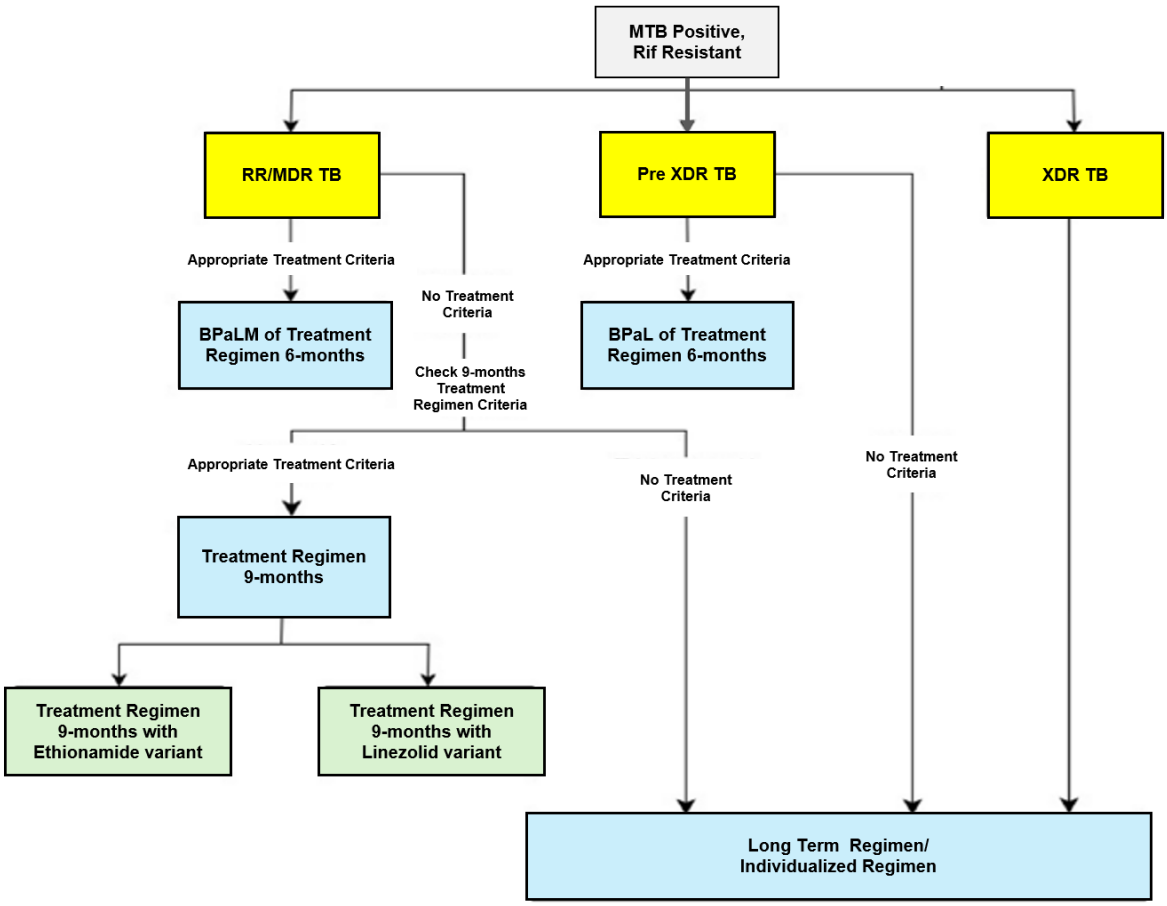


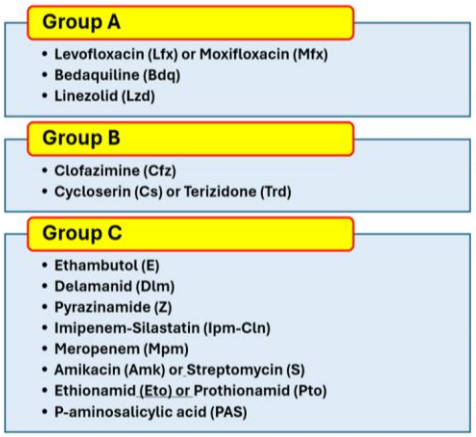
Figure 3. Current Management of DR-TB with BPaL/M Regimen as the Main Priority for DR-TB Treatment with a Shorter Duration of Treatment[18]

REGIMEN of BPaL/M

Criteria for Administration of BPaL/M Regimen

The criteria for using BPaLM or BPaL regimens differ based on the indications for diagnosis and the results of resistance tests of the components of the BPaL/M regimen. There are similarities in the criteria for giving the BPaL/M regimen, including: (1) Adult or adolescent patients >14 years, no matter the presence of HIV; (2) Patients with confirmed PTB or Extra Pulmonary Tuberculosis (EPTB), with the exception of TB that involve the Central Nervous System (CNS), Osteoarticular, and Disseminated/Millary; (3) Have not used Bedaquiline, Pretomanid, Linezolid, or Delamanid for a period more than one month; and (4) Cannot be administered in pregnancy and lactation. Although the two criteria differ by the criteria is BPaLM regimen is recommended for patients diagnosed with RR-TB/MDR-TB, and the BPaL regimen is recommended for patients diagnosed with Pre XDR-TB. Furthermore, the BPaL regimen must be administered in an approach that ensures the drug components are not resistant. It displays the difference between the two regimens.[16,18]

The administration of Moxifloxacin was the only difference between the composition and dosage of OAT in the BPaL/M regimen, as shown in Figure 4.[16-18] BPaLM and BPaL regimens also differ by the duration of treatment. The BPaLM regimen is given at the same time daily for a maximum of 6 months or 26 weeks. Compared to the BPaLM regimen, the duration of the BPaL regimen can be prolonged by 3 months at the time of clinical progress, even if the sputum culture results have not converted by the end of the 6 months of treatment. The BPaL regimen's extended treatment duration aims to achieve a total treatment duration of 9 months or 39 weeks.[16,18,22]



Type of ATD	Dose of Administration
Bedaquiline / Bdq (Dosage: 100 mg tablet)	400 mg once/day for the first 2 weeks daily Followed by 200 mg 3 times/week
Pretomanid / Pa (Dosage: 200 mg tablet)	200 mg once/day daily
Linezolid /Lzd (Dosage: 600 mg tablet)	600 mg once/day daily
Moxifloxacin / Mfx (Dosage: 400 mg tablet)	400 mg once/day daily

Figure 4. Type and Dose of ATD in BPaL/M Regimen[16-18]

Modification of BPaL/M Regimen

The BPaL/M regimen may be modified both before and after treatment. The BPaL/M regimen modification principles are comprised of 4 aspects, especially: (1) Dosage adjustments are just available with Linezolid; (2) Not recommended to change the use of Moxifloxacin to Levofloxacin; (3) The administration of a BPaL regimen is advised in the event that Moxifloxacin has contraindicated; and (4) The administration of Pretomanid and Bedaquiline cannot be permanently discontinued during treatment.[16,18,28] The modified BPaL/M regimen contains 3 primary principles for the administration of Linezolid, that are: (1) The only permitted stoppage of Linezolid is not

recommended within the initial 9 weeks; (2) Linezolid should not be stopped for a duration above 14 days; and (3) Discontinuation of Linezolid for a duration over 14 days within the initial 9 weeks was described as Treatment Failure.[16,28]

The administration of BPaL/M regimen is very important to be considered in detail in Linezolid dose adjustment.[16,28] Linezolid dose adjustment is considered in 3 ways, namely: (1) Can be permanently stopped; (2) Temporarily suspended; and (3) Linezolid administration dose reduction. Indications for permanent discontinuation of Linezolid include a finding of significant toxicity effects in the first 9 weeks of administration of 600mg/day. Where conditions such as optic neuritis, grade 3-4 peripheral neuropathy, recurrent anemia, and severe thrombocytopenia are present, toxicity effects are considered significant. If the remaining treatment time is less than 8 weeks and culture conversion has occurred, there are additional conditions that may result in a permanent end of Linezolid administration.[28,29]

The administration of Linezolid 600 mg/day within the first 9 weeks of treatment may be just stopped if the duration of Linezolid discontinuation is less than 14 days. Any condition that fails to satisfy these criteria will be classified as treatment failure. Linezolid can be reduced to 300 mg/day if it has been administered within the first 9 weeks and there are low toxicity effects, such as grade 1 or 2 peripheral neuropathy and myelosuppression, that have improved after transfusion.[16,30]

Criteria for Treatment Failure of BPaL/M Regimen

The eligibility criteria for each patient are modified prior to the administration of the BPaL/M regimen. Patients who fail to meet the criteria for BPaLM regimens may be considered for BPaL regimens if there is resistance to fluoroquinolones. The subsequent option is STR treatment that is customized to the criteria of Ethionamide or Linezolid variants, as the administration of the BPaLM regimen is contraindicated.[16,18]

The LTR treatment is recommended for patients who fail to meet the criteria for BPaL/M or STR treatment. If there was no sputum conversion at the conclusion of the 6th month of treatment with the BPaLM regimen or the 9th month of treatment with the BPaL regimen, patients were classified as treatment failures.[16] Additionally, treatment failure will be characterized as permanent discontinuation of Bedaquiline or Pretomanid, or the development of resistance to Bedaquiline, Pretomanid, and Linezolid during the treatment period.[28,29] In the event of BPaL/M modification, the criteria for treatment failure also apply if there is substantial toxicity following Linezolid administration and adverse events involving discontinuation of Linezolid for more than 14 days within the first 9 weeks. The patient's treatment is maintained with the LTR treatment following the failure in the BPaL/M regimen.[16,30]

SHORT-TERM REGIMEN (STR)

Generally, the criteria for STR are still unaltered and continue to be applied in accordance with the previously established criteria. The main treatment option for DR-TB management is no longer STR due to recent advances in BPaLM/BPaL regimens (Figure 3).[16] Criteria that are inappropriate for BPaLM/BPaL will be matched with criteria that are acceptable for STR. Patients with RR-TB/MDR-TB may be advised to get STR with the most recent

two variants. If the STR criteria fail to be satisfied, patients will continue to receive LTR treatment.[16,18]

Treatment criteria for STR should be customised for Ethionamide and Linezolid variants according to the most recent WHO recommendations for 2022.[16] In patients with severe peripheral neuropathy, visual impairment, or very low haemoglobin/neutrophils/thrombocytes, STR with Ethionamide variants may be recommended. Pregnancy and breastfeeding are not recommended contraindications to STR with the Ethionamide variant. The STR with the Linezolid variant is considered more effective than Ethionamide in terms of treatment compliance. Comprehensive supervision should be maintained during the administration of Linezolid, as it has the potential to cause toxicity effects.[16,31]

However, the duration of STR treatment with Ethionamide or Linezolid variants remains 9-11 months. Additionally, the two variants maintain similarities in the composition and type of ATD, specifically the administration of Bedaquiline for 4-6 months during the initial stage and the use of the same type of ATD during the advanced stage (Figure 5).[16,31] The two categories of STR variants do not exhibit a significant difference in terms of the duration and advanced stage of treatment. The difference between these two variants is in the initial stage of treatment. The Ethionamide variant involves the administration of Ethionamide for 6 months, and the Linezolid variant involves the administration of Linezolid for 2 months.[31]

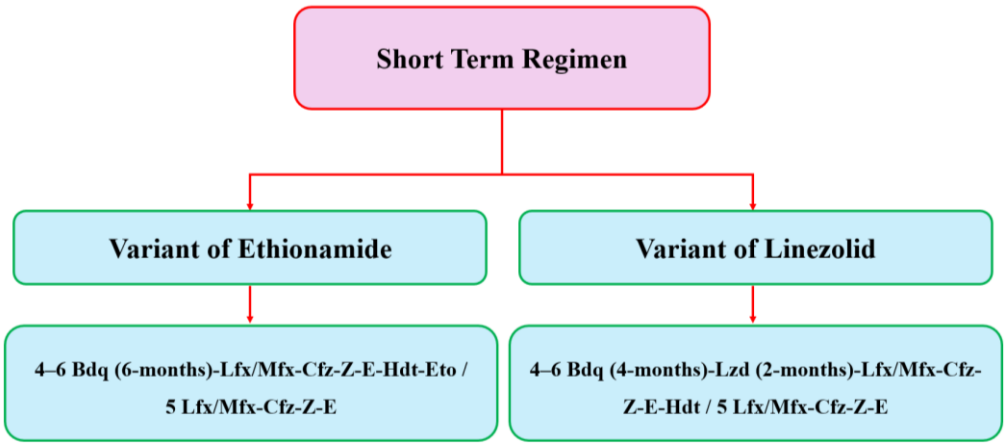


Figure 5. Current Differences of Anti-Tuberculosis Drugs Components in Short-Term Regimens as Alternative Treatment of Contra-Indications in BPaL/M Regimens[16,31]

Treatment with STR is assessed in stages to determine the conversion status of sputum culture. Evaluation of the conversion status to determine the duration of treatment is approximately 9-11 months. The initial phase of treatment is the basis for establishing the parameters used to determine the duration of treatment. Treatment can be administered for 9 months, provided that the sputum culture results at the conclusion of the 4th month have converted, and the following stage continues for 5 months. Patients who have received negative initial AFB or sputum culture results can be administered the initial stage for a period of 4 months. Additionally, for the purpose of ensuring that patients experience proper wellness, clinical and radiological conditions are assessed in stages.[31,32]

Treatment can be administered in 10 or 11 months if no conversion has occurred by the outcome of the 4th month. Therefore, the initial phase of treatment continues to the 5th or 6th month. For confirmation of ATD sensitivity in the treatment regimen

component, it is recommended that second LPA tests and ATD test sensitivity be repeated. Treatment failure is declared if STR treatment is used and LTR treatment is continued after the 5th or 6th month, and there is no sputum culture conversion.[33]

LONG-TERM REGIMEN (LTR)

The initial introduction of DR-TB management started in Indonesia in 2009. The management of DR-TB from 2009-2017 was examined, and it was observed that there was a trend of decreasing treatment success rates, increasing dropout rates, and increasing mortality rates. An oral treatment regimen for the management of DR-TB was issued by the WHO in 2018 in response to these findings. The result is further supported by the 2017 Decree of the Indonesian Minister of Health, which aims to break the link for transmission of DS-TB and DR-TB in the community by conducting DR-TB treatment and expanding the availability of DR-TB health care facilities.[18,34]

Based on the above history, the LTR treatment was initially introduced by the WHO in 2018 as an alternative management option following STR treatment in the treatment of DR-TB. Several studies of clinical trials conducted by the WHO in 2020 to 2022 showed that LTR treatment is still an effective treatment for DR-TB.[1,2,18,34] LTR treatment is the final option in the current management of DR-TB, particularly in patients who have failed treatment after BPaLM, BPaL, and STR treatment, as described in the current management pathway for DR-TB (Figure 3).[33,34]

Individualised regimens are one of the terms used to describe LTR treatment. This is because the ATD components in this regimen can be adjusted in phases depending on the level of the 2nd Line ATD group. The optimal treatment regimen includes 3 Group A drugs and 2 Group B drugs. According to the event that the ideal regimen failed to conform to the criteria for the completion of the 5 drug components in LTR treatment, the use of Group C drugs may be prescribed. The most recommended drug is administered from the highest position of the list. Group C drugs are administered in the following sequence.[16,18,34]

The primary LTR treatment regimen begins with 5 types of ATD that are considered to be effective, and must consist of a minimum of 3 types of ATD after the end of Bedaquiline (Figure 4). The LTR treatment has been customised to the patient's clinical history and treatment, which includes the results of the 2nd Line ATD sensitivity test, a history of disease intolerance, and any comorbidities that may result in ATD interactions with other drugs.[16,33-35] The duration of the LTR treatment regimen was 18-24 months and was modified based on the duration of sputum culture conversion. Individuals who do not achieve culture conversion by the 8th month of treatment are classified as treatment failures and must begin the LTR treatment from the start, with the drug composition adjusted according to the most recent sensitivity test results.[34,35]

STRENGTH AND LIMITATION

The strength of this review article is that it can be used as an updated guide to DR-TB management. This article is expected to provide information to all communities, especially health agencies, in managing DR-TB cases with shorter treatment. The limitations of this review article are subject to change, and the latest scientific developments based on research being developed in the treatment of DR-TB

that is more efficient and effective.

CONCLUSION

The annual increase in cases of DR-TB presents a significant global health burden. The significant prevalence of drug dropout and mortality rates provides a foundation for scientific advancement in enhancing the identification and efficacy of treatment. The MRT examination, using the MTB/XDR cartridge, is an advanced technique for accelerating the identification of PTB cases. It is able to detect the Mtb Complex, which is the main cause of PTB infection. Furthermore, a further benefit is being able to detect both resistance to INH and Fluoroquinolones as a diagnostic approach to monoresistant TB and pre-XDR-TB. This upgrade is anticipated to accelerate the detection of DR-TB in nations with a high prevalence of the disease, such as Indonesia.

Following that, a new strategy for managing DR-TB was implemented, with a shorter treatment period and minimal side effects. The principal treatment for DR-TB has now been superseded by this shift in DR-TB treatment, making STR and LTR useless. According to the WHO Guideline 2022, the BPaL/M regimen has been identified as the primary treatment choice for managing DR-TB. This regimen has been evaluated in multiple clinical trials and is recommended for a treatment period of 6 months. The BPaLM regimen is recommended for cases of RR-TB/MDR-TB, while the BPaL regimen is recommended for cases of Pre XDR TB. The modifications in the BPaL/M regimen specifically apply to the period and dose of Linezolid administration before and after the treatment. Extensive clinical monitoring was conducted to evaluate the adverse effects caused by the administration of Linezolid. The linezolid dose was adjusted during the initial 9 weeks of treatment and was not stopped for more than 14 days.

The administration of STR treatment could be considered for patients who do not meet the criteria of the BPaL/M regimen or who fail treatment with the BPaL/M regimen initially. STR treatment is currently divided into two variants, namely Ethionamide and Linezolid. The administration of both variants is accommodated to the treatment criteria and clinical indications. In the management of DR-TB, LTR treatment is the last option for patients who do not qualify for STR administration or STR treatment failure. It is anticipated that the current approach to the diagnosis and management of DR-TB will be updated in order to enhance the efficacy of DR-TB treatment and decrease the incidence of drug withdrawal, as well as mortality from PTB.

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CONFLICT OF INTEREST

The authors declared there is no conflict of interest.

AUTHOR CONTRIBUTION

Idea and concept: RLS, ETMS, IY, ZAF. Design and manuscript writing: RLS, ETMS. Data collection and processing: ETMS. Control and supervision: RLS, IY,

ZAF. Review and revision: RLS, IY, ZAF. All authors contributed to and approved the final version of the manuscript.

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Synthesis and Characterization of Cu(II)-EDTA Complexes: Antibacterial Studies (*Escherichia coli, Staphylococcus aureus*) and Inhibition of Dengue Virus Serotype 2 in Vero Cell

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Abstract

The Cu(II)-EDTA complex is known to have antibacterial and antiviral potential, but its effectiveness against pathogenic bacteria and dengue virus serotype 2 (DENV-2) still needs to be studied. This study synthesized and characterized the Cu(II)-EDTA complex of $CuSO_4$ precursors, and then tested the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, as well as the antiviral activity against DENV-2 in Vero cells. This study successfully synthesized and characterized the Cu(II)-EDTA complex using $CuSO_4$ as a precursor through the solvothermal method, producing blue crystals with a Cu ratio of 1:1. DSC analysis showed thermal stability up to 250°C with an endothermal peak at 270-300°C. The particles are 6.31 nm in size with a PDI of 0.076, indicating uniform distribution with nanoparticle size (<100 nm). FTIR confirms the formation of the complex through significant shifts in the O-H and C=O bands. SEM shows a layered morphology that can affect the solubility and release of substances. UV-Vis shows maximum absorbance peaks of EDTA at 244 nm and $CuSO_4$ at 740 nm. Antibacterial tests of Cu(II)-EDTA against *E. coli* and *S. aureus* showed that Cu(II)-EDTA had less activity than pure $CuSO_4$. For DENV-2, $CuSO_4$ was more effective with an EC_{50} value of 77.86 µg/mL, lower than Cu(II)-EDTA 356.13 µg/mL, indicating that $CuSO_4$ was better at inhibiting viral replication.

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INTRODUCTION

Transition metals and metalloids have interesting antimicrobial capabilities because they can target bacteria, viruses, and fungi broadly.[5] A prominent example of a transition metal with such properties is copper, which ranks as the third most abundant transition element in the human body. Copper is a bio-essential element used in DNA cleavage and has potential anti-HIV activity.[6] Copper (Cu) is essential for enzyme activity involved in hemoglobin formation and chemical redox reactions in the body. One copper compound, Copper(II) chloride, has the potential to inhibit DENV-2 with an IC_{50} of 0.13 μg/mL. However, excessive copper usage can cause toxicity in the liver, reproductive system, and neurons, as well as trigger stress in the endoplasmic reticulum, which may lead to apoptotic cell death.[7] In the study, the copper complex [Cu(2,4,5-triphenyl-1H-imidazol)$_2$(H_2O)$_2$].Cl_2 showed a decrease in the viability of Vero cells through mitochondria-related cell death, which is crucial in initiating apoptosis by releasing apoptosis-triggering factors and DNA fragmentation.[8]

Dengue fever is a serious global health problem spread by the *Aedes aegypti* and *Aedes albopictus* mosquitoes, especially affecting tropical and subtropical regions. Approximately 2.5 billion individuals are at risk of contracting the disease.[1] In 2023, over 80 countries reported close to five million dengue cases and more than 5000 related deaths.[2] The dengue virus is an RNA virus belonging to the genus Flavivirus in the Flaviviridae family, transmitted through mosquito bites, leading to Dengue Hemorrhagic Fever (DHF. It has four serotypes (DENV1 to DENV-4)[3], with serotype 2 (DENV-2) being a major contributor to fatalities caused by dengue fever.[4]

In addition, copper, historically utilized for its antimicrobial properties, has recently attracted renewed interest with the advancement of nanotechnology. Copper nanoparticles (CuNPs) have a larger surface area and higher toxicity than ordinary metals, making them a more effective choice for antimicrobial applications.[5]

Escherichia coli and *Staphylococcus aureus* are significant and pathogens responsible for a wide range of infections in both humans and animals. *E.coli* is particularly associated with skin and soft tissue infections, surgical wound infections, as well as bone and joint infections.[9] *Escherichia coli (E. coli)* is a facultative aerobic bacterium characterized by its Gram-negative staining and rod-like morphology. Although its presence in the colon is generally harmless as a commensal flora, certain strains of *E. coli* can be the main cause of diseases in humans and animals. Its impact ranges from commensal relationships to digestive tract diseases and extraintestinal pathologies such as diarrhea, urinary tract infections (UTIs), sepsis, pneumonia, and meningitis.[22,23] *Staphylococcus aureus (S. aureus)* is a Gram-positive, spherical-shaped bacterium capable of causing food poisoning, infections, and even death. This bacterium possesses various virulence components that play a role in its ability to cause disease. One of these is surface proteins that enable it to adhere to human tissues, leading to infection of the skin and soft tissue. Moreover, *S. aureus* is capable of producing multiple toxins that result in a range of clinical manifestations, from serious skin infections to food poisoning and staphylococcal scalded skin syndrome.[24,25]

Studies show that Cu^{2+} ions can inhibit *E. coli* and *S. aureus* and increase

when combined or chelated with molecules such as EDTA. EDTA is a commonly used chelation agent due to its ability to form stable complexes with metal ions, such as Cu-EDTA, which has applications in decontamination and treatment of metal poisoning.[10]

As a potent chelating agent, Ethylenediaminetetraacetic acid (EDTA) has been extensively utilized in medical practice, particularly for managing toxicity caused by heavy metals like mercury and lead. The antimicrobial effects of EDTA have been known for decades, effective against bacteria, yeast, and fungi. EDTA works by binding cations such as Mg^{2+} and Ca^{2+}, weakening the microbial cell wall and increasing the efficacy of other antimicrobials. Various EDTA salts, such as disodium, trisodium, and Ca-EDTA, exhibit antibiofilm and antimicrobial activity, with environmental pH being an important factor in their effectiveness. In Gram-negative bacteria, EDTA releases Mg^{2+} and Ca^{2+} ions from the outer cell wall, which increases sensitivity to additional antimicrobial agents.[11]

This study aims to synthesize and characterize Cu(II)-EDTA complexes based on $CuSO_4$, as well as test their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, along with an assessment of their cytotoxicity effects on dengue virus serotype 2 (DENV-2). The results of this research can contribute to the development of more effective antimicrobial and antiviral compounds, with the potential for wide applications in the medical and public health fields.

MATERIALS AND METHODS

Materials

The substances utilized in this study included Anhydrous $CuSO_4$ (Merck, Germany), Na-EDTA (Merck, Germany), sterile aquades, nutrient agar (Oxoid, UK), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), Nalidixic acid (Oxoid, UK), NaCl (Merck, Germany), Minimum Essential Medium Eagle (Sigma-Aldrich, Germany), Fetal Bovine Serum (Sigma-Aldrich, Germany), Dimethyl sulfoxide (Merck, Germany), Viral ToxGloTM Assay reagent (Promega, USA), Vero Cell (ATCC CCL-81TM, USA), trypsin-EDTA (Thermo Fisher Scientific, USA), trypan blue (Thermo Fisher Scientific, USA), DENV-2 Surabaya isolate (Accession number: KT012509).

Synthesis Complex Cu(II)-EDTA

The synthesis of the compounds was carried out using the solvothermal method. That is, the Cu(II) compound used comes from anhydrous $CuSO_4$. The ratio of stoichiometry used (Cu: EDTA) is 1:1. The solution is mixed and then heated at 120°C for 24 hours. Then let it sit for 24 hours until the crystals slowly form[12] Cu(II)-EDTA crystals with blue color were obtained. Characterization of complex used UV-Vis Spectroscopy (Shimadzu UV-1650 PC), Fourier Transform Infra Red Spectroscopy (8400S Shimadzu), Differential Scanning Calorimetry (Perkin Elmer DSC 4000), and Scanning Electron Microscope (HITACHI FLEXSEM 100).

Antibacterial Activity Test

The antibacterial testing method used is the disc diffusion method, utilizing gram-negative (*E. coli*), which is frequently employed as the model bacterium to assess bactericidal efficacy, and gram-positive (*S. aureus*) bacteria, with slight modification[13], *E. coli* and *S. aureus* were subcultured in NA medium and incubated at 37°C for 24 hours. Furthermore, the bacteria were suspended in a 0.9% NaCl solution with a

concentration of 0.5 McFarland (1.5×10^8 CFU/mL). Six blank discs and one nalidixic acid disk (30 µg/disk) for positive control were placed on NA media. Test compound, containing EDTA, $CuSO_4$, Cu(II)-EDTA with concentrations of 250, 200, 150, 100, and 50 mg/mL, respectively. Sterile aquades was used for the negative control. Antibacterial effectiveness is assessed by measuring the inhibition zone that develops after 24 hours of incubation at a temperature of 37°C.

Antidengue Activity Test

The DENV-2 antiviral activity test was conducted based on the method by Sucipto et al (2019)[14] with slight modifications. Vero cell lines were employed for the assay. A confluent monolayer of Vero cells was added into 96 plate at a density of 5×10^4 cells/mL and incubated for 24 hours at 37°C with 5% CO_2. Then the medium was discarded and replace with 50 µl of MEM containing 10% FBS was added; 25 µl of samples (EDTA, and Cu(II)-EDTA) at concentrations of 31.25, 62.5, 125, 250, 500, and 1000 µg/ml; and 25 µl of DENV-2 stock at a concentration of 2 × 103 FFU/mL. Repeated 3 times. Next, the sample was incubated for 48 hours at 37°C with 5% CO_2. After incubation, 100 µl of Viral ToxGlo assay reagent was added and incubated at 37°C with 50% CO_2. The luminescence data obtained was then calculated as % of CPE (cytophatic effect) cells with the formula:

$$\%CPE = \frac{a-b}{c-b} x100\%$$

With a = luminescence of the treatment group; b = luminescence of the medium control; c = luminescence of the DENV-2 and cell control group. Based on the %CPE data, a linear regression graph is plotted with concentration (x) versus %CPE (y), using the equation y = ax + b to calculate the EC_{50} value.

Cytotoxicity Test

The cytotoxicity assessment was performed using the MTT assay method.[14] Vero cell lines were utilized for MTT assays, with confluent monolayers of Vero cells added to 96 plates (5×10^4 cells/mL) and incubated for 24 hours at 37°C with 50% CO_2. Then the medium was discarded and washed with PBS 1× 3 times. Then add 100 µl of MEM 10% FBS; 100 µl of samples (EDTA, and Cu(II)-EDTA) with concentrations of 31.25, 62.5, 125, 250, 500, and 1000 µg/ml; and incubated for 24 hours at 37 °C 5% CO_2. Repeated 3 times. At the end of incubation, the culture medium containing the sample is discarded, and washed with 100 µl of PBS, and 10 µl of MTT reagent is added. Incubate again for 4 hours and wash with PBS. Absorbance is read with a microplate reader at 595 nm. The data obtained is absorbance data, and the % of living cells is calculated by the formula:

$$\%\text{cell viability} = \frac{a-b}{c-b} x100\%$$

Where, a = absorbance with treatment; b = media control absorbance; c = absorbance of cell control. Next, a linear graph of concentration vs % of viability cells was created to determine CC_{50}.

RESULTS AND DISCUSSION

This study successfully synthesized and characterized the Cu(II)-EDTA complex using $CuSO_4$ as a precursor. The solvothermal method produced blue crystals Cu(II)-EDTA with a Cu stoichiometry ratio of 1:1. The results of the characterization were obtained by using Differential Scanning Calorimetry (DSC) to identify phase changes, such as

melting, crystallization, decomposition, or transitions in a material, in this case Cu(II)-EDTA (Figure 1).

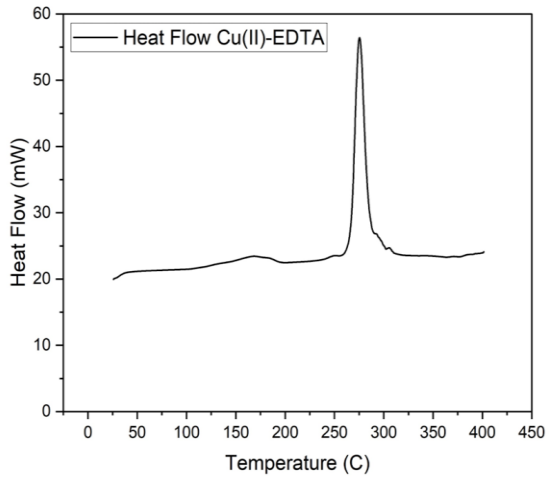

Figure 1. Thermal analysis measurement results of Cu(II)-EDTA at various heating rates using DSC.

The graph shows the thermal profile of Cu(II)-EDTA, with the x-axis indicating the temperature (in °C) and the y-axis indicating the heat flow (in mW). There is a significant endothermic peak around 270-300°C, which indicates a phase change or thermal decomposition process. Cu(II)-EDTA exhibits good thermal stability up to about 250°C, which means it is suitable for use in pharmaceutical formulations that require processing or storage at temperatures below that decomposition point. This knowledge of thermal stability is essential for the development of efficient and effective drug formulations, especially to ensure pharmaceutical effectiveness, safety, and stability of the final product.[15,16]

Particle size characterization is also necessary because the very small particle size facilitates penetration into the cell membrane, thereby increasing intracellular antibacterial activity and reactions. This is important because biofilm microorganisms are more resistant to antibacterial agents than planktonic pathogens, so effective treatment requires higher concentrations of agents.[17] The results obtained a particle size of 6.31 nm with a polydispersity index (PDI) of 0.076. This shows that the synthesized Cu(II)-EDTA is not yet qualified as a nanoparticle measuring <100 nm.[18] In addition, the PDI Cu(II)-EDTA value proves the uniformity of the particle size distribution because the closer the PDI value is to 0, the more uniform the sample size distribution is observed.[19]

In characterizing the functional groups contained in the synthesized molecules, the FTIR (Fourier Transform Infrared Spectroscopy) instrument is used. Here is a detailed analysis of the IR spectra of EDTA and Cu(II)-EDTA (Figure 2).

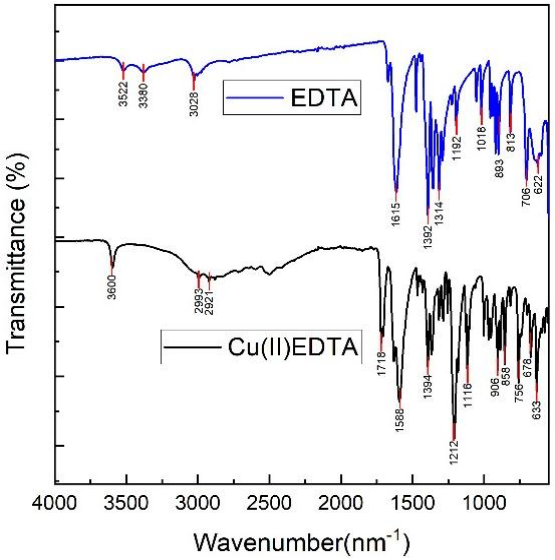

Figure 2. Results of measuring the spectra of EDTA and Cu(II)-EDTA chelates using FTIR

When EDTA is complexed with Cu^{2+} ions, there is a significant band shift. The O-H peak in the EDTA at 3522 cm^{-1} is lost or shifted, suggesting that the coordination of Cu^{2+} ions causes the -OH group to no longer be free. The C=O band at 1615 cm^{-1} in pure EDTA shifted to about 1588 cm^{-1}. This shift suggests that the carboxylate group ($-COO^-$) in EDTA participates in coordination with Cu^{2+} ions. The N-Cu band (stretching) at about 1212 cm^{-1} shows the interaction between the nitrogen atoms in EDTA and the Cu^{2+} ion. This indicates that nitrogen atoms are also involved in the

formation of complexes with Cu(II). This FTIR spectrum confirms that EDTA has successfully complexed with Cu(II) ions, which is indicated by a characteristic band shift in the C=O group, the loss of free O-H bands, and the emergence of new bands related to Cu-N and Cu-O interactions. This shift and change in IR peak intensity indicate a change in chemical structure, from free EDTA to Cu(II)-EDTA complex.

At 5000 magnifications (Figure 3A), the structure of the Cu(II)-EDTA particles is very clear. The particles exhibit an irregular and layered shape, suggesting that this complex has crystalline properties. At 2000 magnifications (Figure 3B), the layered structure and various particle sizes can be seen more clearly. The particles appear to be bound in a complex structure, suggesting that Cu(II)-EDTA is formed through a strong bond between Cu(II) and EDTA ions. At 1000 magnifications (Figure 3C), the overall structure of Cu(II)-EDTA can be clearly observed. The shape of the particles shows the morphology of large aggregates with a thick layer structure. The layered structure and the presence of agglomerations can affect the solubility and release properties of compounds in pharmaceutical applications. Morphology like this could mean that the Cu(II)-EDTA complex may have a slower release because the non-uniform and coated surface can slow diffusion in body fluids.

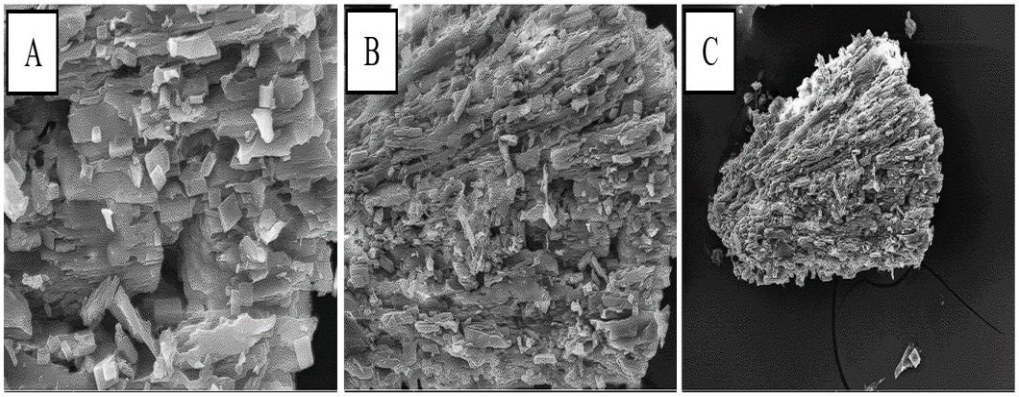

Figure 3. SEM images of Cu(II)-EDTA with magnification (A) 5000 times, (B) 2000 times, and (C) 1000 times

The results of the maximum wavelength measurement using the UV-Vis instrument (Figure 4) show that EDTA has a maximum wavelength in the UV region at about 244 nm, where EDTA absorbs strongly at 230 nm and flows to the absorbance range of 260 nm.[20] Meanwhile, the absorption of $CuSO_4$ is about 740 nm, where this result is similar to the wavelength reported by Hernández-López et al. (2021).[21]

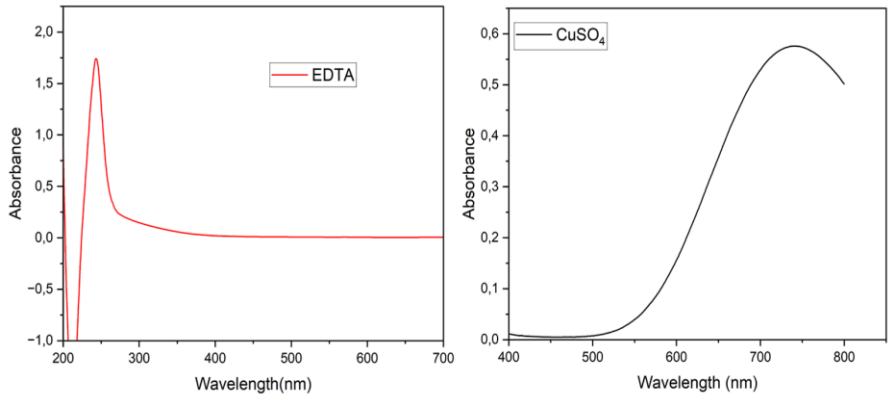

Figure 4. The maximum wavelength of EDTA and $CuSO_4$

An inverse relationship was observed between the concentration of the Cu(II)-EDTA complex and cell viability presented in the graph (Figure 5). The CC_{50} value of 415 µg/ml (Table 1) indicates that Cu(II)-EDTA exhibits a moderate to low level of cytotoxicity. This reduction in cell viability is dose-dependent, as evidenced by the strong correlation between increasing Cu(II)-EDTA concentration and its cytotoxic effects.

Table 1. CC_{50} values indicating cytotoxicity of the tested compounds

Compounds	CC_{50} (µg/ml)
Cu(II)-EDTA	415
$CuSO_4$	High Toxic
EDTA	High Toxic

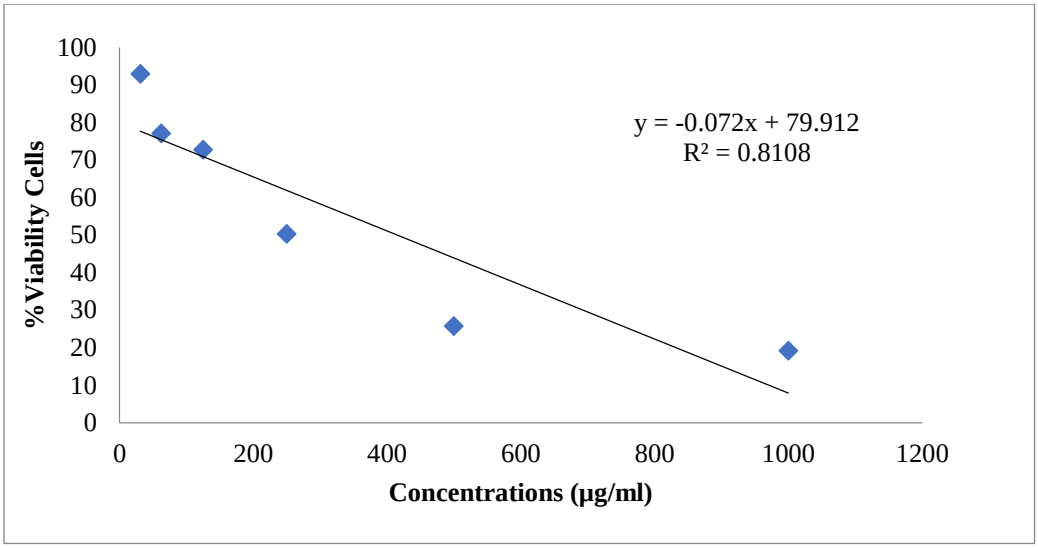

Figure 5. Cytotoxicity curve of Cu(II)-EDTA after treatment using the MTT assay.

The synthesized and characterized Cu(II)-EDTA that have been characterized are then evaluated for antibacterial activity against *E. coli* and *S. aureus*, with the results shown in Table 2 (*E. coli* antibacterial activity) and Table 3 (*S. aureus* antibacterial activity).

Table 2. *E.coli* antibacterial activity test results

Concentration (ppm)	EDTA	$CuSO_4$	Cu(II)-EDTA	K+
	Inhibition Zone ± SD	Inhibition Zone ± SD	Inhibition Zone ± SD	Inhibition Zone ± SD
250,000	24.05 ± 0.82	24.51 ±1.32	11.95 ± 0.15	31.32± 0.29
200,000	22.41 ± 0.30	23.17 ± 1.75	10,51 ± 0.06	
150,000	20.73 ± 0.60	22.17 ± 1.45	10.94 ± 0.83	
100,000	20.08 ± 1.60	20.61 ± 3.06	9.28 ± 1.32	
50,000	13.07 ± 1.99	15.24 ± 4.40	0.00 ± 0.00	

Table 3. *S. aureus* antibacterial activity test results

Concentration (ppm)	EDTA	$CuSO_4$	Cu(II)-EDTA	K+
	Inhibition Zone ± SD	Inhibition Zone ± SD	Inhibition Zone ± SD	Inhibition Zone ± SD
250,000	16.22 ± 2.14	26.35 ±1.09	11.03 ± 0.55	12.11 ± 0.74
200,000	16.04 ± 2.36	24.63 ± 2.10	8.99 ± 1.00	
150,000	15.75 ± 3.89	22.53 ± 0.32	9.40 ± 1.32	
100,000	15.84 ± 2.32	18.60 ± 2.43	7.95 ± 0.37	
50,000	0.00 ± 0.00	13.09 ± 0.63	7.60 ± 0.19	

The antibacterial activity of EDTA against *E. coli* showed the highest inhibitory zone of 24.05 mm at a concentration of 250,000 ppm and the lowest of 13.07 mm at 50,000 ppm, with increased concentrations resulting in a larger inhibitory zone. $CuSO_4$ produced the highest inhibition zone of 24.51 mm at 250,000 ppm and decreased to 15.24 mm at 50,000 ppm, demonstrating consistent antibacterial effects. In contrast, Cu(II)-EDTA showed the lowest activity with an inhibition zone of 11.95 mm at 250,000 ppm and 0 mm at 50,000 ppm, indicating significantly lower effectiveness than EDTA or $CuSO_4$ against *E. coli*.

EDTA showed the highest inhibition zone of 16.22 mm at 250,000 ppm against *S. aureus* (Table 3), but no inhibition zone (0 mm) at 50,000 ppm, indicating its effectiveness only at high concentrations. $CuSO_4$ has strong antibacterial activity with an inhibition zone of 26.35 mm at 250,000 ppm and decreases to 13.09 mm at 50,000 ppm. Cu(II)-EDTA showed a weaker effect, with an inhibition zone of 11.03 mm at 250,000 ppm and 7.60 mm at 50,000 ppm, much lower than $CuSO_4$.

EDTA has limited antibacterial activity due to its ability to chelate cations from the bacterial outer membrane. High concentrations of EDTA, such as 10%, can produce significant inhibitory zones, but lower concentrations indicate minimal effectiveness. EDTA activity remains as long as chelation has not formed a bond with metal ions.[19] In addition, Cu^{2+} ions from $CuSO_4$ produce Reactive Oxygen Species (ROS), such as O_2 and H_2O_2, which cause damage to *S. aureus* and *E. coli* bacterial cells. The reaction of Haber-Weiss and Fenton triggers the formation of hydroxyl radicals (•OH), which are highly reactive and damage the lipids, proteins, and DNA of bacteria. This damage interferes with the integrity of cell membranes, leading to the death of bacteria. This mechanism explains the powerful antibacterial effects of Cu^{2+}.[26] The Cu(II)-EDTA complex has lower antibacterial activity than pure $CuSO_4$ and EDTA because Cu^{2+} ions are tightly bound by EDTA, so the amount of free Cu^{2+} that can produce oxidative stress or attack bacteria is reduced. EDTA may bind Cu^{2+} too strongly, decreasing antibacterial effectiveness. However, at high concentrations, Cu(II)-EDTA still exhibits antibacterial activity, likely due to increased membrane permeability by EDTA and partial release of Cu^{2+} from the complex.

EDTA and $CuSO_4$ were also tested for antiviral activity against dengue virus using DENV-2 because it is more closely related to dengue hemorrhagic fever (DHF) cases, which are more severe compared to other serotypes. Research shows that DENV-2 is significantly more commonly associated with dengue cases than DENV-1. Moreover, DENV-2 and DENV-3 are reported to carry a twofold higher risk of triggering dengue infection compared to DENV-4 [21] The results of the antidengue assay indicated that the EDTA concentration needed to inhibit 50% of the replication of the DENV-2 virus was 356.13 µg/mL. This EC_{50} value indicates that EDTA has low potency as an antiviral agent against DENV-2, as the required concentration is quite high. In addition, $CuSO_4$ is more effective than EDTA in inhibiting DENV-2 replication, due to its lower EC_{50} value (77.86 µg/mL). This indicates that $CuSO_4$ exhibits greater antiviral efficacy against DENV-2 than EDTA.

Based on the slope of the two compounds (Figure 6), it can be seen that $CuSO_4$ has a greater inhibition effect at lower concentrations than EDTA. However, the effectiveness of $CuSO_4$ decreases drastically as its concentration

increases. EDTA showed a much slower decline in effectiveness. This could indicate that EDTA works more slowly or inefficiently in inhibiting DENV-2 replication than $CuSO_4$, but maintains a more stable inhibition at various concentrations.

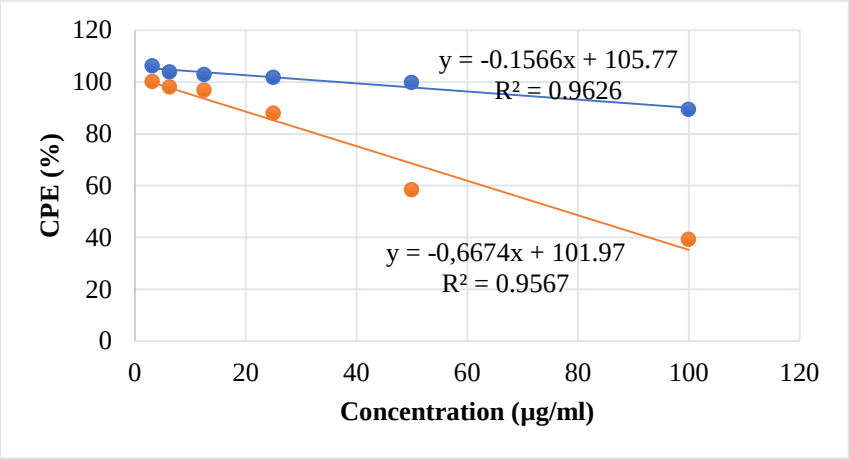

Figure 6. Regression curve of the inhibited DENV-2 after treatment with EDTA (blue line colour) and $CuSO_4$ (orange line colour)

STRENGTH AND LIMITATION

The strength and limitation of this study is there ae not many studies about antiviral from complex compound, especially DENV in Indonesia. The isolate we used for this study were native to Indonesia. This kind of research is certainly essential in Indonesia, as each isolate will have different effects on a drug candidate. Based on the results of this study, it can be used as a referenece for similar reasearch in the future.

CONCLUSIONS

This study succeeded in synthesizing the Cu(II)-EDTA complex using the solvothermal method. The complex has a small particle size and uniform size distribution, but the antibacterial and antiviral activity against DENV-2 is lower compared to $CuSO_4$. $CuSO_4$ was shown to be more effective in inhibiting DENV-2 replication, while the Cu(II)-EDTA complex showed potential as an antibacterial agent, although its effectiveness was lower.

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None to declare.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest to disclose.

AUTHOR CONTRIBUTION

Conception, design, and material: BN and THS; supervision and resources: THS; data collection or processing and literature search: BN, THS, and TJK; analysis or interpretation: TJK and BN; writing manuscript: BN and TJK; and critical review: THS, TJK, HH, and SR.

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Correlation between Probable or Non-Probable Leptospirosis with Laboratory Findings: Based on Leptospirosis Case Definition and Faine Criteria

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Abstract

The incidence of leptospirosis is increasing globally, and developing countries are no exception. Leptospirosis cases are called the tip of the iceberg phenomenon, even though misdiagnosis, underdiagnosis, and underreporting still occur in health services. Thus, it leads to delays in leptospirosis treatment and may result in increased mortality rate from severe leptospirosis infection (Weil's disease). This study was to establish an accurate diagnosis by optimizing the Faine criteria. This study used an analytical observational design with a cross-sectional approach to examine faine criteria and laboratory examinations. We collected data from medical records from the Karanganyar General Hospital and the PKU Muhammadiyah Surakarta Hospital. We processed the data using SPSS version 25. The total number of samples was 42. They were divided into women (19%) and men (81%). Based on the definition category of leptospirosis cases, there were 2.4% probable group (score criteria faine part A 20-25) and 97.6% not-probable group (score criteria faine part A <20). Bivariate analysis (Chi-Square test) showed that there was no significant correlation between Faine Part A criteria and serological tests in both groups (p=0.874) as well as Hb (p=0.522), thrombocytopenia (p=0.265), leukocytosis (p=0.197), and neutrophilia (p=0.710). Loss of sodium and potassium didn't show significant data (hyponatremia p=0.174; hypokalemia p=0.311; hypocalcemia p=0.131) not as in tropical diseases. The approach to diagnosis of leptospirosis cannot be performed using only Part A criteria, Faine, even though the patient was included in the probable definition category, even though the Faine Part A criteria score is 20-25 or ≥26.

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INTRODUCTION

Leptospirosis is an acute infectious disease of humans and animals (zoonosis) caused by the microorganism Leptospira *spp*.[1] Leptospira bacteria are most commonly transmitted to humans through contact with infected animals. This occurs directly, either through direct animal-to-human contact or indirectly through contact with the animal's urine, soil, or water.[2,3]

The World Health Organization (WHO) estimates that approximately 873,000 cases of leptospirosis emerge annually, resulting in over 40,000 deaths. In the United States, the incidence of leptospirosis ranges from 100 to 200 cases per year. In comparison, the incidence of leptospirosis in tropical climates is nearly ten times that of moderate climates.[2] The Indonesian Ministry of Health reports that 920 cases of leptospirosis have been diagnosed, with 112 resulting in death. These cases have been documented in nine provinces: Banten, Jakarta, West Java, Central Java, Yogyakarta, Maluku, South Sulawesi, North Kalimantan, and the rest of Indonesia. These numbers are relatively low in comparison to the annual morbidity rate of leptospirosis in Indonesia, which has been estimated at 39.2 per 100,000 people.[4] However, the case fatality rate during 2018 was 17.8% among an estimated 895 human cases.[5]

The diagnosis of leptospirosis is typically based on clinical features and a history of risk exposure.[6] Conventional laboratory methods for the diagnosis of leptospirosis rely on the examination of patient immune response parameters. The Dark-Ground Microscope (DGM) is the optimal approach for the visualisation of Leptospira organisms on culture media. However, this examination has intrinsic limitations as a diagnostic tool, including the potential for false-negative results due to low concentrations of the organisms in the specimen and false-positive results due to artifacts and the presence of fibrin.[1] Serological analysis employing the microscopic agglutination test (MAT) has been demonstrated to have greater sensitivity in detecting cases of leptospirosis. Due to the inherent complexity of the MAT, rapid screening tests for leptospiral antibodies in acute infections have been developed to facilitate prompt diagnostic confirmation and initiate effective treatment. IgM antibodies, which are produced in the initial phase (after 4-7 days) of the infection, can be identified within the first week of illness, thus providing the opportunity to diagnose the disease and initiate appropriate therapy.[1,7,8]

Three criteria have been established for defining cases of leptospirosis. These are as follows: 1) Suspect cases, 2) Probable cases, and 3) Confirmed cases (Table 1). Furthermore, the World Health Organization (WHO) introduced the Faine criteria for diagnosing leptospirosis cases, which are divided into three parts: 1) Part A (based on clinical history), 2) Part B (based on epidemiologic history), and 3) Part C (laboratory parameters as support for Part B) (Table 2).[9] However, in Indonesia, there has been an increase in leptospirosis cases, a phenomenon that is referred to as “the tip of the iceberg" despite misdiagnosis, underdiagnosis, and underreporting in health services.[10]

Therefore, this study aims to determine the correlation between probable group (score criteria faine part A 20-25) or not-probable group (score criteria faine part A <20) and laboratory findings to facilitate the accurate diagnosis of leptospirosis using the Faine criteria. This will enable earlier diagnosis in primary care facilities, thus preventing the worsening or death of leptospirosis cases.

Table 1. The following three definitions of leptospirosis cases are according to the Book of Internal Medicine, UI Volume I.

Definition	Criteria
1. Suspect Case	• Acute fever with or without headache; • Myalgia; • Weakness (malaise); • Conjunctival hiperemis; • Ciliary suffusion; • Additionally, the individual has a documented history of exposure to a potentially contaminated environment within the last two weeks.
2. Probable Case (Two of the following clinical signs and symptoms were identified)	• Gastrocnemius muscle pain; • jaundice (skin and sclera); • Bleeding manifestation; • Dyspneu; • Oliguria or anuria; • Cardiac arrhythmia; • Cough with or without blood (haemoptysis); • Skin rash. In addition, have laboratory results: • Thrombocytopenia < 100.00 cell/mm • Leucocytosis with neutrophilia >80% • An increase in total bilirubin levels by > 2% or an increase in the levels of other enzymes, including aspartate aminotransferase (AST), amylase, lipase, and creatinine phosphokinase (CPK). • Rapid diagnostic tests (RDTs) are utilized to ascertain the presence of anti-leptospiral IgM antibodies.
3. Confirmed case (Probable cases are accompanied by one of the outcomes below)	• Isolation of Leptospira bacteria from clinical specimens; • The results of the Polymerase Chain Reaction (PCR) are positive; • A shift from a negative to a positive result on the Microscopic Agglutination Test (MAT) indicates seroconversion.

MATERIALS AND METHODS

METHODS

Study Designs and Patients

This study utilized an analytic observational study design with a cross-sectional approach. Data were collected from two hospitals, namely PKU (*Pembina Kesejahteraan Umat*) Muhammadiyah Surakarta Hospital and Karanganyar Regency General Hospital. A non-probability purposive sampling technique was employed to select participants. Data were gathered from.

June 2022 to June 2023. Patient data were collected for individuals ≥18 years of age, hospitalized patients, and those with laboratory results. Patients with a history that have similar and potentially overlapping symptoms or laboratory results, such as uncontrolled diabetes mellitus, uncontrolled hypertension, heart disease, neuroinfectious diseases, and autoimmune diseases, were excluded from the study.[11–15] Additionally, patients with positive laboratory results on dengue fever or typhoid fever serology tests were excluded because these infectious diseases have the same laboratory changes as leptospirosis to avoid overlap and to avoid bias in the category of leptospirosis case definitions.[16–18] Furthermore, the results of the patient's history, such as duration of fever, history of muscle pain, and history of activity in contaminated environments, and physical examination upon admission to the emergency department such as conjunctival suffusion, icteric sclera, epigastric pain, hepatosplenomegaly, gastrocnemius muscle pain, and jaundice were utilized in classifying the patient's Faine criteria.

Patients were categorized as probable or not probable definition. To obtain the data group of confirmed leptospirosis patients, it was ensured that probable group (sample with probable definition category but the Faine Criteria (Part A) score between 20-25) or not probable group (Sample with probable definition category but the Faine Criteria (Part A) score < 20) had confirmed positive results on the IgM/Rapid leptospirosis antibody test. Furthermore, we gathered additional laboratory data, including hemoglobin, platelets, leukocytes, neutrophils, sodium, potassium, and calcium, to contrast the outcomes of probable group (sample with probable definition category but the Faine Criteria (Part A) score between 20-25) or not probable group (Sample with probable definition category but the Faine Criteria (Part A) score < 20) with confirmed positive IgM/rapid results with those of patients with confirmed negative IgM/rapid leptospirosis results.

The Table 1 was used to define which patients are probable cases based on the criteria of each case status. All samples used were probable cases according to the definition of the table, with at least 2 signs and symptoms, and or with IgM/Rapid test results.

Meanwhile, the World Health Organization has established criteria for the diagnosis of leptospirosis, known as the Faine criteria. These criteria are divided into three parts.

In accordance with the Faine criteria Table 2, the score of each sample will be obtained with the score category of Part A, or the score of Part A and Part B was 26 or more. Furthermore, the total score of Parts A, B, and C was 25 or more, and the score of 20-25 was Possible Leptospirosis, so a presumptive diagnosis of leptospirosis can be made.

Table 2. The World Health Organisation's Faine criteria are divided into three parts.

Faine Criteria	Score
1. Part A: Based on Clinical data	
• Headache	2
• Fever	2
• If fever ≥39°C	2
• Conjunctival suffusion	4
• Meningism	4
• Myalgia (especially gastrocnemius muscles)	4
• Conjunctival suffusion + meningism + Myalgia	10
• Jaundice	1
• Albuminuria/Nitrogen retention	2
• Haemoptysis/dyspnea	2
2. Part B: Based on Epidemiological history	
• Rainfall	5
• Contact with contaminated environment	4
• Animal Contact	1
3. Part C: Bacteriological and Laboratory Findings	
• Isolation of leptospira in culture – Diagnosis certain	
- PCR[a]	25
• Positive serology	
- ELISA[b] IgM positive	15
- SAT[c] positive	15
- Other rapid test	15
- MAT[d] – single positive in high titer	15
- MAT – rising titer/seroconversion	25
Presumptive diagnosis of leptospirosis is made of:	
• Part A or Part A and Part B score: 26 or more	
• Total score of Parts A, B, and C: 25 or more	
• Possible Leptospirosis if the score is between 20-25	

[a]Polymerase Chain Reaction; [b]Enzyme Linked Immunosorbant Assay; [c]Slide Agglutination Test; [d]Microscopic Agglutination Test

Statistical Analysis

The characteristics of the study and the laboratory results were described using the format "number (n) and percentage (%)". The data were analyzed univariately to determine associations between the variables. The results were then analyzed bivariately with the chi-square test to assess the correlation between probable/not probable leptospirosis patients with positive

IgM/Rapid test results and laboratory variables, namely hemoglobin, platelets, leukocytes, neutrophils, sodium, potassium, and calcium, using SPSS version 25.

RESULTS AND DISCUSSION

Table 3. Characteristics of the study

Characteristic	Number (n)	Percentage (%)
Gender		
Female	8	19%
Male	34	81%
Serologic Test		
IgM (+)	41	97.6%
Rapid test (+)	1	2.4%
Faine score (Part A)		
Probable (Score 20-25)[a]	1	2.%
Not Probable (Score <20)[b]	41	97.%
Hemoglobin		
Anemic	12	28.%
not anemic	30	71.4%
Thrombocytes		
Thrombocytopenia	23	54.8%
Not thrombocytopenia	19	45.2%
Leukocytes		
Leukocytosis	16	38.1%
Not leukocytosis	26	61.9%
Neutrophil		
Neutrophilia	37	88.1%
Not neutrophilia	5	11.9%
Sodium (Na^+)		
Hyponatremia	15	35.7%
Not hyponatremia	27	64.3%
Potassium (K^+)		
Hypokalemia	21	50%
Not hypokalemia	21	50%
Calcium (Ca^{2+})		
Hypocalcemia	13	31%
Not hypocalcemia	29	69%

[a]Probable Sample with probable definition category but the Faine Criteria (Part A) score between 20-25; [b]Not Probable= Sample with probable definition category but the Faine Criteria (Part A) score < 20.

We obtained a total of 42 samples that met the restriction criteria for the period June 2022 to June 2023 at PKU Muhammadiyah Surakarta Hospital and Karanganyar Regency General Hospital. The 42 samples consisted of women (n=8; 19%) and men (n=34; 81%) (Table 3). Based on the Faine criteria (part A), we found that only one sample whose probable definition category was probable leptospirosis (n=1; 2.4%) and 41 samples whose probable definition category was not probable leptospirosis (n=41; 97.6%). However, all samples we obtained had positive serology test results, both IgM (n=41; 97.6%) and Rapid test (n=1; 2.4%).

Laboratory results indicated the presence of anemia were 12 of 42 samples or 28.6% (n=12; 28.6%), thrombocytopenia were 23 of 42 samples or 54% (n=23; 54%), leukocytosis were 16 of 42 samples or 38.1% (n=16; 38.1%), and neutrophilia were 37 of 42 samples or 88.1% (n=37; 88.1%). The remaining abnormalities observed were as follows: Hyponatremia was 15 of 42 samples or 35.7% (n=15; 35.7%), hypokalemia was 21 of 42 samples or 50% (n=21; 50%), and hypocalcemia was 13 of 42 samples or 31% (n=13; 31%).

The results of bivariate analysis of the relationship between the results of serology examination and probable definition category in Table 4 indicated that the majority of leptospirosis samples with positive IgM test results were included in cases that were not probable leptospirosis by Faine Criteria (part A). Specifically, 40 samples (95.2%) exhibited this pattern. One sample (2.4%) with probable definition category exhibited a positive IgM result, while one additional patient (2.4%) had a positive leptospirosis rapid test result in the not probable definition category. The results of the serological examination analysis yielded a probability (p) value of 0.874 ($p > 0.05$), indicating that there is no significant relationship between the results of the serological examination and probable definition category (Table 4).

Table 4. Bivariate Analysis of Leptospirosis Serology Test Results for Probable Leptospirosis Cases.

Variable	Faine Criteria (Part A)				P value*
	Not Probable		Probable[a]		
	n	%	N	%	
Serology test					
IgM (+)	40	95.2	1	2.4	0.874
Rapid test (+)	1	2.4	0	0	

[a]Probable= Sample with probable definition category but the Faine Criteria (Part A) score between 20-25;
[b]Not Probable= Sample with probable definition category but the Faine Criteria (Part A) score < 20;
*P-value: Bivariate analysis by chi-square test.

Furthermore, the results of bivariate analysis in Table 5 showed that more samples in the not probable definition category had thrombocytopenia (n=23; 54.8%; *p*=0.265) and neutrophilia (n=36; 85.7%; *p*=0.710). Meanwhile, the probable definition category had leukocytosis (n=1; 2.4%: *p*=0.197) and neutrophilia (n=1; 2.4%: *p*=0.710). This analysis showed that leptospirosis samples with no probable definition category can have thrombocytopenia and neutrophilia (Table 5).

Table 5. Bivariate Analysis of Routine Blood Testing Results for Probable Leptospirosis Cases.

Variable	Faine Criteria (Part A)				p value*
	Not Probable[b]		Probable[a]		
	n	%	N	%	
Hemoglobin					
Anemic	12	28.6	0	0	0.522
Not anemic	29	69	1	2.4	
Thrombocytes					
Thrombocytopenia	23	54.8	0	0	
Not Thrombocytopenia	18	42.8	1	2.4	0.265
Leukocytes					
Leukocytosis	15	35.7	1	2.4	
Not leukocytosis	26	61.9	0	0	0.197

Leptospira *spp*. Bacteria, which are the primary vectors for the bacteria.[19] In Indonesia, the increase in leptospirosis cases is referred to as the "tip of the iceberg phenomenon," despite the continued occurrence of misdiagnosis, underdiagnosis, and under-reporting in health services. [10]

A review of score part A faine criteria based on clinical data revealed that the characteristics of samples with leptospirosis showed a higher prevalence of not-probable definition category than probable definition category (Table.3). This discrepancy can be attributed to the incubation period of leptospirosis, which can range from 5 to 14 days or occur between days 2 and 21 after exposure to water or soil. In the leptospirosis phase, symptoms may manifest as a non-specific acute febrile illness or with symptoms such as fever, chills, myalgia, headache, ocular discomfort, nausea, and vomiting.[6,22] Additionally, red eyes and watery eyes may occur. Without serological confirmation, it is challenging to diagnose leptospirosis in this phase in primary healthcare.[6,7]

The diagnosis of leptospirosis is based on identifying the bacterium or its metabolic products in bodily fluids or tissues via serological testing. Serological testing is divided into genus-specific and serogroup-specific.[23] For a leptospirosis antibody test to be valid, antibodies must be present in the body between the third and tenth day following the onset of symptoms. This may result in a negative serology test result in samples collected during the first week of illness. These results should not be interpreted as evidence of the absence of infection and should be retested 7-14 days after the initial examination.[8,19]

Bivariate analysis in Table 4 to determine if there was a significant association between the Faine criteria score A and serology tests. The analysis revealed that there was no statistically significant association between the not-probable and probable definition categories that had positive serology test results (p=0.874). Antibodies are usually detected between the 6th and 10th day of illness, with a peak within 3-4 weeks.[6] Consequently, it is recommended that the serum test be repeated on two separate occasions, with a minimum interval of one to two weeks between each test. This approach was based on the understanding that seroconversion, or the development of antibodies in response to an infection, occurs during the disease.[10] A negative serologic test in the early phase of the disease does not mean that the patient is not infected with leptospirosis cause the early phase of the disease may enable seroconversion to be avoided due to its status as an incubation period, it is important to conduct a re-serum examination to ascertain the increase in titer between two samples (seroconversion) and confirm the diagnosis of leptospirosis. The administration of serum should be performed on two occasions, with a minimum interval of one to two weeks between each administration. This is typically based on the date of onset and the estimated time of seroconversion.[10]

The gold standard test for leptospirosis is the microscopic agglutination test (MAT). The MAT test is preferred over other screening options to avoid misdiagnosis of other tropical infections, including some difficult differential diagnoses due to overlapping clinical presentations such as dengue fever and dengue hemorrhagic fever, influenza, malaria, enteric fever, toxoplasmosis, hepatitis, and others.[9,19] Although the MAT test is the gold standard for leptospirosis, and can differentiate from other differential diagnoses. A study in Thailand showed that the MAT test was not perfect, but quantitative polymerase chain reaction (qPCR) can improve the sensitivity of leptospirosis diagnosis.[24] Furthermore, the MAT test is not available in primary care settings, so Faine criteria part A screening is an option in diagnosis.

The World Health Organization (WHO) has established criteria for leptospirosis screening, the Faine Criteria, consisting of Part A (clinical data; score A), Part B (epidemiologic data; score B), and Part C (serology or laboratory data; score C).[9] In Table 5, 41 samples were categorized as not-probable definition category based on clinical data, and one sample was classified as probable definition category. However, both groups had positive IgM or rapid test serologic results for leptospirosis. The results of the bivariate analysis in Table 4 regarding the results of the serological examination of probable definition category showed that there was no significant results (p=0.874), so this can explain that the leptospirosis diagnostic approach with Faine criteria based on Part A (score 20-25) does not have a significant correlation and still requires evaluation with Part B, especially Part C (serology/laboratory data). However, not all primary health care services have facilities for laboratory examination of both leptospirosis IgM serology and MAT tests, so the Faine criteria part A alone is not recommended when used for the Leptospirosis diagnostic approach in primary health care.

Nonspecific laboratory examinations in leptospirosis can be performed, such as blood analysis, urine analysis, and cerebrospinal fluid.[25,26] Blood analysis showed leukocytosis shifted to the left and

thrombocytopenia. However, this examination cannot be used in the diagnosis of leptospirosis, but rather for differential diagnosis screening and initial observation, and thrombocytopenia is are important laboratory parameter in the context of leptospirosis. It can be used as an early recognition a priori to prevent complications and mortality.[27] Furthermore, studies have indicated that platelet counts of less than 46,000 cells/mm³ are correlated with the occurrence of sepsis, which was a severe form of leptospirosis.[28] As in the results of bivariate analysis, Table 5. The results of hemoglobin, platelets, leukocytes, and neutrophils in both groups showed non-significant results (hemoglobin p=0.522; thrombocytes p=0.265; leukocytes p=0.197; neutrophils p=0.710). Based on the examination results of the two groups, it can be concluded that leptospirosis patients do not always meet the probable febrile criteria and can be a differential diagnosis when thrombocytopenia and neutrophilia are found. If the health facility already has serologic testing as a specific test, the diagnosis of leptospirosis can be confirmed. However, non-specific tests are also important in patients suspected of having leptospirosis, with either a part A+B score (Faine criteria) of probable or non-probable definition category. It is because many patients with leptospirosis present with leukocytosis and thrombocytopenia, which usually do not result in spontaneous bleeding, but may result in gastrointestinal bleeding (melena or hematemesis) or pulmonary bleeding that is difficult to detect.[29] In addition, nonspecific testing can be useful when patients with severe leptospirosis (Weil's Disease) have developed multiorgan liver, kidney, lung, and brain.[29,30]

Hemodynamic changes with decreased systemic vascular resistance, increased cardiac output, and increased renal vascular resistance occur in tropical diseases. Hyponatremia can occur in tropical diseases due to increased levels of antidiuretic hormone (vasopressin), which causes sodium entry into cells, sodium loss, and osmoreceptor resetting.[31] Electrolyte disturbances can occur with renal involvement ranging from mild nonoliguric renal dysfunction to complete renal failure. It is associated with decreased expression of sodium-hydrogen exchanger-3, which leads to decreased reabsorption of sodium and fluid in the proximal tubule.[29] The evaluation of glycolipoprotein interaction with Na/K-ATPase demonstrated that the indication of natural resistance in leptospirosis is not due to the lack of sensitivity of Na/K-ATPase of renal cells, but rather due to the bioavailability of endotoxins in bacterially infected tissues.[32] Consequently, serum sodium and potassium levels are lower among patients with severe leptospirosis than among those with moderate leptospirosis or acute tubular necrosis.[33] Table 6 showed that electrolyte imbalance was not significant for sodium, potassium, or calcium (Na^+ p=0.174; K^+ p=0.311; Ca^{2+} p=0.131). Observations in patients with leptospirosis regarding the loss of sodium and potassium in large amounts occur when the patients are suffering from diarrhea.[31] In Table 6, not all samples were in diarrhea symptoms or diarrhea that had improved with symptomatic treatment that had been given previously due to the delay in diagnosis, thus electrolyte imbalance cannot be used as the principal examination in the diagnostic approach to leptospirosis. However, it is a useful tool for evaluating the impact of the disease and monitoring patient response to treatment.

STRENGTH AND LIMITATION

The findings of this study have the

potential to enhance the attention and diagnosis of leptospirosis cases, which are becoming increasingly prevalent in Indonesia. The results of this study can serve as a reference for primary care physicians to consider the appropriate criteria and improve the quality of support examinations in primary care. A limitation of this study was the restricted sampling frame, which included only two hospitals. Consequently, the number of samples obtained was relatively small. Additionally, the classification of the fainéant criteria for non-probable and probable leptospirosis groups was based solely on medical records, rather than direct interviews. It is recommended that future researchers conduct direct interviews to enhance the accuracy of group classification.

CONCLUSIONS

The approach to diagnosis of leptospirosis cannot be performed using only Faine Part A criteria, even though the patient is included in the probable definition category, even though the Faine Part A criteria score is 20-25 or ≥26. Because the results of this study showed that the Faine criteria part A score <20 showed a positive serological test result, it is not sufficient to diagnose leptospirosis only with Faine criteria part A, but at least use part B or part B and part C in the diagnosis of Leptospirosis. Moreover, there is considerable potential for misdiagnosis due to the possibility of overlap with other tropical diseases, resulting in delays or disruption to patient care. In such cases, primary health services can promptly provide appropriate treatment or first-line treatment, if the patient's Faine criteria part A score is within the range of 21–25. This approach is preferable to the alternative of not treating the patient despite the lack of a confirmed leptospirosis diagnosis.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed to the study by collecting data, analyzing results, and discussing the study.

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The Relationship between Personal Hygiene and the Incidence of Tinea Versicolor among Students at Madrasah Ulumul Quran (MUQ) Pagar Air Islamic Boarding School

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Abstract

Tinea versicolor is an infectious dermatological condition caused by fungi, affecting a substantial proportion of the global population. It is particularly prevalent in tropical regions, including Indonesia. Madrasah Ulumul Quran (MUQ) Pagar Air Islamic Boarding School is a densely populated area where students often exhibit poor hygiene practices, potentially increasing the incidence of the disease. The etiological agent responsible for this infection is the *Malassezia furfur* species, which can be prevented through the adoption of proper personal hygiene behaviors. This study aims to determine the association between personal hygiene and the incidence of Tinea versicolor at MUQ Pagar Air Islamic Boarding School. This is an observational study using a cross-sectional design. Data collection was conducted through interviews using questionnaires. The diagnosis of Tinea versicolor was based on the results of the 10% KOH examination. Sixty students from grades X, XI, and XII participated in the study, of which six were diagnosed with Tinea versicolor. The study found that the majority of the population performed good personal hygiene, with only 10% of the subjects diagnosed with Tinea versicolor. Chi-square analysis revealed a p-value of 0.000 (<0.05), indicating a significant association between personal hygiene and the incidence of Tinea versicolor at MUQ Pagar Air Islamic Boarding School. The majority of students at MUQ Pagar Air Islamic Boarding School practiced good personal hygiene and did not have a Tinea versicolor infection.

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INTRODUCTION

This condition is caused by the fungi *Malassezia furfur* or *Pityrosporum orbiculare*.[1,2] Tinea versicolor affects 20-25% of the global population and is commonly found in tropical regions, such as Indonesia.[3,4] Elevated temperature and humidity favor the proliferation of the fungi responsible for tinea versicolor. The incidence of tinea versicolor tends to increase with age. Young adults -characterized by active sebaceous gland function and higher levels of physical activity -are more frequently affected and report cases more commonly than other age groups. However, existing data present varied findings, and no comprehensive studies have yet been conducted to assess this condition using a multilevel epidemiological approach. Epidemiological estimates suggest that 40-50% of the Indonesian population has experienced tinea versicolor during their lifetime.[5–8]

Tinea versicolor causes skin discoloration, resulting in lighter, darker, or even reddish spots compared to the surrounding skin. The trunk is the most commonly affected area due to the highest concentration of sebaceous glands. Nonetheless, tinea versicolor may also involve other anatomical sites, particularly those with frequent exposure to ultraviolet (UV) radiation, such as the forearms, cervical region, and facial area.[4,9] Although some patients may experience itching, the majority remain asymptomatic. The incidence of the disease is associated with densely populated areas and poor hygiene conditions.[10]

Personal hygiene refers to an individual's attitude and behavior in maintaining and improving personal health to protect against disease. Personal hygiene is a multifaceted domain influenced by complex biopsychosocial factors, including -but not limited to -self-perception, knowledge, social norms, economic conditions, and cultural context. Personal hygiene practices can be implemented by maintaining body cleanliness, such as bathing regularly, brushing teeth, washing hands, and wearing clean clothes.[1,11]

Additionally, the environmental conditions can influence the incidence of infection. For example, densely populated areas are considered a risk factor for tinea versicolor infection. Islamic boarding schools are typically located near residential areas, with relatively small room sizes, limited cleanliness, and poor personal hygiene practices among students, such as sharing of personal items (towels, toiletries, and bedding), which further increases the risk of tinea versicolor infections.[12,13]

The purpose of this study was to examine the relationship between personal hygiene practices and the incidence of tinea versicolor among students at the MUQ Pagar Air Islamic Boarding School in Aceh, Indonesia.

MATERIALS AND METHODS

Materials

The study used a reliable and valid questionnaire to assess personal hygiene. The range of values for the personal hygiene variable was 0-11. Good personal hygiene was defined as a score of >75% (scores of 9-11), while not good personal hygiene was defined as a score of ≤75% (scores of 0-8). The tinea versicolor diagnosis was confirmed definitively in the laboratory using KOH 10% examination.

Methods

The study was conducted using a cross-sectional approach. The population consisted of students from grades X, XI, and XII at MUQ Pagar Air Islamic

Boarding School, totaling 130 students. Using the Slovin formula, the required sample size was calculated to be 58 subjects. Stratified random sampling was employed to select the subjects by dividing the population into strata. The inclusion criteria comprised active students whose participation was approved through written informed consent provided by their parents. The exclusion criteria included students with other pre-existing skin conditions, identified through self-reported responses and clinical findings by the researchers.

RESULTS AND DISCUSSION

The study involved students of grades X, XI, and XII as subjects of the study. Of the 130 female students, 60 met the criteria as the subjects in this study.

Table 1. Student's Personal Hygiene.

Personal Hygiene	Frequency (n)	Percentage (%)
Not good	10	16.7
Good	50	83.3
Total	60	100.0

Table 1 shows that the majority of students at MUQ Pagar Air Islamic Boarding School performed good personal hygiene. Several studies have examined personal hygiene practices among different populations. Desmawati et al. in 2015 at the Al-Kausar Pekanbaru Islamic Boarding School found that 61% of subjects practiced good personal hygiene. Similarly, Ahsani Nadiya et al. in 2019 at the Sa'adatuddarain Islamic Boarding School reported that 54.1% of students exhibited good personal hygiene. Another study by Dewi et al. in 2017 at SMPN 4 Denpasar found that 79% of subjects maintained good personal hygiene. Additionally, a 2021 study by Cep Reza Alam Wahid also showed that the majority of the population met the criteria for good personal hygiene.[14–17]

Table 2. The incidence of Tinea versicolor.

Tinea versicolor	Frequency (n)	Percentage (%)
Yes	6	10.0
No	54	90.0
Total	60	100.0

Table 2 shows that the majority of students at MUQ Pagar Air Islamic Boarding School did not experience tinea versicolor. This finding aligned with studies conducted by Wardana et al. in 2020 at the Darussa'adah Mojo Agung Islamic Boarding School, Central Lampung (78.6% without tinea versicolor), Riska Nazaria et al. in 2017 at Madrasah Tsanawiyah Islamic Boarding School (67.6% without tinea versicolor), and Dwiky Saputra Armansyah in 2020 at Mathla'ul Anwar Islamic Boarding School (80% without tinea versicolor). Additionally, Cep Reza Alam Wahid's study also found that the majority (94.8%) of students at an Islamic boarding school did not have tinea versicolor.[1,3,17–20]

Although various studies may employ varying operational definitions for the diagnosis of tinea versicolor -utilizing methods such as Wood's lamp examination or direct microscopic analysis -all yielded definitive rather than presumptive findings, thereby ensuring the reliability of case identification within the studied population.

Table 3. Bivariate analysis.

Personal Hygiene	Tinea versicolor				Total		p-value
	Yes		No				
	n	%	n	%	n	%	
Not good	6	60	4	40	10	100	0.00
Good	0	0	50	100	50	100	

Bivariate analysis (Chi-square test), as presented in Table 3, yielded a p-value of 0.00 ($p < 0.05$), providing evidence of a statistically significant relationship between personal hygiene and the incidence of tinea versicolor at MUQ Pagar Air Islamic Boarding School. Additional studies,

including those conducted by Wahid C. and Sudiadnyani in similar Islamic boarding school settings, support these findings. Both studies identified personal hygiene as a determinant factor in the occurrence of tinea versicolor, reporting statistically significant associations with p-values of 0.024 and 0.000, respectively. [17,21]

The frequent poor personal hygiene behaviors observed among MUQ Pagar Air students, such as not changing clothes after sweating and not washing hands before or after activities, are likely due to a lack of education and health promotion regarding clean and healthy living practices. Not changing clothes after sweating creates an environment conducive to fungal growth. *Malassezia furfur* thrives on moist skin, altering the skin flora and promoting the development of pathogenic mycelia. Additionally, humid conditions can further increase the virulence of the fungus. Fungal virulence can cause itching, leading to scratching, and the habit of not washing hands before or after activities may facilitate the transfer of mycelia from the keratin layer of the stratum corneum to healthy skin areas, potentially leading to the progression of tinea versicolor.[21–23]

In this study, the majority of students with good personal hygiene can be attributed to the adequate facilities and infrastructure at MUQ Pagar Air Islamic Boarding School, which effectively support students in maintaining good personal hygiene. Additional factors influencing the implementation of personal hygiene practices -particularly among students as a distinct study population - such as institutional regulations and social obligations within dormitory settings, as well as access to routine healthcare services, warrant further investigation. It is also advisable to identify and control for potential confounding variables that may adversely affect the observed outcomes.

Epidemiological studies examining the statistical significance between variables associated with tinea versicolor have generally reported substantial associations, though the findings remain varied. The diverse outcomes regarding tinea versicolor prevalence among student populations across different educational settings suggest the likely involvement of multiple confounding factors influencing the occurrence of this infection.[19,20,24,25]

Malassezia peaks in adolescent (pubertal) and young adult age groups, which aligns with the increased activity of sebaceous glands in these age groups. The yeast density gradually decreases with age. Under normal conditions, *Malassezia* acts as a normal mycobiome, evading the local immune response and maintaining equilibrium, thus not showing significant pathological signs (asymptomatic). However, changes in the proliferation of the saprophytic *Malassezia* yeast into pathogenic hyphae can occur. Various conditions can disrupt this balance, such as immune insufficiency due to chronic metabolic diseases (e.g., diabetes), HIV infection, long-term systemic steroid use, or the use of immunosuppressive agents, which can further promote the pathogenicity of *Malassezia*-related skin diseases. Other predispositions, such as hyperhidrosis and malnutrition, may also be related to the study group in this context.[26,27]

Malassezia spp., by virtue of their pathogenic potential, have been implicated in a broad spectrum of dermatological disorders. These include superficial mycoses such as tinea (pityriasis) versicolor, inflammatory dermatoses including sebaceous gland-associated conditions and seborrheic or atopic dermatitis, as well as immune-mediated disorders like psoriasis. Furthermore, *Malassezia* may co-occur with or

predispose to other fungal infections, such as onychomycosis, and in certain immunocompromised hosts, may contribute to systemic and invasive mycoses. An increased risk of *Malassezia* invasion may result from an elevated yeast population, alterations in epidermal pH, and the mechanical spread of the pathogen due to scratching -all of which are associated with and exacerbated by poor personal hygiene.[26,27]

In tinea versicolor, the skin lesions caused by fungal hyphal invasion arise between and are limited to keratinized cells. Damage to the stratum corneum and depigmentation may occur due to dysregulation of the melanization process. Hypo- or hyperpigmentation is determined by the involvement of azelaic acid produced by *Malassezia*. Azelaic acid inhibits tyrosinase, a component required for melanin synthesis, and this inhibition typically occurs in individuals with darker skin. Conversely, the cytotoxic effects of azelaic acid, which affect the production of bioactive indoles from tryptophan, have implications for melanogenesis, presenting as hyperpigmented lesions, which are more commonly found in individuals with lighter skin.[26,27]

The clinical presentation, highly suggestive of a *Malassezia* infection consistent with tinea versicolor, was subsequently confirmed via direct mycological examination by the researcher. Skin scrapings were subjected to a potassium hydroxide (KOH) 10% preparation, which revealed characteristic morphological features (clusters of round-to-oval, thick-walled yeast cells arranged in grape-like formations, accompanied by short, curved, septate hyphal elements). This classic "spaghetti and meatballs" appearance is pathognomonic for *Malassezia* species and confirms the diagnosis of tinea versicolor among the students.

STRENGTH AND LIMITATION

One of the key strengths of this study is the comprehensive application of microscopy examination using a 10% potassium hydroxide (KOH) solution to establish a working diagnosis of tinea versicolor in all study participants. Unlike many studies that use KOH testing solely to confirm clinically suspected cases, this study employed the 10% KOH diagnostic test uniformly across the entire study population. This approach enhances the accuracy of case identification and reduces the potential for diagnostic bias.

The number of participants included was considered adequate to draw meaningful conclusions. While a larger sample size could have been possible, it was not necessary given the study's duration. The data collected was sufficient to provide valuable insights that can be generalized to a broader population, ensuring the validity and reliability of the study's results.

CONCLUSIONS

The majority of students at MUQ Pagar Air Islamic Boarding School practiced good personal hygiene and did not have a tinea versicolor infection. The study also found a statistically significant relationship between the two measured variables, personal hygiene and the incidence of tinea versicolor.

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ETHICAL CLEARANCE

This study received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Syiah Kuala, registered under the Indonesian National Ethics Committee (KEPPKN) number 1171012P. The approval was issued under reference number 085/EA/FK/2022. Parental consent for students under 18 was obtained through the signing of an informed consent form, which was distributed to the students.

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CONFLICT OF INTEREST

There are no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

TRIP designed the research methodology and provided justifications for the study. MY supervised the writing, data processing, and statistical analysis. HM was responsible for data collection, interviews, and documentation. AW contributed to the publication process and the dissemination of the research findings.

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Resistance Pattern of Anti-TB Drugs in Drug-Resistant TB of Pulmonary Tuberculosis Patients in Dr. Soetomo Academic Hospital, Surabaya, Indonesia

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Abstract

Pulmonary tuberculosis is an infectious disease that can be transmitted through the air due to infection with *Mycobacterium tuberculosis* bacteria. According to the WHO, TB is the second-highest cause of death in infectious diseases in the world. This study aims to determine patterns of anti-TB drug resistance in drug-resistant TB patients in Dr. Soetomo Academic Hospital from January 2022 to December 2023. This was a descriptive retrospective using patient medical record data in Dr. Soetomo Academic Hospital for the period January 2022 - December 2023. This study included 261 drug-resistant pulmonary TB patients, the majority of whom were new TB patients (61.3%). Anti-TB drug resistance was most prevalent in RR-TB (43.7%), with the highest number of new cases (28.4%). Drug susceptibility test showed High-dose Isoniazid (INH^{HD}) had a high resistance rate (56%). Isoniazid (H) had a high resistance rate (66%). Pyrazinamide (Z) showed high sensitivity (66%). Levofloxacin (Lfx) showed high sensitivity (89%). High-dose Moxifloxacin (Mfx^{HD}) high sensitivity level (94%). Moxifloxacin (Mfx) high sensitivity level (92%). Bedaquiline (Bdq) high sensitivity level (98%). Linezolid (Lzd) high sensitivity level (99%). Clofazimine (Cfz) high sensitivity level (97%). Amikacin (Amk) high sensitivity level (100%). Drug-resistant pulmonary TB patients recently show a high drug sensitivity pattern to the second-line anti-TB drugs. MTB has become resistant to Isoniazid. However, it is still sensitive to Pyrazinamide by 66% and Levofloxacin by 89%. Moxifloxacin, Bedaquilin, Linezolid, Clofazimine, and Amikacin have high sensitivity >90%.

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INTRODUCTION

Pulmonary tuberculosis (TB) is an airborne infectious disease caused by infection with *Mycobacterium tuberculosis* bacteria.[1] According to the World Health Organization (WHO), TB is still a global public health problem today.[2] TB is the second-highest cause of death in infectious diseases in the world.[3] The estimated number of people diagnosed with TB worldwide is 10.6 million cases. Up from the previous 2021 of 10.3 million people.[4] Based on the latest data from the WHO Tuberculosis Report 2023, Indonesia currently ranks second with the highest TB caseload after India, with 724 thousand cases and an increase in 2023 of 820 thousand cases.[2,5] Globally in 2022, it is estimated that 410,000 people will experience drug resistance. In Indonesia in 2022, the number of deaths reached 93 thousand, and as many as 12 thousand people were recorded with drug-resistant TB cases. The success rate of drug-sensitive TB treatment is 85% and drug-resistant TB treatment is 55%. This figure has not yet reached the target, indicating that the urgency of commitment needs to be increased and the national TB elimination strategy to achieve the 90% target strengthened by 2024.[6]

Resistance of *Mycobacterium tuberculosis* to anti-TB drugs is a condition where the bacteria cannot be destroyed with anti-TB drugs.[7] Anti-TB drug resistance can be caused by gene mutation or selective pressure. The mechanism of drug resistance is influenced by several factors, including non-compliance of TB patients in taking drugs regularly or not completing the treatment program. [8] The cause of anti-TB drug resistance is due to inadequate use of drugs, which occurs when the treatment given is not in the right way in terms of drug dosage, treatment duration, and is not suitable for the patient's condition. Also, inappropriate treatment regimens can lead to treatment failure of TB patients.[9] Gene mutation is the main mechanism that causes drug resistance to MTB, mutations in certain genes can change the target of drug action, reduce effectiveness, and the bacteria can still survive in extreme conditions.[10] Selective pressure on MTB causes phenotypes to become more favorable in certain environments due to bacterial resistance to antibiotics.[11] Resistance in new patients is patients who have never received treatment before or received anti-TB drugs for less than 1 month. This patient is infected from a previously treated patient.[12] Differences in the characteristics of TB patients mean that drug-resistant TB transmission is more often transmitted from patients who are not on treatment or whose treatment is ineffective. Effective treatment and patient management are important in reducing the transmission of resistant strains to other individuals.[13] TB patients infected by drug-resistant patients have worse clinical outcomes and longer cure times, especially if infected with untreated patients.[14] DR-TB pulmonary TB patients reported include RR-TB, MDR-TB, Pre-XDR-TB, and XDR-TB.[15] RR-TB is determined from the results of the Xpert MTB/RIF examination, in RR-TB patients, followed by MGIT 960 culture examination. The MGIT 960 system is an efficient and rapid TB diagnosis method used for anti-TB drug susceptibility testing.[16] This test records and reports the sensitivity of first-line and second-line anti-TB drugs such as fluoroquinolones (Levofloxacin, Moxifloxacin, and Ofloxacin), aminoglycosides (Amikacin, Kanamycin, and Capreomycin), and Bedaquilin, Linezolid, and Clofazimine.[17,18] The purpose of this study was to determine the pattern of anti-TB drug resistance among DR-TB pulmonary TB patients in Dr.

Soetomo Academic Hospital, Surabaya, from January 2022 to December 2023.

MATERIALS AND METHODS

Materials

The data used in this research were secondary data, obtained from medical records of drug-resistant pulmonary TB patients in new and re-treatment cases, and adult age criteria of 18-65 years. The variables in this study were drug-resistant pulmonary TB patients, age, gender, drug-resistance TB classification, culture MGIT, and Drug-Susceptibility Testing (DST). The sample size was 261 patients who met the inclusion criteria.

Methods

This study used a retrospective descriptive observational design. DR-TB data on RR-TB using Xpert® MTB/RIF and anti-TB drugs sensitivity using BACTEC™ MGIT™ 960 system obtained from sputum of pulmonary TB patients and reported drugs Isoniazid, Pyrazinamide, Levofloxacin, Moxifloxacin, Bedaquilin, Linezolid, Clofazimine, and Amikacin were obtained from pulmonary TB patients' medical records at the Dr. Soetomo Academic Hospital for the period January 1, 2022, to December 31, 2023.

RESULTS AND DISCUSSION

Patient Demographics

Drug-resistant pulmonary TB patients recorded in medical record data at the Dr. Soetomo Academic Hospital from January 1, 2022, to December 31, 2023, totaled 261 patients (Table 1). Demographic characteristics of patients based on gender: 149 patients (57.1%) were male, while 112 patients (42.9%) were female. This is related to the level of physical activity and greater workload in men. Frequent social activities that involve interaction with many people, as well as unhealthy lifestyles such as smoking and drinking alcohol, lead to decreased immunity and increased risk of TB. Men, therefore, tend to be more susceptible to TB infection.[19,20]

Age distribution was grouped based on the age range of 18-24 years as many as 27 patients (10.3%), age 25-34 years as many as 38 patients (14.6%), age 35-44 years as many as 52 patients (19.9%), age 45-54 years as many as 89 patients (34.1%), and age 55-65 years as many as 55 patients (21.1%). Old age is thought to increase the risk of TB disease. This may be due to factors such as the causative agent, individual conditions in the level of immunity, and an unhealthy home environment.[7] Mature age is related to productive working age, and activities related to many people are risk factors for increased transmission from surrounding TB patients.[19,20]

Table 1. Demographics of Drug-Resistant Pulmonary TB Patients with TB Treatment History in Dr. Soetomo Academic Hospital, January 2022 - December 2023

Patient Demographics	TB Treatment History				Total n=261
	New Cases	Relapse	Loss to follow up	Treatment Failure	
Gender					
Male	90 34.5%	37 14.2%	8 3.2%	14 5.3%	149 57.1%
Female	70 26.8%	21 8%	7 2.7%	14 5.3%	112 42.9%
Age					
18-24	24 9.2%	2 0.8%	0 0%	1 0.4%	27 10.3%
25-34	20 7.7%	10 3.9%	4 1.5%	4 1.5%	38 14.6%
35-44	28 10.7%	11 4.2%	5 2%	8 3%	52 19.9%
45-54	58 22.2%	18 6.9%	2 0.8%	11 4.2%	89 34.1%
55-65	30 11.5%	17 6.5%	4 1.5%	4 1.5%	55 21.1%

Drug Susceptibility Test Examination

Sensitivity of anti-TB drugs in patients with drug-resistant pulmonary TB (Table 2) showed that High-dose Isoniazid (INH^{HD}) had a high level of resistance in 135/239 patients (56%). Isoniazid (H) showed high resistance in 100/151 patients (66%). Pyrazinamide (Z) showed sensitivity in 86/131 patients (66%). Levofloxacin (Lfx) showed sensitivity in 213/238 patients (89%). Moxifloxacin (Mfx) showed sensitivity in 11/12 patients (92%). High-dose Moxifloxacin (Mfx^{HD}) showed sensitivity in 215/228 patients (94%). Bedaquilin (Bdq) showed sensitivity in 235/239 patients (98%). Linezolid (Lzd) showed sensitivity in 237/239 patients (99%). Clofazimine (Cfz) showed sensitivity in 232/239 patients (97%). Amikacin (Amk) showed sensitivity in 86/86 patients (100%).

This study revealed that most patients were resistant to Isoniazid of 66%. Isoniazid is the first-line anti-TB drug given to patients diagnosed with TB, is often resistant; and unsupervised and incomplete treatment can increase drug resistance.[21] In addition, they are still sensitive to pyrazinamide (66%). Pyrazinamide has a different mechanism of action from other TB drugs; bacteria are less prone to resistance to Pyrazinamide than Isoniazid and Rifampicin, and Pyrazinamide regimens generally do not require frequent dosing, reducing the potential for resistance.[22] The high sensitivity rates of Levofloxacin, Moxifloxacin, Bedaquilin, Linezolid, Clofazimine, and Amikacin are likely due to the fact that these drugs are used with appropriate indications and are not drugs that are freely used in the community. This requires further research. Levofloxacin and Moxifloxacin are fluoroquinolone class drugs commonly used for drug-resistant TB patients, the sensitivity rate is still high especially in strains that do not have gyrA or gyrB gene mutations, a high level of sensitivity due to the use of the right dose and combination.[23] Second-line anti-TB drugs such as Levofloxacin, Moxifloxacin, Bedaquilin, Linezolid, Clofazimine, and Amikacin have high sensitivity because they have different and more specific mechanisms of action compared to the first-line anti-TB drugs.[24]

The WHO recommends individualized DR-TB treatment based on DST results for first-line and second-line drugs. Nearly a quarter of cases are resistant to at least one first-line drug, so second-line drugs are more widely used in TB treatment, with more side effects and higher costs.[25] DST plays a role in TB management and control, especially in the context of increasing drug resistance. DST enables rapid identification of MTB strains that are resistant to drugs such as isoniazid and rifampicin as first-line drugs, and is important in preventing the spread of DR-TB, thereby facilitating the provision of effective treatment therapy based on specific resistance profiles. First-line anti-TB drugs have a high incidence of drug resistance, possibly because first-line anti-TB drugs are the main TB treatment given to patients who are first diagnosed with TB. The causative factor of this resistance is due to treatment non-compliance and is related to the long duration of treatment, so that the chance of resistance increases. Meanwhile, second-line anti-TB drugs are currently still sensitive and effective as a treatment for TB patients. Factors determining the effectiveness of TB drugs are the ability of bactericidal or bacteriostatic activity that can kill and inhibit the development of bacteria, the ability of drugs to reach the concentration of infection sites, side effects, the ability to overcome drug resistance, and the duration of treatment.[26] TB patients should receive the correct dose. The standard dose for

drugs is Rifampicin 10 mg/kg, maximum 600 mg; Isoniazid 5 mg/kg, maximum 300 mg; Ethambutol 15 mg/kg, and Pyrazinamide 25 mg/kg. Levofloxacin 750-1000mg, Moxifloxacin 400-800mg, Linezolid 600 mg, Bedaquiline 400mg once daily for 2 weeks, followed by 200mg 3 times a week for 24 weeks, Clofazimine the first 2 months 200-300mg then reduced to 100mg, and Amikacin 12-15mg/kg.[27] An indicator of successful TB treatment is if the TB patient completes therapy with symptoms resolved or cured as measured by a negative BTA at the end of treatment and at least one follow-up examination.[28]

Table 2 Drug-Resistant Pulmonary TB based on the Treatment History with Drug Susceptibility Testing (DST) in Dr. Soetomo Academic Hospital, January 2022 - December 2023

TB Treatment History	**Drug Susceptibility Test Results**										
	Sensitivity Pattern	INH HD	H	Lfx	Mfx HD	Mfx	Bdq	Lzd	Cfz	Z	Amk
New Cases	S	67	34	128	131	6	145	143	143	49	49
	R	78	62	16	9	0	0	2	2	30	0
	Total	145	96	144	140	6	145	145	145	79	49
Relapse	S	21	10	48	47	3	53	53	53	22	22
	R	32	21	5	3	0	0	0	0	11	0
	Total	53	31	53	50	3	53	53	53	33	22
Loss to follow up	S	6	3	13	13	1	14	15	13	7	7
	R	9	4	2	1	0	1	0	2	1	0
	Total	15	7	15	14	1	15	15	15	8	7
Treatment Failure	S	10	4	24	24	1	23	26	23	8	8
	R	16	13	2	0	1	3	0	3	3	0
	Total	26	17	26	24	2	26	26	26	11	8
Total	S	104 44%	51 34%	213 89%	215 94%	11 92%	235 98%	237 99%	232 97%	86 66%	86 100%
	R	135 56%	100 66%	25 11%	13 6%	1 8%	4 2%	2 1%	7 3%	45 34%	0 0%

Note : S = Sensitive; R = Resistant; N = Amount

Research conducted in India revealed that, as a country with the highest MDR-TB rate in the world, DR-TB is one of the main obstacles to progress towards TB elimination.[29] In another study in India, many patients were found to have high resistance to fluoroquinolone class drugs at 72.8% and resistance to Second-Line Injectable Drugs (SLID) at 15.7%. Resistance to fluoroquinolones with SLID was 11.5%. Selection of appropriate treatment regimens is needed as a substitute for fluoroquinolones.[30] Research conducted in China, as the country with the third highest number of TB cases after India and Indonesia. Drug-resistant TB strains, especially MDR-TB and XDR-TB, are increasing the dependence on second-line TB drugs. Analysis of all TB drugs showed resistance of 56.8%, requiring increased targeted interventions for drug-resistant TB in China. The country accounts for a quarter of global MDR-TB cases.[31] Research conducted in the Philippines, as the fourth highest TB country, found that patients had SLID resistance of 56%, Fluoroquinolone resistance of 30.7%, MDR-TB patients were found to be 5.3% and XDR-TB as much as 8%. In his study, the addition of the drug Bedaquiline in the treatment regimen against DR-TB assessed its effectiveness, safety, and tolerability. The results of regimens

containing Bedaquiline in MDR/XDR-TB patients were highly effective with good safety and treatment outcomes.[32] Previous research conducted in Dr. Soetomo Hospital, Surabaya, Indonesia, said that the use of the drug Bedaquiline can improve recovery and reduce the risk of death of MDR-TB patients, as well as in patients who cannot be followed up and use in combination with drugs Levofloxacin, Clofazimin, and Linezolid increases the efficacy of therapy.[33] DR-TB in different countries can be influenced by health policy factors and TB treatment; the implementation of the DOTS (Directly Observed Treatment Short-course) program is effective in reducing TB drug resistance.[34]

Classification of Drug-Resistance Patterns

The results of the data analysis of this study showed that the number of drug-resistant TB patients with positive MTB culture (Table 3) results came from new cases as many as 160 patients (61.3%), relapse cases as many as 58 patients (22.2%), loss to follow up cases as many as 15 patients (5.7%), and failed cases as many as 28 patients (10.8%). There is a difference with that revealed by the Ministry of Health of the Republic of Indonesia (2020), that cases of DR-TB are usually more prevalent in TB with re-treatment cases.[35] New cases of drug-resistant TB patients may contain MTB strains in newly diagnosed TB patients who have never received TB drugs or a history of anti-TB drugs for less than one month. These patients are infected with MTB that is resistant to TB drugs, which is called primary resistance. New TB patients who develop resistance can occur due to close contact with DR-TB patients.[36]

This study revealed that drug-resistant pulmonary TB patients in new cases were more numerous, possibly due to social and psychological factors. Patients undergoing re-treatment may not visit the referral hospital, and comorbid conditions could also contribute. Dr. Soetomo's a referral hospital may not cover all drug-resistant TB patients in East Java, Indonesia. This highlights the need for an informative communication approach, intersectoral cooperation, and further research.

Table 3 Classification of Drug Resistance with Pulmonary TB Treatment History in Dr. Soetomo Academic Hospital, January 2022 - December 2023

Drugres istance	Treatment History				Total
	New Cases	Relapse	Loss to follow up	Treatment Failure	
RR	74 28.4%	25 9.5%	5 1.9%	10 3.8%	114 43.7%
MDR	66 25.3%	28 10.7%	6 2.3%	13 5%	113 43.3%
Pre-XDR	16 6.1%	5 1.9%	2 0.7%	2 0.7%	25 9.6%
XDR	4 1.5%	0 0%	2 0.7%	3 1.5%	9 3.4%
Total	160 61.3%	58 22.2%	15 5.7%	28 10.8%	261 100%

Note : RR = Rifampicin Resistant; MDR = Multidrug Resistant; Pre-XDR = Pre-Extensively Drug Resistant; XDR = Extensively Drug Resistant

The results of this study are in line with research at Margono Soekarjo Hospital, Indonesia, showing the majority of new TB patients are 7,3 times more likely to develop resistance. Contact transmission of drug-resistant TB causes new TB cases to experience primary resistance, which causes an increase in drug-resistant cases. Furthermore, relapsed TB patients have a 3.7 times higher risk of developing drug resistance than those who have never received previous treatment.[36]

Diagnosis of TB using the GeneXpert MTB/RIF test to detect drug resistance to rifampicin based on the drug sensitization test. Patients with RR-TB were new cases (43.7%), relapsed cases (9.5%), loss to follow-up cases (1.9%), and

failed cases (3.8%). MDR-TB in new cases (43.3%), relapse cases (10.7%), loss to follow-up cases (2.3%), and failed cases (5%). Pre-XDR-TB in new cases (6.1%), relapse cases (1.9%), loss to follow-up cases and failed cases (0.7%). XDR-TB in new cases (1.5%), loss to follow-up cases (0.7%), and failed cases (1.5%). The highest number of patients with RR-TB was found in the new TB cases group, totaling 74 people (28.4%).

In this study, it was found that the highest number of new cases of pulmonary TB patients experienced RR-TB at the Dr. Soetomo Academic Hospital in 2022-2023. New TB cases are patients who have never received previous treatment or received anti-TB drugs for less than 1 month (<28 doses). RR-TB is rifampicin resistance, without resistance to other anti-TB drugs.[15] The causes of drug-resistant TB are patient non-compliance with treatment regimens, non-completion of treatment, gene mutations, resistant strains transmitted from TB-infected people, and poor immunity.[37] Contact transmission of drug-resistant TB causes new case patients to develop primary resistance, leading to an increase in RR-TB cases.

This study is in line with Wahidah *et al*. (2024), it was found that the majority of anti-TB drug-resistant categories were in RR-TB as many as 85 patients (53.8%). MDR-TB was 59 patients (37.3%), Pre-XDR-TB was 11 patients (7%), and XDR-TB was 3 patients (1.9%). The drugs Rifampicin and Isoniazid are experiencing significant resistance in patients because these drugs are used as first-line anti-TB drugs and are the most effective in eliminating TB.[29] Research conducted at Dr. Mohammad Hoesim Palembang General Hospital found that 52 subjects (56.5%) had RR-TB, 33 subjects (35.8%) had MDR-TB, 6 subjects (6.5%) had Pre-XDR-TB, and 1 subject (1.2%) had XDR-TB.[38]

All countries must be committed to ending TB globally with the End TB Strategy and SDGs, promptly diagnose TB, record and report TB incidence, and DR-TB cases.[39] In addition, implementation is important to intensify education on patient adherence to complete treatment.[40] Long-term research services are needed to continue to determine anti-TB drug resistance patterns, to determine trends, and diagnostic capabilities.

STRENGTH AND LIMITATION

The strength of this study lies in the use of Drug Susceptibility Testing (DST) for anti-TB drug sensitivity. However, the limitation of this study was that the data were collected over a limited period of time, so it did not include long-term changes in the variables studied and unmeasured variables that might affect the results of the study, such as close contact with DR-TB and comorbidities.

CONCLUSIONS

This study showed that drug-resistant pulmonary TB patients recently show a high drug sensitivity pattern to the second-line anti-TB drugs. MTB has become resistant to Isoniazid. However, it is still sensitive to Pyrazinamide by 66% and Levofloxacin by 89%. Moxifloxacin, Bedaquilin, Linezolid, Clofazimine, and Amikacin have high sensitivity >90%.

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ETHICAL CLEARANCE

All the protocols and the use of medical records for the data on this research are approved by Dr. Soetomo Surabaya General Hospital ethics committee (Ref. No.1477/LOE/301.4.2/X/2023).

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CONFLICT OF INTEREST

All of the authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION

Every author has equally contributed to this research, from the design, data analysis, and interpretation, critically revising it, and giving their final approval of the article.

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Intervention Model for Pulmonary Tuberculosis (TB) with a Positive Acid-Fast Bacilli (AFB+) in Peukan Bada Sub-district, Aceh Besar Regency

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Abstract

Pulmonary tuberculosis (TB) with positive Acid-Fast Bacilli (AFB+) remains one of the most transmissible infectious diseases worldwide. This disease poses a significant public health challenge in many countries. This study aimed to develop a risk-factor-based intervention model to reduce the incidence of Pulmonary TB (AFB+). A case-control approach was employed, with the case group comprising people diagnosed with Pulmonary TB (AFB+), and the control group consisting of non-TB individuals from the same neighborhoods. Binary logistic regression was used for bivariate analysis, and multivariate analysis utilized logistic regression. This study found that the social determinants model accounted for 34.9% of the variance in the incidence of Pulmonary TB (AFB+) (R^2 = 0.349). The biological determinants model showed an R^2 of 0.127, indicating that this model explains 12.7% of the variance in the disease. The third model, which focused on behavioral determinants, had an R^2 of 0.312, meaning that behavioral factors accounted for 31.2% of the variance. The fourth model, examining the physical condition of housing, showed an R^2 of 0.425, indicating that 42.5% of the variance in Pulmonary TB (AFB+) is explained by variables related to housing conditions. In conclusion, the physical condition of housing emerged as the strongest predictor of Pulmonary TB (AFB+). These findings suggest that improving housing conditions should be a key component of public health strategies to reduce the incidence of Pulmonary TB (AFB+). Targeted interventions to improve the household environment are crucial for reducing the risk and transmission of Pulmonary TB (AFB+).

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INTRODUCTION

Tuberculosis (TB) is one of the deadliest infectious diseases in the world, posing a significant public health problem, especially in countries that have high TB burdens.[1] Every year, around 10 million people are diagnosed with TB, leading to 1.5 million deaths annually.[1] The disease is caused by *Mycobacterium tuberculosis*, which primarily affects the lungs (pulmonary TB).[2] Among those with pulmonary TB, people who tested positive for Acid-Fast Bacilli (AFB+) are the most infectious, and they need immediate and appropriate treatments to prevent further transmission.[3]

To deal with this issue, the World Health Organization (WHO) initiated the "End TB Strategy Program", aiming to reduce TB incidence by 80% and TB mortality by 90% in 2035.[4] Indonesia, which ranks third globally for the TB burden, has adopted and implemented various national strategies in line with this global initiative.[1] In Indonesia, several policies and strategies have been developed by the Ministry of Health of the Republic of Indonesia to control TB, including the Directly Observed Treatment (DOTS), Short-Course (DOTS) program, case detection through active screening, and enhancing the laboratory-based surveillance system.[5] Despite these efforts, significant challenges persist, particularly in ensuring patient adherence to medication and combating the social stigma related to TB.

At the regional level, TB burden is one of the public health concerns in Aceh Province. Data from the Aceh Provincial Health Office showed that the TB incidence steadily increased, making this disease control a top priority in regional health planning.[6] In Aceh Besar Regency, the number of TB cases rose from 331 in 2020 to 355 in 2021, with a further increase to 437 cases in 2022. Peukan Bada Sub-district has emerged as the area with the highest number of confirmed TB cases. Various factors contribute to challenges in addressing TB in this area, including limited access to healthcare facilities, lack of knowledge of TB, and low medication adherence.[7,8]

Due to these challenges, developing an intervention model for pulmonary TB (AFB+) in Peukan Bada Sub-district to identify an effective strategy for controlling TB transmission is highly important. This model takes into consideration local variables, such as risk factors and population characteristics. By utilizing local epidemiological data, the model can project the impact of the implemented intervention on reducing Pulmonary TB (AFB+) cases over a specific period.

The objective of this study is to develop more targeted and evidence-based interventions, which focus on improving early detection, enhancing medication adherence, and reducing pulmonary TB (AFB+) in Peukan Bada Sub-district. This model is predicted to be able to contribute to achieving the TB elimination target more quickly and effectively. This model is also anticipated to support sustainable health program planning at the community level by taking into account local risk factors and social and economic characteristics of the community in Peukan Bada.

MATERIALS AND METHODS

Study Design

This study utilized a case-control design to identify Pulmonary TB (AFB+) risk factors by comparing exposure to risk factors between case and control groups. Age and gender were matched, and the sample ratio was 1:1.

Population

The population consisted of 46 people, including 23 confirmed Pulmonary TB (AFB+) patients in the Peukan Bada Public Health Care Center's working area in 2023, representing the total number of TB (AFB+) cases in the area. The control group was 23 non-infected individuals residing in the same neighborhood.

Variable

Variables in this study were the incidence of pulmonary TB (AFB+), education, knowledge, food security, nutrition, smoking, close contact, housing density, humidity, lighting, and temperature.

Instrument

Interviews using a structured questionnaire were used to collect data. Only three variables were tested for validity and reliability. Validity was assessed using item-total correlation, with an r-table value of 0.209 as the threshold. Only one question from each variable (knowledge and stigma) was invalid and therefore excluded from further analysis. All items in the prevention behavior variable were valid.

The reliability test showed high internal consistency for all tested variables, with Cronbach's alpha values of 0.902 for knowledge, 0.943 for stigma, and 0.945 for prevention behavior.

Statistical Analysis

The data were analyzed using SPSS version 25. Univariate analysis was done to calculate the frequency distribution. In bivariate analysis, binary logistic regression was utilized to identify risk factors of pulmonary TB (AFB+) with a *P*-value < 0.005, and logistic regression was used in the multivariate stage.

RESULTS AND DISCUSSION

Respondent's Characteristics

The number of respondents (Table 1) in this study was 46 people. More than half of the respondents were males (56.5%), adults (39.1%), elderly (39.1%), and married (80.4%).

Table 1. Respondents' characteristics

Characteristics	n	%
Gender		
Male	26	56.5
Female	20	43.5
Age		
Adolescent (17-25 years old)	10	21.7
Adult (26-45 years old)	18	39.1
Elderly (>45 years old)	18	39.1
Marital status		
Married	37	80.4
Single	8	17.4
Widow/widower	1	2.2

Univariate Analysis

The univariate analysis (Table 2) reveals that 37% of respondents attended primary education, more than half had incomes below the regional standard, and 52.2% reported experiencing a low level of stigma. As many as 43.5% of respondents had poor knowledge related to Pulmonary TB (AFB+), 47.8% of respondents reported having poor food security, and 19.6% of them were undernutrition. The analysis also shows that 41.3% of respondents were smokers, 15.2% reported close contact with TB patients, 41.3% resided in high-density housing, 32.6% experienced poor humidity in their house, 32.6% had inadequate lighting, and 32.6% faced unsuitable temperatures in their homes.

Table 2. The result of univariate analyses of Pulmonary TB (AFB+)

Variables	n	%
Educational level		
High	16	34.8
Secondary	13	28.3
Primary	17	37.0
Income		
≥ Regional standard	19	41.3
< Regional standard	27	58.7
Stigmatization		
Low	24	52.2
High	22	47.8
Knowledge		
Good	26	56.5
Poor	20	43.5
Food security		
Good	24	52.2
Poor	22	47.8
Nutritional status		
Normal	30	65.2
Overnutrition	7	15.2
Undernutrition	9	19.6
Smoking Status		
No	27	58.7
Yes	19	41.3
Close contact		
No	39	84.8
Yes	7	15.2
Housing density		
Low	27	58.7
High	19	41.3
Humidity		
Good	31	67.4
Poor	15	32.6
Lighting		
Sufficient	31	67.4
Insufficient	15	32.6
Temperature		
Good	31	67.4
Unsuitable	15	32.6

Bivariate Analysis

The bivariate analysis of health determinants (Table 3) identifies several significant factors associated with Pulmonary TB (AFB+). These included primary education (*P*-value= 0.041; OR= 0.192; CI= 0.040-0.936), low income (*P*-value= 0.036; OR= 3.683; CI= 1.062-12.771), high level of stigma (*P*-value= 0.018; OR= 4.286; CI= 1.246-14.735), inadequate knowledge about TB (*P*-value= 0.017; OR= 4.407; CI= 1.260-15.414), and poor food security (*P*-value= 0.018; OR= 4.286; CI= 1.246-14.735).

The analysis of biological determinants found a significant association between comorbidity (*P*-value= 0.04; OR= 7.765; CI= 0.852-70.752) and Pulmonary TB (AFB+). Additionally, behavioural determinants linked to the disease included inadequate nutrition (*P*-value= 0.027; OR= 0.050; CI= 0.004-0.706), smoking (*P*-value= 0.036; OR= 3.683; CI= 1.062-12.771), and close contact with TB patients (*P*-value= 0.040; OR= 7.765; CI= 0.852-70.752).

The analysis also revealed that physical housing conditions were significantly associated with Pulmonary TB (AFB+), including high housing density (*P*-value= 0.036; OR= 3.683; CI= 1.062-12.771), poor humidity (*P*-value= 0.028; OR= 2.298; CI= 0.949-5.567), insufficient lighting (*P*-value= 0.028; OR= 4.352; CI= 1.125-16.854), and unsuitable temperature (*P*-value= 0.028; OR= 4.352; CI= 1.125-16.854).

Multivariate Analysis

The result of multivariate analysis (Table 4) in model 1 (social determinants) revealed that low income (OR= 1.701; CI= 0.138-20.916), high stigmatization (OR= 1.860; CI= 0.073-47.916), poor knowledge (OR= 2.698; CI= 0.659-11.045), and poor food security (OR= 1.198; CI= 0.041-34.940) were the most significant social determinant risk factors influencing Pulmonary TB (AFB+) incidence. This model has an R-square value of 34.9%, indicating that it accounts for 34.9% of the variability in the incidence of Pulmonary TB (AFB+).

Model 2 (biological determinants) revealed that comorbidity (OR = 7.765; CI = 0.852–70.752) was a factor influencing the incidence of Pulmonary TB (AFB+). This model had an R-squared value of 12.7%, suggesting that comorbidity explains 12.7% of the variation in the incidence of Pulmonary TB (AFB+).

Table 3. Bivariate analysis of the incidence of pulmonary TB (AFB+)

Variables	Cases (n=23)		Control (n=23)		*P*-value	OR (95% CI)
	n	%	n	%		
Social Determinants						
Education level						
Higher	5	21.7	11	47.8		
Secondary	5	21.7	8	34.8	0.012	0.140 (0.030-0.653)
Primary	13	56.5	4	17.4	0.041*	0.192 (0.040-0.936)
Income						
≥Regional standard	6	26.1	13	56.5		
< Regional standard	17	73.9	10	43.5	0.036*	3.683 (1.062-12.771)
Stigmatization						
Low	8	34.8	16	69.6		
High	15	65.2	7	30.4	0.018*	4.286 (1.246-14.735)
Knowledge						
Good	9	39.1	17	73.9		
Poor	14	60.9	6	26.1	0.017*	4.407 (1.260-15.414)
Food Security						
Good	8	34.8	16	69.6		
Poor	15	65.2	7	30.4	0.018*	4.286 (1.246-14.735)
Biological Determinants						
Gender						
Female	10	43.5	10	43.5		
Male	13	56.5	13	56.5	1.000	1.000 (0.312-3.209)
Age						
Adolescent (17-25 years old)	5	21.7	5	21.7		
Adult (26-45 years old)	9	39.1	9	39.1	1.000	1.000 (0.213-4.693)
Elderly (>45 years old)	9	39.1	9	39.1	1.000	1.000 (0.271-3.694)
Comorbidity						
No	17	73.9	6	26.1		
Yes	6	26.1	1	4.3	0.040*	7.765 (0.852-70.752)
Behavioral Determinants						
Nutrition						
Normal	13	56.5	17	73.9		
Overnutrition	2	8.7	5	21.7	0.037	0.096 (0.011-0.863)
Undernutrition	8	34.8	1	4.3	0.027*	0.050 (0.004-0.706)
Smoking						
No	10	43.5	17	73.9		
Yes	13	56.5	6	26.1	0.036*	3.683 (1.062-12.771)
Close contact						
No	17	73.9	22	95.7		
Yes	6	26.1	1	4.3	0.040*	7.765 (0.852-70.752)
The Physical Condition of The Housing						
Housing Density						
Low	10	43.5	17	73.9		
High	13	56.5	6	26.1	0.036*	3.683 (1.062-12.771)
Humidity						
Good	19	82.6	12	52.2		
Poor	4	17.4	11	47.8	0.028*	2.298 (0.949-5.567)
Lighting						
Sufficient	12	52.2	19	82.6		
Insufficient	11	47.8	4	17.4	0.028*	4.354 (1.125-16.854)
Temperature						
Good	12	52.2	19	82.6		
Unsuitable	11	47.8	4	17.4	0.028*	4.354 (1.125-16.854)

**P*-value < 0.05: Significant

Table 4. The result of the multivariate analysis of the incidence of pulmonary TB (AFB+)

Variables	Model 1 AOR (95% CI)	Model 2 AOR (95% CI)	Model 3 AOR (95% CI)	Model 4 AOR (95% CI)
Social Determinants				
Education level				
High	Ref			
Secondary	0.195 (0.034-1.128)			
Primary	0.303 (0.053-1.724)			
Income				
≥ Regional Standard	Ref			
< Regional Standard	1.701 (0.138-20.916)*			
Stigmatization				
Low	Ref			
High	1.860 (0.073-47.162)*			
Knowledge				
Good	Ref			
Poor	2.698 (0.659-11.045)*			
Food Security				
Good	Ref			
Poor	1.198 (0.041-34.940)*			
Biological Determinants				
Comorbidity				
No		Ref		
Yes		7.765 (0.852-70.752)*		
Behavioral Determinants				
Nutritional status				
Normal			Ref	
Overnutrition			0.147 (0.015-1.466)	
Undernutrition			0.068 (0.004-1.095)	
Smoking				
No			Ref	
Yes			2.141 (0.531-8.633)*	
Close contact				
No			Ref	
Yes			6.008 (0.528-68.430)*	
Physical Condition of Housing				
Housing density				
Low				Ref
High				3.674 (0.761-17.743)*
Humidity				
Good				Ref
Poor				0.065 (0.006-0.696)
Lighting				
Sufficient				Ref
Insufficient				1.493 (0.281-7.921)*
Temperature				
Good				Ref
Unsuitable				8.721 (0.805-94.452)*
R2	**34.9%**	**12.7%**	**31.2%**	**42.5%**

*P-value < 0.05: Significant

In the third model (behavioral determinants), smoking habit (OR = 2.141; CI = 0.531–8.633) and history of close contact (OR = 6.008; CI = 0.528–68.430) significantly influenced the incidence of Pulmonary TB (AFB+), with the model explaining 31.2% of the variation in TB

incidence. These findings underscore the importance of behavioral factors in shaping TB risk within the studied population.

Model 4 (the physical condition of housing determinants) revealed that high-density housing (OR= 3.674; CI= 0.761-17.743), insufficient lighting (OR= 1.493; CI= 0.281-7.921), and unsuitable temperature (OR= 8.721; CI= 0.805-94.452) significantly influenced the incidence of Pulmonary TB (AFB+). This model shows an R-squared of 42.5%, indicating 42.5% of Pulmonary TB (AFB+) is explained by variables in this model. This model is reasonably good at explaining almost half of the variation in TB incidence based on the examined risk factors.

Discussion

The relationship between education and the incidence of pulmonary TB (AFB+)

Respondents with a higher level of education tend to have better knowledge related to TB, including its transmission methods, symptoms, and the importance of proper treatment. Their understanding of TB encourages them to seek medical treatment immediately if they experience TB symptoms. Education level is also associated with healthier lifestyle choices. People with a good educational background are more likely to adopt good personal hygiene, ensure adequate ventilation at home, and practice a lifestyle that minimizes the risk of TB infection.[9]

People having a high level of education have more access to healthcare services to prevent TB and to seek treatment. They are more aware of the existence and the significance of TB programs offered by governments or health organizations.[10] Furthermore, a higher education level is also linked to a better economic status, which allows individuals to access healthcare facilities and medications easily.[11]

Highly educated people commonly live in areas with improved sanitation and housing, further reducing the risk of TB transmission.[12] Education has a significant role in reducing the stigma associated with TB. People who have a better knowledge related to TB are less likely to discriminate against TB patients. This encourages TB patients to seek medical care without fear of judgment or social exclusion.[12] Education plays a crucial role in reducing pulmonary TB incidence by improving knowledge, awareness, and access to healthcare resources. Pulmonary TB incidence is also influenced by economic and social conditions. Thus, interventions aimed to reduce TB prevalence often include an educational component and efforts to improve health literacy.

The relationship between income and the incidence of pulmonary TB (AFB+)

Individuals with low income may face significant barriers to accessing pulmonary TB healthcare services, including delays in diagnosis and treatment. This situation can lead to an increase in transmission.[13] Additionally, low income is often associated with inadequate nutritional status, which compromises the immune system and raises the risk of becoming infected with TB or developing active TB.[14]

Furthermore, low-income individuals are more likely to reside in densely populated and unhealthy environments, such as slum areas or locations with insufficient ventilation, which further facilitates the spread of TB.[15] Particular occupations or economic conditions may also elevate exposure to TB risk factors, such as close contact with infected persons, working in high-risk environments, or being employed

in informal sectors lacking adequate social protections.[16]

Moreover, low income is associated with lower educational attainment, which negatively affects knowledge and awareness regarding TB. A limited understanding of TB symptoms, the significance of treatment, and preventive measures can exacerbate transmission rates.[17] Additionally, low-income individuals may not have the opportunity to take time off work to isolate when ill, thereby increasing the likelihood of TB transmission.[10] Low income influences various factors that elevate the risk of TB infection, ranging from environmental conditions to healthcare access, all of which contribute to an increased TB risk. Therefore, effective TB prevention strategies should employ a multi-sectoral approach that addresses the socio-economic determinants of health.

The relationship between stigmatization and the incidence of pulmonary TB (AFB+)

TB Stigma causes a TB sufferer to be reluctant to seek medical treatment. The fear of social exclusion delays visiting a healthcare facility.[18] Stigmatizing TB patients is less likely to adhere to their medical regimen because they may feel embarrassed to pick up a prescription publicly or to attend regular medical appointments. Consequently, this results in medical failure and an increase in drug resistance.[19]

Stigmatization can also cause TB patients to experience social isolation. TB patients may face discrimination, such as from their families, friends, and communities. This influences their mental health status and reduces motivation to recover.[20] When a TB sufferer delays medication due to stigmatization, the risk of TB transmission increases significantly, and this worsens TB incidence in the community.[21]

Stigmatization also reduces social support for TB patients. Patients receiving adequate social support have more motivation to recover, to adhere to their medication regimes, and to complete their treatment.[22] Furthermore, stigmatization can lessen the effectiveness of public health programs. Early detection and treatment initiatives may be less successful if the people in the community are reluctant to participate due to stigma.[23] To overcome this, a holistic approach, such as mass education to dispel myths and baseless fears in the community, psychosocial support for TB patients, and health policies that promote equitable and stigma-free access to healthcare services, is highly required.

The relationship between knowledge and the incidence of pulmonary TB (AFB+)

Knowledge related to TB among people and healthcare workers influences the early detection of TB cases in the community. This is highly important because the earlier TB suspects receive diagnoses, the higher the chance for them to recover from TB and lower the risk of transmitting TB in the community.[24] Having sufficient knowledge related to TB helps individuals to identify early symptoms of TB, access timely medical treatment, and reduce the risk of TB transmission, including the understanding of transmission methods, symptoms, and preventive measures, such as vaccination and good hygiene practices.[25]

An effective education program is required to increase people's awareness about TB and encourage people to actively participate in TB prevention and control programs.[26] Insights resulting from studies related to TB contribute significantly to the design of a more effective health policy

and increase advocacy for better resources in eliminating this disease.[27]

Lack of knowledge about TB results in social stigmatization of the sufferer, and this hampers efforts to find new cases and provide adequate treatment.[20] Knowing that TB is caused by *Mycobacterium tuberculosis* bacteria and is spread through the air can encourage individuals to adopt preventive methods, such as wearing masks and having adequate ventilation.[7] Generally, sufficient knowledge related to TB not only directly influences individuals in preventing and managing this disease but also contributes to the efforts to control this disease in a broader context at the community and national levels.

The relationship between food security and the incidence of pulmonary TB (AFB+)

Good food security ensures access to enough nutritious food, which is essential for maintaining a strong immune system and fighting off pathogens.[28] Inadequate nutrition weakens the immune system and increases the risk of TB infection. Poor or unstable food security often leads to malnutrition, which negatively affects the immune system's ability to protect the body from TB infection.[29]

Poor or inadequate food security is able to influence individuals' ability to meet their nutritional needs. Food rich in nutrients, such as vitamins and minerals, can support the immune system to eliminate infection, including TB.[30] Moreover, Poor food security is commonly associated with inadequate access to healthcare services. This delays diagnoses and prompt treatment of TB.[29]

Furthermore, poor food security is commonly associated with poverty and unhealthy living conditions. These factors elevate the risk of TB spreading, particularly in densely populated areas or regions with limited healthcare access.[31] Efforts to improve food security can involve public health programs that can also strengthen efforts to prevent and control infectious diseases such as TB. Therefore, good food security contributes to reducing Pulmonary TB risk by enhancing nutrition, strengthening the immune system, and supporting overall public health initiatives.

The relationship Between Comorbidity and the incidence of pulmonary TB (AFB+)

Comorbidity and TB incidence are influenced by various physiological mechanisms, immunological factors, and other elements that affect individual vulnerability to TB infection.[23] Comorbidities weaken the immune system, making the body more susceptible to infection. For example, HIV/AIDS significantly weakens the immune system, thereby increasing the risk of TB infection.[32] Comorbidities such as diabetes can influence the immune system's ability to prevent infection, including TB. Diabetes causes changes in how the immune system responds to infectious diseases, increasing the body's vulnerability to pulmonary TB infection.[33] Some comorbidities, such as chronic kidney disease or cancer, may require immunosuppressive medication, which weakens the immune system and increases the risk of TB.[34]

Lung diseases, such as chronic obstructive pulmonary disease or lung fibrosis, damage lung tissues and create a supportive environment where TB bacteria can easily multiply.[35] Unhealthy lifestyles, such as smoking and excessive alcohol consumption, also increase the risk of TB infection. For instance, smoking damages lung tissues and disrupts the lung's defense mechanisms against infection.[36]

Some comorbidities are commonly found in the low socioeconomic population, which also has a high risk of TB infection. Factors such as housing density, lack of access to healthcare, and malnutrition are worsening the situation.[37] Some individuals may have a genetic vulnerability to TB infection, which can be influenced by other comorbidities.[38] Overall, comorbidities affect the incidence of TB in multifaceted ways, influencing the immune system, metabolic changes, medications, and lifestyle and socioeconomic factors. Taking a comprehensive approach to treating TB patients with comorbidities is crucial for improving medication outcomes and reducing further transmission.

The association between nutrition status and the incidence of Pulmonary TB (AFB+)

Lack of adequate nutritional intake, such as protein, vitamins A and D, and minerals, including Zinc, weakens the immune system and makes an individual more susceptible to TB infection after exposure to *Mycobacterium tuberculosis* [39]. Vitamin D plays an important role in how the body responds to infection. Vitamin D deficiency has been associated with an increase in the risk of developing TB.[40]

Malnourished individuals, including people suffering from chronic malnutrition and a medical condition causing significant weight loss, are more susceptible to TB.[41] Adequate nutrition intake strengthens the immune system, especially for people living with TB infection.[42] TB sufferers experience loss of appetite and weight loss, leading to malnutrition. Malnutrition delays the healing and recovery processes of TB patients.[29] A sufficient nutrition intake and adequate nutrition status are essential in TB prevention and management. An effective TB prevention program should consider these aspects to improve the overall public health status.

The association between smoking and the incidence of pulmonary TB (AFB+)

A smoking habit damages the mucosa and cilia in the lungs. These lung components act as an initial defense against infection. Thus, smoking makes the lungs more susceptible to TB infection.[37,43] Nicotin and other cigarette contents suppress the immune system, allowing *Mycobacterium tuberculosis* to multiply and cause an active infection.[25] Cigarettes cause chronic inflammation and damage lung tissues, which creates a more favorable environment for the growth of *Mycobacterium tuberculosis*.[43]

TB-infected smokers are more likely to experience complications and have a greater mortality rate compared to non-smokers.[44,25] Smoking disrupts the body's response to TB medication, therefore delaying the recovery process and causing ineffectiveness of TB medication.[45] Smoking is a significant risk factor for TB infection and the development of active TB. Thus, implementing an effective intervention to reduce smoking habits in the population prevents TB transmission in the community.

The association between close contact and the incidence of pulmonary TB (AFB+)

Pulmonary TB spreads through small droplets containing *Mycobacterium tuberculosis*, which are released when an active TB sufferer (especially AFB+) coughs, sneezes, or talks. Individuals in close contact with pulmonary TB (AFB+) sufferers, such as family members or friends living together or working closely with them, have a higher risk of being exposed to this bacterium.[46] The longer the

duration of contact with TB sufferers, the greater the chance of being infected by *Mycobacterium tuberculosis*. Repeated or continued close contact with an infected TB individual increases the risk of transmission.[47] Densely populated areas, poor ventilation, and inadequate sanitation facilities also elevate the risk of TB transmission, particularly in close contact.[48]

People with weakened immune systems, such as children, the elderly, or individuals with medical conditions like HIV/AIDS, are more vulnerable to TB infection after close contact with TB sufferers.[49] It is recommended that individuals who have been in close contact with people with Pulmonary TB (AFB+) undergo TB screening, such as a Tuberculin skin test or blood test, to determine the likelihood of infection.[50]

If close contact is not identified and intervened upon, the risk of TB transmission in the community increases. This results in more cases of active TB, which becomes a source of new transmissions.[51] Close contact with Pulmonary TB (AFB+) is the main risk factor in TB transmission. Thus, case identification, screening, and intervention in close contact with individuals need to be done to control TB cases in the community.

The association between housing density and the incidence of pulmonary TB (AFB+)

Living in dense housing means that many people live in a small space, increasing the likelihood of close and prolonged contact between individuals. Tuberculosis (TB) is an infectious disease that spreads through the air when someone with active TB coughs, sneezes, or talks. In a high-density environment, there is a significantly increased chance of an individual inhaling droplets containing *Mycobacterium tuberculosis*.[52] In such crowded living conditions, it is challenging to isolate TB-infected individuals from other family members, which in turn raises the risk of TB transmission within the household.[53]

A dense room tends to have poor ventilation, which reduces airflow and increases the concentration of TB bacteria in the air. This raises the risk of TB transmission, especially if an occupant is suffering from active TB.[54] High-density housing is often linked to poor lifestyle, such as poor hygiene, inadequate sanitation, and lack of access to health facilities. All of these factors weaken an individual's immune system, making them more susceptible to being infected by *Mycobacterium tuberculosis*.[55]

Living in densely populated areas with low socioeconomic status can limit people's access to healthcare services. This can result in delays in diagnosing and treating tuberculosis (TB), as well as prolonging the period during which an infected individual can spread the disease to others.[56] When one person in a community is infected with *Mycobacterium tuberculosis*, there is a higher risk of transmission to others in the community. This can lead to a cluster of TB cases that are difficult to control if living conditions do not improve.[57] High residential density is a significant risk factor for TB transmission. To reduce TB in densely populated areas, it's important to decrease residential density, improve ventilation, provide better access to healthcare services, and promote health awareness related to TB.

The association between humidity and the incidence of pulmonary TB (AFB+)

Bacteria that cause TB live longer in humid air. In high humidity conditions, droplets or aerosol particles containing *Mycobacterium tuberculosis* bacteria remain

suspended in the air longer, increasing the risk of transmission if inhaled by susceptible individuals.[58] An environment with low humidity causes dryness in the respiratory tract and makes people more susceptible to infection, including TB. On the other hand, high humidity can cause breathing to become heavier or trigger other conditions that affect the respiratory system.[59]

In high-humidity areas, such as tropical regions, there are more cases of TB. This is connected to other factors like crowded living spaces, inadequate ventilation, and low socioeconomic status, all of which worsen sanitation conditions and make it easier for TB to spread.[60] High humidity is linked to poor living conditions, like overcrowded houses and insufficient ventilation, which increase TB transmission because infected and non-infected people live closely together for extended periods in closed spaces.[61] Overall, the relationship between humidity and TB incidence is complex and influenced by various environmental, social, and biological factors. Humidity affects how long bacteria can survive in the air and can also impact respiratory health, ultimately contributing to TB transmission patterns.

The association between Lighting and the incidence of pulmonary TB (AFB+)

Vitamin D, which is produced by the body through exposure to sunlight, is essential in supporting the immune system. A deficiency of vitamin D weakens the immune system, increasing the risk of developing TB. People who live in environments with low natural light, for example, crowded areas or homes that do not have enough access to sunlight, may be more susceptible to vitamin D deficiency.[57]

Poor lighting is often associated with poor ventilation, especially in enclosed environments such as homes or workplaces. Poor ventilation can lead to the accumulation of moist, stagnant air, which increases the risk of spreading *Mycobacterium tuberculosis*. TB is spread through droplets in the air, and rooms with inadequate ventilation are more likely to become a place for the spread of TB.[52]

Damp, dark environments with insufficient lighting are a supportive environment for microorganisms to grow, including bacteria that cause respiratory infections such as TB.[61] Poor lighting is commonly found in low socioeconomic households, where access to health facilities, adequate lighting, and ventilation may be limited. This increases the risk of TB transmission, especially in densely populated areas.[62] Although poor lighting does not directly cause TB, it contributes to the risk of TB transmission. Therefore, adequate lighting, in addition to good ventilation, plays a crucial role in TB prevention and control programs.

The association between temperature and the incidence of pulmonary TB (AFB+)

Mycobacterium tuberculosis, which causes TB, lives in low temperatures and high humidity. Low temperature increases the bacteria's resistance in the environment, allowing them to survive longer in the air or on surfaces, increasing the chance of infection.[63] In some countries, TB cases increase during the winter. This is due to a combination of factors, such as people spending more time indoors with poor ventilation, which increases the risk of transmission through droplets containing TB bacteria in the air.[58]

Extremely low temperature affects the human immune system, making it more susceptible to infections, including TB. The respiratory system is more vulnerable to infection by bacteria in cold temperatures due to reduced activity as the body's defense mechanism.[64] In tropical areas with higher temperatures, although the incidence of TB is also high, the main risk factors are more related to population density, poverty, and poor sanitation, rather than temperature.[65]

In regions with cold climates, people tend to spend more time indoors with limited ventilation. This increases the risk of TB transmission because poor air circulation prolongs the exposure time to bacteria in the air.[66] In countries with colder climates, homes often have less ventilation during the winter, increasing the risk of transmission of TB. In addition, crowded living conditions and a lack of access to adequate health facilities can also worsen the situation.[67] Temperature may play a role in influencing TB incidence by interacting with environmental factors and human behavior. However, it is important to note that TB is a complex disease, influenced by various factors such as social, economic, and biological elements that contribute to its transmission.

STRENGTH AND LIMITATION

This study has a number of significant strengths. It employs a comprehensive approach that was used to analyse various determinants of Pulmonary TB (AFB+), including social, biological, behavioral factors, and physical condition of housing. The use of Logistic regression analysis in the multivariate stage allows for the identification of influencing factors while controlling for confounding variables. In addition, this study also has a high local relevance because it was focused on a high TB burden area. Therefore, findings of this study can be utilized in planning and development of public health programs in Peukan Bada Sub-district of Aceh Besar Regency. However, this study also has limitations. The Sample size of this study is considered small, limiting the generalization of the results.

CONCLUSIONS

Our study reveals that various factors are linked to the incidence of Pulmonary TB (AFB+). These factors include health determinants (such as education, income, stigma, lack of knowledge, and food security), biological determinants (comorbidities), behavioral determinants (nutrition, smoking, and close contact), and physical condition of housing determinants (housing density, humidity, lighting, and temperature).

To address this issue, it is essential to enhance community outreach and education programs, improve sanitation, offer healthy home renovation programs, build stronger partnerships with the private sector and NGOs, provide comprehensive health worker training, launch campaigns to reduce stigma, and involve local communities in planning and executing TB intervention programs.

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CONFLICT OF INTEREST

The authors stated that there is no conflict of interest regarding this manuscript.

AUTHOR CONTRIBUTION

F.F. designed the research, data, and wrote the manuscript. W was responsible for literature review, manuscript editing, and translation.

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Effect of Fetal Bovine Serum Concentration on Detection and Morphological Identification of *Blastocystis hominis* in vitro

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Abstract

Diarrhea significantly contributes to the high rates of illness and death among young children. Diarrhea can be caused by bacterial infections, viruses, or even parasites. *Blastocystis hominis* causes parasitic diarrhea, which can be identified by microscopy, culture, and molecular methods. Previous reports have modified the Jones' culture medium using three different serums, such as human plasma, donkey serum, and horse serum (in Jones' medium). This research replaces horse serum with fetal bovine serum for detection tests, morphological observation, and diagnosis of *B. hominis*. The research encompasses five experimental groups, each subjected to varying concentrations of fetal bovine serum: 2%, 10%, 20%, 30%, and 40%. Detection analysis is conducted using the Mc-Nemar test, while the Wilcoxon test is applied to evaluate ordinal data from morphological assessments. Diagnostic tests and metrics such as accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are performed using MedCalc® software. The findings demonstrate that serum concentrations of 2%, 10%, 20%, and 30% produced effective results in detection tests, morphological identification, and diagnostic evaluations of B. hominis, exhibiting high sensitivity, specificity, PPV, NPV, and accuracy. Fetal bovine serum can be used at a concentration of 2% in a Jones' medium that has been modified. This depends on the results of detection tests, morphology, and diagnosis.

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INTRODUCTION

Diarrhea is a medical condition characterized by increased intestinal activity and the frequent production of feces with a loose consistency, occurring at least three times a day.[1] Diarrhea represents a major public health challenge, especially among young children, for whom it remains one of the primary contributors to both morbidity and mortality. It is recognized as the second most common cause of death in this age group, surpassed only by pneumonia [2]. It is responsible for significant child mortality globally, with 444,000 deaths annually, equating to 1,200 deaths each day [3]. Based on the Indonesian Health Profile 2022, diarrhea caused a mortality rate of 6.6% in children aged 29 days to 11 months and a mortality rate of 5.8% in youths aged 12-59 years [4]. The incidence of diarrhea among adults aged 70 years and above was approximately half that of infants aged below 5 years [5]. Diarrhea during early infancy can significantly impair the absorption of essential nutrients, such as fats, vitamins, proteins, carbohydrates, electrolytes, minerals, and water, potentially leading to malnutrition [6 7]. This disruption in nutrient absorption can have severe consequences for the growth and development of infants, highlighting the importance of addressing the underlying causes of diarrhea to prevent malnutrition and ensure the well-being of young children. In the past, severe dehydration and significant fluid loss were the primary causes of mortality connected to diarrhea. Presently, there is a potential that infection will play a more substantial role in the overall number of deaths associated with diarrhea [8].

Diarrhea, an infectious condition caused by various agents such as bacteria, viruses, and parasites, is often associated with the intestinal protozoan Blastocystis hominis, which commonly infects humans and animals [910]. It is known that *B. hominis* comprises up to 22 subtypes, which can overlap between humans and animals [11]. According to the CDC (2019), the life cycle of *B. hominis* remains a subject of debate [12]. Infections caused by *B. hominis* have shown increased transmission rates due to poor sanitation, close contact with pets, reliance on water supplies directly sourced from wells and rivers [13]. Recent studies indicate that in Europe, the majority of human *Blastocystis* infections are attributable to subtypes ST1, ST2, ST3, and ST4, which collectively represent approximately 90% of detected cases. On a global level, ST3 emerges as the most commonly identified subtype, particularly among individuals presenting with symptoms, while ST1 and ST2 also occur frequently but at lower rates [14].

Blastocystis hominis is classified as a protist due to its cellular structure, which includes one or more nuclei, rough and smooth endoplasmic reticulum, Golgi apparatus, and organelles such as mitochondria; it also exhibits sensitivity to antiprotozoal medications [15]. There are 6 forms of *B. hominis*, namely vacuolar, avacuolar, multivacuolar, ameboid, granular, and cyst. The pathological progression of the disease, which transforms the condition of patients from asymptomatic to symptomatic, occurs due to the morphological shift of *B. hominis* from the vacuolar form to the amoeboid form [16]. Appropriate management can be provided if the etiology and clinical manifestations in patients with diarrhea are clearly identified [17]. Inadequate management may allow *B. hominis* to persist, leading to chronic diarrhea and further complications such as malnutrition.

Confirmatory tests that can be conducted to validate the diagnosis of *B. hominis* using microscopy, culture, immunoserological, and molecular [18].

Previous reports have demonstrated that the combination of culture methods and immunoserological assays is the most sensitive approach for detecting *B. hominis* [19]. Sari et al., (2018) found that the level of culture sensitivity in Jones' medium is greater when compared to polymerase chain reaction (PCR) [20]. Modification culture in Jones' medium was done by Hassan et al. (2016) by culturing the samples in different culture media supplemented with human plasma, donkey serum, and horse serum, with horse serum as the primary serum. This research modified the use of horse serum using fetal bovine serum [21].

Fetal bovine serum is the most prevalent serum used for cell culture in laboratories worldwide [22]. This serum is commonly employed in cell culture because of its high concentration of growth factors, making fetal bovine serum more prevalent in cancer cell culture, such as of colorectal cancer and breast cancer [23]. Fetal bovine serum has high levels of growth hormones and low levels of γ-globulins, which restrict cell proliferation [24]. Currently, there is a lack of studies regarding the optimal dosage that should be used for the Jones' culture medium. Improper levels of fetal bovine serum can complicate research by causing wrinkles, which hinder the identification, detection, and analysis of *B. hominis* morphology. This investigation aimed to determine the most effective concentration of fetal bovine serum for the in vitro cultivation of *B. hominis*. The study concentrated on several critical components, such as parasite detection, comprehensive morphological analysis, and the assessment of the culture method's sensitivity and specificity. By systematically varying the concentrations of fetal bovine serum, the study aimed to establish the most effective conditions for maintaining the viability and integrity of *B. hominis* in a controlled laboratory environment.

MATERIALS AND METHODS

The specimens were collected from the regional public hospital and the community health clinic in Buleleng, between May and November 2023. Research was conducted on 35 samples of diarrhea patients. Participants in this study gave informed consent, consenting to the collection of their fecal samples immediately following defecation, which were then deposited into sterile containers. These specimens were then transported in an ice box directly to the Parasitology Laboratory at the Faculty of Medicine, Universitas Pendidikan Ganesha, for further processing, including direct smear and in vitro culture.

The research begins with preparing the stock solution based on Hassan et al. (2016) by mixing 1.244 grams of disodium phosphate (Na_2HPO_4) into 131.25 mL of distilled water, and 0.397 grams of monopotassium phosphate (KH_2PO_4) into 43.75 mL of distilled water. Fetal bovine serum (HiMedia Laboratories Pvt. Ltd, Brazil) at various concentrations was added to the prepared stock solution. Table 1 shows the different treatment variations of bovine fetal serum concentrations based on adjustments made to the Jones' medium. The cultures were observed for 24, 48, and 72 hours. The samples were examined under a microscope using high-power duplicates (400x).

Preparations of *B. hominis* culture will be observed under a microscope and verified immediately by the parasitological analysts. The morphological identification was evaluated in 100 fields of view by morphological observations that were categorized as: (1) absence of parasite; (2)

presence of parasites with morphology characterized by wrinkled walls; and (3) presence of parasites with perfectly rounded wall morphologies. Diagnostic testing is conducted on culture findings that display the ideal morphology of *B. hominis*. The culture findings will be assessed by five observers utilizing five duplicate samples, resulting in a total of 125 test samples for the optimal culture test group. A direct microscopic examination with identical replication would be compared with the diagnostic test.

Table 1. Modification of the concentration of fetal bovine serum in *B. hominis* in vitro culture

Test Groups	Concentration of Fetal bovine serum
P1	2% (0.1 mL)
P2	10% (0.5 mL)
P3	20% (1.0 mL)
P4	30% (1.5 mL)
P5	40% (2.0 mL)
K (-)	Aquades
K (+)	Fecal + examined microscopically

Data Analysis

The Mc-Nemar test method is used to analyze the detection test in this study. The Wilcoxon test will be used to examine the ordinal data obtained from the morphological test. MedCalc® software is utilized to conduct diagnostic tests and assess accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A variable is deemed statistically significant when the $P < 0.05$.

RESULTS AND DISCUSSION

Of the 35 samples that were confirmed positive for diarrhea, five of them specifically gave positive results on microscopic examination for *B. hominis*. These samples were sent for testing by five competent laboratories. The testing process extended over a period of 24, 48, and 72 hours. The samples underwent a series of tests, including a detection test, a morphological test, and a diagnostic test.

Table 2. Detection of *B. hominis* in vitro using different concentrations of fetal bovine serum

Test Groups	Negative N (%)	Positive N (%)	P Value*
Positive control**	0 (0)	25 (100)	-
Negative control	25 (100)	0 (0)	<0.01
P1 2%	1 (4)	24 (96)	1.00
P2 10%	2 (8)	23 (92)	0.50
P3 20%	1 (4)	24 (96)	1.00
P4 30%	2 (8)	23 (92)	0.50
P5 40%	15 (60)	10 (40)	<0.01

*Difference in proportion of detection test results using Mc-Nemar test

According to the data presented in Table 2, the morphological outcomes for the P1, P2, P3, and P4 test groups with the golden standard did not differ ($P > 0.05$). Statistically significant differences were seen in the P5 test groups ($P < 0.01$). The P1, P2, P3, and P4 test groups exhibited no visible differences in morphology compared to the positive control group. The only group that exhibited significant differences.

Table 3 indicates that the morphological characteristics of B. hominis cell walls in the P1, P2, P3, and P4 test groups did not significantly differ from those observed in the gold standard group ($P > 0.05$). These test groups showed no discernible morphological differences when compared to the positive control group. However, statistically significant differences were noted in the P5 test group ($P < 0.01$).

Table 3. Morphological observations of *B. hominis* in in vitro cultures were conducted using varying concentrations of fetal bovine serum.

Test groups	Negative N (%)	Wrin-kled N (%)	Ideal N (%)	P Value*
Positive control**	0 (0)	0 (0)	25 (100)	-
Negative control	25 (100)	0 (0)	0 (0)	<0.01
P1 2%	1 (4)	0 (0)	24 (96)	0.317
P2 10%	2 (8)	0 (0)	23 (92)	0.157
P3 20%	1 (4)	0 (0)	24 (96)	0.317
P4 30%	2 (8)	0 (0)	23 (92)	0.157
P5 40%	15 (60)	10 (40)	0 (0)	<0.01

*Difference in proportion of detection test results using the Wilcoxon test
**This positive control is used as the gold standard

Table 4 demonstrates that the P1, P2, P3, and P4 test groups achieved the highest values in sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy in the in vitro diagnostic test of *B. hominis*. In contrast, the P5 group showed the lowest sensitivity and accuracy compared to the other test groups.

Table 4. Sensitivity and specificity of the *B. hominis* in vitro diagnostic test using different concentrations

Test Groups	Sn (%)[a]	Sp (%)[b]	PPV[c]	NPV[d]	Acc[e] (%)
Positive control	**	**	**	**	**
Negative control	-	-	-	-	-
P1 2%	100	100	100	100	100
P2 10%	100	100	100	100	100
P3 20%	100	100	100	100	100
P4 30%	100	100	100	100	100
P5 40%	40	~	100	0	40

~ = untestable value, [a]Sn=Sensitivity, [b]Sp=Specificity, [c]PPV=positive predictive value, [d]NPV=negative predictive value, [e]Acc=Accuracy. **Used as a gold standard

In this study, differences in pH levels were observed among the test groups. The P5 group exhibited a pH of 6.15, which was more acidic compared to the P1 and P2 groups, with pH values of 7.23, and the P3 and P4 groups, with pH values of 7.15. A prior research conducted by Farah Haziqah et al [25] highlighted the critical role of acidity or pH levels in influencing the viability and morphology of *B. hominis* cell walls. Physiologically, *B. hominis* requires a neutral pH environment for optimal growth, typically ranging from 7.0 to 7.5 [26] Deviations from this optimal pH range, whether too acidic or too alkaline, can adversely affect the growth and morphology of this microorganism. Extreme pH levels can disrupt cell membrane integrity, leading to leakage of intracellular components and organelle dysfunction [27].

At extreme pH levels, essential metabolic enzymes critical for the survival of *Blastocystis* may undergo denaturation or reduced activity, leading to impaired growth and replication [28]. In acidic environments, the activity of digestive enzymes tends to increase, creating stress conditions for organisms such as *B. hominis* [29]. In this study, low pH levels resulted in significant changes in cell wall morphology, which appeared wrinkled and irregular. These wrinkled cell walls indicate structural damage caused by prolonged exposure to unstable environmental conditions. Such changes reflect the parasite's inability to maintain cell membrane integrity under suboptimal conditions. These findings are particularly important as they suggest that increased acidity significantly impacts the viability and morphological structure of *Blastocystis hominis*. The significant variations in pH levels observed could account for the discrepancies in detection outcomes within the P5 group compared to other groups. This underscores the pivotal

role of environmental factors, particularly pH, in preserving the viability and morphological features of *B. hominis*.

STRENGTH AND LIMITATION

The study presents significant strengths, serving as an innovative and valuable contribution to parasitology by offering a practical, efficient, and cost-effective diagnostic alternative. Its streamlined methodology facilitates implementation, particularly in resource-constrained regions. However, the research has limitations as it concentrates just on general morphological characteristics such as complete grown structures and wrinkled cell walls, excluding more intricate morphological aspects.

CONCLUSIONS

Fetal bovine serum exhibits good detection and identification capabilities for *B. hominis* morphology at concentrations of 2%, 10%, 20%, and 30%. Fetal bovine serum can be used as a modality to diagnose *B. hominis* . Based on the clinical findings from detection, morphological, and diagnostic tests, it is recommended to utilize fetal bovine serum in modified medium at a concentration of 2% (0.1 mL). 2% concentration offers significant advantages in terms of material use efficiency and practicality in applications. Despite that, for an extensive review of morphology, a serum concentration of 20% (1.0 mL) is the ideal medium to stimulate growth. This research aims to serve as a reference for future studies and as a clinical guide for diagnosing *B. hominis* in patients with diarrhea.

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ETHICAL CLEARANCE

The Ethics Committee of Universitas Pendidikan Ganesha approved the research protocol, as indicated by reference number 099/UN48.24.11/LT/2024.

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CONFLICT OF INTEREST

No conflicts of interest are declared by the authors. Furthermore, the funding agencies had no involvement in any stage of the research process. This includes the planning and design of the study, the implementation and execution of the research methods, and the preparation of the manuscript.

AUTHOR CONTRIBUTION

The research design was conceptualised by PSAJ and MBP. PSAJ, KES, KIAS, and KIM conducted the clinical study and data collection. PSAJ, KIAS, and KIM wrote the article. The article was reviewed and revised by MBP and MKWG.

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