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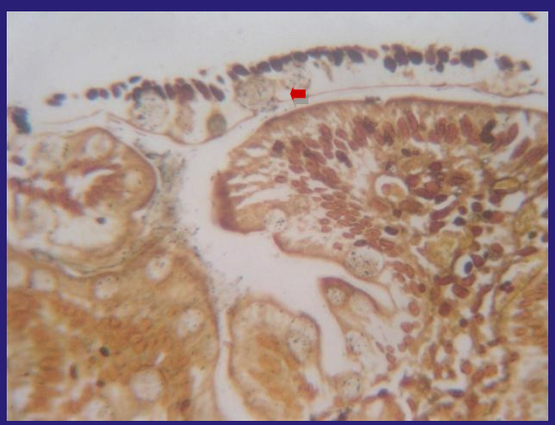



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# *Indonesian Journal of* Tropical and Infectious Disease

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Scientific Journal of Tropical and Infectious Disease

# Incidence of Candidemia in Neutropenia with Administration of Broad-Spectrum Antibiotics in Pediatric Patients with Acute Lymphoblastic Leukemia

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## Abstract

Candidemia is one of the main causes of morbidity and mortality in patients with hematological malignancies. However, the difficulty in establishing a definitive diagnosis causes these high rates. Therefore, a rapid diagnosis process is needed for the early stages of infection as the initial clinical management in pediatric patients with malignancy, especially accompanied by neutropenia. This study aims to determine the risk factors for candidemia in children using PCR (Polymerase Chain Reaction). A cross-sectional study design was used to determine the relationship between neutropenia and broad-spectrum antibiotics with the incidence of candidemia in pediatric patients with acute lymphoblastic leukemia (ALL). The results were analyzed statistically. 33 pediatric patients who met the inclusion criteria, 22 (66.67%) were positive for candida. The sample was dominated by male (66.67%) with a mean age of 4.5 years and had undergone the standard (14 patients) and high-risk (19 patients) chemotherapy protocols. The correlation test revealed no significant correlation between the administration of broad-spectrum antibiotics and the incidence of candidemia in pediatric patients with ALL (p= 0.052), neutropenia recorded a notable relationship to those patients (p=0.033). This study shows that neutropenia is a risk factor that affects the prevalence of candidemia in pediatric patients with ALL. Children with ALL who have severe neutropenia are at an increased risk of developing candidemia.

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## INTRODUCTION

Candidemia is a leading cause of morbidity and mortality among patients with hematologic malignancies, and its incidence has been on the rise in recent years.[1] In the United States, the occurrence of candidemia in children varies from 0.35 to 0.81 cases per 1000 hospitalized patients. Among hospitalized children with hematological malignancies, 1.25 episodes per 1000 cases were reported, with *Candida albicans* being the most common cause.[2] Kalista et al. reported that the prevalence of candidemia in pediatric patients with hematological malignancies and solid tumors at RSCM was 8.7% and 23.1%, respectively.[3] The mortality rate associated with candidemia is as high as 60% in children with hematological malignancies.

Pediatric patients with blood malignancies often undergo extended treatment courses, particularly chemotherapy. Prolonged exposure to high chemotherapy doses can lead to neutropenia in pediatric patients, and the use of broad-spectrum antibiotics can increase the incidence of infections, particularly those caused by *Candida* spp..[1]

The mortality rate due to candidemia was significantly higher in patients with neutropenia, exceeding that in non-neutropenic patients by 13.6%.[5] Challenges in establishing a definitive diagnosis contribute to these elevated rates. Consequently, a prompt diagnosis in the early stages of infection is crucial for initial clinical management in patients with neutropenia. One method to achieve this is Polymerase Chain Reaction (PCR) for pediatric patients suspected of having candidemia, as it demonstrates reasonably high sensitivity compared to the culture method.[6]

## MATERIALS AND METHODS

### Research Subject

The population in this study were hospitalized pediatric patients with acute lymphoblastic leukemia and neutropenia who were undergoing chemotherapy and had received broad-spectrum antibiotics. A total of 29 pediatric patients with acute lymphoblastic leukemia who met the inclusion and exclusion criteria were selected as samples based on the Receiver Operating Characteristic (ROC) diagnostic test formula.

### Methods

### Research design

This study is a preliminary study. This study used a cross-sectional design to determine the relationship between neutropenia and broad-spectrum antibiotics with the incidence of candidemia in pediatric patients with acute lymphoblastic leukemia (ALL). Samples were taken by consecutive sampling from patients who were suspected of candidemia in February - May 2022 at the pediatric ward (IRNA IV) of Dr. Saiful Anwar Hospital, Malang.

### Inclusion Criteria

The inclusion criteria in this study were patients with ALL who experienced neutropenia, had received broad-spectrum antibiotic therapy for at least 5 days, and were not taking antifungal drugs (at least 1 month before the study). The degree of neutropenia used to classify each case was as follows: mild neutropenia (absolute neutrophil count (ANC) of 1000-1500 cells/mm$^3$), moderate neutropenia (ANC of 500-999 cells/mm$^3$) and severe neutropenia (ANC od <500 cells/mm$^3$).

### Exclusion Criteria

Exclusion criteria in this study were pediatric patients with malignancies other than ALL and immunodeficiency conditions due to other causes.

### Research procedure

Blood samples (5 ml) were drawn twice, namely when the patient came to determine the degree of neutropenia and during a conventional PCR examination. Blood samples were centrifuged at 3000 rpm for 10 minutes, and the serum was stored at 4 °C for a follow-up examination. PCR assay with the serum, performed at the start of the study, used 1 pair of

primers to amplify the target genome sequence.

**Data Analysis**

The data in this study were not normally distributed, so the follow-up test used was the Mann-Whitney, Kruskal-Wallis, Fisher, and Chi Square tests with = 0.05 and Confidence Interval (CI) of 95%, run by SPSS for Windows 25 software. The relationship between neutropenia with the incidence of candidemia in pediatric patients with ALL was measured using the Fisher correlation test, while administration of broad-spectrum antibiotics was measured using Chi Square.

## RESULTS AND DISCUSSION

**Results**

Three pediatric patients who met the inclusion criteria (Table 1), 22 (66.67%) were positive for candida. The sample was dominated by male (66.67%) with a mean age of 4.5 years and had undergone the standard (14 patients) and high-risk (19 patients) chemotherapy protocols. The majority of patients had good nutritional status (22 patients), followed by malnourished (5 patients), undernourished (3 patients), overweight (1 patient), and obese (2 subjects). As many as 18 patients were still in the induction phase of chemotherapy, 7 in the consolidation phase, 2 in the intensification phase, and 4 in the maintenance phase. During the chemotherapy, 5 patients experienced moderate neutropenia (4 patients with negative candida and 1 patient with positive candida in PCR), and 28 patients have severe neutropenia (7 patients with negative candida and 21 patients with positive candida in PCR). Ceftriaxone was administrated to 24 patients with severe neutropenia; Cotrimoxazole for 5 patients with moderate neutropenia; Cloxacillin with gentamycin for 1 patient with positive candida in PCR; Meropenem for 2 patients with positive candida in PCR; Cefoperazone for 1 patient with positive candida in PCR. A total of 6 patients with positive candida in PCR experienced febrile neutropenia.

No patient was recorded to have very severe neutropenia (ANC od <500 cells/mm$^3$). In contrast to patients with severe neutropenia, patients with moderate neutropenia (ANC of 500-999 cells/mm$^3$) typically have negative candida findings. This study showed a significant relationship (p value of 0.033, in which a p value of < 0.05 is considered significant) between neutropenia and candida results on PCR, whereas the correlation test unveiled no notable correlation (p value of 1.000) between antibiotic therapy and candidemia.

**Discussion**

Mortality rate of 35–38%, fungal infections are a significant cause of morbidity and mortality in patients treated for hematological malignancies.[7] Wang et al. reported 123 episodes of children with fungal infections in Queensland, Melbourne, Perth, and Sydney pediatric hospitals, of which 119 were pediatric patients with ALL.[8]

Cases of candidemia continue to increase along with the increasing number of patients with impaired immunity and the use of invasive devices. In patients with malignancy, decreased immunity and damage to the gastrointestinal mucosal barrier are risk factors for developing candidiasis, including candidemia. Candidemia was defined as the presence of Candida spp. in blood.[9,10] Marwa et al. found the incidence of candidemia in pediatric patients with acute lymphoblastic leukemia in Egypt reached 75%.[11]

**Table 1.** Patient Characteristics

| Characteristics | Negative Candida | Positive Candida | p |
|---|---|---|---|
| Sex, f(%) | | | |
| Male | 8 (36.4) | 14 (63.6) | 0.709 |
| Female | 3 (27.3) | 8 (72.7) | |
| Age (years), median (min-max) | 5 (2-13) | 4.5 (1.5 – 15) | 0.908 |
| Nutritional Status, f (%) | | | |
| Malnourished | 3 (60) | 2 (40) | 0.307 |
| Undernourished | 1 (33.3) | 2 (66.7) | |
| Well-nourished | 6 (27.3) | 16 (72.7) | |
| Overweight | 1 (100) | 0 (0) | |
| Obese | 0 (0) | 2 (100) | |
| Diagnosis | | | |
| ALL-L2 | 8 (32) | 17 (68) | 1.000 |
| ALL-L2 (relapse) | 3 (37.5) | 5 (62.5%) | |
| Antibiotic Therapy | | | |
| Ceftriaxone | 6 (25) | 18 (75%) | 0.103 |
| Cloxacillin + gentamisin | 0 (0) | 1 (100%) | |
| Cotrimoxazole | 4 (80) | 1 (20%) | |
| Meropenem | 0 (0) | 2 (100%) | |
| Cefoperazone | 1 (100) | 0 (0%) | |
| | 0 (0) | 1 (100%) | |
| Chemotherapy Type | | | |
| Standard | 5 (35.7) | 9 (64.3%) | 1.000 |
| High-risk | 6 (31.6) | 13 (68.4%) | |
| Chemotherapy Phase | | | |
| Induction | 8 (44.4) | 10 (55.6%) | 0.339 |
| Consolidation | 1 (14.3) | 6 (85.7%) | |
| Intensification | 0 (0) | 2 (100%) | |
| Maintenance | 2 (50) | 2 (50%) | |
| ANC Total, median (min-max) | 420 (70-950) | 350 (10-790) | 0.302 |
| Neutropenia duration (days), median (min-max) | 7 (4-14) | 6 (3-21) | 0.861 |
| Febrile Neutropenia, f (%) | 2 (25) | 6 (75) | 0.687 |

This study found that severe neutropenia was associated with the incidence of candidemia in pediatric patients with ALL. This is in line with the findings of previous studies, which stated that the risk factors for candidemia in children with acute lymphoblastic disease included severe neutropenia (neutrophil count <500/ul) for more than 10 days along with the presence of a bacterial infection that is resistant to previous broad-spectrum antibiotics.[12] The prolonged duration of neutropenia is associated with a longer period of chemotherapy, leading to a higher risk for candidaemia.[13]

Gastrointestinal bacterial colonization is often affected during treatment with chemotherapy,[14] either due to damage to the mucosal barrier or the use of broad-spectrum antibiotics and other antimicrobials. The use of beta-lactam and cephalosporins antibiotics in leukemia patients can increase the risk of MDRO, including broad-spectrum beta-lactamase resistance (ESBL).[15]

**Polymerase Chain Reaction (PCR)**

Non-culture-based methods, such as DNA detection by PCR, have been developed to aid in the rapid diagnosis of candidemia, enabling faster initiate empiric antifungal therapy and reducing mortality in pediatric patients with acute leukemia.[20,21] El-Ashry and Ragab[18] reported that the RT-PCR test has a sensitivity level of 100% compared to the results of blood cultures. This is supported by Gupta et al. [19] that stated the sensitivity, specificity, and positive and negative predictive values of RT PCR were 82.7%, 54%, 72.9%, and 67.5%, respectively. Furthermore, Ratridewi et al. noted that the sensitivity and specificity of PCR reached 69.2% and 71% in diagnosing systemic candidiasis in children with malignancy with severe neutropenia, respectively

(Figure 1). However, a need to further assess other potential risk factors in the incidence of candidemia in malignancy cases have to be considered clinically.[22, 23,24,25]

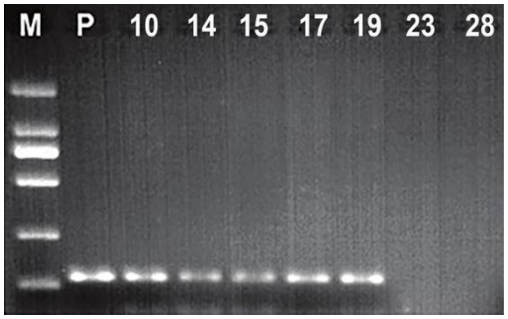


**Figure 1.** PCR results for candida in pediatric patients with Acute Lymphoblastic Leukemia (ALL) at Dr. Saiful Anwar Hospital, Malang. Marker gene URA 3, ARG 4, HIS 1, LEU 2, SAT1 was used.

## STRENGTH AND LIMITATION

The current research was limited by its study design, short time-period and low sample size in a single center. Further large-scale research with long-term follow up is needed to evaluate the contribution of risk factors and the other preexisting medical conditions in ALL children with candidemia.

## CONCLUSIONS

Candidemia is one of the leading causes of morbidity and mortality in patients with hematologic malignancies, with an increasing incidence over the last few decades. The risk factors for candidemia in pediatric patients with acute leukemia are prolonged neutropenia along with the presence of resistant bacterial infections. The main problem that leads to a high mortality rate is difficulties in establishing a definitive diagnosis. Thus, PCR examination can be used as an effective and efficient diagnostic tool for candidemia with high sensitivity and specificity values.

## FUNDING

This study did not receive funding

## ETHICAL CLEARANCE

This study was approved by the Research Ethics Committee of Saiful Anwar General Hospital Malang (Reference letter number 400/085/K.3/10.7/2022).

## CONFLICT OF INTEREST

There was no conflict of interest in this study.

## AUTHOR CONTRIBUTION

IR is the main author and contributed the most in writing the study. CC oversaw data collection and contributed in writing the manuscript. SLW oversaw data analysis and contributed in writing the manuscript. SN contributed in enriching the discussion and overall writing of the manuscript.

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Case Report

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Scientific Journal of Tropical and Infectious Disease

# Mebendazole treatment in ascariasis re-infection of two-year-old boy in rural Ambon: a case report and literature review

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## Abstract

Ascariasis is currently a health problem in developing countries, especially in rural areas. Successful control of ascariasis is highly dependent on therapeutic interventions, environmental, and individual hygiene practices. Ascariasis is generally asymptomatic but can cause severe problems if treated improperly. Treatment is available, but reinfection may occur. This case aims to emphasize the usage of mebendazole treatment in ascariasis reinfection. A two-year-old boy came to the hospital with mucus diarrhea and worms in the stool. Two months ago, he had the same symptoms and experienced improvement after taking pyrantel pamoate at the previous hospital. The patient was diagnosed with acute diarrhea with mild to moderate dehydration, re-infection ascariasis, and malnutrition. Mebendazole 100 mg was administered twice daily for 3 days. Treatment with mebendazole was repeated twice with an interval of one month after the previous therapy due to the presence of *Ascaris lumbricoides* eggs in fecal examination. Fecal examination in the third month revealed the absence of *Ascaris lumbricoides* egg. Mebendazole can be used as therapy for ascariasis reinfections. However, repeated therapy is required in some cases. By integrating repeated therapy with comprehensive control measures, including health education and improved sanitation infrastructure, sustainable progress in combating ascariasis can be achieved.

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## INTRODUCTION

The most prevalent worm infection is ascariasis, which was projected to have 804 million cases worldwide in 2013.[1,2] The most critical parasite in the nematode class and common name for roundworms, *Ascaris lumbricoides*, is the cause of this disease. Ascariasis most often occurs in children living in tropical and developing countries. This occurs due to soil contamination by human waste or the disposal or use of untreated waste, as well as air or food contaminated by ingested eggs.[3] Key factors associated with higher prevalence are poor socio-economic conditions, poor hand hygiene, poor sanitation practices such as not washing hands after defecation, and soil-transmitted helminths (STH) infections in mothers during pregnancy. While infections can occur at any age, they are most common during early childhood, particularly in children under five years old, commonly referred to as toddlers. In Indonesia, the prevalence of worm infections ranges from 2.5% to 62%, indicating a significant variability. This range signifies a relatively high prevalence, and this number is still relatively high, and this is a public health problem in Indonesia.[4,5]

Although this infection can be treated with antiparasitic drugs, sometimes this infection can recur, especially in children who have recovered previously.[2] The phenomenon of ascariasis reinfection is when someone infected with the *Ascaris lumbricoides* worm suffers the infection again after a period of healing. This reinfection can occur several times in a person's life.[6] Ascariasis reinfection remains a public health problem, especially in areas with poor sanitation. Understanding the factors contributing to reinfection and implementing appropriate preventive measures are essential control measures in ascariasis infections. This case describes a two-year-old child who experienced ascariasis reinfection as an illustration of this phenomenon.

## CASE REPORT

A two-year-old boy came with his mother to the hospital emergency department with complaints of diarrhea six times a day since three days ago. Worms and mucus accompanied the diarrhea. The patient's mother also complained that the patient was fussier than usual and vomited when he ate and drank. The patient sometimes coughs without mucus. There was no fever. The patient had experienced a similar complaint, which was worm defecation, two months ago and had been hospitalized. The patient was given pyrantel pamoate at the previous hospital during treatment, and his condition improved.

His general condition looked weak on physical examination, and his consciousness was compos mentis. The patient's weight was 9400 grams, height 83 cm, with nutritional status <-2 SD, according to weight for age. Vital signs were within normal limits. The eyes appeared glazed, and other examinations were normal. Laboratory result at admission was shown on Table 1. Abdominal ultrasound examination at Figure 1 showed a hypoechoic tubular structure with parallel echogenic lines in the left paracolic area.

The patient was diagnosed with ascariasis reinfection with acute diarrhea, mild to moderate dehydration, and malnutrition. During treatment, the patient did not defecate but vomited worms approximately 7 times, with a total of around 50 worms. The patient was treated, rehydrated with fluids and Zinc 1 x 20 mg, and an enema was given on the third day of treatment, 30 minutes post enema, the

**Table 1.** Laboratory result at admission.

| | Result | Normal value |
|---|---|---|
| Hemoglobin | 12.3 | 11.5-14.5 |
| Leucocyte | 12.2 | 6-17 |
| Platelet | 389 | 150-400 |
| Eosinophils | 10.7 | 1-5 |
| Neutrophils | 68.7 | 25-60 |
| Lymphocytes | 14.6% | 25-50 |
| NLR | 4.79 | ≦3.13 |
| Feces | Macroscopic: blood (+), worms (+) identified as *Ascaris lumbricoides* and positive 3 (+++) worm eggs | |

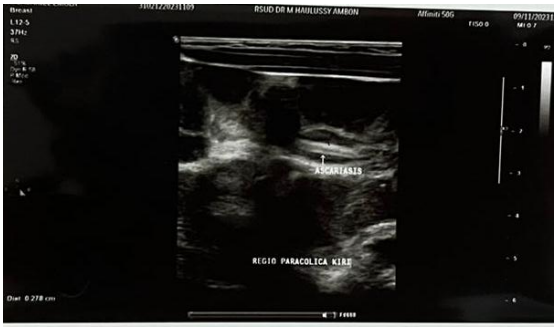


**Figure 1.** Abdominal ultrasound of a 2-year-old patient with ascariasis.

patient defecated with lumps of worms. After that, Mebendazole was given 2 x 100 mg for 3 days on day 4 of treatment. The patient was discharged on day 6 with an improved condition. The patient was treated with mebendazole again 2 times at 1-month intervals after the previous treatment because worm eggs were still found in the fecal examination. At the end of the third month, the patient's stool examination appears greenish-yellow, soft, and well-formed in consistency. The stool contains fat but does not contain blood, mucus, pus, undigested meat fibers, harmful bacteria, viruses, fungi, or parasites. At the outpatient clinic, a Denver II examination was carried out. There was a failure in 2 components: language and personal social. The patient was given an additional diagnosis, suspecting growth and development delay.

## DISCUSSION

Approximately 1.4 billion individuals globally, constituting around 25% of the world's population, are estimated to be infected with *Ascaris lumbricoides*. Ascariasis primarily occurs in tropical and semitropical regions globally. The affected body area typically spans from the stomach to the ileocecal valve, with approximately 99% of cases inhabiting the jejunum and proximal ileum.[7,8] The genus *Ascaris* has 17 species, and *A. lumbricoides* has high host specificity towards humans, although it can sometimes be found in pigs.[9,10] As previously mentioned, ascariasis is the most significant type of worm infection, with the size of male worms around 10 to 30 cm and female worms around 22 to 35 cm.[11]

When the host ingests eggs found in stool-contaminated soil, infection occurs. The larvae are released into the duodenum and pass through the intestinal mucosa to reach the circulation. After that, the larvae penetrate the duodenum wall and migrate into the circulatory system or lymph channels, carrying them to the heart and lungs. In the lungs, the larvae penetrate the blood vessel walls, then the alveolar walls, enter the alveolar cavity, and then move into the trachea through the bronchioles and bronchi. From the trachea, the larvae move to the pharynx and cause stimulation in the pharynx, which ultimately triggers coughing so that the larvae are swallowed back into the esophagus and then into the small intestine. Within the small intestine, the larvae undergo a transformation into adult worms. The time it takes from when the infective eggs are swallowed until the adult worms start laying eggs is around 2-3

months. The female worms can generate up to 200,000 eggs a day when they copulate with the males, which can be expelled in feces and combined with soil trash. In two to eight weeks, the eggs develop into infectious forms in wet, shady, and warm environments, and they can stay viable in the soil for up to 17 months. The infectious cycle can be restarted by consuming them.[2,9].

Re-infection is associated with a higher incidence of a type 1 hypersensitivity reaction, involving considerable pulmonary eosinophilic infiltrates and marked peripheral eosinophilia.[12] Dun et al., done a study at Myanmar, stated that RR for the six-month reinfection period was statistically significant for *A. lumbricoides* infection in school-aged children after given mass drug administration (MDA) (RR = 2.67,95% CI 1.37–5.21).[13]

While the majority cases of ascariasis in pediatric children show long-term signs of malnutrition and developmental retardation, some patients may not exhibit any symptoms at all. When symptoms do occur, the most typical ones are bloating, nausea, vomiting, anorexia, and intermittent diarrhea. Clinical symptoms of ascariasis generally occur during the larval migration phase. During migration, larvae can cause responses in the tissues they pass through. For example, when the larvae reach the lungs, the antigens produced by the larvae can induce an inflammatory response that appears as infiltrates on chest radiographs and generally disappears within three weeks. Symptoms of pneumonia include wheezing, dyspnea, dry cough, fever, and phlegm may be mixed with blood in cases of more severe infections. When pneumonia is accompanied by an increased number of eosinophils and high levels of IgE in the blood, the condition is known as Loeffler's syndrome.[10,14] When the larvae die in the liver, this can cause the formation of eosinophil-rich granulomas. The patient's condition indicates the migration phase of larvae to the lungs, characterized by a dry cough that lasts for quite a long time but comes and goes and is accompanied by an increase in eosinophils in laboratory results of 10.7%. However, the diagnosis of Loeffler syndrome cannot be confirmed because a chest x-ray was not taken.

In the intestinal phase, digestive symptoms are usually subtle and caused by adult worms inhabiting the digestive tract. When symptoms appear, they tend to be general and non-specific, such as nausea, low intake, digestive problems such as diarrhea or constipation, lethargy, and difficulty concentrating, which can significantly affect a child's development.[14,15] *Ascaris* infection can also trigger lactose intolerance and interfere with vitamin A and essential micronutrient absorption. Chronic infections can result in growth failure in children due to decreased appetite, digestive disorders, and malabsorption problems.[16,17] In our case, at the intestinal phase, complaints were found, including diarrhea, vomiting, constipation, and even suspicion of developmental delay and poor nutritional status, which may be related to the underlying worm infection.

More severe impacts occur when adult worms clump together in the intestines, causing intestinal obstruction (ileus). In addition, adult worms can migrate into the lumen of the appendix and cause acute appendicitis or gangrene. Suppose adult worms enter and block the bile ducts. In that case, it can trigger problems such as colic, cholecystitis, cholangitis, pancreatitis, and liver abscess.[7,16,18] Apart from migrating to these organs, adult worms can also exit through the anus, mouth or nose. This worm migration is often triggered by factors such as high fever or the use of certain

medications.

In diagnosing cases of ascariasis, the gold standard diagnostic test is still a direct wet stool examination to look for eggs and parasites. In *Ascaris* infection, the infertile eggs have characteristics of brownish color with an elongated oval shape (both ends are slightly flat), have layered walls (2 or 3) with a thick, winding outer layer that is very rough/irregular (albumin layer), and an inner layer relatively smooth (hyaline layer). It's crucial to highlight that the stool may test negative as the worms migrate and reach maturity, typically occurring within approximately 20 to 30 days.[14] Egg laying begins only after the worms reach maturity. Occasionally, adult worms may be visible in stool or expelled from the rectum, but they can also be discharged through coughing or in urine. Both *Ascaris lumbricoides* parasites and eggs were found in the patient's stool examination. In addition, a history of bowel movements with worms had also been previously reported by the parents. During follow-up at the hospital, the patient vomited worms and expelled lumps of worms after 30 minutes post-enema, as shown in Figure 2.

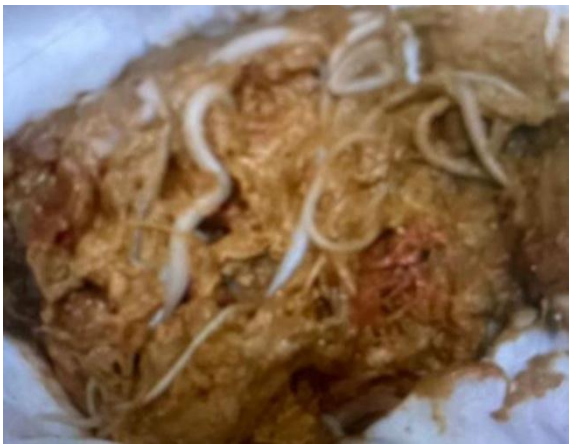

**Figure 2.** Stool of a 2-year-old patient with *Ascaris*.

Peripheral eosinophilia is one of the hallmarks of helminth infection.[19] Eosinophilia is usually present in the early stage, increasing several days after symptom onset and remaining high for a few weeks. Sputum analysis may demonstrate eosinophilia and Charcot-Leyden crystals. Eosinophil counts are usually 5 to 12 percent but can be as high as 30 to 50 percennt.[12] Neutrophil, lymphocyte ratio (NLR) has recently been shown to be superior due to its better stability compared with the other parameters that can be altered by various physiological, pathological, and physical factors. However, no previous work has been done on the predictive ability of NLR in helminthic infection. NLR represents a combination of two markers where neutrophils constitute the active nonspecific inflammatory mediator that initiates the first line of defense, while lymphocytes reflect the regulatory or protective component of inflammation.[20]

During the phase of active migration from the intestines to the lungs, larvae can be observed in sputum, and eosinophilia can be evident in a comprehensive blood count examination. Abdominal X-rays may show sensitivity in detecting signs like whirlpools indicative of volvulus or intussusception, although they may lack specificity.[2,8] Gallbladder worms and bile ducts can be detected using Computed Tomography (CT) scans and Ultrasonography (USG). ERCP (Endoscopic Retrograde Cholangiopancreatography) serves as both a diagnostic and treatment option. On the other hand, ERCP reports with ascariasis abnormalities are more common in affluent nations and sometimes result from misdiagnosis of other conditions.[21,22] In our patient, an increase in eosinophils was found in the complete blood count, and the ultrasound showed the presence of adult worms in the left paracolic area.

Serological diagnosis has been proposed as well. Infection with *A. lumbricoides* prompts the production of antibodies, whose levels can vary depending on the extent of exposure and the severity of the infection, especially in areas with high endemicity. The humoral response to *Ascaris* may be influenced by co-infections, age, atopy, genetic predisposition, and dietary status. In those who live in endemic locations, total immunoglobulin (Ig) titer is correlated with worm burden. Prior research has demonstrated that specific and sensitive markers for chronic *A. lumbricoides* infection, such as IgG4, may be identified and that these markers positively correlate with the infection's severity. These results align with other parasitic infections, albeit investigations on *Ascaris* show more inconsistent outcomes. Antibodies against *Ascaris* frequently cross-react with epitopes from other helminths. It's crucial to standardize *Ascaris* antigens, encompassing recombinant antigens, allergens linked with *Ascaris*, and antigens from different *Ascaris* species, to facilitate research and diagnostic accuracy.[14,23,24]

Previous research has not assessed the community's application of serological diagnostics for ascariasis. Antibody levels against *Ascaris* are mostly linked to infections during the larval stage of the parasite. They may endure for several months post-treatment, especially in areas prone to frequent reinfection. Consequently, the presence of anti-*Ascaris* antibodies might exaggerate the count of individuals requiring treatment in mass control programs, and they are generally not deemed suitable for identifying active *Ascaris* infections. Numerous commercial diagnostic assays are available to identify IgG and IgM antibodies against *Ascaris lumbricoides*. Nonetheless, they most frequently cross-react with other helminths and are based on the helminth antigens of somatic *A. lumbricoides*. Antigen detection indicates the current infection, whereas antibody detection can indicate the current infection and any previous infections or exposures.[2]

As control programs aim to eliminate soil-transmitted helminths (STH) in children, antibodies can serve as a valuable indicator of childhood infections, particularly in regions where children are regularly exposed to enteric pathogens.[2,14] Meanwhile, biomedical target markers for *A. lumbricoides* infections have also been reported. Products of fatty acids resulting from *A. lumbricoides* infection can be identified in urine using gas-liquid chromatography, and their concentrations correspond with the severity of the worm infestation. Nevertheless, there are currently no commercially available tests for this purpose.[25]

Even mild symptoms of ascariasis necessitate treatment to avert complications arising from parasite migration. However, owing to the heightened risk of pneumonitis, medical intervention is not recommended during the phase of active migration through the lungs. In our patient at the beginning of treatment, we did not immediately treat the patient with anthelmintic drugs. Therapy with anthelmintic drugs is given post-enema, and the patient defecates worm lumps. This follows studies to prevent parasite migration, which can worsen symptoms such as intestinal obstruction. In cases of partial intestinal obstruction, insertion of a nasogastric tube is recommended, and oral intake should be withheld; instead, intravenous fluids and piperazine should be administered. In situations of complete intestinal obstruction, surgical intervention such as laparotomy may be necessary to extract the worms. If necrosis, resection and re-anastomosis may

be necessary. After surgery, medical antiparasitic treatment should be initiated to eradicate any remaining eggs.[9,14] The patient's condition did not lead to intestinal obstruction, so surgery was unnecessary.

The recommended medication for medical therapy is 400 mg of albendazole in a single dose. The second therapeutic option includes Mebendazole at 100 mg twice a day for three days, a single dose of 500 mg, or Ivermectin at 100 to 200 micrograms per kilogram once. Treatment options for pregnancy include piperazine at a dose of 50 mg/kg per day for five days or 75 mg/kg in one dose or the recommended medication and pyrantel pamoate at a dose of 11 mg/kg up to a maximum of 1 g. Since medical therapy targets adult worms, repeating the treatment after one to three months is best. It gives any larvae that may be present time to mature and become therapeutically responsive. Levomisole and nitazoxanide are examples of substitute agents.[9,26] Research carried out in Asia and Africa demonstrated that a single dose of albendazole treatment achieved a cure rate of over 95%, with a gradual decline in egg count observed in the subsequent weeks across 995 cases. Nonetheless, patient relocation is vital to prevent recurrence.[9,12] Besides albendazole, mebendazole is an equivalent alternative to albendazole in treating *Ascaris* infection.[27] The patient was treated with pyrantel pamoate once, followed by mebendazole 3 times over 5 months. Although it is not the first line, pyrantel pamoate is known to be a neuromuscular blocking agent that causes paralysis in worms. After it was proven to be an ascariasis reinfection, Mebendazole 100 mg twice daily was given for three days. This is in line with the literature, which states that Mebendazole works by inhibiting the energy formation of worms, resulting in their death. This repeated treatment aligns with the literature because both drugs are active against adult worms but insufficient against larvae.

Anthelmintic resistance may occur in the treatment of ascariasis. In a study about worm infection in Rwanda, the efficacy of deworming school children with albendazole against *Ascaris* infection was highly variable and, overall, inadequate. In Rwanda, with very high rates of helminth treatment coverage, these findings are considered a warning sign of the emergence of the spread of resistance. The β-tubulin genotype does not explain the inappropriate efficacy of albendazole.[28] This particularly emphasizes the need for continuous monitoring of the results of routine deworming.[29,30]

Before confirming a diagnosis of anthelmintic resistance, several factors need to be assessed. Initially, it's crucial to recognize that various illnesses can manifest clinical symptoms like parasitic infections. Additionally, failure of anthelmintic treatment to manage nematodes may occur due to factors unrelated to resistance. Failure in this situation is often caused by problems such as underdosing due to incorrect weight estimation. Both in vivo and in vitro approaches are utilized to detect and monitor resistance. In vivo, the Fecal Egg Count Reduction Test (FECRT) compares the number of worm eggs in animals before and after treatment to assess efficacy. Resistance is identified when two criteria are fulfilled: the percentage reduction in egg count is less than 95%, and the lower limit of the 95% confidence interval is 90% or lower. Different approaches can be utilized to evaluate resistance, encompassing in vitro methods like Egg Hatch Assays (EHA), larval development assessments, larval motility evaluations, and polymerase chain reaction (PCR) tests. Nevertheless, EHA isn't adequate for assessing tetrahydropyrimidines, imidazothiazoles,

and macrocyclic lactones due to their lack of ovicidal properties. The examination involved depositing fresh eggs into the compartments of a multiwell plate, and then determining the $LD_{50}$. In the larval development test, the ability of the larvae to survive and develop in different concentrations of anthelmintic drugs is examined. Variations in $LD_{50}$ are reported depending on the time of infection, mainly when macrocyclic lactones are used. In the locomotion test, larvae are incubated in various drug concentrations, and larval movements are counted after light stimulation. In the PCR test, the genotype of adult worms or larvae that are resistant (rr) or susceptible (rS and SS) can be detected using PCR.[31]

Nitazoxanide, a recently developed antiprotozoal agent, has demonstrated effectiveness against various parasites, including *A. lumbricoides*. It has been suggested as a promising candidate for treating soil-transmitted helminthiasis in humans, prompting the need for additional research.[27] The process of developing new anthelmintic drugs to counter resistance is both slow and costly. Hence, it is crucial to utilize current anthelmintics judiciously to mitigate the effects of resistance. Various management strategies exist to prevent parasite infections or maintain low infection pressure, including pasture and refugia management. This will reduce the need for the use of anthelmintic drugs, which can delay the development of resistance. Necessary actions needed to slow the development of resistance include using appropriate doses of anthelmintic drugs, reducing dependence on anthelmintics, maintaining worm populations susceptible to anthelmintics, and regular anthelmintic resistance testing. Combinations of anthelmintics with related spectrums of activity and different modes of action have been recommended to slow the development of anthelmintic resistance. Developing an efficient vaccine against intestinal parasites would allow the use of antiparasitic drugs less frequently. However, currently, there is only one commercially available vaccine for Dictyocaulus viviparus.[30,31] The recurrence of ascariasis is a significant public health problem, especially in the pediatric population. Reinfections can reduce a child's overall quality of life and decrease cognitive and developmental outcomes due to malnutrition and anemia. This is caused by decreased food intake and malabsorption of nutrients, which is proven in patients when fecal examination finds food residues (+) and fat (+). Patients with poor nutritional status but not yet anemic can be seen from the blood test results of Hb 12.3g/dL. It is also possible that anemia has not occurred in the patient because the worms have not yet attached themselves to the intestinal mucosa, which will cause gastrointestinal bleeding.

Stool testing may be performed two months following treatment of patients in non-endemic areas to ensure successful clearance.[12] Conterno et al. stated that the egg reduction rate (ERR) measured up to 60 days after the treatment was high in all treated groups, regardless of the anthelmintic used (range 96% to 100%).[27]

Several causes and predisposing factors play an essential role in the incidence of reinfection infections. Children who live in areas with poor sanitation or have limited access to clean water are at higher risk of reinfection. Additionally, conditions such as a weakened immune system or nutritional deficiencies can make a person more susceptible to reinfection after recovering from previous ascariasis. Inadequacy or imperfection in treatment when treating the initial infection, as well as increased drug resistance due to genotype or widespread

use of certain drugs, can also increase the risk of ascariasis reinfection. Over time, surviving worms can grow into adult worms and release eggs that cause new infections.[4,5] Enhancing basic sanitation and ensuring access to clean drinking water are essential in regions with poor sanitation and low socioeconomic status. Essential measures for preventing ascariasis include avoiding contact with soil, wearing appropriate footwear, and education. In our patient, ascariasis reinfection was likely caused by inadequate previous treatment with pyrantel pamoate, economic factors where the patient's family came from a family with a lower middle socioeconomic background. The patient's father's job is a pedicab driver. According to his mother, he has poor nutritional intake because he rarely consumes protein. Besides that, poor environmental sanitation is due to living in rural areas with non- permanent house buildings as shown in Figure 3 and a lack of family knowledge about preventing ascariasis, for example, using footwear and poor hand hygiene.

**Figure 3.** Home environment of a 2-year-old patient with ascariasis.

## STRENGTH AND LIMITATION

The strength of this study is the review of the existing literature regarding mebendazole treatment for ascariasis, most of which focused on albendazole as the first-line treatment. The limitation of this study is the challenge in determining the effectiveness of mebendazole treatment for ascariasis reinfection compared with alternative treatments or no treatment. Additionally, this study did not involve other family members of the patients.

## CONCLUSIONS

Ascariasis reinfection may occur in children. Inadequate treatment when treating the initial infection can increase the risk of ascariasis reinfection, impacting the child's quality of life, growth, and development. Adequate treatment, improved sanitation, proper personal hygiene, and maintaining balanced nutrition in children are needed to prevent reinfection worm infections.

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## CONFLICT OF INTEREST

The authors confirm that they are not associated with any organization or entity that has a financial interest in the subject matter or materials discussed in this manuscript.

## AUTHOR CONTRIBUTION

MTT as the main author and contribute to preparing the manuscript. MRP and DH reviewed the paper and suggested changes. All authors read and approved the final manuscript.

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# Predation Time for Halfmoon and Multicolor Plakat of Varieties of Betta Fish Against *Aedes aegypti* Larvae in Different Water Volume

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## Abstract

One biological strategy for controlling mosquito vectors is using larvivorous fish as larvae predators. Larvivorous fish are an alternative to overcome the problem of larval resistance to temephos. Among the many varieties of Betta fish, the specific predation rates associated with each variety and their behavior in different water volumes remain unclear. This study aims to analyze the differences in predation time for halfmoon and a multicolor plakatof varieties of Betta fish against *Aedes aegypti* larvae in different water volumes.. The research was conducted as a laboratory experiment using a post-test-only design with five replications. Four treatment groups were established, each consisting of one aquarium filled with a specific water volume, one fish, and 25 *Ae. aegypti* larvae. The tests began at 12:00 WIB, and the predation time was recorded until all larvae were consumed. The findings showed that all All varieties of Betta fish can typically predate *Ae. aegypti* larvae. Halfmoon and multicolor plakat have the same predation ability against *Ae. aegypti* larvae ($p > 0.05$). The Mann Whitney's results indicated that Betta fish placed in water with a water volume of 1 and 3 liters had no significantly different predation against *Ae. aegypti* larvae ($p > 0.05$). However, the Kruskall-Wallis test results showed a significant difference in predation abilities between the two varieties when exposed to different water volumes ($p < 0.05$) . The multicolor plakat variety displayed the fastest predation time in 1 liter of water, whereas the halfmoon variety predated more quickly in 3 liters of water.

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## INTRODUCTION

The *Aedes aegypti* mosquito significantly impacts human health by disrupting daily life, feeding on human blood, and serving as a vector for various diseases, including dengue, chikungunya, yellow fever, filariasis, Zika, and West Nile. Dengue hemorrhagic fever (DHF) is among the most severe public health challenges in Indonesia, caused by the dengue virus, which has four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4.[1-4] *Aedes aegypti* spreads the virus from person to person, with the first dengue fever cases in Indonesia reported in Jakarta and Surabaya in 1968. Since then, dengue has been found in all provinces, with infection rates increasing during the rainy season. DHF continues to cause outbreaks and fatalities across numerous districts and cities, with cases rising annually.[5-6]

Currently, there are no human dengue vaccines or antiviral treatments available.[7] Without effective therapeutic drugs, the best approach to preventing and managing the transmission of Aedes-borne arboviruses involves eliminating mosquito habitats and targeting larvae and adult mosquitoes through physical, biological, and chemical control methods.[4,8,9] Chemical insecticides, such as temephos, have been widely used for *Aedes aegypti* management in Indonesia since 1980. Temephos is commonly applied to water reservoirs, which are critical habitats for *Aedes aegypti* larvae.[10,11]

However, prolonged use of temephos has several drawbacks, including the development of resistance in *Aedes aegypti* larvae and environmental pollution due to its persistence.[10,12,13] Resistance to temephos and other insecticides has been reported in Indonesia and several other countries, including Singapore, Malaysia, Thailand, India, and Peru.[14] Despite these challenges, comprehensive data on insecticide resistance across all Indonesian provinces remains limited.[15] To address resistance, strategies such as insecticide rotation and alternative biological vector management should be considered.[16]

The Ministry of Health Indonesia has implemented several strategies to reduce dengue hemorrhagic fever (DHF), including the PSN 3M Plus program (draining, covering, burying, or recycling), using mosquito repellents, keeping larvivorous fish, applying larvicide, and promoting the G1R1J movement (1 House 1 Jumantik).[17-19] Among these, keeping larvivorous fish is emphasized as an effective and user-friendly biological control method.17 Several species, including *Gambusia affinis*, *Poecilia reticulata*, and *Betta splendens*, have been identified as potential predators of *Aedes aegypti* larvae.[20-22] Notably, Betta splendens thrives in low to moderate temperatures, making it well-suited for various regions in Indonesia.[22]

*Betta splendens*, commonly known as Siamese fighting fish or "betta," is a freshwater species renowned for its physical diversity and aggressive behavior.[23] Over 600 years of domestication have led to variations in pigmentation, body size, and fin shapes, with popular strains including halfmoon, crown tail, double tail, and plakat.[24,25] The diverse color patterns and tail shapes of B. splendens can lead to species selection errors, and thus far, predation studies have focused on *B. splendens* as a species without distinguishing between varieties.[28-30] Specific information on which varieties are more effective at preying on mosquito larvae remains unclear and needs further exploration.

While *B. splendens* is recognized for its larvivorous potential, research on the differences in predation efficiency among its varieties is limited. Our previous study

identified the crown tail (Serit) variety as a promising predator, with an average feeding time of 3 minutes and 30 seconds to consume mosquito larvae, and a maximum feeding duration of 4 minutes.[31] In this study, we aim to investigate the predation efficiency of two other betta varieties—halfmoon and multicolor plakat—against *Aedes aegypti* larvae in different water volumes to determine their potential as effective biological control agents.

## MATERIALS AND METHODS

### Samples Preparation

Male halfmoon and multicolor plakat variants of *B. splendens*, measuring 3.5-5.5 cm from mouth to tail fin, were palced in an aquarium and fed twice daily. Acclimatization lasted for one week to allow the fish to adjust and avoid stress in their new surroundings. Fish were fasted for one day before testing to ensure their predation ability was not compromised by previous satiety. The Service Unit Identification at the Faculty of Marine and Fisheries, Universitas Airlangga, uses the identification certificate 56/ULMKILP/UA.FPK/09/2023 to identify the fish. Third-instar *Ae. aegypti* larvae were procured from the Entomology Laboratory, Health Polytechnic, Ministry of Health Surabaya.

### Fish Predation Testing

This study used four treatment groups. The first group contains the halfmoon variety in an aquarium with a water volume of 1 liter. The second group of halfmoon varieties in an aquarium with a water volume of 3 liters. The third group of multicolor plakat varieties in an aquarium with a water volume of 1 liter. The fourth group of multicolor plakat varieties in an aquarium with a water volume of 3 liters. Each group had five replications and a total of 20 aquariums. The glass aquarium measures 14 cm long, 14 cm wide, and 24 cm high. Each aquarium contained 25 individual *Ae. aegypti* larvae. The research was carried out during the day (12.00 WIB).

When the larvae reached the water, the timer was activated. The time taken for the *Betta* fish to predate all of the *Ae. aegypti* larvae was recorded.

### Data Analysis

The data were examined using SPSS™ 25 statistical software. Data analysis was performed using Mann-Whitney and Kruskal-Wallis tests, with post-hoc follow-up tests being included. Statistically significant differences were determined when the p-value was less than 0.05.

## RESULTS AND DISCUSSION

All betta fish in this study can generally predate all *Ae. aegypti* larvae. The Shapiro-Wilk normality test revealed that the predation data for multicolor plakat had a normal distribution with a p-value of 0.129 ($p > 0.05$). However, the halfmoon data was not normally distributed with a p-value of 0.027 ($p < 0.05$). The Levene test revealed that the two varieties of Betta fish had a homogenous variance in predation time, with a p-value of 0.225 ($p > 0.05$). The results of the test for differences in halfmoon and multicolor plakat predation times on *Ae. aegypti* larvae were analyzed using the Mann-Whitney test (Table 1).

**Tabel 1.** Mann-Whitney Analysis Result

| Variety | Mean | SD | *p* |
|---|---|---|---|
| Halfmoon | 4,85 | 2,40 | 0,519 |
| Multicolor plakat | 4,85 | 3,51 | |

Mann-Whitney (* = Significantly different at 5% significance level)
SD = Standard Deviation

Table 1 shows that the halfmoon has an average predation againts *Ae. aegypti* larvae of 4.85 minutes with a median of 3.75 minutes. On average, multicolor plakat predation time is 4.85 minutes, with a median of 4.25 minutes. Descriptively, it shows that the variety of *Betta* fish with the fastest predation time against *Ae. aegypti* larvae is the halfmoon because it has a lower median, even though the average predation time is the same as the multicolor plakat. The Mann-Whitney test showed that the two types of fish studied had no significantly different predation abilities in preying on *Ae. aegypti* larvae, as indicated by a p-value 0.519 ($p > 0.05$). Halfmoon (Figure 1) and multicolor plakat (Figure 2) have the same predation ability against *Ae. aegypti* larvae.

**Figure 1.** Halfmoon variety

**Figure 2.** Multicolor plakat variety

Results of the Shapiro Wilk normality test during predation of *Betta* fish in a 1 Liter aquarium ($p = 0.012$) and a 3 Liters aquarium ($p = 0.092$). The results of the normality test showed that *Betta* fish placed in a 3 Liters aquarium had normal distribution ($p > 0.05$), while *Betta* fish placed in a 1 Liter aquarium had data that was not normally distributed ($p < 0.05$). The results of the homogeneity test using the Levene test ($p = 0.592$) showed that both aquarium volumes had homogeneous predation time variances ($p > 0.05$). The test for differences in predation time for *Betta* fish on *Aedes* larvae based on aquarium volumes of 1 liter and 3 liters was analyzed using the Mann-Whitney test (Table 2).

**Table 2.** Mann-Whitney Analysis Result

| Volume | Median | Mean | SD | *p* |
|---|---|---|---|---|
| 1 Liter | 2,75 | 4,05 | 2,95 | 0,111 |
| 3 Liters | 5,00 | 5,65 | 2,83 | |

Mann-Whitney (* = Significantly different at 5% significance level)
SD = Standard Deviation

Table 2 shows that *Betta* fish placed in a 1 liter water had an average predation time against *Ae. aegypti* larvae of 4.05 minutes with a median of 2.75 minutes. On average, *Betta* fish placed in a 3 liters water have a predation time of 5.65 minutes with a median of 5 minutes. Descriptively, it shows that the *Betta* fish had the fastest predation time against *Ae. aegypti* larvae are those placed in 1 liter water because they have a lower mean and median.

Mann Whitney's results showed that the two volumes of water studied had no significantly different predation abilities against *Ae. aegypti* larvae, as indicated by a value of p = 0.111 (p > 0.05). These results mean that *Betta* fish placed in water with a volume of 1 and 3 liters have the same predation ability in preying on *Ae. aegypti* larvae.

The difference in predation time for halfmoon and multicolor plakat placed in 1 liter and 3 liters water against *Aedes* larvae was analyzed using the Kruskall Wallis test and followed by the Mann-Whitney test. Shapiro Wilk normality test results during predation of halfmoon in a 1 liter water (p = 0.311), halfmoon in a 3 liters water (p = 0.421), multicolor plakat in a 1 liter aquarium (p = 0.146), and multicolor plakat in a 3 liters aquarium (p = 0.216). The normality test results showed that all groups of varieties of *Betta* fish had a normal distribution (p > 0.05). The results of the homogeneity test using the Levene test (p = 0.003) showed that the predation time data for the two varieties in 1 liter and 3 liters water had a variance in predation time that was not homogeneous (p < 0.05). The results of the test for differences in predation time based on the types of halfmoon and multicolor plakat in 1 liter and 3 liters water against *Ae. aegypti* larvae were analyzed using the Kruskall Wallis test.

Multicolor plakat in 1 liter water has the fastest predation time against *Ae. aegypti* larvae. Halfmoon has a faster predation time when placed in 3 liters of water. The results of the Kruskall Wallis analysis showed that the two groups of fish with different water volumes had significantly different predation abilities against *Ae. aegypti* larvae, as indicated by the p-value = 0.005 (p < 0.05) (Table 3).

**Table 3.** *Kruskall Wallis* Analysis Result

| Variety | Volume water (L) | Mean | *p* |
|---|---|---|---|
| Halfmoon | 1 | 6,10 | |
| Halfmoon | 3 | 3,60 | |
| Multicolor plakat | 1 | 2,00 | 0,005* |
| Multicolor plakat | 3 | 7,00 | |

Mann-Whitney (* = Significantly different at 5% significance level)

Table 4 shows that further tests using the Mann-Whitney test to find out which group had the fastest predation time compared to other groups showed that halfmoon in a 1 liter water volume and halfmoon in a 3 liters water volume had predation abilities that were not significantly different (p>0.05). Halfmoon in 1 liter water had a significantly different and longer predation time than the multicolor plakat in 1 liter water (p < 0.05). Halfmoon in 3 liters water had a long predation time that was significantly different from multicolor plakat in 1 liter water (p = 0.014) and multicolor plakat in 3 liters water (p = 0.009) but was faster than multicolor plakat in 3 liters water (p < 0.05). Multicolor plakat in 1 liter water had a significantly different predation time than in 3 liters water (p = 0.009). Multicolor plakat in 1 liter water had a faster predation time than multicolor plakat in 3 liters water (p < 0.05).

**Table 4** Mann Whitney Test Results of Predation Time of Halfmoon and Multicolor Plakat Fish in 1 liter and 3 liters Volumes Against *Ae. aegypti* Larvae

| Group | A | B | C | D |
|---|---|---|---|---|
| **A** | - | - | - | - |
| **B** | 0,293 | - | - | - |
| **C** | **0,016*** | **0,014*** | - | - |
| **D** | 0,690 | **0,009*** | **0,009*** | - |

* = Significantly different at 5% significance level)
A = Halfmoon in 1 liter water
B = Halfmoon in 3 liters water
C = Multicolor plakat in 1 liter water
D = Multicolor plakat in 3 liters water

Overall, the combination of *Betta* fish varieties and water volume had a significant effect the predation time of *Ae. aegypti* larvae. The *Betta* fish varieties have the best predation time against *Ae. aegypti* larvae are multicolor plakat, placed in 1 liter water, followed by halfmoon in 3 liters of water. Lastly, multicolor plakat in 3 liters of water and halfmoon in 1 liter water have the same capabilities.

The variety capabilities of *Betta* fish in this study are like previous studies. The Serit variety (crown tail *Betta*) of *Betta* fish has potential as a larvivorous fish because it can predate all 25 individual third instar *Ae. aegypti* larvae each replication.[31] The results of different larval predation abilities based on varieties are also like the results of previous studies. The multicolor plakat variant of *Betta fish* suggested a faster predation time compared to the single-colored plakat variant of *Betta fish*. A single-colored plakat variant can consume 25 *Ae. aegypti* larvae in an average time of 3.4 minutes. The multicolor plakat variant can consume 25 *Ae. aegypti* larvae in an average of 2.7 minutes.[32] Male and female fish consumed more mosquito larvae during the daytime than at night. The availability of light influenced the predator's eating rate since it made it easier to seek for and attack the prey. The water volume, prey species, number of fish predators available, prey densities, and sex of the prey all had an impact on predation activities.[33] Water volume also has an impact on predation and feeding rates. When 2 liters of water were utilized in a previous study, predation and feeding rates fell. The fish spent more time feeding and looking for mosquito larvae. The feeding rate was reduced as the volume of water increased, but it increased when the number of predators and prey densities increased.[33] With the increase in the volume of water, the predation rate lowered, but the prey consumption increased linearly with the prey density irrespective of water volume. The consumption of mosquito larvae at a particular prey density is reduced with an increased volume of water, possibly due to the evasion tactics of the mosquitoes.[34]

This study predicted that the size of the tail fin influences the swimming speed and prey of larvae. Body size and tail type affected swimming speed.[35] Fish's capacity to swim is influenced by the morphology of their tail fins, body shape, and habitat. The tail beat is a significant factor in fish swimming speed. Furthermore, it determines fish swimming endurance, which can be used to distinguish between maximum sustained, protracted swimming speed and maximum (burst) swimming speed. The fish's ability to swim is mainly determined by its tail fin. The movement of a fish's caudal fin relates to energy intake and body metabolism.[36]

*Betta splendens* fish are the most effective predators at medium and lower temperatures. They have adapted to lower temperature levels. Due to temperature variations ranging from standard to low, this species' predatory efficiency remains reasonably steady.[22] Male *Betta* fish were chosen in this study because male fish have more beautiful bodies, so they are more popular than female *Betta* fish, they are sold and kept more often.[37]

Previous research reported that an average predation ability of 85.87% discovered a significant difference in the quantity of *Ae. aegypti* larvae while bathing before and after adding *Betta* fish. The acceptance rate and sustainability of utilizing *Betta* fish as a biocontrol agent achieved 96.67%, and the community has a very favorable opinion of their use. Taste, color, and scent did not alter the properties of the water.[30]

Larvivorous fishes are not only implemented in bathtubs or water reservoirs in the house. Larvivorous fishes can thrive in both artificial and natural environments, including water tanks, lakes, fountains, pools, cattle troughs, swimming pools, water storage tanks, seepage, irrigation cisterns, canals, shallow pools, small dams, rice fields, ponds, riverbed pools, slow-moving streams, swamps, and temporary water collection systems.[38]

The use of larvivorous fish is a simple approach of eliminating vector mosquitos.[22] Effectiveness of *Betta* Fish *Betta splendens*, as a biological predator of *Ae. aegypti* larvae, can be utilized to control *Ae. aegypti* larvae in a safer, faster, more cost-effective, and community-wide manner. The efficient use of this fish is likely to reduce the amount of Dengue Hemorrhagic Fever patient occurrences.[29] Aside from that, larvivorous fishes can be helpful to on a vast scale that is commercially viable and pollution-free.[20]

## STRENGTH AND LIMITATION

The advantage of this research is that the ability of *Betta* fish against *Ae. aegypti* larvae are more apparent based on variety. We can determine which varieties are more appropriate to implement in the 3M Plus program. A limitation of this study is that no fish other than *Betta* were compared as a control group. The varieties studied still use two varieties, so further research is needed on other varieties of *Betta* fish in the future.

## CONCLUSIONS

The combination of *Betta* fish varieties and water volume had a significant effect on the predation time of *Ae. aegypti* larvae.

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## ETHICAL CLEARANCE

The research protocol was approved by the Health Research Ethics Committee of the Hang Tuah Medical Faculty, Surabaya, number I/120/UHT.KEPK.03/IX/2023.

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## CONFLICT OF INTEREST

The authors declare that this manuscript was approved by all authors, and that no competing interests exist.

## AUTHOR CONTRIBUTION

Conception, design, and/or analysis and interpretation of data, drafting the article: HA. Discussion, and critical revision for important intellectual content: IR and HTHS. Review: HA.

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Review Article

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# The Role of Host Genetics Regulating Proteins in HIV-1 Susceptibility: Epidemiological and Demographic Insights on HIV-1 in Indonesia (2022)

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ART therapy



## Abstract

Human Immunodeficiency Virus type 1 (HIV-1) remains a global public health concern, marking 52,995 cases in Indonesia alone, dominated with CRF01_AE strain which is classified as an X4 strain or a virus that uses CXCR4 co-receptor. This highlights the urgent needs to develop therapies that utilize CXCR4 inhibitors to modulate HIV-1 infection and replication. The aims of this study were to assess the epidemiological and demographic insights on HIV-1 in Indonesia in 2022, and connecting it to the dominated strain to further assess various host genetics known to promote HIV-1 infection, focusing on the co-receptors CCR5 and CXCR4. A systematic review was conducted, analyzing published studies and the 2022 HIV/AIDS report from the Ministry of Public Health of Indonesia. Additionally, the study evaluated the therapeutic potential of CXCR4 antagonists, including AMD3100, AMD070, BPRCX807, and MCo-CVX-5c, known for their anti-HIV-1 activity. Among the listed antagonists, AMD070 and MCo-CVX–5c are advancing among the others, leading to a potential most advanced combination antiretroviral therapy (cART). This research contributed to the development of personalized treatment strategies for HIV-1 by providing insights into the genetic factors influencing co-receptor regulation and HIV-1 susceptibility.

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## INTRODUCTION

HIV-1 is a complex virus capable of infecting and replicating in a variety of cell types, including CD4+ T cells, macrophages, and dendritic cells.[1] This virus has been a global focus due to its complexity and impact on public health. In Indonesia, the trend of HIV-1 cases has fluctuated significantly over the years. The population of people living with HIV (PHIV) rose from thousands to tens of thousands, reaching a peak in 2019 with 50,282 individuals diagnosed across the country. [1]

Efforts to prevent and treat HIV-1 in Indonesia by the Ministry of Health Republic of Indonesia have been quite thorough to the point they were able to suppress the rate of HIV-1 in previous years; these efforts include awareness campaigns, treatment programs, and prevention strategies. However, the fluctuating trend suggests that additional challenges persist, such as inadequate distribution about HIV-1 prevention and inconsistent access to healthcare. The continued presence and spread of HIV-1 highlight the need further research in creating more strategies of prevention and treatment to address these gaps effectively.[2]

The study of host genetics has revealed that host genetics can influence HIV-1 by affecting viral entry, replication, immune response, and disease progression. To illustrate, genetic variants in the CCR5 gene, which encodes the co-receptor used by HIV-1 to enter cells have been shown to influence HIV-1 susceptibility. [3] Individuals with a homozygous deletion of CCR5Δ32 are resistant to HIV-1 infection, whereas those with a heterozygous deletion have a lower risk of infection and a slower disease progression. Other genetic variants in the CCR5 gene and other viral entry genes, such as CXCR4 and CD4, have also been linked to HIV-1 susceptibility. [3]

Interestingly, while CCR5 is predominantly used in the early stages of HIV infection, the virus's shift to CXCR4 usage is often associated with accelerated disease progression.[4] Understanding and targeting CXCR4 offers a promising avenue to intervene in this critical stage of HIV progression. In addition to that, the exploration of CXCR4 antagonists provides insights into a broader spectrum of HIV-1 strains. CXCR4-tropic viruses, although less common in early-stage infections, play a significant role in the later stages and in cases of treatment failure.

The objective of this study is to explore the role of CXCR4 antagonists and their impact on HIV-1 pathogenesis, this research aims to identify novel therapeutic strategies and contribute to the broader understanding of HIV-1 infection dynamics. The outcomes of this study could provide valuable insights for designing more effective prevention and treatment strategies, particularly in regions like Indonesia, where HIV-1 remains a significant public health challenge.

## MATERIALS AND METHODS

### Materials

The systematic review was conducted on PubMed and Google Scholar ranging from 2013-2023 as well as the 2022 quarterly and annual report on HIV AIDS by the Ministry of Health Republic of Indonesia. Additionally, BioRender was utilized to create schematic illustrations of host genetics and other molecules, indicated by figures without citation.

### Methods

#### Eligibility Criteria

This study was a systematic review using data from studies that have been

published from 2013-2023 regarding host genetics regulating proteins that are susceptible to HIV-1. The exclusion criteria were the following: used language other than Indonesia or English, could not be accessed, did not show data of interest, did not specify X5 or X4 strain, did not identify year or location, CXCR4 antagonists without anti-HIV activity, and host genetics other than regulating proteins and are promoting HIV-1 resistance.

**Literature Review Process**

Literature search was done on PubMed and Google Scholar with keywords: "Human Immunodeficiency Virus type 1 OR HIV-1 OR CCR5 OR CXCR4 OR X5 strain OR X4 strain OR Antiretroviral Therapy OR ARV OR Host Genetics Regulating Proteins OR CXCR4 Antagonists". The search was set to obtain studies from 2013-2023.

**Study Inclusion and Data Extraction**

A total of 45 research articles were collected based on search results from each database and were imported into Zotero to remove duplicates. Next, title, abstract, and results were screened based according to predefined inclusion and exclusion criteria. The data from studies included that were extracted are: first author, publication year, study area, and specimen tested. Research articles lacking sufficient details and results were excluded, leaving a final selection of 35 articles.

The main outcomes in this review, focusing on the trends and distribution of HIV-1 in Indonesia in 2022, were presented in bar graphs and percentages, categorized by region. In addition, the potential CXCR4 antagonists were thoroughly compared based on their efficacy and therapeutic potential.

## RESULTS AND DISCUSSION

**Rate of HIV-1 in Indonesia (2022)**

According to the Annual HIV AIDS report by The Ministry of Health Republic of Indonesia in 2022 as shown on Figure 1, the first quarter of 2022 (January—March) had 10,525 cases diagnosed. It then increased to 11,000 in the second quarter (April—June), the third quarter (July—September) continued the upward trend with 12,588 cases. There was a more substantial rise in the fourth quarter (October—December), where the number of diagnosed cases reached 18,782.

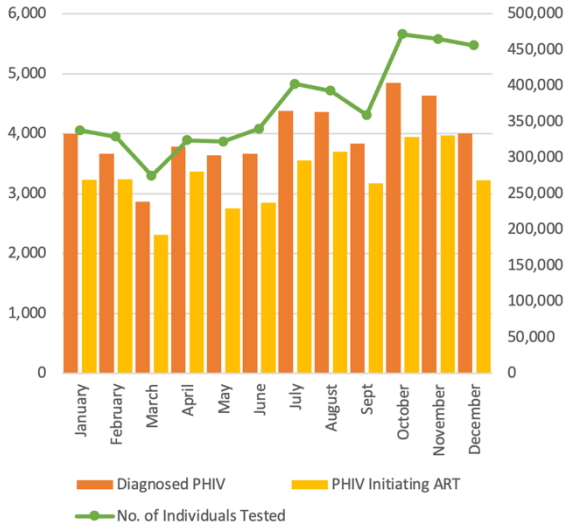


**Figure 1**. HIV-1 in Indonesia year 2022[5]

The data reveal a consistent increase in the number of HIV diagnoses throughout the year, with the highest number recorded in the last quarter. The increasing trend could reflect various factors, such as increased testing and reporting, spread of HIV or increase rate of HIV infection, or heightened awareness and diagnostic activities. However, the data may not be representing accurately as there are nine districts/cities which have not submitted any HIV data to the Ministry of Health Republic of Indonesia to the last quarter or December 2022, as attached in Table 1.

**Table 1.** List of districts/cities which have not submitted their HIV data as per December 2022[5]

| Province | District/City |
|---|---|
| East Nusa Tenggara | Alor, Central Sumba |
| North Sulawesi | Siau Tagulandang Biaro Islands |
| Southeast Sulawesi | North Konawe |
| North Maluku | Tailabu Island |
| West Papua | Maybrat, Pegunungan Arfak |
| Papua | Nduga |

The case distribution, as shown in Figure 2 displays the number contributed by each province for the diagnosed people with HIV (PHIV) and the PHIV initiating ART in Indonesia in 2022. However, it can be identified that the top 3 provinces contributing to the diagnosed PHIV (West Java, East Java, and Central java) are different, in terms of order, or province with the top 3 provinces contributing to PHIV initiating ART as shown in Figure 3 (West Java, East Java, and Central Java).

The different order can be attributed to several factors, including the access to healthcare, awareness and education, stigma and discrimination, and economic factors. The access to healthcare can be one of the causes for the factors where some regions may have better healthcare infrastructure and access to medical services, awareness, and education where regions with higher awareness or better public health campaigns about HIV can lead to more people getting tested and starting treatment. Stigma and discrimination which leads regions with higher stigma may lead to fewer people initiating ART despite being diagnosed, and, lastly, economic factors where wealthier regions may have a higher percentage of diagnosed individuals and starting ART due to better financial access to healthcare services.

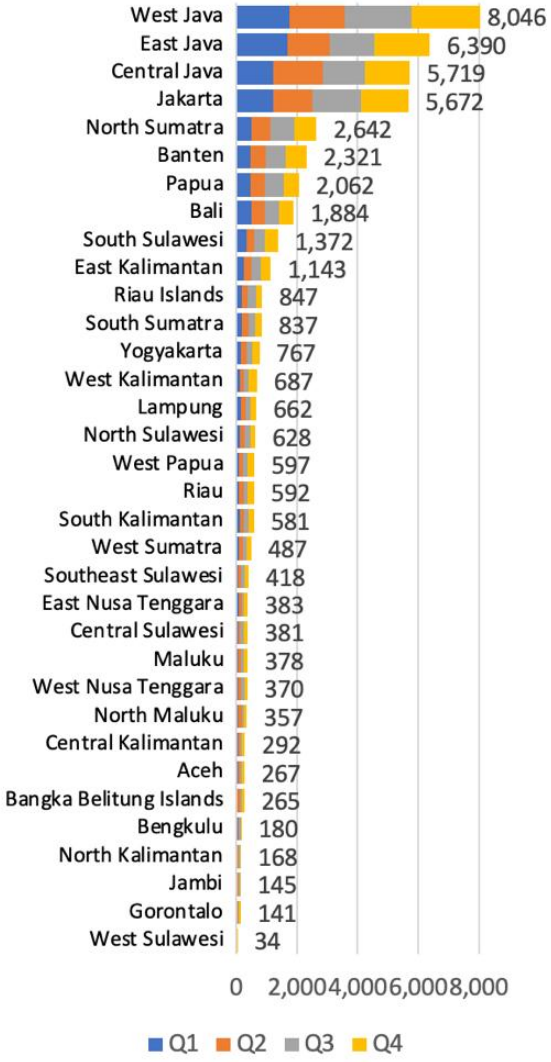


**Figure 2.** Diagnosed people with HIV in Indonesia year 2022[5]

According to Figure 4 on the age distribution for PHIV in 2022, the majority is within the range of 25-49 years old for 66.6%, followed by 20-24 years old for 17.7%, over 50 years old for 9.3%, 15-19 years old for 3.9%, and both below 4% to the range of 4-15 years old for 1.2% each. Regarding gender, the PHIV is dominated by male for 71% and female 29%.

The potential reasons for the age distribution can be affected by several factors, including sexual activity, awareness and education, and mother-to-child transmission. The dominant age falls within the 25-49 years old which is typically the most sexually active age range, hence leading to a higher risk of HIV transmission due to a higher rate of

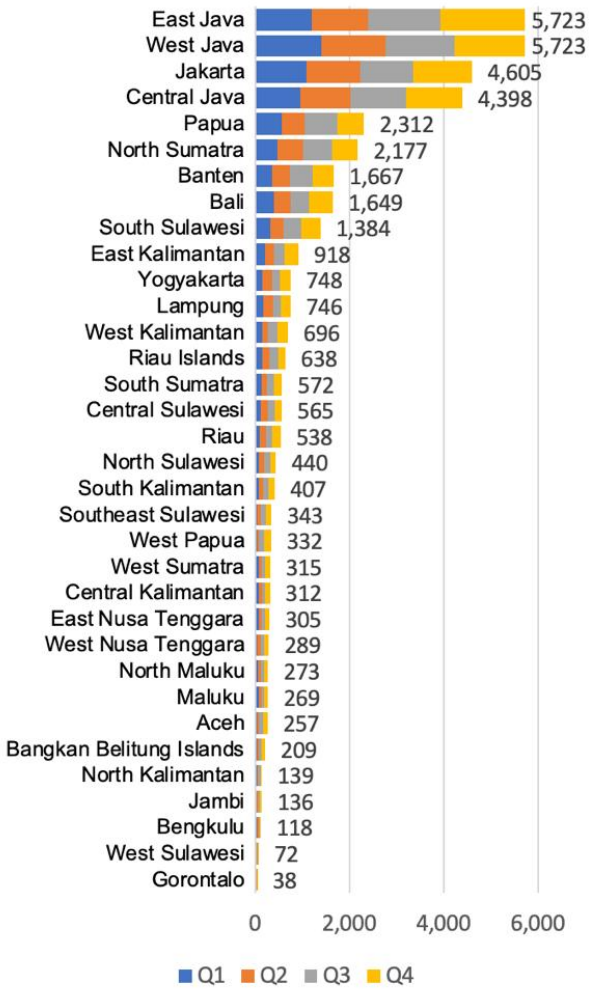


**Figure 3.** People with HIV initiating ART in Indonesia year 2022[5]

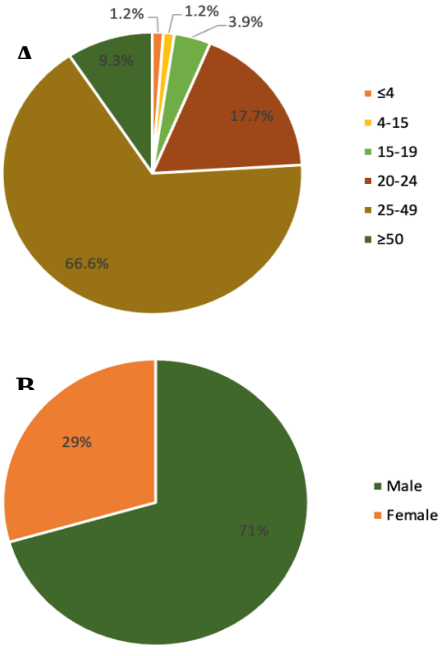


**Figure 4.** (A) Age distribution and, (B) gender distribution of people with HIV year 2022[5]

change of partner and potentially unprotected sexual encounters. Whereas younger age groups may have access to education about HIV prevention, lowering the rate of HIV. Lastly, mother-to-child transmission, which is the reason as to how a very young age group can also be infected with HIV.

As for the gender distribution factors, the higher percentage of males could be due to riskier behaviors that are more prevalent among men, such as unprotected sex with multiple partners, hiring sex workers, or engaging in sex with other men, which is a significant risk factor for HIV. Biological factors also play a role in this as women are biologically more susceptible to acquire HIV during heterosexual intercourse than men, and lastly is occupational exposure where men are most likely to work in industries with higher mobility where they may engage with commercial sex workers.

Regarding the population of PHIV, as shown in Figure 5 this is dominated with MSM or men who have sex with men counting for 27.8%, followed by others/unknown for 27.3%, pregnant women for 15.8%, TB (Tuberculosis) patients 12.4%, transgenders 6%, clients of sex workers 4.3%, sex workers 3.1%, high risk couples 1.1%, correctional institution residents 1%, and the least is drug users at 0.5%. TB patients are involved as the bacteria (Mycobacterium tuberculosis) can increase the viral load in people living with HIV, which may increase the infectiousness and eventually accelerate to AIDS.[6] Drug users are also involved as they are most likely being involved in the activity of sharing needles, increasing the HIV rate from the blood transmission.

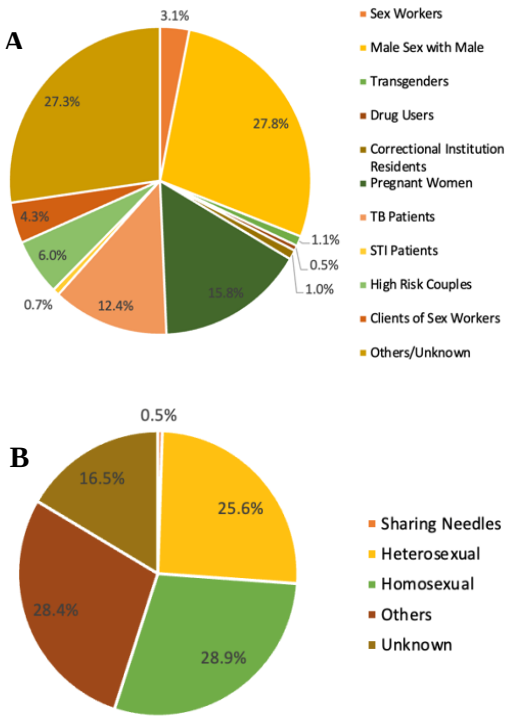


**Figure 5.** (A) Population and, (B) risk factors of people with HIV in Indonesia year 2022[5]

As for the risk factor, the majority was caused by homosexual intercourse for 28.9%, followed by others (such as blood transfusion, mother-to-child transmission, or traditional practices involving exposure to blood) comprising 28.4%, heterosexual for 25.6%, unknown factor for 16.5%, and sharing needles for 0.5%.

According to Figure 6, the testing number increased up to 761,496 in the last quarter, which could be due to enhanced public health campaigns, improved access to prenatal care, or increased adherence to testing protocols which often recommend HIV screening for all pregnant women. The number of pregnant positive HIV fluctuates from the first to the last quarter which can also be caused by the number of HIV tests. The gap between the number of pregnant women diagnosed with HIV and those receiving ART could be due to the access to treatment, such as limited availability of medication or distance to healthcare facilities, and stigma where they can fear for receiving discrimination. In addition to that, lack of awareness may also be the factor as in the lack of knowledge about the importance of ART for their health and their babies.

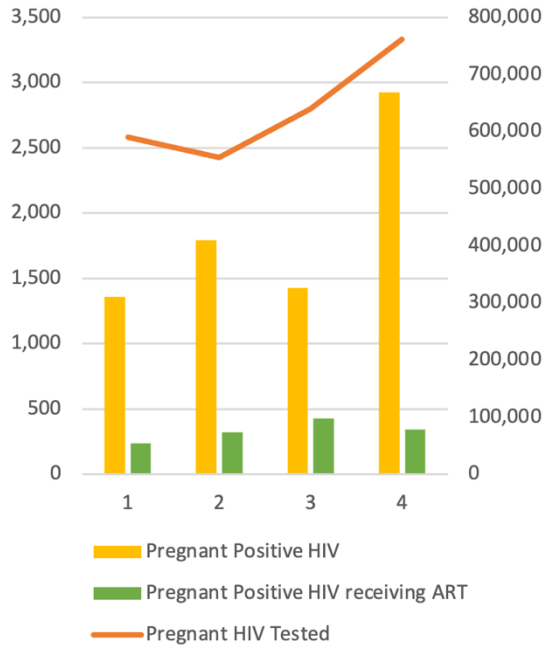


**Figure 6.** Population of pregnant positive HIV in Indonesia 2022[5]

**Table 2**. Coverage of HIV diagnosis and ART initiation[5]

| Year | Diagnosed PHIV | ART Initiated PHIV |
|---|---|---|
| 2019 | 50,282 | 38,998 |
| 2020 | 41,987 | 32,925 |
| 2021 | 36,902 | 30,160 |
| 2022 | 52,992 | 42,610 |

PHIV : People with HIV
ART : Antiretroviral Therapy

Table 2 displays data over a four-year period from 2019 to 2022 concerning the number of HIV diagnoses and the initiation of antiretroviral therapy (ART) in Indonesia. In 2019, there were 50,282 HIV diagnoses with 77.6% of those diagnosed starting ART. The following year, 2020, showed a decrease in diagnoses to 41,987 but an increase in the percentage of individuals starting ART to 78.4%.

In 2021, the number of diagnoses decreased further to 32,925, yet the percentage of people beginning ART rose significantly to 81.7%. In contrast, 2022 increased in HIV diagnoses to 52,995, while the percentage of patients initiating ART decreased slightly to 80.4%. The graph highlights a trend where, despite the fluctuations in the number of diagnoses, a consistently high and increasing proportion of diagnosed individuals are starting treatment each year, except for a small decline in 2022.

Regarding the molecular epidemiology of HIV-1 in Indonesia, a recent study investigated HIV-1 subtyping and the identification of HIV Drug Resistance (HIVDR) in 105 individuals infected with HIV-1 who lived in different cities between 2018 and 2019. The results, shown in Table 3 revealed that CRF01_AE is the predominant HIV-1 strain causing the epidemic, accounting for 81.9% of infection cases. Subtype B follows with a prevalence of 12.4%, while CRF02_AG, CRF52_01B, and a recombinant strain between CRF01_AE and CRF02_AG account for 3.8%, 1%, and 1.0% of cases, respectively.[7] This is corroborated by a study carried out in 2022 and 2023, which indicates the prevalence of CRF01_AE in Medan and Makassar.[8,9]

**Table 3**. HIV-1 subtype prevalence in Indonesian cities in 2018-2019[7]

| Subtype | Infection Case |
|---|---|
| CRF01_AE | 81.9% |
| Subtype B | 12.4% |
| CRF02_AG | 3.8% |
| CRF52_01B | 1% |
| CRF01_AE / CRF03_AG | 1.0% |

Subtype AE is known to be a part of X4 tropic virus, which is supported by research conducted in 2019 regarding co-receptor tropism and genetic characteristics. Among the 16 X4-tropic viruses studied, 12 of them are CRF01_AE, two CRF55_01B, one subtype B and one URF. The determination that CRF01_AE is a part of X4-tropic viruses was made through the analysis of co-receptor usage in different genotypes of HIV-1.[10] In the study, the genotypes of the viruses were determined by analyzing the pol genes of the subjects. Hence, the increased rate of HIV-1 and the majority of AE subtype led to a higher urgency to conduct more research in enhancing the available treatment or to create new avenue within the treatment strategies, specifying to ones that are targeting CXCR4.

## Role of Host Genetics Regulating Proteins in HIV-1 Susceptibility

The complication of the host genetics study lies within their variations, analogous to how CCR5 highly contributes to HIV-1 susceptibility but CCR5-Δ32 is well known to be resistant to HIV-1; other host genetics also have their own kinds and lead to different results.[15]

Among the host genetics studied for this research, five host genetics are widely known to promote HIV-1 susceptibility, including cyclophilin A (CypA), certain alleles of apolipoprotein E (APOE) and Human Leukocyte Antigen (HLA), CCR5, and CXCR4 with the summarized elaboration displayed in Table 4.

### Cyclophilin A

Cyclophilin A (CypA) plays a significant role in promoting HIV-1 susceptibility through its interaction with the HIV-1 capsid protein. This interaction is crucial for the virus's ability to infect human primary cells, such as peripheral blood mononuclear cells (PBMCs) and CD4+ T cells. The mechanism by which CypA enhances HIV-1 infections involves several

**Table 4.** Summarized host genetics regulating proteins in HIV-1 susceptibility

| No | Host Genetic | Role in promoting HIV Susceptibility | Mechanism of Action | References |
|---|---|---|---|---|
| 1 | Cyclophilin A (CypA) | The virus' uncoating process | Prevents TRIM5α from binding to the viral capsid and promote delayed uncoating leading to a successful viral integration | Selyutina et al.[10] |
| 2 | Apolipoprotein E (ApoE) | Viral entry and LTR transactivation | ApoE4 helps easier viral entry and is less effective in restricting Tat-mediated long terminal repeat (LTR) transactivation--a viral protein necessary for HIV-1 thus allowing more Tat protein to be converted into LTR for HIV formation | Chen et al.[13] |
| 3 | Human Leukocyte Antigen (HLA) | HIV suppressive and non-suppressive immune response | The non-suppressive immune response or non-protective HLA B allele (HLA-B*35) producing monofunctional CTL with limited recognition for infected cells | Lunardi et al.[14] |
| 4 | CCR5/CXCR4 | Viral entry | The chemokine receptor for HIV viral entry | Chen et al.[13] Lunardi et al.[14] |

key steps, including CypA-Capsid interaction, protection from TRIM5α restriction, influence on reverse transcription, and differential effects in cell types.[10]

The contact between CypA and the HIV-1 capsid is crucial for the successful infection of human primary cells, as CypA binds to the capsid.

Research has demonstrated that administering Cyclosporin A (CsA), a substance that hinders the binding of CypA to the HIV-1 capsid, effectively suppresses HIV-1 infection in both PBMCs and CD4+ T cells.[13] The next key step is its protection against TRIM5α restriction, CypA binding to the HIV-1 core is thought to protect the virus from the restriction factor human tripartite motif 5 alpha (TRIM5α), which is known to inhibit HIV-1 infection by recognizing and binding to the viral capsid, leading to its premature disassembly and degradation. By binding to the capsid, CypA prevents TRIM5α from exerting its restrictive effects as a host genetic promoting HIV-1 resistance.[10]

Moreover, studies have shown that when the interaction between CypA and capsid protein is disrupted in CD4+ T cells, it greatly reduces the efficiency of reverse transcription, which is a critical step in the HIV-1 life cycle. This implies that CypA helps the virus's early stages of replication in addition to shielding it from restriction factors. Finally, compared to Jurkat cells (a human T cell line), the effect of CypA on HIV-1 infection appears to be more pronounced in primary human lymphocytes. This suggests that CypA's role in promoting HIV-1 susceptibility may be especially significant in the context of primary human cells.[11]

Figure 7 displays a comparative view of the presence of Cyclophilin A (A) and the absence of Cyclophilin A (B), where, in the presence of CypA, it blocks the TRIM5α in binding to the HIV, leading to delayed uncoating and a successful integration, promoting HIV disease progression. Whereas in the absence of CypA, TRIM5α would take place in binding to the capsid of HIV, being

recognized by the innate immune response and causing the virus to undergo premature uncoating and the failure of integration, preventing the HIV disease progression.[12]

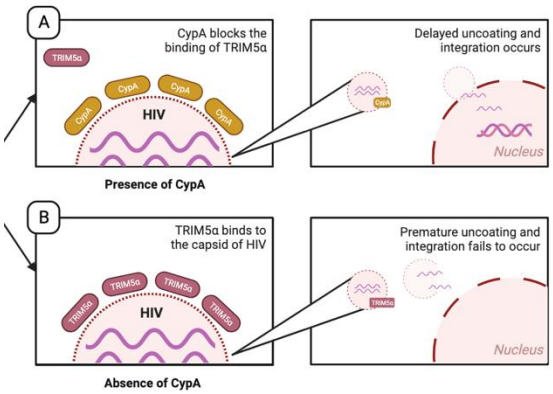


**Figure 7**. Cyclophilin A mechanism of action in promoting HIV disease progression

**Apolipoprotien E (APOE)**

Apolipoprotein E (APOE) is a protein that is responsible for regulating the breakdown and use of fats in the body. However, it also has a notable impact on the health and diseases of the nervous system. In the case of HIV-1, APOE has been found to influence the progression of the disease and the development of HIV-associated neurocognitive disorders (HAND). APOE exists in three main forms, known as isoforms - APOE2, APOE3, and APOE4. These isoforms differ from each other by a single amino acid substitution, specifically an interchange between arginine and cysteine at two positions.[13], as shown in Figure 8. These isoforms have been studied for their potential impact on HIV-1 pathogenesis.

APOE has a well-established role in neurological disorders, particularly Alzheimer's disease and viral diseases. In the context of HIV, the presence of the APOE4 allele has been correlated with an increased risk of developing neurocognitive impairments. APOE4 may exacerbate neuronal damage caused by HIV-1, leading to a more rapid decline in cognitive function.[13]

The differential impact of APOE isoforms on HIV-1 pathogenesis is a subject of ongoing research. APOE2, for instance, is thought to have a protective effect against HIV-related neurocognitive disorders, potentially due to its anti-inflammatory properties. Understanding these variations is crucial for developing targeted interventions to mitigate the neurocognitive impacts of HIV-1 and for tailoring antiretroviral therapy based on individual genetic profiles.[13]

Figure 8 depicts the comparison of ApoE3 and ApoE4 in HIV infectivity, where (A) shows the virus with ApoE4 which interacts with its receptor (HSPG and LDLR) accelerate the contact between HIV and cell membrane which facilitates virus cell entry, whereas the ApoE3 depicted in (B) has a lesser ability in bringing the virus inside the target cell. Figure (C) illustrates that ApoE4 has reduced efficacy compared to ApoE3 (D) in inhibiting Tat-induced long terminal repeat (LTR) transactivation, a crucial viral protein essential for HIV-1 replication, therefore promoting more Tat protein to enter the nucleus for producing LTR required for the virus' formation.

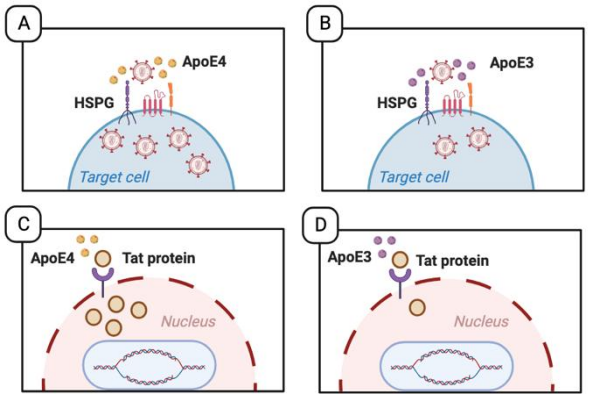


**Figure 8**. Comparison of ApoE3 and ApoE4 mechanism in accelerating HIV infection

**Human Leukocyte Antigen (HLA)**

Human Leukocyte Antigen (HLA) molecules are essential for the immune system's capacity to identify and react to infections, such as HIV-1. The HLA system exhibits a high degree of polymorphism, resulting in a wide range of distinct HLA alleles throughout the human population. Each allele has the ability to deliver a unique collection of peptides to T cells. The diversity is essential for the immune system's capacity to effectively counteract a broad spectrum of infections.[14]

HLA Class I molecules, which include HLA-A, HLA-B, and HLA-C, present endogenous peptides, including those derived from viral proteins, on the surface of infected cells. Cytotoxic T lymphocytes (CTLs) recognize these peptide-HLA complexes and can kill the infected cells, thereby controlling the infection. The variability in HLA molecules, particularly in the peptide-binding groove, affects the repertoire of viral epitopes that can be presented. HLA is widely known to promote HIV resistance. However, specific HLA alleles have been linked to greater susceptibility to HIV infection and accelerated disease advancement.[14]

One approach involves the presentation of viral peptides that have a reduced ability to stimulate a robust immune response. HLA-B*35-Px has been linked to rapid HIV-1 disease advancement, as an example. This genotype exhibits a more restricted spectrum of HIV peptides to the immune system, potentially constraining the efficacy of the immunological response. [14]

Another method occurs through the selection of viral escape mutants. The immunological pressure exerted by cytotoxic T lymphocytes (CTLs) might result in the emergence of viral variations that possess alterations in the epitopes they exhibit. This enables the variants to avoid detection by the immune system.16 HLA alleles like HLA-B27 and HLA-B57 are linked to a slower course of disease because the escape mutants they choose usually have a negative impact on the virus's ability to survive and reproduce, resulting in reduced fitness.

However, other HLA alleles may select escape mutants that do not impair viral fitness, thus promoting viral persistence and disease progression. [13] Furthermore, certain HLA alleles may influence the immune response in ways that promote HIV replication. For example, HLA-B*35-Px has been associated with increased inhibitory immunoregulatory impulses, which could potentially enhance HIV replication.[14]

Figure 9 displays the schematic representation of the protective and non-protective HLA B alleles in HIV infectivity, where Figure (A) shows the protective HLA B alleles producing polyfunctional CTL which recognizes more diverse infected cells whereas Figure (B) shows the non-protective HLA B alleles producing monofunctional CTL and its limited ability to recognize diverse types of infected cells.

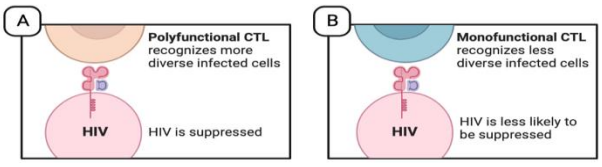


**Figure 9**. Illustration of the protective and non-protective HLA B allele

**CCR5**

Next is CCR5 (C-C Chemokine Receptor type 5) which is the necessary receptor for HIV-1 to enter certain immune cells. The HIV-1 virion's surface glycoproteins known as the viral envelope use the chemokine receptor family's CCR5

or CXCR4 co-receptors in addition to the primary receptor CD4 to enter target host cells. Env encodes the envelope glycoproteins, which bind non-covalently to the transmembrane gp31 subunit and surface gp120 subunits of the virion to form trimmers at the lipid membrane.[15]

During the initial stage of viral entry, the gp120 attaches to one or more CD4 primary receptors, causing structural modifications in gp41 and revealing a previously hidden binding site for chemokine receptors. The V3 loop residues of gp120 engage in interactions with the N terminus, which in turn interacts with the bridging sheet of gp120. Additionally, these V3 loop residues interact with residues present in the chemokine receptor binding pocket, as well as in ECL1 and ECL2 of the co-receptor, either CCR5 or CXCR4.[15]

Gp41 and gp120 approach the target membrane through sequential binding to CD4 and a co-receptor. This causes the gp41 domains to go through a complex folding process that results in the formation of a fusion intermediate involving a six-helix bundle. As a result, the target cell membrane's lipid bilayer can be penetrated by gp41's extremely hydrophobic fusion peptide, causing the two membranes to fuse and create a pore that lets the viral capsid enter the infected cells' cytoplasm.[15]

Pre-integration latency can occur when HIV-1 attacks inactive and inexperienced T cells, thereby obstructing the integration of HIV-1 into the genetic material of the host by reverse transcription and then progressing to active or memory cells. In either case, memory cells have the ability to be reactivated, or naïve cells can be stimulated, resulting in the production of infected effector and memory cells.

This process helps to preserve the transcription and translation of HIV-1. Thus, cells that express CCR5 make up the largest portion of HIV-1 latency and serve as a hidden storage of the virus. These can occur due to the infection of quiescent memory T cells, active memory T cells that remain in a memory T cell state, activated thymocytes undergoing transition to naïve T cells, or activated T cells that return to a quiescent memory T cell state.[15]

The identification of the CCR5-Δ32 mutation has offered additional understanding regarding the involvement of CCR5 in determining vulnerability to HIV-1. The mutation leads to the formation of a shortened receptor that is absent from the cell surface, as depicted in Figure 10. Consequently, persons who have two copies of this mutation are immune to HIV-1 infection, whereas those who have one copy experience a slower pace of disease progression.[16]

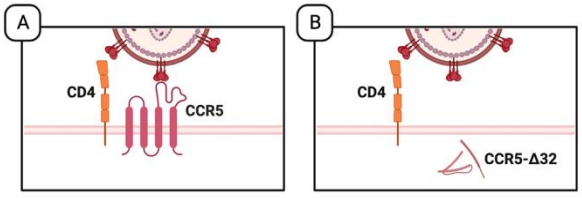


**Figure 10**. The illustrated comparison between CCR5 and CCR532

The discovery has sparked significant interest in developing therapeutic strategies that explicitly focus on CCR5, either by inhibiting its interaction with gp120 or by mimicking the effects of the Δ32 mutation. The CCR5-Δ32 mutation provides a unique perspective on the relationship between host genetics and susceptibility to HIV-1. This mutation is caused by a 32 base pair deletion in the CCR5 gene, leads to the truncation of the receptor and its inability to be generated on the cell surface.[17]

**CXCR4**

As previously mentioned, not only CCR5 but CXCR4 (C-X-C chemokine receptor type 4) is also the co-receptor for

HIV-1 entry as shown on Table 5. The different strains of HIV-1 lead to different chemokine receptor utilization. As shown in Table 3, R5 tropic uses CCR5, and X4 tropic uses CXCR4 as their co-receptor. The primary targets of HIV-1 early infection are monocyte-derived macrophages and memory CD4 cells, which are mostly infected by exclusively R5 strains. In contrast, exclusively X4 strains predominate at a later stage and favor naïve and resting T cells.

**Table 5**. Phenotypic classification of HIV-1 based on co-receptor use[18]

| Chemokine Receptor | Natural Ligands | HIV-1 |
|---|---|---|
| CCR5 | CCL3, CCL4, CCL5 | R5 |
| CXCR4 | CXCL12 | X4 |
| CCR5, CXCR4 | | R5X4 |
| CCR2, CCR3, CCR5 | | R2R3R5 |

The primary cause of early infections is R5 tropic viruses, which have a greater attraction to CD4 and a higher level of CCR5 surface expression compared to CXCR4 on CD4+ memory T cells and immature dendritic cells. This influences how effectively the virus can enter the cells. Previous investigations have shown that the transmission of HIV-1 by R5 strains is more effective than X4 strains, as is the multiplication of the virus.[19]

The chemokine receptor CXCR4 plays a crucial role in the vulnerability and advancement of HIV-1 infection. HIV-1 exploits this receptor, in addition to CCR5, to enter CD4+ T cells (CD4TL). The interaction between HIV-1 and CXCR4 plays a vital role in regulating the virulence of the virus, especially in relation to its capacity to induce AIDS.

Research has indicated that viruses that utilize CXCR4 (X4-tropic viruses) tend to exhibit higher levels of pathogenicity compared to those that utilize CCR5. The strains that use CXCR4 are associated with a quicker decline of CD4TL cells and a faster development of AIDS. It is worth mentioning that there is a significant range in the severity of CXCR4-using viruses, suggesting a complicated interaction between viral and host variables in the advancement of the disease.[20]

Therefore, the emergence of CXCR4-tropic HIV-1 viruses represents a significant turning point in the infection's progression. The ability of these viruses to infect a broader range of cells, and the resultant immune system damage they cause are central to understanding the accelerated progression toward AIDS. This knowledge not only aids in prognostication but also underscores the need for targeted therapeutic strategies to combat CXCR4-tropic HIV-1 strains.

**Discovery of CXCR4 Antagonists**

The clinical implications of the CXCR4 usage by HIV-1 have led to the development of CXCR4 antagonists as potential therapeutic agents. These drugs aim to block the interaction between the virus and CXCR4, thus preventing the virus from entering cells. However, their effectiveness is limited to cases where the virus predominantly uses CXCR4 for entry.[21]

Understanding the role of CXCR4 in HIV-1 infection and its implications for disease progression is crucial for developing targeted therapies and managing late-stage HIV-1 infection.

As research continues, further insights into the molecular mechanisms governing the CCR5-to-CXCR4 switch and the role of CXCR4 in HIV pathogenesis are expected to emerge, offering potential new strategies for intervention.

**Table 6.** Comparison of CXCR4 antagonists

| No | Antagonist | Class | Anti-HIV ($IC_{50}$) | Toxicity ($CC_{50}$) | Pharmaco-kinetics | Development Status | Disease Treatment | References |
|---|---|---|---|---|---|---|---|---|
| 1 | AMD3100 (Plerixafor) | Small molecule | 3 nM | >500 µM | Subcutaneous | Terminated in clinical trial for HIV | Stem cell mobilization | Wang et al.[24] Huang et al.[25] |
| 2 | AMD070 (Mavorixafor) | Small molecule | 10 nM | >100 µM | Oral | Phase 2 | HIV | Huang et al.[25] |
| 3 | BPRCX807 | Small molecule | 40.4 ± 8.0 nM | NS | Subcutaneous | Investigational primarily for anticancer effects | Mainly for cancer | Dogra et al.[34] |
| 4 | MCo-CVX-5c | Peptide | 2 nM | >100 µM | Subcutaneous | Preclinical | HIV | Chaudhuri et al.[32] |

NS: Not Specified

CXCR4 is not only known in HIV-1 but also several diseases such as carcinoma and hepatitis, however, up to this day, there has not been a single CXCR4 antagonist that is approved by the FDA for the HIV-1 treatment.[21] The list of CXCR4 antagonists is as listed in Table 6 which compares the class, type, mechanism of action, anti-HIV ($IC_{50}$), toxicity ($CC_{50}$), pharmacokinetics, and their potential effectiveness as HIV treatment. There are over 30 CXCR4 antagonists at the present time, including Tachyplesin 1, T22, MSX-11, miR-146, and many more.[22] However, many still lack data and information required for comparison and some are reportedly to have inability to treat HIV-1, hence four CXCR4 antagonists were chosen (AMD3100 (Plerixafor), AMD070 (Mavorixafor), BPRCX807, and MCo-CVX-5c) for a more in-depth analysis rather than a broad overview with limited information, as shown in Table 4.

**AMD3100**

AMD3100 (Plerixafor) is a small non-peptide chemical that inhibits the CXCR4 receptor. The nitrogen atom that has gained a proton on the ring interacts with the carboxylic acid group on CXCR4, which restricts the binding of CXCL12 to CXCR4. This interaction prevents downstream signaling and controls several physiological activities.[23]

AMD3100 functions as a selective inhibitor of CXCR4 by disrupting its interaction with CXCR4, as depicted in Figure 11. It attaches to CXCR4 and hinders the attachment of CXCL12, thereby impeding the transmission of signals and movement of cells toward the chemical gradient.

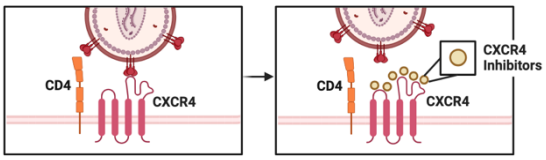


**Figure 11.** Illustration of CXCR4 Inhibitors

AMD3100 has demonstrated limited efficacy as a partial agonist against the normal form of CXCR4 and can enhance the function of a mutant form of CXCR4 that is constantly active, but only at high dosages.[23] In addition to its investigation for HIV-1, AMD3100 was also studied for its potential in treating WHIM syndrome (a rare immunodeficiency disorder characterized by panleukopenia), brain tumors, and

autoimmune diseases. Furthermore, it has been demonstrated to possess the capacity to synergize with other anti-cancer treatments for conditions such as cervical cancer, pancreatic illnesses, mesothelioma, ovarian cancer, hepatocellular carcinoma, and numerous others.

AMD3100 is proven to be effective, hence approved by the Food and Drug Administration (FDA) for the application in stem cell mobilization and radiation-induced injury.[24] AMD100 is no longer being developed for HIV-1 treatment, specifically as an antiretroviral therapy. This is due to the fact that it has failed to inhibit the infection of macrophage tropic (R5) HIV-1 strains, making it unsuitable for use as a monotherapy. Additionally, AMD100 has poor oral bioavailability and has been associated with serious side effects, such as cardiac disturbance. A clinical study was halted when it was discovered that extended use of AMD3100 caused premature ventricular contractions in 2 out of 40 patients.[24-28]

**AMD070**

AMD070, also referred to as AMD11070 or Mavorixafor, is a potent inhibitor of CXCR4, a protein involved in HIV-1 replication. It has a high level of tolerance and may be taken orally, making it an effective blocker of X4 HIV-1 replication. In a prior study, it was found that AMD070 significantly decreased the migration and invasion of oral cancer cells that rely on the CXCL12/CXCR4 pathway. In addition, it was shown that the combination of AMD070 and the light-absorbing material indocyanine green (ICG) created nanobubbles that hindered the interaction between CXCL12 and CXCR4 in breast cancer cells. As a result, this limited the growth of cancer cells and promoted programmed cell death, known as apoptosis.[26]

The pharmacological profile of AMD070 also showed potent inhibition of X4 HIV-1 replication and the gp120/CXCR4 interaction, which are crucial steps in HIV infection process. By inhibiting this interaction, AMD070 prevents HIV from entering CD4 cells, thus potentially inhibiting the progression of infection.[29]

Furthermore, AMD070 has demonstrated safety and efficacy in clinical trials for HIV-1 treatment. The fact that it has been tested in human clinical trials for this specific application and shown promising results is a strong indicator of its potential effectiveness. Safety in clinical trials is a critical factor, and demonstrating efficacy in this context is a clear signal of therapeutic potential.[30]

The clinical trial results for AMD070, known as AMD11070 in its trial phases, indicate its potential effectiveness in treating HIV-1 infection, particularly in patients harboring CXCR4-tropic virus. Within the tolerability and viral load reduction study conducted by Mosi et al. (2012), through clinical testing it was determined that the medicine exhibited good tolerability and was orally bioavailable in healthy volunteers. In a proof-of-concept clinical experiment including HIV-infected persons with X4 virus infection, 4 out of 9 patients experienced a reduction of more than 1log10 in X4 viral levels.[30]

**MCo-CVX-5c**

Next is MCo-CVX-5c, it is a cyclotide-based CXCR4 antagonist that Has demonstrated encouraging outcomes in impeding the penetration and duplication of CXCR4-tropic in human lymphocyte MT4 cells in a manner that is dependent on the dosage. Cyclotide analogs, including Mco-CVX-5c, have

become important lead candidates against CXCR4-mediated HIV-1 entry, it is shown to inhibit CXCL12-activation of CXCR4 and HIV infection by binding to CXCR4 with high affinity, thereby blocking the entry of HIV-1 into cells.[31]

Among all CXCR4 antagonists compared, MCo-CVX-5c provides the most recent research regarding its anti-HIV-1 activity. In addition to that, among the MCo-CVX-5c derivatives, MCo-CVX-PP is proven to have the highest potential as entry inhibitor in HIV-1 treatment since its $IC_{50}$ value is 7.9 ± 0.5 nM being the most potent compared to its derivatives and AMD3100.[32]

**BPRCX807**

BPRCX807 exhibits greater efficacy compared to AMD3100, both when used as an individual drug and when combined with sorafenib, for antiangiogenic therapy. A research examining the use of a CXCR4 antagonist for treating hepatocellular carcinoma compared the benefits of BPRCX807 with the already licensed CXCR4 antagonist, AMD3100.

BPRCX807 exhibits a greater maximum tolerated dosage (MTD) in comparison to AMD3100 (75 mg/kg vs 15 mg/kg, respectively), indicating that BPRCX807 is much safer than AMD3100. Pharmacokinetic investigations have shown that BPRCX807 has significantly higher maximum concentration (Cmax) and blood exposure (area under the curve [AUC]) compared to AMD3100, demonstrating superior bioavailability and systemic exposure of BPRCX807.[33]

BPRCX807 has demonstrated the ability to hinder the formation of new blood vessels, enhance the infiltration of cytotoxic T cells, reduce the infiltration of tumor-associated macrophages (TAMs), and alter the polarization of TAMs in an orthotopic HCA-1 model. This leads to a transformation of the tumor microenvironment from immune suppression to anti-tumor immune response.

In addition to that, BPRCX807 exhibited exceptional selectivity for CXCR4, completely inhibiting its activity (100% inhibition), while only causing a minimal inhibition (5%) of other chemokine receptors. This indicates that BPRCX807 is a highly specific antagonist of CXCR4 with functional specificity. Ultimately, BPRCX807 has been proven to effectively hinder the movement of HCC cells through the CXCL12/CXCR4 axis, suggesting its potential to reduce metastasis.[34-35]

## STRENGTH AND LIMITATION

The strength of this study is its ability to represent the HIV-1 cases trendline in Indonesia and the dominated strain, as well as the comprehensive comparison of host genetics and CXCR4 antagonists for HIV-1. The limitation of this study is the lack of available research conducted within the area of the developed clinical trials for the CXCR4 antagonists.

## CONCLUSIONS

In conclusion, as HIV-1 cases keeps increasing especially in Indonesia with X4 strain dominating, CXCR4 antagonists that are highly potential for development and to further be utilized as HIV-1 antiretroviral therapy are AMD070 and MCo-CVX-5c.

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**CONFLICT OF INTEREST**

The authors confirm that they have no conflict of interest.

**AUTHOR CONTRIBUTION**

Writer, literature searcher, collecting data from literature: SAFS. Review and supervision: NSN.

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Original Article

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# Antibiotic-Resistant Genes and Polymorphisms of *bla$_{TEM1}$* gene in Multidrug-resistant *Escherichia coli* from Chicken Eggs and Cloacal Swabs in Sleman, Yogyakarta: The Impact on Public Health

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## Abstract

Antimicrobial resistance in pathogenic bacteria is a serious problem in public health. Antibiotic-resistant pathogens are the cause of many deaths. *Escherichia coli* (*E. coli)* is one of the bacteria that experienced multi-drug resistance (MDR). Infection of *Escherichia coli* in humans occurs through transmission of fecal-oral. This study**,** conducted at the Veterinary Public Health Laboratory of Gadjah Mada University, aimed to assess MDR *E. coli* prevalence in 200 chicken egg samples sourced from poultry farms and supermarkets, alongside 63 cloacal swab samples from broiler poultry in Sleman, Yogyakarta. The study focused on detecting resistance genes including *tetA, aadA1, aph(3)IIa, and bla$_{TEM}$1*, also analyzing polymorphisms in the *bla$_{TEM1}$* gene associated with antibiotic resistance. Identification technique of *E. coli* positivity refers to the Indonesian National Standard (SNI) 2897:2008, then *E. coli* identification was performed using the Analytical Profile Index (API) Test 20E Kit. Antibiotic sensitivity was determined by the Kirby Bauer method. Detection of antibiotic resistance genes in *E. coli* were determine using Polymerase Chain Reaction (PCR) method. Sequencing and analysis of polymorphism and phylogenetic were performed only in *bla$_{TEM1.}$* There were 12 samples identified as having *E. coli* (1 from chicken eggs and 11 from cloacal swabs), resistance percentages were highest for erythromycin (100%), ampicillin (91.7%), ciprofloxacin (91.7%), sulfamethoxazole (83.3%), streptomycin (83.3%) gentamicin (75%), tetracycline (41.7%), and chloramphenicol (25%). respectively. All of 12 *E. coli* samples were bacteria with MDR. Resistant genes were prevalent, notably *bla$_{TEM1}$* and *aadA1* (100% each), with *aph(3)IIa* and *tetA* genes also detected in 58.3% of samples each. Sequencing of the *bla$_{TEM1}$* gene revealed polymorphisms in isolate A8. However, these did not alter its antibiotic resistance phenotype. Sequences of *E. coli* isolates showed similarities to strains from Vietnam, China, and India, countries with high antibiotic consumption, particularly ampicillin.

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## INTRODUCTION

Antimicrobial resistance (AMR) in bacteria, especially pathogenic bacteria, is a serious problem in public health, both human and veterinary. Antimicrobial resistance is one of the threats to human health, which can cause death. Mortality caused by infection with AMR pathogens was estimated at 4.95 million deaths worldwide in 2019, with three infectious diseases dominating the cause of death, which are associated with AMR pathogens: thoracic, infection of the lower respiratory tract, bloodstream infections, and intra-abdominal infections. Pathogens with antimicrobial resistance that have caused more than 250,000 deaths include *E. coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. *Escherichia coli* alone is the pathogen that causes many deaths due to AMR[1].

*Escherichia coli* is a normal bacterium found in the digestive tract of humans and animals. However, pathogenic *E. coli* can infect humans and cause gastrointestinal disorders. The transmission route is generally, through contamination of food or beverages consumed by humans (food borne diseases). Therefore, *E. coli* is used as an indicator to indicate contamination and health risks in food[2]. In addition, *E. coli* may be associated with extraintestinal disorders such as urinary tract infections, which is one of the nosocomial bacterial diseases[3].

Serious diarrhea may occur as a result of food-borne pathogens caused by harmful strains of *E. coli*. One of the bacteria that causes food poisoning is pathogenic *E. coli*, which can be found in a wide range of countries, including developing ones like Indonesia. The latest data obtained from Japan shows that 3,000 elementary school students experienced food poisoning, and one of the causes is the contamination of pathogenic *E. coli in* food[4]. A study conducted by Djaja, Puteri, and Wispriyono [5], in the canteen of one of the universities in Jakarta, also showed the presence of *E. coli* contamination in food (42%).

Food contamination by pathogenic *E. coli* may occur during any of the steps in the farm-to-table continuum, coming from a neighbouring environment polluted by *E. coli*. This is certainly a problem that needs to be considered, especially if the pathogen *E. coli* that contaminates is MDR *E. coli*[6]. Previous research showed that there was *E. coli* with MDR that produced extended-spectrum beta-lactamase (ESBL) in beef in Surabaya traditional market, where the $bla_{TEM}$ and $bla_{CTX-M}$ genes are genes encoding ESBL in *E. coli* and are often found in food of animal origin[7]. The other important resistance genes obtained in *E. coli* are *tetA, aadA1, and aph(3)IIa, that are* resistance gene for tetracycline, streptomycin and aminoglycoside respectively. *Escherichia coli* bacteria can be found in animal products due to contamination from animal faeces[8].

*Escherichia coli* from livestock faeces have the potential to contaminate livestock products and the surrounding environment if hygiene in animal husbandry and the management of livestock waste disposal systems are poor. *E. coli* can contaminate livestock products such as meat, chicken eggs, and milk, which the products are the sources of protein that are mostly consumed by the Indonesian people, especially chicken eggs. The consumption of chicken eggs in Indonesia consistently rises annually, with egg consumption reaching 20.02 kg/capita per year in 2022[9].

Some food processing and serving in Indonesian society still needs attention to improve in cleanliness and hygiene. People

sometimes do not wash their hands after managing raw chicken eggs or other animal products, then managing the food being served, is one of the factors of *E. coli* contamination in food. Based on research conducted by Lee *et al*[10], the bacteria found in eggshells are *E. coli* and become a food borne outbreak pathogen that is often found compared to Salmonella or other pathogenic bacteria. These bacteria can come from chicken feces, and then contaminate chicken eggs through the pores of the eggs. This should be a concern especially if these bacteria have developed antibiotic resistance and carry antibiotic-resistant genes that could potentially be transmitted to other pathogens. Therefore, this study aimed is to determine the profile of antibiotic resistance genes, which are *tetA*, *aadA1*, *aph(3)IIa*, and *bla*$_{TEM1}$ in *E. coli* isolated from chicken eggs and cloacal swabs from several farms in Sleman, Yogyakarta which have the potential to be transmitted to humans.

## MATERIALS AND METHODS

### Materials

This study used 200 chicken eggs collected from four layer farms and three supermarkets, along with 63 cloacal swabs obtained from three broiler farms and three-layer farms, as samples.

The materials drug needed in the study are antibiotic disks ampicillin, chloramphenicol, sulfamethoxazole, tetracycline, ciprofloxacin, erythromycin, aminoglycoside antibiotics (streptomycin and gentamicin). As mention in the previous study, streptomycin and gentamicin are aminoglycoside drugs[11].

The other materials needed in this research are *E. coli* isolates, Macfarland 0.5, Brain Heart Infuse (BHI) medium (Thermo Fisher Scientific, USA), Chromocult Coliform Agar (CCA) medium (Sigma-Aldrich, USA), Eosin Methylene Blue Agar (EMBA) medium (Sigma-Aldrich, USA), MacConkey Agar (MCA) medium (Thermo Fisher Scientific, USA), Mueller Hinton agar (MHA) medium (Thermo Fisher Scientific, USA), Nutrient Agar (NA) medium (Thermo Fisher Scientific, USA), NaCl 0.9% (Himedia, USA), aluminum foil, plastic bag, cotton swab, label paper, sterile swab, tissue, Analytical Profile Index (API) Test 20E Kit (Biomerieux, USA, Catalog No. 20100), 70% alcohol (Onemed, Indonesia), absolute alcohol (Sigma-Aldrich, USA), Tris/Borate/EDTA (TBE) buffer (Sigma-Aldrich, USA), QIAamp® DNA Mini Kit (QIAGEN, Germany, Catalog No. 51104), and primers for antibiotic-resistant gene detection, Gotaq® Green Master Mix (Promega Corporation, Hollow Road-Madison, USA, Catalog No. M7112), agarose powder (Sigma-Aldrich, USA), and FluoroVue™ Nucleic Acid Gel Stain (SMOBIO Technology Inc., Taiwan).

The various equipment used are an autoclave, incubator, micropipette, bunsen, petri dish, cover glass, erlenmeyer, Eppendorf tube, beaker glass, object glass, measuring cup, hot plate equipped with a magnetic stirrer, incubator, test tube rack, test tube, scale, centrifuge (DLAB D2012 plus High-Speed Mini Centrifuge), refrigerator (Polytron), Polymerase Chain Reaction (PCR) machine (Swift™ MiniPro® Thermal Cycler), electrophoresis gel tank (Fisherbrand™ Horizontal Electrophoresis System), gel documentation (SMOBIO B-Box Blue Light LED epi-illuminator, AC 100 – 240V, 50/60Hz), scale (Precisa 205 A), vortex (Gemmy VM-300), and Sequencer Machine.

### Methods

#### Identification of *Escherichia coli*

Identification technique of *E. coli* positivity from chicken eggs and cloacal

swabs used the procedure from Indonesian National Standard (SNI) 2897:2008[12]. *Escherichia coli* morphology of the samples were identified on selective media EMBA, MCA, and CCA, followed by biochemical tests using API Test 20E Kit (Biomerieux, USA, Catalog No. 20100) that can for testing β-galactosidase enzyme hydrolysis activity to the substrate o–nitrophenyl–b-D-galactopyranoside (ONP-G), decarboxylation of the amino acid arginine by arginine decarboxylase that also known as arginine dihydrolase (ADH), decarboxylation of the amino acid lysine by lysine decarboxylase (LDC), decarboxylation of the amino acid ornithine (ODC), citrate utilization (CIT), production of hydrogen sulfide ($H_2S$), urease (URE), tryptophan deaminase (TDA), Indole test (IND), Voges-Proskauer test (VP), production of the enzyme gelatinase (GEL), fermentation of glucose (GLU), mannose (MAN), inositol (INO), sorbitol (SOR), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY), and arabinose (ARA), for clarification that the microbials are exactly *E. coli*.

**Antibiotic Susceptibility Test and Multidrug-resistant Classification**

*Escherichia coli* isolates are rejuvenated on BHI medium and then incubated for 24 hours. Afterwards, *Escherichia coli* isolate is suspended by inoculating 10 ml of sterile saline (0.9% NaCl). Then, the bacterial suspension was homogenized by vortex and compared its turbidity to a MacFarland 0.5 standard solution. Antibiotic susceptibility test was performed using the disk diffusion method (Kirby-Bauer)[13] on MHA and incubated at 37 °C for 16 – 18 hours. Antibiotics used were ampicillin (10 µg), chloramphenicol (30 µg), sulfamethoxazole (23.75 µg), tetracycline (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), streptomycin (10 µg), gentamicin (10 µg). The size of the inhibition zone diameter is used to determine antibiotic sensitivity into three results statuses: sensitive, intermediate, and resistance, which varies for each antibiotic according to the Clinical and Laboratory Standards Institute guidelines[14].

Multiple antibiotic resistance (MAR) indication was also performed in this study to assess the risk level of *Escherichia coli* isolates. If the MAR index is greater than 0.2, considered high risk, that means the isolate is multi-drug resistant. The MAR index is determined using the following formula[15].

$$MAR\ Index = \frac{Total\ of\ resistant\ antibiotic\ in\ every\ isolate}{Total\ antibiotics\ tested} \quad (1)$$

**Detection of Antibiotic Resistance Genes in *Escherichia coli***

Bacterial genomic DNA was extracted using the QIAamp® DNA Mini Kit (Catalog No. 51104) as follows: One milliliter of *E. coli* stock was cultured in 5 ml of BHI medium at $37^0$ C overnight. The culture was harvested by transferring 1 ml fresh culture into a 1.5 ml Eppendorf micro centrifuge tube, followed by centrifugation at 7500 rpm for 5 minutes to get the pellet of the bacterial cells. The supernatant was discarded, and this process was repeated for each fresh culture batch. Then, add Buffer ATL (supplied with QIAamp DNA mini kit) a total volume of 180 µl to the bacterial pellet. Add 20 µl proteinase K, mix by vortexing, and incubate at 56°C (10 – 30 min). Vortex occasionally during incubation to disperse the sample, and briefly centrifuge the 1.5 ml microcentrifuge be to remove droplets from the inside of the lid.

Mixture the sample add with 200 µl of Buffer AL for 15 seconds in a pulsing vortex, and then place it in an incubator at 70 °C for 10 minutes. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove

droplets from the lid. Add 200 µl of ethanol (96-100%) to the sample and pulse-vortex for 15 seconds to mix. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove droplets from the lid. Afterwards, gradually pour the mixture onto the QIAamp mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap and centrifuge for 1 minute at 8000 rpm. Place the QIAamp Mini Spin Column into the clean 2 ml collection tube provided and discard the tube containing the filtrate. Carefully open the end of the QIAamp Mini spin column and add 500 µl of Buffer AW1 without wetting the rim. Close the cap and centrifuge for 1 minute at 8000 rpm. After that, transfer the QIAamp mini spin column to a 2 ml collection tube (provided) and eliminate the collection tube that holds the filtrate. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at maximum speed (14,000 rpm) for 3 minutes. Place the QIAamp mini spin column into a new 2 ml collection tube (not included) and discard the old collection tube containing the filtrate. Centrifuge for 1 minute at maximum speed. Place the QIAamp mini spin column into a clean 1.5 mL micro centrifuge tube (not included) and discard the collection tube containing the filtrate. Carefully add 200 µL of Buffer AE to the QIAamp Mini spin column. Incubate for 1 min at room temperature and centrifuge for 1 min at 8000 rpm[16]. Removed QIAamp Mini spin column and the DNA solution in 1.5 ml microcentrifuge tube was kept at minus 20°C.

Polymerase Chain Reaction is conducted using specific primers, to detect antibiotic resistance genes in *Escherichia coli*. There are four types of resistant genes to the drugs tested in this study, i.e to tetracycline (*tetA*), to streptomycin (*aadA1*), to aminoglycoside (*aph(3)IIa*), and to β-lactamase ($bla_{TEM1}$. The primers used were *tetA*[17], *aadA1*[18], *aph(3)11a*[19] and $bla_{TEM1}$[20] (**Table 1)**. Briefly, reactions were performed in a total volume of 30 µL, using Promega Green Gotaq Master-mix (Catalog No. M7112). PCR optimization conditions, denaturation started at 95°C for 5 min followed by the 35 cycles with denaturation (95°C for 1 min), annealing (55°C for 1 min), extension (72°C for 1 min), and after 35 cycles, add an extension (72°C for 2 min). PCR results were visualized using gel electrophoresis and documented with UV light or gel documentation system[19].

**Sequencing and Analysis of Phylogenetic and Polymorphism of $bla_{TEM1}$ gene**

Sequencing analysis was performed only in $bla_{TEM1}$ gene, as understandable that the gene is one of the responsible genes in extended-spectrum beta-lactamase (ESBL) *E.coli*[21]. The writer used the sequencing service from PT. Genetika Science Indonesia for $bla_{TEM1}$ gene sequencing analysis.

Phylogenetic and polymorphism analyses are performed using MEGA X software, where $bla_{TEM1}$ gene sequences are compared with nine control $bla_{TEM1}$ genes obtained from gene banks: Malaysia (NZ PKNA01000132), Singapore (NZ CP102064), Thailand (NZ RKKJ01000503), Vietnam (AP027949), India (MT174046 and KP724850), China (NG 050209. 1), Norway (NG 050185), and Hamburg (NG 050185).

## RESULT AND DISCUSSION

### Result

### Identification of *E. coli*

Based on a pilot study conducted on 200 chicken eggs and 63 cloacal swabs in Sleman, Yogyakarta, 15 isolates (4 samples from chicken eggs and 11 samples from cloacal swabs) were suspected as

**Table 1. Antibiotic-resistant Gene Primers**

| Resistance Gene | Primer | Nucleotide Sequence 5' – 3' | Product size (bp) | Annealing Temperature ($^0$ C) | Reference Source |
|---|---|---|---|---|---|
| *tetA* | F | GGTTCACTCGAACGACGTCA | 577 | 57 | [17] |
| | R | CTGTCCGACAAGTTGCATGA | | | |
| *aadA1* | F | TATCAGAGGTAGTTGGCGTCAT | 793 | 58 | [18] |
| | R | GTTCCATAGCGTTAAGGTTTCATT | | | |
| *aph(3)11a* | F | TCTGAAACATGGCAAAGGTAG | 484 | 55 | [19] |
| | R | AGCCGTTTCTGTAATGAAGGA | | | |
| *bla*$_{TEM-1}$ | F | CATTTCCGTGTCGCCCTTAT | 582 | 55 | [20] |
| | R | TCCATAGTTGCCTGACTCCC | | | |

*Escherichia coli* based on the result of morphological characteristics on selective media (mentioned in method).

The macroscopic characteristics were determined on EMBA media, the colonies that grew were metallic green in colour which identified them as *E. coli*. Furthermore, the isolates were also cultured on CCA media, and the colonies that grew were violet/dark blue, which means they were positive for *E. coli*. Also, MCA was used and the positive *E. coli* colonies formed are in pink colour (Table 2).

**Table 2.** Morphological Identification of *E. coli* on Selective Media

| No | Isolate code | Source | Selective Media | | |
|---|---|---|---|---|---|
| | | | EMBA* | CCA** | MCA*** |
| 1 | KP7 | egg | green metallic | violet | pink |
| 2 | KP3 | egg | green metallic | violet | pink |
| 3 | KP2 | egg | green metallic | dark blue | pink |
| 4 | KP8 | egg | green metallic | dark blue | pink |
| 5 | B6Ka | cloacal swab | green metallic | dark blue | pink |
| 6 | A7 | cloacal swab | green metallic | dark blue | pink |
| 7 | C3 | cloacal swab | green metallic | dark blue | pink |
| 8 | C2 | cloacal swab | green metallic | dark blue | Pink |
| 9 | A1Ka | cloacal swab | green metallic | violet | Pink |
| 10 | A8 | cloacal swab | green metallic | dark blue | Pink |
| 11 | B11 | cloacal swab | green metallic | dark blue | Pink |
| 12 | B10 | cloacal swab | green metallic | dark blue | Pink |
| 13 | C9 | cloacal swab | green metallic | dark blue | Pink |
| 14 | A3 | cloacal swab | green metallic | dark blue | pink |
| 15 | C4 | cloacal swab | green metallic | dark blue | Pink |

*__EMBA test:__ positive *E. coli* = green metallic; negative *E. coli* = brown/pink

**__CCA est:__ positive *E. coli* = violet/dark blue; negative *E. coli* = colorless

***__MCA test:__ positive *E. coli* = pink; negative *E. coli* = colorless

Despite that, after doing API 20E test there were only 12 isolates, which are 1 isolate (KP7) from chicken egg and 11 isolates from cloacal swabs, that were *E. coli* positive. The result of API 20E test was presented in Table 3**.**

**Table 3.** API 20E Test result

| No | Isolate Code | Source | API 20E Test Result |
|---|---|---|---|
| 1 | KP7 | Egg | *Escherichia coli* |
| 2 | KP3 | Egg | *Hafnia alvei* |
| 3 | KP2 | Egg | *Enterobacter cloacae* |
| 4 | KP8 | Egg | *Enterobacter cloacae* |
| 5 | B6Ka | cloacal swab | *Escherichia coli* |
| 6 | A7 | cloacal swab | *Escherichia coli* |
| 7 | C3 | cloacal swab | *Escherichia coli* |
| 8 | C2 | cloacal swab | *Escherichia coli* |
| 9 | A1Ka | cloacal swab | *Escherichia coli* |
| 10 | A8 | cloacal swab | *Escherichia coli* |
| 11 | B11 | cloacal swab | *Escherichia coli* |
| 12 | B10 | cloacal swab | *Escherichia coli* |
| 13 | C9 | cloacal swab | *Escherichia coli* |
| 14 | A3 | cloacal swab | *Escherichia coli* |
| 15 | C4 | cloacal swab | *Escherichia coli* |

**Antibiotic Susceptibility Test and Multidrug-resistant Classification**

Susceptibility analysis of *E. coli* isolates obtained from one chicken egg (KP7) and 11 cloacal swab samples showed the highest resistance to the antibiotics erythromycin (100%), followed by ampicillin and ciprofloxacin (91.7%); then sulfamethoxazole, streptomycin, gentamicin, tetracycline, and chloramphenicol (in percentage order as follows: 83.3%; 83.3%; 75%; 41.7%; and 25% respectively. Multidrug-resistant *E. coli* analysis was performed on isolates acquired from chicken egg and cloacal swabs, and all isolates (12 or 100%) were categorized as MDR *E. coli*. All *E. coli* isolate from egg and cloacal swab samples were categorized as multi-drug-resistant based on the MAR index result, also they showed resistance to three or more classes of antibiotics tested.

Multidrug-resistant *E. coli* in this study were resistant to 5-6 classes of antibiotic tested on average, and there were even three isolates derived from cloacal swab samples (B6Ka, A8, and C4) experiencing resistance to all antibiotics tested. Moreover, the MAR index was also determined to analyze the risk level of *E. coli* isolates, and the MAR index value of 12 *E. coli* in this study was more than 0.2, which means that the all of *E. coli* isolates are at high risk when infecting humans or animals, because they experience multiple resistance to antibiotics (Table. 4).

The intermediate category was also observed in few samples (A7 & KP7). Antibiotics with intermediate status in A7 and KP7 are chloramphenicol and streptomycin respectively. It means that bacteria can be eliminated in body compartments that easily reached by the drug, while with the same dose antibiotic may not effectively treat the bacteria if they are infecting other body organs. Furthermore, in some cases of infectious bacterial infections, higher doses are required for treatment. The intermediate result is considered sensitive in the antibiogram, because in the antibiotic susceptibility test showed a zone of inhibition[22].

**Table 4.** Detail of MDR *E. coli* obtained from Chicken Egg and Cloacal swabs

| Sample code | Resistance to** | | | | | | | | MAR Index | MDR*** |
|---|---|---|---|---|---|---|---|---|---|---|
| | AMP* | C* | SMX | TE* | CIP* | E* | Aminoglycoside drugs | | | |
| | | | | | | | S* | GM* | | |
| | 10 µg | 30 µg | 23.75 µg | 30 µg | 5 µg | 15 µg | 10 µg | 10 µg | | |
| KP7 | R | S | S | S | S | R | I | S | 0.25 | + |
| B6Ka | R | R | R | R | R | R | R | R | 1.00 | + |
| A7 | R | I | R | S | R | R | R | R | 0.88 | + |
| C3 | R | S | R | S | R | R | R | R | 0.75 | + |
| C2 | R | S | R | S | R | R | R | R | 0.75 | + |
| A1Ka | R | S | R | S | R | R | R | R | 0.75 | + |
| A8 | R | R | R | R | R | R | R | R | 1.00 | + |
| B11 | R | S | R | R | R | R | S | S | 0.63 | + |
| B10 | S | S | R | R | R | R | R | S | 0.63 | + |
| C9 | R | S | S | S | R | R | R | R | 0.63 | + |
| A3 | R | S | R | S | R | R | R | R | 0.75 | + |
| C4 | R | R | R | R | R | R | R | R | 1.00 | + |
| **% of R** | **91.7%** | **25%** | **83.3%** | **41.7%** | **91.7%** | **100%** | **83.3%** | **75%** | | **100%** |

*** AMP:** ampicillin, **C:** chloramphenicol, **SMX**: sulfamethoxazole, **TE:** tetracycline, **CIP:** ciprofloxacin, **S:** streptomycin , **E:** erythromycin,
**GM :** gentamicin.
** Antibiotic susceptibility status; **R**: resistance**,** S**:** susceptibility, **I**: intermediate (calculate as sensitive**)**
******* Multidrug-resistance: **negative MDR (-)** if score less than 0.2 ; **positive MDR** (+) if score more than 0.2**.**

**Detection of Antibiotic Resistance Genes in *Escherichia coli***

Detection of resistant genes was also carried out to see the presence of resistant genes supporting antibiotic resistance in *E. coli* isolated from cloacal swabs and chicken eggs (Figure 1). Four types of resistant genes tested in this study, were *tetA*, *aadA1*, *aph(3)IIa*, and $bla_{TEM1}$. The results (Figure 2) performing DNA extraction and PCR with specific resistant gene primers showed that all *E. coli* isolates from both chicken egg and cloacal swabs had *aadA1* and $bla_{TEM1}$ resistant genes (100%), while *tetA* and *aph(3)IIa* are found in 7 isolates (58.3%).

The presence of resistant genes in *E. coli* isolates supports and matches the phenotype profile of antibiotic resistance in these isolates, whereas ampicillin (β-lactamase) and streptomycin are common antibiotics resistant to *E. coli* in this experiment, according to their antibiotic susceptibility test. However, there are differences in the molecular analysis of resistant genes for these two classes of antibiotics. The resistant genes *aadA1* and $bla_{TEM1}$ are found in all *E. coli* isolates (Table 5), but the antibiotic susceptibility results (Table 4) showed two isolates, one is still susceptible to ampicillin (isolate B10) but has the resistant gene to ampicillin and the other one is susceptible to streptomycin (isolate B11) but has the resistant gene to streptomycin. This allows those two *E. coli* isolates to be resistant if ampicillin and streptomycin are overused because resistant genes that support resistance to those two antibiotics are discovered in their genome.[23]

**Table 5.** PCR Result of Resistance Gene in *E. coli*

| No | Gene Resistance | *E. coli* Isolate* | | | | | | | | | | | Percentage of positive gene resistance (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | B10 | B11 | C9 | B6Ka | C4 | A1Ka | C3 | A8 | A3 | A7 | KP7 | C2 | |
| 1 | *tetA* | - | + | - | - | + | - | - | + | + | + | + | + | 58.3 |
| 2 | *aadA1* | + | + | + | + | + | + | + | + | + | + | + | + | 100 |
| 3 | *aph(3)IIa* | + | + | - | + | - | - | - | - | + | + | + | + | 58.3 |
| 4 | $bla_{TEM1}$ | + | + | + | + | + | + | + | + | + | + | + | + | 100 |

***Positivity of gene resistance**: positive (+); negative (-)

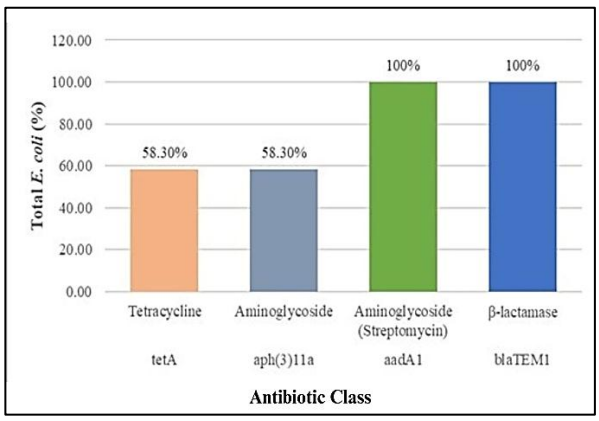


**Figure 1.** Percentage Prevalence of Antibiotic Resistance Genes in *E. coli* from chicken egg and cloacal swabs

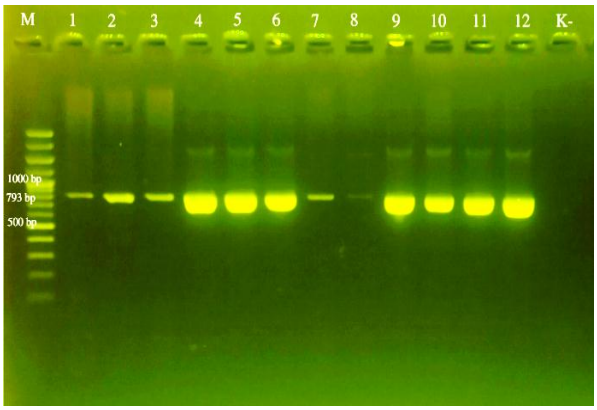


**Figure 2.** Electrophoresis of $bla_{TEM1}$ resistant gene PCR positives results, with a band size is 793 bp. Lane: M : Marker (100 bp); 1: B10; 2: B11; 3: C9; 4: B6Ka; 5: C4; 6: A1Ka; 7: C3; 8: A8; 9: A3; 10: A7; 11: KP7; 12: C2; K- : negative control.

**Sequencing and Analysis of Phylogenetic and Polymorphism of *bla*$_{TEM1}$ Gene**

Sequencing was performed in this study, only on the *bla*$_{TEM1}$-resistant gene. The sequencing results showed genomic profiles in the twelve samples of *E. coli* that were resistant to β-lactamase class antibiotics. After editing and alignment using MEGA X software, then BLAST on the NCBI website https://blast.ncbi.nlm.nih.gov/Blast.cgi), it is known that 100% of *E. coli* in this research are *E. coli* resistant to β-lactamase class antibiotics.

The alignment process was performed to examine the presence of genetic variation in each gene sequence of *Escherichia coli* isolates. Alignment is done by comparing the gene sequences of all isolates with nine *bla*$_{TEM1}$ genes of *E. coli* obtained from gene banks as mentioned in the method.

Construction of phylogenetic trees performed in this study (Figure 3) showed that the twelve *E. coli* obtained had close similarities with *Escherichia coli* from Vietnam, India, China, Norway, and Hamburg (branch length 0.0000 - 0.0038), while *E. coli* from Singapore, Malaysia, and Thailand have distant similarities with the twelve *E. coli* in the study (branch length 1.7954 and 2.1368).

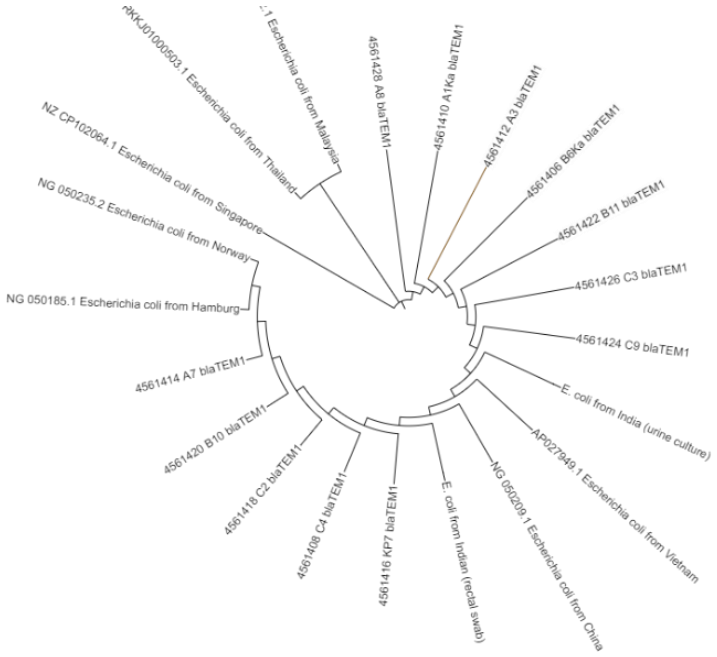


**Figure 3.** Phylogenetic tree of twelve *Escherichia coli* isolates (obtained from chicken egg and cloacal swabs). The phylogenetic tree was reconstructed using the neighbour-joining tree algorithm.

The alignment results show that there is one *E. coli* (A8) has a different nucleotide base from the other isolates. The nucleotide base in isolate A8 has genetic variations in the nucleotide 137$^{th}$ of the sequence. The codon formed in other *E. coli* is AGT (AGU: encodes serine amino acid), while in isolate A8 the codon formed is AAT (AAU), there is a substitution of guanine to adenine, so that the coded amino acid at those sites changes to asparagine. However, the difference in nucleotide bases in the isolates A8 did not affect the beta-lactamase class antibiotic resistance phenotype, which in this study the antibiotic used was ampicillin.

**Discussion**

Antibiotic resistance, especially in pathogenic bacteria, poses a serious threat to public health. *Escherichia coli* is one of the important bacteria that is a concern in human health, as well as veterinary. *Escherichia coli* bacteria are known to have developed resistance to many antibiotics, such as colistin, erythromycin, streptomycin, ciprofloxacin, sulfamethoxazole, and gentamicin[13]. This research was conducted to determine the antibiotic resistance profile and resistant genes in *E. coli* bacteria obtained from chicken eggs and cloacal swabs from broiler farms, as well as chicken eggs obtained from farms and supermarkets in the Sleman area, Yogyakarta, that potentially be transmitted to humans. It is known that *E. coli* when contaminating food, water, or the environment[7] and then infecting humans can cause several diseases such as intestinal (diarrhoea) and extraintestinal (urinary tract infection/bladder infection, sepsis, neonatal meningitis in humans and animals)[24].

The antibiotic susceptibility test profile of the twelve *E. coli* obtained in this study is shown in the result. Erythromycin is the highest antibiotic that has been resistant to *E. coli,* followed by ampicillin and ciprofloxacin.

Antibiotics are commonly used to treat *E. coli* infections in humans including ciprofloxacin (first-line therapy), penicillin (ampicillin), and sulfamethoxazole[25]. Ciprofloxacin is the best antibiotic in the treatment of *E. coli,* but it has experienced resistance[26].

Ciprofloxacin resistance is widely occurring, especially in broiler farms. An experiment by Kiiti *et al*.[27] showed that *E. coli* in broiler and layer chickens have the highest resistance to ampicillin (100%), sulfamethoxazole (89.2%), ciprofloxacin (68.6%), chloramphenicol (53.9%), ceftriaxone (46.6%), ertapenem (30.4%), and gentamicin (10.3%). Antibiotic resistance in *E. coli* is also clinically prevalent. Previous study showed antibiotics that have developed resistance and are primarily used to treat *E. coli* infection are ciprofloxacin[28], cefazolin, amoxicillin, cefuroxime, ceftriaxone, ceftazidime, gentamicin, and sulfonamide[24].

Multidrug-resistant *E. coli* in this study was discovered 100%, from 12 isolates of *E. coli*, only 1 isolate (KP7) has the lowest MAR index (0,25). Even tough, this isolate only resistant to two antibiotics out of 8 antibiotics tested, but its MAR result was more than 0,2 that means this isolate has a high risk as multidrug-resistant bacteria. *Escherichia coli* is one of the bacteria that present multidrug-resistance, this can impact human or animal *E. coli* infection treatment. Previous studies showed that *E. coli* obtained from pig farm and barbeque beef have MDR percentages of 57.3% and 87%[13,29] whereas the percentage of MDR *E. coli* from urine samples of urinary tract infection (UTIs) patients is 97.5%[30].

Antibiotic resistance and MDR that occur in *E. coli* or other bacteria are caused by the factor of unwise or excessive use of antibiotics[23]. Data from the WHO Regional Office for South-East Asia showed that the antibiotic use in South East Asia countries was high, and there was a misused of antibiotic in some countries[31]. The irrational use of antibiotics still occurs in many regions in Indonesia, recent research conducted by Hanifa[4], with the subject of a study was a health facility in the Loa Janan area, East Kalimantan. This result study showed that 27 cases (33.75%) of the antibiotics usage included in the category of irrational

antibiotic administration with details: short duration of consumption (12.50%), using less effective antibiotics (8.75%), and using antibiotics without an indication (12.50%). Another study conducted by Sholih, Muhtadi, and Saidah[32], also showed irrational use of antibiotics in Bandung regional hospitals, with drug utilization (DU) values reaching 90% for penicillin, cephalosporin, quinolone, and macrolide antibiotics.

Based on data obtained by Nguyen *et al*,[33] ampicillin usage, especially in Vietnam, is very high. Based on those data, antibiotics that are often purchased in rural Vietnam are extended-spectrum penicillins (amoxicillin and ampicillin), and first-generation cephalosporins (cefalexin). Similarly, in China and India, consumption of extended-spectrum penicillin antibiotics is also high. The use of extended-spectrum penicillin antibiotics (amoxicillin and ampicillin) in India almost reached 3 billion of antibiotic usage during the period 2000 – 2010[25], while in China extended-spectrum penicillin became the most frequently utilized antibiotic at about 21.21% of total antibiotic consumption[34]. The consumption of ampicillin in Indonesia itself, especially in the human health sector, reached 28.0 defined daily doses (DDD)/100 patient per days[35].

Livestock products such as meat, eggs, and chicken can be a medium for transmitting antibiotic-resistant pathogenic bacteria if these products are contaminated by them[36]. This can occur due to a lack of hygiene during product processing or farm location cleanliness[38]. Bacteria in the farm environment can also experience antibiotic resistance by the presence of resistant genes obtained from genetic transfer, or gene mutations that occur[38]. The resistance genes obtained in this study showed that *E. coli* samples from egg and cloacal swabs carry *tetA, aadA1, aph(3)IIa and* $bla_{TEM1}$ genes , with the two highest (100%) resistance genes are *aadA1* and $bla_{TEM1}$ while the two lower (58.3%) resistance genes are *tetA* and *aph(3)IIa.*

β-lactamase resistant genes (such as $bla_{TEM1}$, $bla_{CTXM}$, $bla_{OXA}$) are widely found in *E. coli*, where these genes can cause it extended-spectrum β-lactamase (ESBL)[39]. The percentage of β-lactamase resistant genes found in previous studies ranged from 42.86% to 98.2%[40]. In the other research arranged by Deku *et al.*[41], isolating *E. coli* from various clinical samples, such as urine, blood, sputum, and vaginal swabs also obtained ESBL genes, which the majority (83.9%) of ESBL *E. coli* had $bla_{TEM1}$ gene.

Resistant genes discovered in *E. coli* isolates in this experiment also support the results of antibiotic susceptibility tests of these isolates. This also shows that one factor contributing to the occurrence of antibiotic-drug-resistant in pathogens is antibiotic-resistant genes. In a study carried out by Aworh *et al.*[42], testing the susceptibility of *E. coli* obtained from faeces samples of farm workers, chicken faeces, farm waste, and water around the farm, the highest resistance occurred to tetracycline, ampicillin, sulfamethoxazole/ trimethoprim, streptomycin, nalidixic acid, and gentamicin respectively. The resistance that occurs is supported by the discovery of resistant genes to the aminoglycoside class of antibiotics (streptomycin and gentamicin), and six types of β-lactamase resistant genes.

β-lactamase resistant genes are an important concern because the presence of these genes in bacteria can lead to the production of ESBL, that result in bacteria resistant to β-lactamase class antibiotics, and need a higher doses of antibiotics to treat infections from ESBL bacteria. The bacteria produce ESBL widely found in hospital facilities, and bacteria that are often produce

ESBLs are *E. coli* and *Klebsiella pneumonia*, that can cause of nosocomial infections.[21]

Resistant genes found in pathogenic bacteria are obtained from a horizontal genetic transfer process from other bacteria found in the surrounding environment[43]. Thus, resistant genes in *E. coli* obtained in this research are likely can be transferred to other pathogenic bacteria also. Antibiotic-resistant genes (ARGs) are transferred across bacteria by horizontal gene transfer, which is mediated by mobile genetic elements (MGE), such as integrons located on transposons and plasmids[44]. Based on the latest research [45,46], the $bla_{TEM1}$ gene is known to be found in the plasmid of *Escherichia coli* bacteria, so it has the potential to be transferred horizontally to other pathogenic bacteria.

*Escherichia coli* carrying antibiotic-resistant genes can pollute the environment, and then the resistant genes can be transferred to other pathogenic bacteria, which can cause problems in public health. This can be happened because *E. coli* acts as a donor and as a recipient of resistance genes[47]. Thus, the detection of antibiotic-resistant genes in bacteria is important in the livestock, agriculture, or aquatic sectors that have the potential to transmit antibiotic-resistant bacteria through food (foodborne disease). The result study of Wintersdorff *et al.*[48] also found that MGE can spread to the environment. This result study is expected to be a reference for policymakers to give more attention to the use of antibiotics in every sector, which can increase antibiotic-resistant bacteria.

The β-lactamase TEM1 gene ($bla_{TEM1}$) sequenced in this study was carried out to ascertain the genomic profile of the isolated *E. coli* and the potential for gene changes in the sequence of DNA pair bases. The BLAST process on the NCBI website was carried out on the twelve samples, it was found that all *E. coli* isolates had close similarity with *E. coli* strains from Vietnam, India, China, Norway, and Hamburg in Gene-Bank.

Phylogenetic analysis was conducted to examine the relatedness of the twelve *E. coli* samples, which were aligned with nine *E. coli* $bla_{TEM1}$ controls, e.g. *E. coli* from Malaysia, Singapore, Vietnam, Thailand, China, India, Norway, and Hamburg. Neighbour-joining tree reconstruction was used in the phylogenetic analysis. The twelve *E. coli* samples in this study have very close similarities with *E. coli* $bla_{TEM1}$ strains from Vietnam, India, and China. These three countries are the countries with the three highest sales and consumption of FDC (fixed dose combination) antibiotics, and ampicillin β is the most widely sold antibiotic without a license from the FDA (Food and Drug Administration) when compared to Indonesia which is ranked 30$^{th}$.[49] Moreover, Vietnam, China, and India are countries that use most antibiotics in food-producing animals, including Indonesia.[50]

There was a difference in the nucleotide sequence of A8 isolate compared with the other 11 isolates. The substitution of nitrogen base occurred in the 137$^{th}$ nucleotide of the sequence of A8 isolate causing a different amino acid in its sequence, so the genetic code serine amino acid change to be genetic code for asparagine amino acid, while the other isolates have serine as their amino acid. Changes in amino acids of isolate A8 did not affect the results of β-lactamase class antibiotic resistance phenotypes tested (ampicillin), where isolate A8 is still resistant to ampicillin antibiotics with no inhibition zone formed. Serine amino acid in the $bla_{TEM1}$ gene is one of the amino acid that support β-lactam antibiotic degradation by producing serine-β-

lactamases (SBLs) enzyme. SBLs utilize a catalytic serine residue to initiate a nucleophilic attack on the β-lactam carbonyl, similar to the mechanism of serine-dependent protease.[51]

However, asparagine (Asn) has a similar function as serine, which can hydrolyze β-lactam antibiotics, also affect catalytic mechanism of β-lactam antibiotics[52]. Asparagine is critical for penicillin and cephalosporin hydrolysis, which can lead to resistance against these antibiotics[53]. Thus, the polymorphism of amino acid serine to asparagine does not affect the β-lactam antibiotic resistance phenotype of *E. coli* isolates.

Human mobilization can also affect the similarity of *E. coli* strains in Indonesia with other countries, especially with China and Vietnam, both are countries that have high mobilization to Indonesia or vice versa. Although human mobility from Indonesia to Malaysia, Singapore, and Thailand is also high, *E. coli* $bla_{TEM1}$ strains from the three countries have a distant similarity with *E. coli* in Indonesia. This happens because the consumption of ampicillin antibiotics in public from those three countries is lower than in Indonesia.[49]

## STRENGTH AND LIMITATION

The strength of the study obtained gave molecular information about resistant genes in *E. coli* collected from egg and swab cloacal of broiler chicken in Yogyakarta, Indonesia, and polymorphism of $bla_{TEM1}$ gene. The limitation of the study was sample collection only done in supermarkets and farms and only from livestock. Samples collection needs to be expanded in human and environmental samples.

## CONCLUSIONS

This study obtained 12 *Escherichia coli* isolates that are resistant to antibiotics tested with the highest percentage are erythromycin (100%), follow by ciprofloxacin (91.7%), ampicillin (91.7%), sulfamethoxazole (83.3%), streptomycin (83.3%), gentamicin (75%), tetracycline (41.7%), and chloramphenicol (25%). Resistant genes were also found in the samples obtained, with the highest percentage of *aadA1* and $bla_{TEM1}$ genes (100% each), followed by *tetA* and *aph(3)IIa* genes (58.3% each). It is important to pay attention to this result study because a the resistant gene $bla_{TEM1}$ that causes ESBL *E. coli* which has an important influence on human health, was found in 100% *E.coli* in this study and all of 12 *E. coli* samples were bacteria with MDR. One of the *E. coli* samples has a polymorphism in its gene, causing changes in the amino acid sequences, which is serine change with asparagine. The polymorphism did not affect the phenotype of *E. coli* resistance to beta-lactamase class antibiotics tested (ampicillin). This is because both amino acids have the same function, which can hydrolyze beta-lactamase antibiotics. Phylogenetic analysis showed that all the *E. coli* samples have close similarity with *E. coli* strains from China, Vietnam, and India. This happened because it was influenced by several factors, including high human mobility from the three countries to Indonesia. In addition, these three countries are known to have the highest consumption of ampicillin antibiotics (beta-lactam class) in the human or animal health sector. Detection of resistant genes and analysis of gene sequences in pathogens is crucial, to provide information on AMR data, which in Indonesia itself is still very limited. Therefore, this study is expected to provide

information on antibiotic-resistant genes in bacteria that could potentially become human pathogens from other sectors outside the health sector, which will help paramedics recognize the possibility of AMR transmission from the environment to the human health sector.

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**CONFLICT OF INTEREST**

The authors emphasize that they have no conflict of interest.

**AUTHOR CONTRIBUTION**

Experimental design: NIN. Materials preparation: NIN. Research implementation: NIN. Research supervision: WA, KP. Manuscript writing: NIN. Manuscript editing: NIN.

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Scientific Journal of Tropical and Infectious Disease

# Description of Mothers' Knowledge, Attitudes, and Behavior Regarding Deworming The Children Against Soil-Transmitted Helminthiasis at The Lampaseh Health Center in Banda Aceh City

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## Abstract

Helminth infections are a significant public health problem in developing countries, including Indonesia, where the prevalence ranges from 60% to 90%. One common helminth infection is Soil-Transmitted Helminthiasis (STH), which particularly affects children. One of risk factors is children who lack personal hygiene, especially when they are playing with soil. The increased incidence of STH can be prevented by deworming programs, and the role of parents, especially maternal parenting. This study aims to determine the description of the level of knowledge, attitudes and behavior of mothers on deworming the children against STH at the Lampaseh Health Center, Banda Aceh City, Indonesia. This research is descriptive with a cross-sectional design. The sample consists of mothers with children aged 2-12 years, selected using accidental sampling techniques. Data were collected through interviews using structured questionnaires. The results showed that the level of maternal knowledge of deworming the children against STH categorized as good (95.1%), quite good (3.9%), and less good (1.0%) as well as the attitude of mothers was categorized into good (51.5%), quite good (46.6%), and less good (1.9%). However, the mother's behavior was good (69.9%) and less good (30.1%). The conclusion was that the knowledge, attitudes, and behavior of mothers on deworming the children against STH need to be maintained and improved.

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## INTRODUCTION

Worm infection in human is a disease caused by parasite which can be transmitted to human through the soil.[1] A diagnosis of worm infection is confirmed when finding worm eggs in a stool examination.[2] Helminth infections are a significant public health issue in developing countries. This is due to the fact that urbanization and socioeconomic factors in developed countries consistently help in controlling the spread of helminth infections.[3] One common type of worm infection is intestinal worm infection, which involves soil media in its spread and is known as Soil Transmitted Helminth (STH).[4] The types of STH include roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*).[5]

In general, the incidence of worm infection in Indonesia is still quite high, especially among people who have economic limitations and low levels of sanitation. The prevalence rate of intestinal worm infections ranges from 2.5% to 62%.[3] According to the information from Riskesdas in 2013, the prevalence rate of worm infection in children is 22.6%.[6] The most affected age groups are those between 5-14 years old, with 21% of cases.[7] Aceh Province has the second-highest rate of worm infections in Indonesia, with a prevalence of around 59.2%.[8]

School-age children are susceptible to various infections. This situation arises because children often play on the ground, interact with their friends, share games, hug each other, and involve themselves in various social activities during their development.[9] Children who affected by worm infections and when the symptoms are undetected can cause various health problems. Although helminth infections rarely cause death, they can affect the health and productivity of patients through decreased nutritional status.[10] Long-term effects of infection typically manifest as malnourishment, impaired growth and development and cognitive impairment in children, as the parasites absorb essential nutrients needed during the growth phase.[11] The high prevalence of worm infection in Indonesia is due to the tropical climate that favors the development of endemic diseases, including intestinal worms, and various risk factors such as age, socio-economic environment, behavior, and cultural practices.[12,13] Common transmission routes among children include going barefoot, direct contact of feet with the ground, and neglecting hand hygiene, hence it is necessary to examine and care for them.[14]

To combat this issue, Indonesia has implemented several initiatives aimed at preventing helminth infections. These include campaigns promoting healthy lifestyles and good sanitation, mass deworming treatments, and parental education.[6] One of Indonesia's initiatives in preventing worm infection in children is through the Mass Preventive Drug Administration Program (POPM). Deworming aims to treat, eradicate, and prevent infestations by worms that can inhibit child growth and prevent further infections.[15] The drug used in the implementation of mass deworming programs is a single dose of Albendazole or Mebendazole, which is available as chewable tablets and syrup. Simultaneously, deworming the children under five every two years along with the program of the distribution of vitamin A. Likewise elementary school children are in receive both service twice a year, in February and August at their schools.[16] In addition, the care of the child depends on the parenting style provided by the mother.

Therefore, less positive knowledge, attitudes, and behaviors of mothers can have a negative impact on parenting, especially in an effort to prevent intestinal worms infection.[5]

The working areas of Lampaseh Health Center (Puskesmas Lampaseh) of Banda Aceh City in Aceh Province, Indonesia covers six villages, namely Lampaseh Kota, Merduati, Keudah, Peulanggahan, Gampong Jawa, and Gampong Pande.[17] In the working area of Lampaseh Health Center, especially in Gampong Java, there is a garbage dump land, which is often used by children for playing. The age period of 2-12 years is a period when children tend to enjoy playing, especially on the ground, and the potential for STH infection is closely related to the sanitary conditions of the environment. Therefore, children living around the region are at risk of getting STH infection.

The distribution of deworming at Lampaseh Health Center to primary and preschool age children has reached a satisfactory level, which is 91.9%.[18] Therefore, the description of mothers' knowledge, attitudes, and behavior on deworming the children against STH in the operational areas of Lampaseh Health Center, Banda Aceh City, is needed to be reported which can be used as a reference for mothers in other areas in assisting government programs for the treatment and prevention of STH infections in Indonesia.

## MATERIALS AND METHODS

This research is a descriptive approach by applying a cross-sectional research design. The focus of this study was on collecting variable data at one point in time by only once observations and analysis. Measurements were made against certain characteristics or variables at the time of examination. The purpose of this study was to collect data on the description of mothers' knowledge, attitude, and behaviors on deworming the children against STH at the operational areas of Lampesh Health Center. The Subjects of this study involved mothers who had children aged 2-12 years who lived within operational areas or visited the Lampaseh Health Center and met the established inclusion criteria. The sampling method used was incidental sampling. The research was carried out in the operational area of the Lampaseh Health Center in Banda Aceh City. The survey was carried out from October to November 2023, involving a total of 103 respondents. In this study, the measurement scales for knowledge and behavior were assessed using the Guttman scale, where respondents received a score of 1 for correct answers and 0 for incorrect answers. Meanwhile, the attitude measurement scale used the Likert scale, where respondents received a score of 5 for strongly agreeing and a score of 1 for strongly disagreeing. The collected data were then summed and classified into three categories: less good (<56%), quite good (56-75%), and good (76-100%).[19] The income variable was subsequently divided into two categories: high income for those earning more than Rp. 750,000 and low income for those earning less than Rp. 750,000.[20] The data was analyzed using an univariate approach, especially descriptive analysis.

The formula used to calculate the percentage of data is as follows:

$$P = \frac{F_1}{n} \times 100\%$$

where $P$ is percentage; $f1$ is the observed frequency; n is number of respondents.

## RESULTS AND DISCUSSION

### Characteristics of Respondents

The general characteristics of respondents are shown in Table 1 below.

**Table 1. Characteristics of Respondents**

| Category | Frequency (n=103) | Percentage (%) |
|---|---|---|
| **Mother's Age** | | |
| 24-30 | 34 | 33 |
| 31-40 | 60 | 58.3 |
| 41-48 | 9 | 8.7 |
| **Education** | | |
| Junior high school | 1 | 1 |
| Senior high school | 63 | 61.2 |
| Undergraduate | 39 | 37.9 |
| **Working Status** | | |
| Working | 19 | 18.4 |
| Not Working | 84 | 81.6 |
| **Income** | | |
| High | 22 | 21.4 |
| Low | 81 | 78.6 |

Based on the data in Table 1, 60 mothers (58.3%) are in the age group of 31 to 40 years old. The majority of respondents achieved the high level of education in their educational journey (61.2%). Meanwhile, the majority of respondents, consisting of 84 mothers (81.6%), stated that they were unemployed. Of the total respondents, 81 mothers (78.6%) have a relatively low income level.

Research findings related to maternal age characteristics indicated that most mothers are in the age group of 31-40 years (Table 2). Age is one of the determining factors in determining a person's health behavior. Individuals who follow a conventional lifestyle may be considered to have an edge in experience, knowledge, skills, and decision-making abilities as they age. The results of the study on the education level of respondents showed that the majority, as many as 63 people, had high school education. This reflects that most participants have a relatively high level of education. A person's level of education can have an impact on his ability to understand information, and the achievement of that level of education has a direct correlation with the quality of education received.[21] In addition, parents who have a high level of education and awareness of hygiene and health practices tend to provide better education and health attention to their children than children who have parents with lower levels of education.[22] Based on characteristic of occupation showed that the majority of mothers, 84 respondents, were unemployed. Type of work has a significant correlation with the incidence of helminthiasis because work has a close relationship with family income, and family conditions have a major impact on the level of hygiene and sanitation in the home environment.[23] The characteristics of income showed that 81 respondents have a relatively low income. The socioeconomic characteristics of a person have a major impact on the prevalence of helminthiasis, and the socioeconomic factors have an effect on a person's well-being, involving financial resources, housing conditions, access to nutritious food, and adherence to hygiene standards.[24]

Maternal knowledge related to the characteristics of respondents on deworming the children against STH showed that 57 respondents (95%) had good knowledge in the age range of 31-40 years, 60 respondents (95.2%) had good knowledge with the last level of high school education, 81 respondents (96.4%) had good knowledge and did not work, and 78 respondents (96.3%) had good knowledge with a low-income level.

**Table 2. Distribution of Knowledge Frequency Based on Respondent Characteristics**

| Category | Knowledge | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Good | | Quite good | | Less good | | | |
| | n | % | n | % | n | % | n | % |
| **Mother's Age** | | | | | | | | |
| 24-30 | 32 | 94.1 | 2 | 5.9 | 0 | 0 | 34 | 100 |
| 31-40 | 57 | 95 | 2 | 3.3 | 1 | 1.7 | 60 | 100 |
| 41-48 | 9 | 100 | 0 | 0 | 0 | 0 | 9 | 100 |
| **Education** | | | | | | | | |
| Junior high school | 1 | 100 | 0 | 0 | 0 | 0 | 1 | 100 |
| Senior high school | 60 | 95.2 | 2 | 3.2 | 1 | 1.6 | 63 | 100 |
| Undergraduate | 37 | 94,9 | 2 | 5.1 | 0 | 0 | 39 | 100 |
| **Working Status** | | | | | | | | |
| Working | 17 | 89.5 | 2 | 10.5 | 0 | 0 | 19 | 100 |
| Not Working | 81 | 96.4 | 2 | 2.4 | 1 | 1.2 | 84 | 100 |
| **Income** | | | | | | | | |
| High | 20 | 90.9 | 2 | 9.1 | 0 | 0 | 22 | 100 |
| Low | 78 | 96.3 | 2 | 2.5 | 1 | 1.2 | 81 | 100 |

## Knowledge Frequency Distribution

Table 3 shows that the distribution of respondents' level of knowledge regarding deworming the children showed that 98 out of 103 participants (95.1%), answered questionnaire that reflected a high level of knowledge. Based on the results of questionnaires filled out by respondents regarding knowledge on deworming the children against STH are categorized as good.[19] Knowledge that is considered good in mothers is assumed to come from several factors that affect the level of knowledge. Factors that can affect a mother's knowledge include education level, occupation, experience, income, age, and the amount of information she has. The existence of higher education in a person can affect the way the individual receives information, which in turn can increase their insight and understanding of a disease.[25] The higher a person's level of education, the easier it is for them to access and receive information, which in turn increases the amount of knowledge he has.[26] A person's knowledge is influenced by age, which plays a role in influencing the individual's comprehension ability and thinking patterns. Increasing age causes an increase in the ability to capture information and develop a more complex mindset, supported by a growing body of experience. Therefore, the productive age category, which is the stage of active adulthood, tends to have a high level of recall of information.[27] Thus, parents who have a high level of education, extensive experience, and older age tend to impart more abundant and measurable knowledge than parents who are younger and have a low level of education. [28]

**Table 3. Level of Knowledge Frequency Distribution**

| Knowledge | Frequency (n) | Percentage (%) |
|---|---|---|
| Good | 98 | 95.1 |
| Quite good | 4 | 3.9 |
| Less good | 1 | 1.0 |
| **Total** | 103 | 100.0 |

In addition, employment and income also affect a person's knowledge.[29] The type and context of work can play a role in influencing the incidence of helminthiasis, and having good knowledge can help lower the incidence rate of the disease. Work also has a close relationship with family income level, which is an important

**Table 4. Distribution of attitude frequency based on respondent characteristics**

| Category | Attitude | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|
| | Good | | Quite good | | Less good | | | |
| | n | % | n | % | n | % | n | % |
| **Mother's Age** | | | | | | | | |
| 24-30 | 17 | 50 | 16 | 47.1 | 1 | 2.9 | 34 | 100 |
| 31-40 | 33 | 55 | 26 | 43.3 | 1 | 1.7 | 60 | 100 |
| 41-48 | 3 | 33.3 | 6 | 66.7 | 0 | 0 | 9 | 100 |
| **Education** | | | | | | | | |
| Junior high school | 1 | 100 | 0 | 0 | 0 | 0 | 1 | 100 |
| Senior high school | 31 | 49.2 | 30 | 47.6 | 2 | 3.2 | 63 | 100 |
| Undergraduate | 21 | 53.8 | 18 | 46.2 | 0 | 0 | 39 | 100 |
| **Working Status** | | | | | | | | |
| Working | 10 | 52.6 | 9 | 47.4 | 0 | 0 | 19 | 100 |
| Not Working | 43 | 51.2 | 39 | 46.4 | 2 | 2.4 | 84 | 100 |
| **Income** | | | | | | | | |
| High | 10 | 45.5 | 12 | 54.5 | 0 | 0 | 22 | 100 |
| Low | 43 | 53.1 | 36 | 44.4 | 2 | 2.5 | 81 | 100 |

factor in forming a healthy lifestyle in the family environment. A nutritious diet and personal hygiene can be preventive measures against diseases, including intestinal worm infections.

Based on the age of mothers who categorized into the productive age, the knowledge of mothers at the Lampaseh Health Center are relatively obtained from high level of maternal education, such as a high school, and from various sources of information, including health workers at Public Health Center, Integrated Healthcare Center, and educational institutions such as elementary schools. Good knowledge in a mother is expected to be able to reduce the incidence of worm infection in her child and encourage her to be more concerned in deworming. This knowledge involves an understanding of healthy and clean-living behaviors as well as in-depth knowledge of deworming and drug administration.

## Attitude frequency distribution

Table 4 shows that the distribution of the mother's attitude on deworming the children against STH children is that 33 respondents (55%) aged 31–40 years have a good attitude; 31 respondents (49.2%) who were high school-educated had good attitudes; 43 respondents (51.2%) who did not work had a good attitude; and 43 respondents (53.1%) who had low incomes had good attitudes.

**Table 5. Distribution of Attitude Frequency**

| Attitude | Frequency (n) | Percentage (%) |
|---|---|---|
| Good | 53 | 51.5 |
| Quite good | 48 | 46.6 |
| Less good | 2 | 1.9 |
| **Total** | 103 | 100.0 |

Table 5 presents the distribution of the frequency of maternal attitudes on deworming the children against STH. The data revealed that out of 103 respondents, as many as 53 people (51.5%) gave positive responses to the questionnaire that showed a good attitude. A competent mother's attitude is believed to be formed by personal experiences, cultural factors, and the influence of important individuals, such as health professionals and mass media, including health worker brochures and billboards. The experience that respondents have is closely related to the knowledge they gain because later this experience will have a direct effect on what can be realized. Educational institutions are also related to the respondents' personal experiences. Through educational institutions, respondents can find out about worms and how to prevent them, so that respondents' can lead to good behavior that can prevent worms from occurring.[30] But there are still those who have a pretty good and less good attitude. This is because respondents lack awareness or sensitivity to moral principles, despite having good knowledge. Acquiring extensive knowledge can enhance an individual's understanding, while cultivating a positive attitude includes both emotional and social factors.[30] A mother's attitude refers to her perspective and beliefs about the purpose and benefits of deworming the children. The attitude of the mother also plays an important role in the prevention of intestinal worms in children. Attitudes are influenced by knowledge, therefore assessing attitudes needs to consider respondents' knowledge. Therefore, having the power of knowledge is closely related to a positive attitude.[31]

## Frequency Distribution of Behavior

Table 6 shows the distribution of maternal behavior on deworming the children against STH based on the characteristics of respondents. A total of 39 respondents (65%) showed good behavior in the age range of 31-40 years; 41 respondents (65.1%) behaved well with their level of high school education; 57 respondents (67.9%) showed good behavior despite not working; and 54 respondents (66.7%) behaved well despite having low incomes.

**Table 6. Distribution of Frequency of Behavior Based on Respondent Characteristics**

| Category | Behaviour | | | | Total | |
|---|---|---|---|---|---|---|
| | Good | | Less good | | Total | |
| | n | % | n | % | n | % |
| **Mother's Age** | | | | | | |
| 24-30 | 26 | 76.5 | 8 | 23.5 | 34 | 100 |
| 31-40 | 39 | 65 | 21 | 35 | 60 | 100 |
| 41-48 | 7 | 77.8 | 2 | 22.2 | 9 | 100 |
| **Education** | | | | | | |
| Junior high school | 1 | 100 | 0 | 0 | 1 | 100 |
| Senior high school | 41 | 65.1 | 22 | 34.9 | 63 | 100 |
| Undergraduate | 30 | 76.9 | 9 | 23.1 | 39 | 100 |
| **Working Status** | | | | | | |
| Working | 15 | 78.9 | 4 | 21.1 | 19 | 100 |
| Not Working | 57 | 67.9 | 27 | 32.1 | 84 | 100 |
| **Income** | | | | | | |
| High | 18 | 81.8 | 3 | 18.2 | 22 | 100 |
| Low | 54 | 66.7 | 27 | 33.3 | 81 | 100 |

**Table 7. Distribution of Behavioral Frequencies**

| Behaviour | Frequency (n) | Percentage (%) |
|---|---|---|
| Good | 72 | 69.9 |
| Less good | 31 | 30,1 |
| **Total** | 103 | 100,0 |

Table 7 provides an overview of the frequency distribution of maternal behavior related to the deworming the children. The data showed that 72 out of 103 respondents (69.9%) answered the questionnaire with a good level of maternal behavior. Maternal behavior plays an important role in a child's life in maintaining health. Mothers who are not used to deworming their children will have a higher risk of experiencing worms compared to mothers who are used to deworming their children.[32] Effective maternal behavior is expected to be achieved through good understanding, adequate information support, availability of resources, and implementation of worm eradication programs launched by the government.

Of the total number of respondents, 31 people showed poor behavior. In detail, 24 respondents did not deworming periodically every 6 months as a preventive measure, while 4 respondents did not deworming at all. In addition, 4 respondents did not carry out deworming, either at the recommended time or as a preventive measure. This is due to the mother's dependence on deworming from the staff during counseling and not deworming the child. They only rely on clean and healthy living practices and only seek medical help at the Health Center when symptoms become severe.

## STRENGTH AND LIMITATION

This study serves as an additional reference source and basis for further investigations in the field of public health, especially regarding efforts to eradicate worm infections in the age group of children. A weakness to note in this study is the inability of researchers to directly observe the practices carried out by respondents. As a result, the data collected can only depend on the perceptions reported by respondents through questionnaires. The use of different measurement scales, particularly the Guttman scale and Likert scale, creates obstacles in comparing variables, potentially leading to inaccuracies in responses and lack of clarity in interpreting the overall score of the questionnaire.

## CONCLUSIONS

Mothers' knowledge on deworming the children against STH at Lampaseh Health Center is mostly at a good level (95.1%). Mothers' attitudes on deworming the children against STH at Lampaseh Health Center were mostly in the positive category (51.5%) showing very good attitudes. Meanwhile, maternal behavior on deworming the children against STH at Lampaseh Health Center are categorized as good behavior (69.9%). However, health promotion regarding STH is still needed so that mothers are aware of this disease.

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## ETHICAL CLEARANCE

This research was carried out after obtaining approval from the Health Research Ethics Committee of the Faculty of Medicine, Syiah Kuala University with ethical approval number 154/EA/FK/2023. As a follow-up to the ethical approval, researchers maintain the confidentiality of data by not including the full name of the research subject. All subject data are used only once, for the purposes of this research alone and not used for other purposes.

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This study did not receive any funding.

## CONFLICT OF INTEREST

We affirm that there are no conflicts of interest between authors in this study

## AUTHOR CONTRIBUTION

SU and TRM designed the study. RY was responsible for data collection, analysis, and interpretation. RY also wrote the original manuscript. TM, NM, and TAZ contributed to proofreading the manuscript. All authors read and approved the final version of the manuscript.

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# Red Laser-Activated Silver Nanoparticles from Green Synthesis Extract of Butterfly Pea for Antimicrobial Photodynamic Therapy Against *Staphylococcus aureus*

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## Abstract

This study investigated the potential of photodynamic therapy (PDT) using green-synthesized silver nanoparticles (AgNPs) derived from butterfly pea extract (*Clitoria ternatea L.*) to combat *Staphylococcus aureus* (*S. aureus*). The use of a red diode laser as a method for enhancing the antimicrobial activity of AgNPs presents a novel approach to treating bacterial infections. The red diode laser is crucial, as it activates the AgNPs, enhancing their antimicrobial properties. This combination of light, natural extract, and nanoparticles underscores the innovative approach of using PDT in treating bacterial infections. By integrating these elements, the study aims to provide insights into effective, biocompatible treatments for antibiotic-resistant bacteria. The primary objective of this study is to synthesize and characterize AgNPs using butterfly pea extract and evaluate their effectiveness against *S. aureus* when combined with red laser irradiation. Silver nanoparticles were synthesized using an environmentally friendly method that processes butterfly pea extract as the reducing agent for the synthesis of the nanoparticles. Using UV-Vis spectrophotometry to track the creation of silver nanoparticles (AgNPs), it was determined that the butterfly pea extract was an effective source of nanoparticles. The particle size distribution and peak absorbance wavelength were determined by characterization utilizing a Particle Size Analyzer (PSA). Tryptic soy agar (TSA) plates were used to investigate the antibacterial activity of AgNPs against *Staphylococcus aureus (S. aureus)*. The effectiveness of photoinactivation against S. aureus was evaluated by exposing AgNPs at a concentration of 1 mM to a red diode laser for 90 seconds. The results showed that the produced AgNPs had potential antibacterial capabilities when combined with red light therapy. The results demonstrated that the synthesized silver nanoparticles can effectively kill or inhibit the growth of *Staphylococcus aureus (S. aureus)* when exposed to a red diode laser for 90 seconds. The findings suggest that photodynamic therapy using green-synthesized AgNPs and red laser irradiation could be a promising approach to controlling bacterial infections like *S. aureus*. Further research is recommended to explore the underlying mechanisms of photoinactivation and to optimize treatment parameters for in vivo applications on experimental animals.

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## INTRODUCTION

*Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium responsible for many clinical diseases. Infections caused by this pathogen are prevalent in both community and hospital settings. Treatment remains challenging due to the emergence of multidrug-resistant strains, such as Methicillin-Resistant *Staphylococcus aureus* (M.R.S.A.). According to WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) 2020 data, 40% of *S. aureus* strains are resistant to MRSA.[1]

One promising approach is the development of nanotechnology-based antimicrobial therapies, particularly the green synthesis of nanoparticles. Nanotechnology involves the production of materials at the nanoscale, typically ranging from 1 to 100 nm.[2] Nanoparticles can be produced from gold, silver, platinum, and palladium materials. Silver nanoparticles (AgNPs) are especially favoured due to their ease of production, unique properties, and strong affinity for binding to various biomolecules, making them effective in eradicating bacteria while being non-toxic to humans, animals, and plants.[3]

The benefits of nanoparticles include increased reactivity and focused medication administration with fewer adverse effects because of their tiny size and large surface area. Technology and medicine are progressing because of their special qualities, which are useful in imaging, diagnostics, and environmental applications. Silver nanoparticles are synthesized by introducing reducing agents through physical, chemical, biological, and green synthesis methods. Green synthesis is a method using organisms such as algae, plants, bacteria, fungi, and their metabolities. The method of synthesis with plant extracts is effective in preventing oxidation and aggregation of synthesized AgNPs while reducing cost and chemical usage.[4] The success of silver nanoparticle formation depends on the presence of many phytochemicals of plant extract which is fully responsible for AgNPs synthesis.[5] A green synthesis approach is employed to mitigate the dangers associated with these reductants,[6] utilizing natural materials such as plant extracts derived from roots, stems, leaves, seeds, fruits, and flowers.[7]

One of the plant extracts with functional benefits for the human body is the butterfly pea (*Clitoria ternatea L.*). The butterfly pea (*Clitoria ternatea L.*), which has antibacterial, antidiabetic, anti-obesity, anticancer, and anti-inflammatory qualities, is one of the plant extracts with practical uses. When these advantages are delivered as nanoparticles, their bioavailability and therapeutic efficacy increase. All parts of the butterfly pea plant are claimed to offer various health benefits. For instance, the petals are known for their antioxidant, antidiabetic, anti-obesity, anticancer, anti-inflammatory, and antibiotic properties.[8] Butterfly pea also exhibits pharmacological potential as an antimicrobial, antidepressant, anthelmintic, anticancer, and antidiabetic agent, with anthocyanin pigments that are water-soluble and produce colors ranging from red to blue.[9]

Using natural extracts as reducing and stabilizing agents in the synthesis of AgNPs is environmentally friendly and enhances the biocompatibility of the nanoparticles produced. Photodynamic therapy (PDT) is a promising alternative that combines light of specific wavelengths with photosensitizers and oxygen to generate reactive oxygen species (ROS) capable of killing microorganisms. In this context, silver nanoparticles (AgNPs) have shown significant potential as photosensitizers due

to their antimicrobial properties and ability to produce ROS when light activates them. The red laser-activated synthesis of butterfly pea (*Clitoria ternatea L.*) extract could be a novel approach to enhancing the effectiveness of photodynamic antimicrobial therapy.[10] *S. aureus* is efficiently eliminated by red laser-activated PDT because of its deep tissue penetration and enhanced generation of ROS. Strong photosensitizer properties of the butterfly pea extract improve antibacterial activity, especially against resistant bacteria like MRSA.[11]

This study aims to determine the antimicrobial effectiveness of AgNPs derived from butterfly pea extract (*Clitoria ternatea L.*) through the photodynamic therapy of bacteria by combining red laser light. This combination is expected to produce reactive oxygen species that cause biological damage to the target *S. aureus*. This study's long-term benefit is providing valuable knowledge to stakeholders about using silver nanoparticles from butterfly pea extract as photosensitizers in photodynamic therapy to reduce *S. aureus* bacteria.

## MATERIALS AND METHODS

This research was conducted at the Biophysics Laboratory of Universitas Airlangga from March to May 2024. The materials used in this experiment included butterfly pea (*Clitoria ternatea L.*) extract, distilled water, liquid $AgNO_3$ (at concentrations of 1 mM, 1.5 mM, 2 mM, and 3 mM), tryptic soy agar (TSA), tryptic soy broth (TSB), *S. aureus* bacteria, and physiological saline (distilled water and pure NaCl solution).

The important materials and tools utilized in this experiment are clearly listed, and each item is associated with a trademark which included CNC Red-Ozone Diode Laser, Total Plate Counter, Biobase dynamic light scattering nanometer particle size analyzer, and UV-Vis spectrophotometer.

CNC Red-Ozone Diode Laser, which is essential for causing silver nanoparticles to activate. This laser's emission produces reactive oxygen species (ROS) at certain wavelengths, which increases the antibacterial efficiency against *Staphylococcus aureus*. Provide comprehensive diode laser specs to guarantee maximum treatment efficacy and repeatability.

The mechanism of this research method is described as follows in Figure 1.

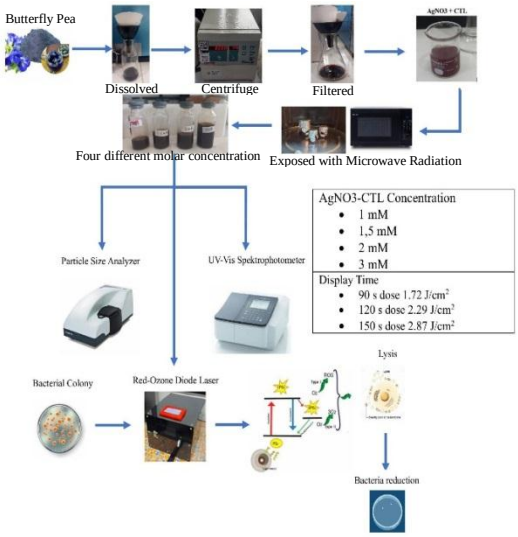


**Figure 1.** Mechanism of Research Methods

### Silver Nanoparticles Butterfly Pea

The process of making silver nanoparticles concentrated using the extract from butterfly peas (*Clitoria ternatea L.*) as a reducing agent. A clear extract solution was produced after dissolving 25 grams of butterfly pea powder in 50 milliliters of distilled water and centrifuging the mixture to get rid of any debris. Rather than going into depth about how the concentrated extract was made, it was decreased to 12 milliliters and used to create silver nanoparticles. The emphasis of the final AgNPs is their

ability to attack *Staphylococcus aureus* effectively.

To determine the effect of $AgNO_3$ concentration, the filtered solution was mixed with $AgNO_3$ at four different molar concentrations: 1 mM, 1.5 mM, 2 mM, and 3 mM. Butterfly pea extract as the filtered solution was produced in a 1:9 ratio to $AgNO_3$ for each molar concentration. After that, the mixture was exposed to 450 watts of microwave radiation for five minutes, which produced silver nanoparticles.

**AgNPs-CTL Characterization Method**

Reactive oxygen species are produced when a diode laser is used because the light it generates has certain wavelengths that silver nanoparticles can absorb.[10] These very reactive chemicals, known as ROS, can harm proteins, nucleic acids, and bacterial cell membranes, eventually leading to bacterial inactivation.[11] This technique demonstrates how well photodynamic treatment targets and eradicates bacteria while improving antibacterial results when combined with diode lasers and silver nanoparticles.[12]

UV-Vis spectrum analysis was conducted using a U.V-Vis spectrophotometer (Shimadzu, 1800) covering a wavelength range of 300 to 1100 nm. The reduction of pure Ag+ ions was monitored by observing the spectrum at room temperature. The maximum characteristic peak of AgNPs is observed at around 400–500 nm.[9]

The test involved placing silver nanoparticles in a glass bottle, sealing it, and storing it in a dark room at room temperature. The extract preparation process involves using butterfly pea powder. The powder is dissolved in distilled water and stirred until thoroughly mixed. Then, it is centrifuged and filtered to obtain a clear extract solution. This standardized extract is then used to synthesize silver nanoparticles, ensuring consistency and reproducibility in subsequent experiments. The stability of the silver nanoparticles was assessed by checking the color change and monitoring the wavelength of the absorbance peak with UV-Vis daily for one week.

The absorbance test was continuously conducted from the first to the seventh day to determine the stability of the silver nanoparticles over time. The stability of the colloidal silver nanoparticles can be inferred from changes in the absorbance peak.[13] A shift in the absorption peak to a longer wavelength indicates low stability due to agglomeration, characterized by a color change and a corresponding shift in the peak wavelength.[12]

The next step in nanoparticle characterization was measuring the particle size distribution using the dynamic light scattering (DLS) method with a particle size analyzer (PSA). This method is considered more accurate than SEM or TEM as it provides the particle size distribution within the sample. PSA can measure particles ranging from 0.6 nanometers to 7 micrometres. The principle of DLS is based on the Brownian motion of suspended particles, which are generated from thermal collisions with the solvent, causing fluctuations in the intensity of the light scattered by a laser.

The analysis of these fluctuations determines the speed of Brownian motion and particle size using the Stokes-Einstein equation. PSA works by emitting light scattered by the particles in the sample, where the scattering intensity is inversely proportional to the particle size. The measurement results are processed into digital data for mathematical analysis.

Particle size and distribution measurements were conducted at the Institute of Biological Sciences and

Engineering (LIHTR), Airlangga University, using a Particle Size Analyzer (PSA). The testing involved inserting a sample of silver nanoparticles (AgNPs) into the PSA and setting the wavelength and absorbance value according to the highest result from the previous absorbance test with a UV-Vis Spectrophotometer. The measurement results included the single particle size distribution, average size, and Polydispersity Index (PdI) values.

Measurements using PSA provide average particle size distribution results, assuming a spherical shape, with the size expressed in radii.[14] PSA can measure particle sizes ranging from 0.6 nanometers to 7 micrometres. This revision improves the clarity and readability of the original text while preserving the citation placement.[15]

**Antibacterial Activity Test**

This test assessed the antibacterial properties of AgNPs-CTL using the disk diffusion method, a standard approach for evaluating antibacterial activity. This approach was carried out aerobically since *S. aureus* bacteria normally prefer situations with plenty of oxygen, which makes it perfect for evaluating the efficacy of antibiotics in settings that closely mimic human illnesses. The process began by culturing the bacteria in sterile Tryptic Soy Broth (TSB) and incubating them for 24 hours. Following the culturing process, bacterial growth was monitored to ensure it reached the target density. Then, 50 µL of the bacterial culture was equally distributed onto 9 cm diameter Petri plates filled with Tryptic Soy Agar (TSA) medium to guarantee constant bacterial coverage for the following tests.

After the media surface had dried, ten microliters of AgNPs were added to four 0.6-cm-diameter paper discs. These discs were then placed over the TSA medium in the Petri dishes that contained TSB and *S. aureus* bacteria. The discs were placed equidistantly within the dish, aligned in four quadrants, and incubated for 24 hours. The antibacterial activity of the butterfly pea flower extract was indicated by the presence of an inhibition zone around the paper discs, and the diameter of these zones was measured after incubation.

To prepare the bacterial culture of *S. aureus*, a sterile solution, was made by mixing 0.3 g of TSB with 10 mL of distilled water and adding *S. aureus* bacteria. Subsequently, 5 mL of the sterile TSB solution was transferred into a test tube, and one dose of bacterial isolate was mixed into it, followed by a vortex homogenization. Once homogeneous, the TSB in the reaction tube was poured into a 25 mL Erlenmeyer flask and incubated for 24 hours.

**Irradiation of Bacteria with Laser**

After the bacteria are grown with the photosensitizer (silver nanoparticles) for 30 minutes, the bacteria on the microplate are ready for light exposure using a red diode laser. The irradiation is performed perpendicular to the sample at a fixed ideal distance of 10 mm from the light source. A red laser with a wavelength of 600 nm was used, characterized by a Jasco CT-10 monochromator, to measure the peak wavelength. The OMM-6810B-220V power meter was used to measure the power output, which came out to be 2.49 mW. Diode laser radiation was applied for 90, 120, and 150 seconds at different intervals The irradiation stage is crucial because it must maximize the antibacterial impact while limiting harm to adjacent tissues and achieving optimal bacterial elimination via energy density optimization. The objective is to identify the optimal irradiation settings in photodynamic antimicrobial therapy to

improve treatment effectiveness.[30] Different time intervals resulted in different energy densities for each treatment.

The first step involved irradiation preparation by mixing 500 microliters of bacterial culture with the photosensitizer and diluting it in 4.5 mL of sterile physiological water, then vortexing to achieve homogeneity. Fifty microliters of the dilution and 50 microliters of AgNPs-CTL were added (1 mM, 1.5 mM, 2 mM, and 3 mM). This process was repeated five times for each AgNPs-CTL concentration, resulting in 20 wells in the microplate. The same procedure was performed for the control group (without AgNPs-CTL).

Once the microplate was prepared, it was incubated for 30 minutes at 37°C, followed by red laser irradiation for 90, 120, and 150 seconds. The irradiated samples were poured into 3.5 cm diameter Petri dishes containing sterile TSA media. The process was homogenized by forming a figure-eight pattern in each treatment group, followed by a 24-hour incubation at 37°C with the Petri dishes placed upside down. The final step involved reading the *S. aureus* antibacterial test results using a colony counter.

**Data analysis**

The efficacy of red laser irradiation and silver nanoparticles in suppressing Staphylococcus aureus growth was assessed by analyzing data from bacterial tests and silver nanoparticle characterization. The observation data were analyzed statistically using the Two-Way ANOVA Factorial test in IBM SPSS. The Two-Way ANOVA Factorial test is a parametric statistical test used to compare the means of multiple samples, mainly when two or more factors categorize the samples.

## RESULTS AND DISCUSSION

This research uses butterfly pea flower extract in the green method of synthesizing silver nanoparticles with varying solution concentrations of 1 mM, 1.5 mM, 2 mM, and 3 mM. It uses photodynamic red laser light therapy to test the effectiveness of S. aureus bacteria at varying durations of exposure. different, namely 90 seconds, 120 seconds, and 150 seconds.

**Synthesis of Silver Nanoparticles from Butterfly Flower Extract**

Figure 2 shows the Butterfly Flower Extract Solution.

**Figure 2.** Butterfly Flower Extract Solution

Silver nanoparticles (AgNPs) were synthesised using a natural bioreductant from butterfly pea flower extract.[16] Butterfly pea flower extract contains a phenolic concentration of 16.20 µg GAE/100% concentration and a total flavonoid content of 4.88 µg QE/100%. Butterfly pea flower extract contains active compounds such as flavonoids, tannins, saponins, anthraquinones, terpenoids, and alkaloids.[8]

The anthocyanin pigment in butterfly pea flowers is water-soluble, producing colors that range from red to blue. This is evident when a solution of butterfly pea flower extract mixed with distilled water appears purplish-blue.

The color changed to brownish-yellow when $AgNO_3$ solutions with varying molarity concentrations were added and irradiated in a microwave for 5 minutes. The formation of silver nanoparticle colloids can be visually observed, with the colloid transitioning from yellow to brownish after adding butterfly pea flower extract, as shown in the following picture.

Visually, the colloidal silver nanoparticles formed are brown. The concentration variations were 1 mM, 1.5 mM, 2 mM, and 3 mM. Higher concentrations of silver nitrate precursor resulted in more pronounced color changes.[17] This occurs because higher concentrations of silver nitrate led to the formation of more silver nanoparticles in the presence of bioreactors from butterfly pea flower extract. The mechanism for reducing silver ions into colloidal silver nanoparticles can be seen in equation (1).

$$Ag^{+} + e^{-} \rightarrow Ag^{0} \qquad (1)$$

The electron source used for $Ag^{+}$ ion reduction is predicted to come from phenol compounds which have conjugated double bonds. These phenolic compounds are functional groups attached to the structure of the flavonoid compounds contained in butterfly pea flower extract. Butterfly pea flowers contain a total of phenolic compounds ranging from 53-460 mg or the equivalent of acid gallate/g dry extract as well as compounds such as tannins, saponins, triterpenoids, phenols, flavonoids, flavonol glycosides, alkaloids, anthraquinones and steroids.[18]

Flavonoid compounds can be used as natural reducing agents in the synthesis of silver nanoparticles (AgNps). Phenolic compounds in flavonoids function as natural bioreductors for the formation of $Ag^{+}$ ions into $Ag^{0}$. Apart from functioning as a natural bioreductors, butterfly pea flower extract also functions as a capping agent for silver nanoparticles so that the particle size remains stable on the nanometer scale.

**Characterization of Nanoparticles using UV-Vis**

The stability of AgNPs-CTL from a physical perspective can be observed visually through changes in the color of the solution. The color change in the green synthesis AgNPs-CTL is a crucial indicator of the reduction of silver to nanoparticles, as shown in Figure 2. According to Figure 2, the color changes from brownish-yellow to a darker brownish hue. There was no visible agglomeration in the sample, indicating that the particle size in the solution was small and stable and did not tend to form large clumps. This information is crucial for ensuring the effectiveness of nanoparticles in various applications. Over time, as the solution is left in the dark at room temperature, the color of AgNPs-CTL changes from yellow-brown to dark brown.

The formation of silver nanoparticles is marked not only by a change in solution color but also by the appearance of $\lambda maks$.[13] Silver nanoparticles exhibit the Surface Plasmon Resonance (SPR) phenomenon,[19] which can be observed in the spectrum of wavelength and absorbance peak measurements using a UV-Vis spectrophotometer. The absorbance and wavelength were measured using a UV-Vis spectrophotometer to confirm the ongoing reduction reaction. The formation of silver nanoparticles is characterized by an absorbance peak and a wavelength in the range of 385-515 nm. AgNPs-CTL UV-Vis analysis with variations (1 mM, 1.5 mM, 2 mM, 3 mM) is illustrated in Figure 3a (first day) and Figure 3b (seventh day).

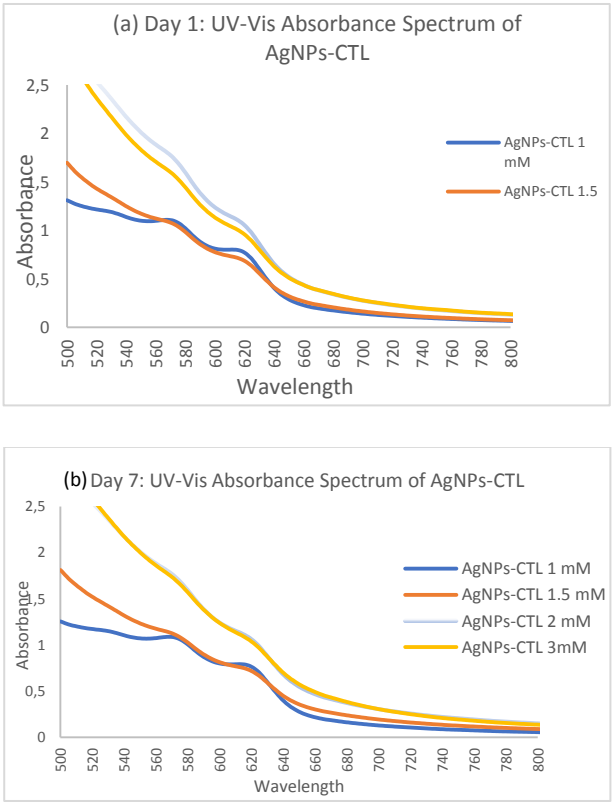


**Figure 3**. UV-Vis Spectrum of Solution Synthesis of Silver Nanoparticles $AgNO_3$ concentration 1 mM, 1.5 mM, 2 mM, 3 mM first day (a) and seventh day

The stability of the absorbance and wavelength of AgNPs-CTL was analyzed using a UV-Vis spectrophotometer on day 1 and day 7 after synthesis. The absorbance peak and wavelength of AgNPs-CTL with different concentrations (1 mM, 1.5 mM, 2 mM, 3 mM) show two peak wavelengths with the following descriptions: (1) 1 mM has peak wavelengths of 574 and 618 nm with absorbances of 1.052 and 0.708, (2) 1.5 mM has peak wavelengths of 571 and 621 nm with absorbances of 1.075 and 0.678, (3) 2 mM has peak wavelengths of 575 and 618 nm with absorbances of 1.699 and 0.982, and (4) 3 mM has peak wavelengths of 575 and 619 nm with absorbances of 1.531 and 0.972.

According to Figure 3, on the seventh day of measurement, there are two peaks in wavelength and absorbance as follows: (1) 1 mM has peak wavelengths of 573 and 612 nm with absorbances of 1.074 and 0.71; (2) 1.5 mM has peak wavelengths of 571 and 619 nm with absorbances of 1.176 and 0.741; (3) 2 mM has peak wavelengths of 575 and 618 nm with absorbances of 1.699 and 0.982; and (4) 3 mM has peak wavelengths of 576 and 619 nm with absorbances of 1.732 and 1.051.

The UV-Vis test results indicate that the wavelength stability of AgNPs-CTL is within the range of 570–620 nm. Non-agglomerated AgNPs-CTL improves the antibacterial effectiveness due to their superior stability and reactivity. Large clumps decrease the efficacy of therapy because they have less surface area and responsiveness. The first wavelength range of 570 nm shows the characteristic value of silver nanoparticles. The second wavelength range of 620 nm represents absorption used as a reference for measuring red diode laser irradiation, which has a wavelength spectrum of 620–759 nm.[12] This observation, as depicted in Figure 3, shows no significant shift in wavelength, indicating relative stability. Meanwhile, the decrease in absorbance at a concentration of 1 mM suggests a change in the composition of the solution, potentially due to the oxidation of phytochemicals in the extract that may have contributed to the absorption at 570 nm.[20] Table 1 shows the PSA Test Results.

**Table 1.** PSA Test Results

| Concentration AgNPs-CTL | Average (nm) | Standar Deviation (nm) |
|---|---|---|
| **1 mM** | 5.35 | 4.90 |
| **3 mM** | 4.04 | 3.22 |

**Particle Size Analyzer Characterization**

The Particle Size Analyzer (PSA) test aims to determine the particle size distribution using the Dynamic Light Scattering (DLS) method, which utilizes infrared scattering. DLS is also known as photon correlation spectroscopy. The particle size measured by DLS is the diameter of a circle of particles that diffuse at the same speed during the measurement. In this study, we tested AgNPs-CTL at 1 mM and 3 mM concentrations.

From the PSA test results, as shown in Table 1, the average particle diameter size for AgNPs-CTL 1 mM is 5.35 nm, while for AgNPs-CTL 3 mM, it is 4.04 nm. These results indicate that AgNPs-CTL 1 mM and 3 mM fall within the nanoparticle category, as their sizes are between 1-100 nm. These findings correspond with the nanoparticle category for the other two molarities tested.

Figure 4 shows the PSA Test Results AgNPs-CTL 1 mM and 3 mM. Figure 4(a) illustrates the particle size distribution of AgNPs-CTL 1 mM, which ranges from 1.22 nm to 18.23 nm. Figure 4(b) shows the particle size distribution of AgNPs-CTL 3 mM, which ranges from 1.22 nm to 12.22 nm. The distribution indicates that most of the particles are concentrated in the smaller size range, as evidenced by the peaks at low diameter values.

**Antibacterial Activity Test**

Antibacterial test against *Staphylococcus aureus* was carried out to determine the diameter of the inhibition zone for the growth of bacterial colonies. Each sample was tested using the disk diffusion method. The results showed an inhibition zone with antibacterial AgNPs-CTL of 1 mM, 1.5 mM, 2 mM, and 3 mM, respectively, for *Staphylococcus aureus*, a Gram-positive bacterium. Measurement of the inhibition zone of AgNPs-CTL with *Staphylococcus aureus* showed good antibacterial activity with an average inhibition zone of 1.40 mm.

Figure 5 shows photographic images of bacterial inhibition zones against *S. aureus*, produced by silver nanoparticles prepared with different molar ratios of $AgNO_3$. These results show good antibacterial activity with an average inhibition zone diameter of 1.51 mm.

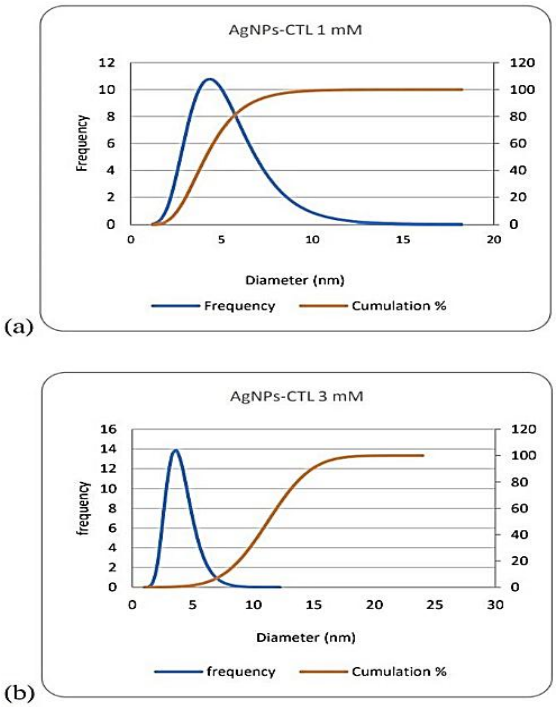


**Figure 4.** PSA Test Results AgNPs-CTL 1 mM and 3 mM

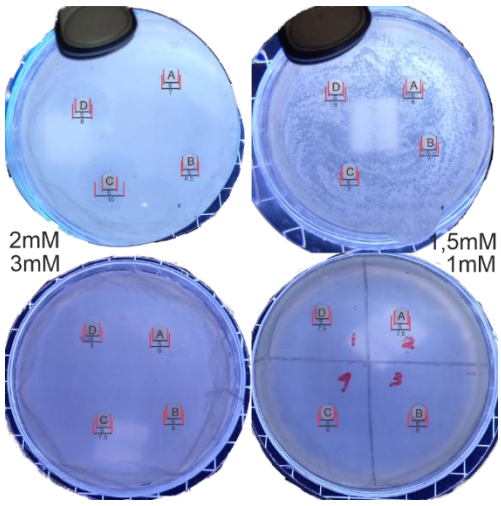


**Figure 5.** Disc Diffusion Method Results

Based on the inhibition zone test results presented in Table 1, it can be seen that the average diameter of the inhibition zone is the highest there is inhibition of AgNPs-CTL of *S. aureus* bacteria with a concentration variation of 3 mM, which is 1.8 mm. This shows that AgNPs-CTL has good antibacterial effectiveness.[21] Figure 5 shows the transparent zone (inhibition zone) formed using the disc diffusion method, indicating the antibacterial effectiveness of different treatments against *Staphylococcus aureus.*

**Laser Irradiation Results**

The laser irradiation process was carried out with varying times, namely 90, 120 and 150 seconds. These different time intervals result in different energy densities for each treatment. The effectiveness of this laser irradiation can be observed by reducing the number of bacterial colonies compared to the control group.

The addition of AgNPs-CTL to bacterial samples shows that the synthesis used is antibacterial. Bacterial growth results were obtained by calculating the number of bacterial colonies grown with AgNPs-CTL in a plate.

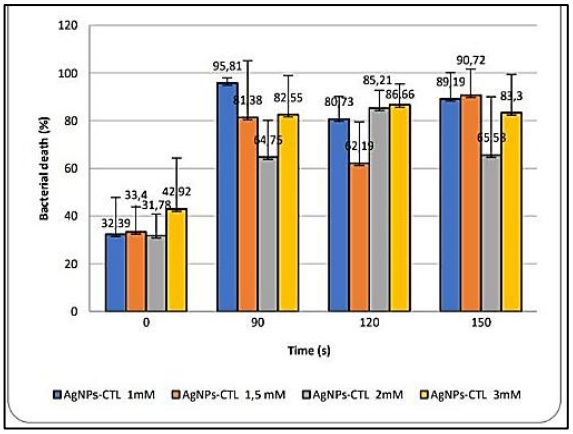


**Figure 6.** Percentage of Death of *Staphylococcus aureus* Bacteria

Figure 6 shows the percentage increase in death of *Staphylococcus aureus* bacteria. It can be seen that compared to the addition of AgNPs-CTL without irradiation (0s), the addition of AgNPs-CTL becomes effective with laser irradiation. There was an increase in bacterial death at each variation in AgNPs-CTL concentration.

**Analysis of the Effectiveness of Red Laser against Staphylococcus aureus Bacteria**

The observation data obtained were then analyzed statistically using the Two-Way ANOVA Factorial test on IBM SPSS. Based on the results of the statistical analysis in Table 2, a significance value of ($p = 0.094$) (where $\alpha = 0.05$) was obtained from the data normality test, indicating that the data is usually distributed. The subsequent analysis using the Two-Way ANOVA Factorial test yielded a significance value of ($p = 0.043$) for the variations in AgNPs-CTL concentration and ($p = 0.039$) for the interaction between AgNPs-CTL concentration and exposure time, both of which are less than ($\alpha = 0.05$). This indicates significant differences in both cases. A post hoc test determined which treatment groups showed significant differences. The results revealed that AgNPs-CTL concentrations of 1 mM and two mM produced significantly different outcomes, with the highest percentage of bacterial death being 88.58% for the one mM concentration.

Additionally, the interaction between AgNPs-CTL concentration and exposure time showed that the highest percentage of bacterial death was achieved with a combination of 1 mM for 90 seconds (1A), yielding a value of 95.81%.

Other notable results included combinations of 1.5 mM for 150 seconds (2C) with 90.72% bacterial death and 1 mM for 150 seconds (1C) with 89.19% bacterial death.

These results suggest that lower concentrations with shorter exposure times can effectively kill bacteria. Overall, based on this analysis, the most optimal treatment combination for bacterial inactivation is a concentration of 1 mM for 90 seconds.

Conversely, for variations in exposure time, the Two-Way ANOVA Factorial test showed a significance value of ($p = 0.790$) (where ($\alpha = 0.05$), indicating that variations in exposure time do not produce a significant difference.

Photodynamic inactivation involves three main aspects: visible light, reactive oxygen species (R.O.S.), and photosensitizers that act as light sensitizers. This process requires an alignment between the absorption spectrum of visible light and the photosensitizer. Lasers are commonly used as light sources in photodynamic inactivation due to their advantages, such as uniform (monochromatic) and parallel light emission.[23] This research used a red laser diode with a wavelength range of 620 – 759 nm.

The photochemical process in photodynamic inactivation consists of two types: type I and type II. In the type I pathway, electron transfer occurs between excited sensitizer molecules and biological molecules, producing radical ions in the form of reactive oxygen species (ROS). Meanwhile, in the type II pathway, energy is transferred from the excited triplet photosensitizer to the triplet oxygen, producing excited singlet oxygen. ROS can damage the structure of bacterial cell walls, causing bacterial cell lysis. This is the initial stage in bacterial cell death.[24]

ROS and triplet oxygen radicals formed from photochemical processes initiate the peroxidation of unsaturated fatty acids, forming hydroperoxides. Next, singlet and triplet oxygen radicals break hydrogen bonds in saturated fatty acids, producing toxic hydroperoxides, known as lipid peroxidation. This process damages the structure of the bacterial cell wall, resulting in cell lysis.[25]

This research uses *Staphylococcus aureus* as a Gram-positive bacterium with a thick peptidoglycan layer, making it more stress-resistant. A red laser was used to irradiate it for 90, 120, and 150 seconds. The red laser generates reactive oxygen species (ROS) that penetrate the cell wall, damaging proteins, DNA, and lipids, thus effectively inhibiting S. aureus growth. Longer irradiation times enhance the antibacterial effect by increasing ROS production. [26]

This is because *Staphylococcus aureus*, a Gram-positive bacterium, has cell walls consisting of peptidoglycan, teichoic acid and neuraminic acid.[26,27] This polysaccharide-rich cell wall structure is more susceptible to damage during the photoinactivation process.[28,29] So, in this case, there was a significant increase in deaths, namely 42.92%.

Based on Table 2, it is evident that the addition of AgNPs with laser significantly affects the death percentage of *S. aureus*. This is attributed to the fact that the addition of AgNPs can induce bacterial dysfunction and death. *Staphylococcus aureus* bacteria were subjected to the addition of extract, AgNPs, extract+ laser, and AgNPs + laser. According to Figure 6, the highest percentage of death of *Staphylococcus aureus* bacteria in AgNPs-CTL 1mM was observed with AgNPs+laser treatment, reaching 95.81%. Thus, it can be concluded that the addition of AgNPs with a laser significantly affects the death of bacteria on plates containing *Staphylococcus aureus* bacteria.

Based on the results of calculating bacterial death above, statistical analysis can be conducted using SPSS. In this research, two statistical tests were carried

**Table 2. Factorial Test Results**

| Treatment | Group | N | Death Bacteria (%) | | Faktorial Test Result | |
|---|---|---|---|---|---|---|
| | | | Average | SD | Signification | Conclusion |
| **Concentration AgNPs-CTL** | 1 mM (1)[2] | 15 | 88.58 | 8.66 | P = 0.043 | There are different meanings |
| | 1,5 mM (2)[1,2] | 15 | 78.10 | 24.09 | | |
| | 2 mM (3)[1] | 15 | 71.85 | 18.36 | | |
| | 3 mM (4)[1,2] | 15 | 84.17 | 12.10 | | |
| **Total** | | 60 | | | | |
| **Time** | 90 s (A) | 20 | 82.20 | 11.61 | P = 0.790 | There is no difference meaning |
| | 120 s (B) | 20 | 78.70 | 11.83 | | |
| | 150 s (C) | 20 | 81.12 | 14.18 | | |
| **Total** | | 60 | | | | |
| **Interaction** | 1A[(4)] | 5 | 95.81 | 6.43 | P = 0.039 | There are different meanings |
| | 1B[(1,2,3,4)] | 5 | 80.73 | 6.40 | | |
| | 1C[(3,4)] | 5 | 89.19 | 21.92 | | |
| | 2A[(1,2,3,4)] | 5 | 81.38 | 11.71 | | |
| | 2B[(1)] | 5 | 62.19 | 8.10 | | |
| | 2C[(4)] | 5 | 90.72 | 26.46 | | |
| | 3A[(1,2)] | 5 | 64.75 | 5.65 | | |
| | 3B[(1,2,3,4)] | 5 | 85.21 | 7.11 | | |
| | 3C[(1,2,3)] | 5 | 65.58 | 1.98 | | |
| | 4A[(1,2,3,4)] | 5 | 82.55 | 24.01 | | |
| | 4B[(2,3,4)] | 5 | 86.66 | 15.18 | | |
| | 4C[(1,2,3,4)] | 5 | 83.30 | 15.56 | | |
| **Total** | | 60 | | | | |

out: the normality test and one-way ANOVA.

Based on the results of the normality test using One-Sample Kolmogorov-Smirnov, a significance value of 0.60 was obtained, indicating that the data is usually distributed since p > 0.05. Subsequently, the data was analyzed again with one-way ANOVA, and the results can be seen in Table 2.

Based on Table 2, the addition of AgNPs with laser has a significant effect on the death percentage of *Staphylococcus aureus* bacteria, with a significance value of 0.00. This is attributed to the fact that the addition of AgNPs can induce bacterial dysfunction and death.

## CONCLUSIONS

Silver nanoparticles synthesized from butterfly pea flower extract have been successfully employed in bacterial photoinactivation. In vitro tests have shown antibacterial activity. AgNPs-CTL at a concentration of 1 mM significantly increased the death rate of *S. aureus* bacteria by 42.92%. Red laser irradiation resulted in 95.81% bacterial death for *S. aureus* when using AgNPs-CTL at a concentration of 1 mM with an exposure time of 90 seconds.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

No conflict of interest.

## AUTHOR CONTRIBUTION

SDA: Conceptualization, methodology, validation, writing original draft preparation, supervision, funding acquisition GRAF: Conceptualization, methodology, writing original draft preparation, software UMUS: Conceptualization, Sources, software RA: Conceptualization, writing review and editing. AHZ: Conceptualization, methodology, validation, supervision. AKY: Conceptualization, methodology, validation.

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# Clinical Patterns and Demographic Characteristics of Dermatophytosis in Surabaya

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## Abstract

Dermatophytosis, the most common fungal infection in humans, significantly impacts quality of life due to its clinical and cosmetic effects. Its high prevalence underscores the need to evaluate patient profiles to improve management strategies. This study aim is to identify the most prevalent type of dermatophytosis, patient demographics, clinical characteristics, laboratory investigations, and therapy in dermatophytosis into the clinical and epidemiological characteristics of dermatophytosis in a tropical, high-burden region. This descriptive retrospective study used total sampling of medical records of dermatophytosis patients from January 2017 to December 2022. Tinea corporis and tinea cruris was the most common, while tinea manuum is the least common dermatophytosis. Female adults were the most affected group. Common clinical features for each type included alopecia for tinea capitis, erythematous macules for other types, and nail dystrophy for tinea unguium. *Trichophyton mentagrophytes* was the commonest pathogen in 2017. Most of the therapies followed Clinical Practice Guidelines with extensive use of griseofulvin and ketoconazole cream. Further research should explore therapeutic outcomes, preventive measures, and factors influencing recurrence and adherence to treatment.

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## INTRODUCTION

Mycoses are divided into 3 forms: superficial, involving the stratum corneum, hair, and nails; subcutaneous, involving the dermis and/or subcutaneous tissue; and systemic, showing hematogenous spread from the stratum corneum, hair, and nails. subcutaneous tissue; and systemic, which shows hematogenous spread of opportunistic pathogens in immunocompromised hosts. Superficial dermatomycosis is a fungal infection that is limited to the body surface, such as the stratum corneum, hair, nails, and the mucous membrane surfaces of the oral cavity and vulva.[1]

Dermatophytosis superficial fungal infections affect 20-15% of the world's population and are the most common fungal infections in humans.[2] Dermatophytosis is an infection by dermatophyte fungi that attack tissues with keratin, such as the stratum corneum of the epidermis, hair, and nails.[3] Dermatophytosis is caused by dermatophyte fungi, which are keratinolytic fungi classified into three genera, which are *Microsporum*, *Trichophyton*, and *Epidermophyton*.[3] Dermatophytosis develops primarily in keratinized areas of the body, including the scalp (tinea capitis), body skin (tinea corporis), groin skin (tinea cruris), feet (tinea pedis), hands (tinea manuum), and nails (tinea unguium).[5]

In the last two decades, there has been a significant escalation in human dermatophytosis cases as a consequence of socioeconomic problems, immigration from tropical countries, and contact with animals, particularly pets.[4] Age and the usage of immunosuppressant drugs are also predisposing factors for mortality due to dermatophytosis in humans.[6] One of the risk and predisposing factors for tinea infection is a humid environment. Other risk factors include diabetes mellitus, hypertension, atherosclerosis, usage of occlusive footwear, use of public bathing facilities or public sports facilities, and repeated trauma on the hands, usually related to occupation.[7]

Indonesia is a tropical country with year-round sunshine. On the other hand, most of Indonesia's territory is mostly water, hence Indonesia has a high rate of rainfall. This condition causes Indonesia to have a humid environment. In tropical and subtropical regions, where humidity levels are consistently high, dermatophytosis is more prevalent. Dermatophytes thrive in high humidity environments, typically above 60% relative humidity.[35] Combination of high temperatures (around 35°C) and high humidity (95%-100%) creates the most favorable conditions for dermatophyte penetration into the skin.[36] This is attributed to the ideal conditions for fungal growth during periods of increased sweating and moisture on the skin. [20-21]

Dermatophytosis can be both clinically and cosmetically disruptive, thereby decreasing the quality of life. Psychosocial issues such as embarrassment, decreased self-esteem, anxiety, and depression are often more prevalent in dermatophytosis patients rather than physical symptoms.[8] The large number of dermatophytosis cases each year indicates that there is still a need for ongoing evaluation of these cases. This study is a continuation of the previous study on the profile of dermatophytosis at Dr. Soetomo General Academic Hospital Surabaya.

## MATERIALS AND METHODS

This research is a descriptive retrospective study using the total sampling method. The research instrument used was the medical records of dermatophytosis patients in the

dermatology and venereology Outpatient Unit at Dr. Soetomo General Academic Hospital Surabaya for the period January 1, 2017 to December 31, 2022. the variables of this study consisted of age, gender, occupation, domicile, classification of dermatophytosis, chief complaint, clinical presentation, predisposing factors, laboratory examination, and management of dermatophytosis.

The inclusion criteria of this study were patients with a diagnosis of dermatophytosis who examined themselves at the dermatology and venereology Outpatient Unit at Dr. Soetomo General Academic Hospital Surabaya from January 2017 to December 2022 with appropriate medical record data, that includes patient identification, medical history, diagnosis, treatment, and laboratory results. It should ensure consistency between electronic medical records (EMR) and manual records in the outpatient unit. The study was conducted at the Dermatology and Venereology Outpatient Unit and Information and Communication Technology Installation of Dr. Soetomo General Academic Hospital Surabaya from September 2023 to February 2024. This research has been reviewed by the Ethics Committee at Dr. Soetomo General Academic Hospital.

## RESULTS AND DISCUSSION

A total of 1100 dermatophytosis patient data were obtained from 2017 to 2022, however, only 864 patients were eligible according to the inclusion criteria. A total of 236 other patients were excluded from the study due to missing medical records, inappropriate diagnosis data, or discrepancies between electronic medical records and paper-based records. Multiple cases of dermatophytosis were found in some patients, resulting in a total of 930 cases of dermatophytosis.

The number of new cases of dermatophytosis was 281 (32.52%) in 2017, 184 patients (21.30%) in 2018, 172 patients (19.91%) in 2019, 88 patients (10.19%) in 2020, 80 patients (9.26%) in 2021, and 59 patients (6.83%) in 2022.

### Patient Demographics

According to the demographic data of the patients as shown in Table 1, the most common age group was the adult group with 678 patients (72.9%), followed by the pediatric age group with 109 patients (11.7%). Tinea facialis, tinea corporis, tinea cruris, tinea pedis, tinea manuum, and unguium were dominated by the adult age group. However, tinea capitis was dominated by the child age group with 46 patients (4.9% of all dermatophytosis cases, 95.8% of tinea capitis cases).

Overall, there were more female patients than male patients. Tinea facialis, tinea corporis, tinea cruris, tinea pedis, and tinea unguium were dominated by female patients. However, for tinea capitis and tinea manuum, male patients outnumbered female patients.

Occupational classification was based on the International Standard Classification of Occupations (ISCO). The most common occupation was private employees, with 297 patients (31.9%). Meanwhile, the most common occupational classification was others, totaling 744 patients (80.0%) followed by the professional group as many as 16 patients (3.5%).

Regarding the domicile of the patients, most of the patients originated from Surabaya. The ratio of male to female patients was 1:1.26. Tinea facialis, tinea corporis, tinea cruris, tinea pedis, and tinea unguium were dominated by female patients. There were however more male

patients than female patients for tinea capitis and tinea manuum.

In this study, it was found that most of the patients with dermatophytosis were adult patients. This may be since the adult age group has the largest age range among other age groups. Besides that, adults are often more physically active, leading to increased sweating. Many adults work in warm, humid environments, such as outdoor jobs, which promote fungal infections due to increased perspiration. Adults also may be more likely to seek medical attention for persistent symptoms like itching or discomfort compared to children. This increased awareness and response to symptoms can lead to a higher reported incidence of dermatophytosis among adults. [3,10,23]

In addition, in this study also found that the least age groups were neonates, infants, and children. This is due to the division of Outpatient Unit of RSUD Dr. Soetomo Surabaya. There is a separate pediatric polyclinic from the dermatology and venereology polyclinic. Infant and child patients tend to come to the pediatric polyclinic so the number of infants and children who come to the dermatology and venereology outpatient unit are few.

The higher prevalence of tinea capitis in children and the male sex, in addition to prepubertal factors such as fungistatic fatty acid levels, is most likely also due to hormonal factors and low levels of progesterone, which cause steroid-mediated inhibition of dermatophyte growth. Rare cases of tinea capitis in adults may be due to the fungistatic characteristics of long-chain fatty acids in post-pubertal sebum, hair follicle maturation, and the immune system after adulthood which can protect the body from fungal invasion.[9]

Housewives are increasingly found to have active infections. The heated kitchen environment with increased sweating supports the growth of dermatophyte fungi, making housewives more vulnerable.[10] In several other studies, an increase in the frequency of dermatophytosis was found in student groups. School and sports activities and the use of school uniforms and footwear for a long time may be contributing factor to the occurrence of dermatophytosis in students.[11-13]

Tinea pedis is common in athletes, especially those who often walk barefoot or in people with occupations that use occlusive footwear for long periods, such as military personnel). Prolonged exposure to moist environments, use of occlusive footwear, and shared communal spaces, creates ideal conditions for fungal growth. Additionally, inadequate foot drying and minor skin trauma further compromise the skin barrier, increasing susceptibility to infection. [14-15] In this study, different results were obtained. Tinea pedis was not found in the military personnel occupational groups, and athletes did not appear as one of the occupational groups with dermatophytosis. Several possibilities can cause these results, including the type of work that is less explored in the anamnesis or there are indeed epidemiological differences in patients who are the subject of the study. patients who became the subject of the study.

Although RSUD Dr. Soetomo Surabaya is a referral hospital, patients from Surabaya still outnumber patients from cities other than Surabaya. Dermatophytosis is a disease of general practitioners' competence to perform clinical management, such as the ability to correctly identify, diagnose, manage, and monitor diseases or medical conditions, to provide appropriate treatment.

**Table 1.** Dermatophytosis patient in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital Surabaya from January 2017-December 2022 demographics

| Variable | Tinea capitis (n=48) | Tinea facialis (n=26) | Tinea corporis (n=423) | Tinea cruris (n=386) | Tinea pedis (n=17) | Tinea manuum (n=6) | Tinea unguium (n=24) |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | |
| Infant | 0 (0.0%) | 0 (0.0%) | 1 (0.2%) | 2 (0.5%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Child | 46 (95.8%) | 9 (34.6%) | 31 (7.3%) | 20 (5.1%) | 3 (17.6%) | 0 (0.0%) | 0 (0.0%) |
| Adolescent | 1 (2.0%) | 3 (11.5%) | 30 (7.1%) | 33 (8.5%) | 0 (0.0%) | 0 (0.0%) | 1 (4.1%) |
| Adult | 1 (2.0%) | 14 (53.8%) | 326 (77.1%) | 302 (78.2%) | 11 (64.7%) | 5 (83.3%) | 19 (79.1%) |
| Elderly | 0 (0.0%) | 0 (0.0%) | 35 (8.3%) | 29 (7.5%) | 3 (17.6%) | 1 (16.7%) | 4 (16.7%) |
| **Gender** | | | | | | | |
| Male | 26 (54.2%) | 10 (38.5%) | 173 (40.9%) | 179 (46.4%) | 8 (47.1%) | 5 (83.3%) | 11 (45.8%) |
| Female | 22 (45.8%) | 16 (61.5%) | 250 (59.1%) | 207 (53.6%) | 9 (52.9%) | 1 (16.7%) | 13 (54.2%) |
| **Occupation (classification based on International Standard Classification of Occupations)** | | | | | | | |
| Professionals | 0 (0.0%) | 1 (3.8%) | 21 (4.9%) | 7 (1.8%) | 2 (11.7%) | 0 (0.0%) | 2 (8.3%) |
| Managers | 0 (0.0%) | 0 (0.0%) | 17 (4.0%) | 12 (3.1%) | 0 (0.0%) | 1 (16.6%) | 1 (4.1%) |
| Skilled agricultural, | 0 (0.0%) | 0 (0.0%) | 4 (0.9%) | 4 (1.0%) | 1 (5.8%) | 0 (0.0%) | 0 (0.0%) |
| Plant and machine | 0 (0.0%) | 0 (0.0%) | 4 (0.9%) | 2 (0.5%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Elementary occupations | 0 (0.0%) | 0 (0.0%) | 2 (0.5%) | 2 (0.5%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Service and sales workers | 0 (0.0%) | 0 (0.0%) | 1 (0.2%) | 1 (0.2%) | 0 (0.0%) | 1 (16.6%) | 0 (0.0%) |
| Armed forces | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 2 (0.5%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Clerical support workers | 0 (0.0%) | 0 (0.0%) | 1 (0.2%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Others | 42 (87.5) | 22 (84.6%) | 328 (77.5%) | 324 (83.9%) | 9 (52.9%) | 4 (66.6%) | 15 (62.5%) |
| **Domicile** | | | | | | | |
| Surabaya | 36 (75%) | 13 (50%) | 265 (62.6%) | 243 (62.9%) | 7 (41.1%) | 4 (66.65) | 18 (75%) |
| Others | 12 (25%) | 10 (38.4%) | 141 (33.3%) | 129 (33.4%) | 7 (41.1%) | 2 (33.3%) | 6 (25%) |
| **Total** | 48 (5.2%) | 26 (2.8%) | 423 (45.5%) | 386 (41.5%) | 17 (1.8%) | 6 (0.6%) | 24 (2.6%) |

### Classification of Dermatophytosis

As presented in Table 2, dermatophytosis with the most frequent cases was tinea corporis, with 358 cases (41.4%), followed by tinea cruris with 324 cases (37.5%). Dermatophytosis with the least number of cases was tinea manuum with 6 cases (0.7%), as well as tinea corporis et facialis, tinea corporis et capitis, and tinea cruris et unguium, each with only 1 case (0.1%).

This study shows the same results as previous research which was conducted at RSUD Dr. Soetomo Surabaya. Tinea corporis and tinea cruris are the types of dermatophytosis with the highest incidence rate in the study of 2011-2016. In previous studies, it was also found that tinea manuum is tinea with the lowest incidence rate. Different results were obtained in 2011-2013, which was found as many as 2 patients who experienced tinea barbae, while in 2014-2016 there were no tinea barbae patients at all.[16-17] Another study conducted in Africa obtained similar results. Tinea corporis was found to be the most common dermatophytosis, especially in adulthood.[18]

**Table 2.** Distribution of dermatophytosis classification in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital Surabaya from January 2017-December 2022

| Classification of Dermatophytosis | n | % |
|---|---|---|
| Tinea corporis | 358 | 41.4 |
| Tinea cruris | 324 | 37.5 |
| Tinea corporis et cruris | 61 | 7.1 |
| Tinea capitis | 47 | 5.4 |
| Tinea facialis | 25 | 2.9 |
| Tinea unguium | 21 | 2.4 |
| Tinea pedis | 17 | 2.0 |
| Tinea manuum | 6 | 0.7 |
| Tinea corporis et unguium | 2 | 0.2 |
| Tinea corporis et facialis | 1 | 0.1 |
| Tinea corporis et capitis | 1 | 0.1 |
| Tinea cruris et unguium | 1 | 0.1 |
| Tinea barbae | 0 | 0.0 |

### CHIEF COMPLAINTS

The most common chief complaint was itching in 608 cases (65.4%), followed by red patches in 499 cases (53.7%). Some of the complaints were found only in specific types of dermatophytosis, for example, complaints on nails (damaged nails, yellowing nails, white nails, thickening nails, and blackish nails) were only found in tinea unguium. In addition, complaints such as hair loss, baldness, and dandruff are only found in tinea capitis. Details of chief complaints of dermatophytosis patients are shown in Table 3.

**Table 3.** Distribution of dermatophytosis chief complaints in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital from January 2017-December 2022

| Chief complaint | n | % |
|---|---|---|
| Itching | 608 | 65.4 |
| Red patches | 499 | 53.7 |
| Black patches | 83 | 8.9 |
| White patches | 11 | 1.2 |
| Patches | 66 | 7.1 |
| Brown patches | 4 | 0.4 |
| Hair loss | 29 | 3.1 |
| Dandruff | 1 | 0.1 |
| Damaged nails | 15 | 1.6 |
| Yellowing nails | 1 | 0.1 |
| White nails | 1 | 0.1 |
| Thickening nails | 1 | 0.1 |
| Wounds | 7 | 0.8 |

The results of this study are in line with research conducted at Dr. Soetomo Hospital Surabaya in the previous period. In the period 2014-2016, the most common complaint was itching, followed by red patches. The study also found that the most common complaints of tinea capitis were baldness.[16] Itching is more common complained than spotting because itching causes a disturbing sensation, so that it can reduce quality of life. In addition, itching is also more associated with allergic or infectious diseases, so it attracts more attention so that patients tend to seek treatment immediately. Persistent itching may also lead to irritation or infection secondary to scratching. This finding aligns with other studies showing that itching is the primary symptom of dermatophytosis, significantly affecting quality of life and driving patients to seek prompt medical care due to its impact on daily activities and productivity. [22]

## Clinical Appearance

The most common clinical features of tinea capitis were alopecia and thin scales (n=29, 60.42%) followed by erythematous macules (n=2, 27.08%). Among the patients with tinea faciei (n=26), the most common clinical features were erythematous macules (n=21, 80.77%), followed by thin scales (n=15, 57.69%), and active margins (n=12, 46.15%). In tinea corporis (n=358), the most common clinical features were erythematous macules (n=304, 71.87%), followed by macules with active margin (n=274, 64.78%) and thin scales (n=240, 56.74%). In patients with tinea cruris, there were more various clinical features than other types of tinea. The most common clinical appearance was erythematous macules (n=274, 70.98%), followed by active marginal lesions (n=234, 60.62%) and thin scales (n=226, 58.55%). The most common clinical feature in tinea pedis was erythematous macules (n=11, 64.71%), followed by thin scales (n=9, 52.94%). Among tinea manuum patients, the most common symptoms were erythematous macules and thin scales (n=4, 66.67%). Among the 26 patients with tinea unguium, the most common clinical feature was dystrophy (n=9, 37.50%), followed by dyschromia and hyperkeratosis (n=8, 33.33%). Particular details of the clinical appearance of each type of dermatophytosis are presented in Table 4. Most patients had overlapping symptoms, so data could not be fully accumulated.

**Table 4.** Distribution of tinea capitis clinical appearance in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital from January 2017-December 2022

| Clinical presentation | n | % |
|---|---|---|
| **Tinea capitis** | | |
| Alopecia | 29 | 60.42% |
| Thin scales | 29 | 60.42% |
| Erythematous macule | 23 | 27.08% |
| Poorly demarcated margin | 6 | 12.50% |
| Gray patch | 4 | 8.33% |
| Crust | 4 | 8.33% |
| Well-defined lesion margin | 4 | 8.33% |
| Others | 17 | 35.42% |
| **Tinea facialis** | | |
| Erythematous macule | 21 | 80.77% |
| Thin scales | 15 | 57.69% |
| Active margin | 12 | 46.15% |
| Polycyclic | 11 | 42.31% |
| Central healing | 9 | 34.62% |

| | | |
|---|---|---|
| Others | 15 | 57.69% |
| **Tinea corporis** | | |
| Erythematous macule | 304 | 71.87% |
| Active margin | 274 | 64.78% |
| Thin scales | 240 | 56.74% |
| Central healing | 176 | 41.61% |
| Polycyclic | 173 | 40.90% |
| Well-defined lesion margin | 80 | 18.91% |
| Hyperpigmented macule | 73 | 17.26% |
| Poorly demarcated margin | 36 | 8.51% |
| Erythematous and hyperpigmented macule | 21 | 4.96% |
| Papules | 11 | 2.60% |
| Others | 40 | 9.46% |
| **Tinea cruris** | | |
| Erythematous macule | 274 | 70.98% |
| Active margin | 234 | 60.62% |
| Thin scales | 226 | 58.55% |
| Polycyclic | 190 | 49.22% |
| Central healing | 167 | 43.26% |
| Hyperpigmented macule | 79 | 20.47% |
| Well-defined lesion margin | 76 | 19.69% |
| Others | 101 | 26.17% |
| **Tinea pedis** | | |
| Erythematous macule | 11 | 64.71% |
| Thin scales | 9 | 52.94% |
| Polycyclic | 8 | 47.06% |
| Others | 17 | 100% |
| **Tinea manuum** | | |
| Erythematous macule | 4 | 66.67% |
| Thin scales | 4 | 66.67% |
| Active margin | 2 | 33.33% |
| Others | 6 | 100% |
| **Tinea unguium** | | |
| Dystrophy | 9 | 37.50% |
| Dyschromia | 8 | 33.33% |
| Hyperkeratosis | 8 | 33.33% |
| Onycholysis | 2 | 8.33% |
| Others | 12 | 50% |

## Predisposing Factors

The most prevalent predisposing factor was warm humid conditions (n=182,19.6%), followed by frequent friction and maceration in warm climates (15.1%). The predisposing factor of warm and humid conditions was found to be the most prevalent in patients with tinea corporis, while friction and frequent maceration in warm climates were most common in patients with tinea cruris. In tinea capitis, the most common predisposing factor found was infected pets (n=27, 2.9%). The distribution and percentage of predisposing factors are presented in Table 5 below.

*Microsporum canis*, which originates from cats, is one of the most common causative agents of zoophilic infections leading to tinea capitis.[24] The transmission of *Microsporum canis* from cats to humans typically occurs through direct contact with an infected animal's fur or by touching objects that have come into contact with the cat's spores.[25] In tinea manuum and tinea pedis, the most common predisposing factor was excessive sweating.

People who work outdoors in a hot and humid environment are more susceptible to being infected with dermatophyte fungi. Outdoor workers are at heightened risk of fungal infections due to prolonged exposure to warm, humid environments, increased sweating, and limited hygiene facilities. Factors such as tight-fitting, non-breathable clothing and footwear exacerbate moisture retention, creating ideal conditions for fungal growth and skin maceration, which facilitates fungal penetration. [10,20,26]

**Table 5.** Dermatophytosis predisposing factors in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital from January 2017-December 2022

| Predisposing factor | n | % |
|---|---|---|
| Warm and humid conditions | 200 | 21.5 |
| Frequent friction and maceration in warm climates | 140 | 15.1 |
| Wearing tight clothing | 80 | 8.6 |
| Excessive sweating | 78 | 8.3 |
| Infected pets | 67 | 7.2 |
| Contact with patients | 33 | 3.5 |
| Immunocompromised | 25 | 2.7 |
| Immunosuppressant consumption | 21 | 2.3 |
| Lack of hygiene | 14 | 1.5 |
| Tinea pedis co-infection | 1 | 0.1 |
| Persistent use of socks and shoes | 1 | 0.1 |
| Use of towels or objects infected with fungus | 1 | 0.1 |
| Obesity | 1 | 0.1 |

In addition, the use of tight clothing that causes skin to become damp also increases the prevalence, recurrence, and chronicity of dermatophytosis.[10] This is in line with the results of this study, namely the most predisposing factor is a warm humid environment. Underlying chronic conditions are also a predisposing factor for dermatophytosis. predisposing factors of dermatophytosis, including diabetes mellitus, patients who received immunosuppressant therapy, chronic hepatitis C infection, and HIV.[18]

## Laboratory Examination

Microscopic examination with 10-20% KOH is used to diagnose dermatophytosis which involves the nails, hair, or fingernails. Findings from the microscopic examination using 10-20% KOH can include hyphae, blastophores, and septa. Some patients were also found to be negative for the KOH microscopic examination, so the diagnosis of dermatophytosis was established based on favorable clinical findings. Microscopic examination using 10-20% KOH findings details as shown in Table 6.

**Table 6.** Microscopic examination using 10-20% KOH results in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital from January 2017-December 2022

| Diagnosis | Positive result (%) | | | | | | Negative result | n (%) |
|---|---|---|---|---|---|---|---|---|
| | Hyphae | Blastospore | Septa | Hypha + Blastospore | Hypha + Arthroconidia | Hypha + Septa | | |
| Tinea corporis | 341 (36.7%) | 1 (0.1%) | 7 (0.8%) | 5 (0.5%) | 13 (1.4%) | 1 (0.1%) | 45 (4.8%) | 423 (45.5%) |
| Tinea cruris | 311 (33.4%) | 1 (0.1%) | 4 (0.4%) | 3 (0.3%) | 10 (1.1%) | 1 (0.1%) | 41 (4.4%) | 386 (41.5%) |
| Tinea capitis | 27 (2.9%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 4 (0.4%) | 0 (0.0%) | 14 (1.5%) | 48 (5.2%) |
| Tinea faciei | 19 (2.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (0.1%) | 0 (0.0%) | 5 (0.5%) | 26 (2.8%) |
| Tinea unguium | 11 (1.2%) | 1 (0.1%) | 0 (0.0%) | 0 (0.0%) | 1 (0.1%) | 0 (0.0%) | 7 (0.8%) | 24 (2.6%) |
| Tinea pedis | 8 (0.9%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 2 (0.2%) | 0 (0.0%) | 2 (0.2%) | 17 (1.8%) |
| Tinea manuum | 5 (0.5%) | 0 (0.0%) | 1 (0.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 6 (0.6%) |

The most common result of microscopic examination with 10-20% KOH was visible hyphae (n=722, 77.6%). The least examination results found were blastopores, which were in 3 patients (0.3%) and hyphae with septa in 2 patients (0.2%). Of all dermatophytosis patients, there were some patients in whom no data were found from the microscopic examination with 10-20% KOH (n=38, 4.1%).

Wood's lamp fluorescence examination is an additional examination for tinea that occurs at the location of the hair pads so it can only be applied on tinea capitis and tinea barbae. Wood's lamp examination showed positive results (fluorescent greenish-yellow) in 21 patients with tinea capitis (43.8%) and showed negative results in 2 patients (4.2%). In the other 25 patients with tinea capitis, no data was obtained on the results of the Wood's lamp fluorescence examination.

Data of species causing dermatophytosis or culture results were only obtained in 2017, as shown in Table 7. Culture examination is not routinely done, so only a small number of patients were examined for fungal culture. The most common species of dermatophytosis was *Tricophyton mentagrophytes*. *Tricophyton mentagrophytes* was found in cases of tinea corporis, cruris, and capitis. *Tricophyton rubrum* was found in tinea cruris and tinea corporis. *Tricophyton ferrugineum* was found in tinea capitis and corporis. *Epidermophyton floccosum* is found in tinea cruris, *Microsporum canis* was found in tinea capitis, *Tricophyton veruccosum* was found in tinea corporis, and *Microsporum audouinii* was found in tinea capitis.

KOH examination is performed for rapid identification of dermatophytosis. In dermatophytosis involving skin, hair and nails, septa and hyphae are visible on microscopic examination with 10-20% KOH. In dermatophytosis, the hyphae are long and branched. Since microscopic examination with KOH can present false-negative results in up to 15% of cases, patients suspected of dermatophytosis based on clinical findings should be treated.[19]

**Table 7.** Dermatophytosis culture results in Mycology Division Dermatology and Venereology Outpatient Unit Dr. Soetomo General Academic Hospital on 2017

| Species | n | % |
|---|---|---|
| *Tricophyton mentagrophytes* | 7 | 2.5 |
| *Tricophyton rubrum* | 5 | 1.8 |
| *Tricophyton ferrugineum* | 2 | 0.7 |
| *Epidermophyton floccosum* | 3 | 1.1 |
| *Microsporum canis* | 2 | 0.7 |
| *Tricophyton veruccosum* | 1 | 0.4 |
| *Microsporum audouinii* | 1 | 0.4 |

Wood's lamp fluorescence examination is an examination performed on dermatophytosis involving hair-bearing areas, such as the head of hair and beard. Examination with Wood's lamp will fluoresce greenish yellow in several types of ectotrics dermatophyte fungi such as *Microsprosum canis, Microsporum audouinii, Microsporum distortum, and Microsporum ferrugineum*, while endotrics organisms will not fluoresce.[19]

Data on the species causing dermatophytosis or culture results were only obtained in 2017. This shows that improvements need to be made to the registration and archiving of patient medical records data in the Mycology Division of Dermatology and Venereology

Outpatient Unit of Dr. Soetomo Surabaya Hospital so that further research can be more accurate results with better medical record data. Culture examination is also not a routine examination so that only a small proportion of patients are examined for fungal culture.

**Treatment**

Systemic therapy given to patients with tinea capitis in the Mycology division of the Dermatology and Venereology Outpatient Unit of RSUD Dr. Soetomo Surabaya in the period January 2017 to December 2022 consists of griseofulvin and ketoconazole while topical therapy given consists of ketoconazole shampoo and 2% ketoconazole cream. In tinea capitis, topical therapy is only an adjunct to systemic therapy. The provision of therapy in patients with tinea capitis is mostly following the 2021 PERDOSKI Clinical Practice Guidelines. [27]

The most frequent systemic therapy given to patients with tinea faciei was griseofulvin (n=19, 73.1%). Topical therapy was also given to patients with tinea faciei, and the most commonly given to patients was 2% ketoconazole cream (n=5, 19.2%). In patients with tinea faciei, the systemic therapy that was mostly given was griseofulvin. The most commonly prescribed systemic therapy is different from that listed in the 2021 PERDOSKI Clinical Practice Guidelines. Although terbinafine, itraconazole, and fluconazole are considered more effective and have better pharmacokinetic profiles, griseofulvin is also effective for dermatophytosis, including tinea faciei. Griseofulvin is likely to be preferred based on drug availability and lower cost compared to newer antifungal drugs such as terbinafine, itraconazole, and fluconazole. Griseofulvin is an effective treatment, it has a higher cost per mycologically cured infection compared to terbinafine and itraconazole. However, griseofulvin remains a viable option due to its availability and established use in clinical practice, particularly in cases where cost is a significant concern for patients or healthcare systems.[30]

The most common systemic therapy given to patients with tinea corporis is also griseofulvin, while the most common topical therapy given is ketoconazole cream. The most common topical therapy given was 2% ketoconazole cream (n=37, 8.7%), followed by 10% urea cream which was given to (n=22, 5.2%). In patients with tinea corporis in the Mycology Division of Dermatology and Venereology Outpatient Unit RSUD Dr. Soetomo Surabaya from January 2017 to December 2022, the topical and systemic therapies most commonly given were alternative therapies. The drug that is the main choice based on 2021 PERDOSKI Clinical Practice Guidelines, namely terbinafin, is a newer drug with a broader spectrum and can be given in a shorter treatment duration. However, it is more expensive than griseofulvin so it is less of an option at RSUD Dr. Soetomo Surabaya.

Griseofulvin is classified as a fungistatic agent, meaning it inhibits the growth and reproduction of fungi rather than killing them outright. This mechanism is particularly effective against dermatophytes that cause tinea infections, including *Trichophyton*, *Microsporum*, and *Epidermophyton* species. [28] Griseofulvin also has extensive experience contributes to its continued use in practice, as clinicians are familiar with its dosing regimens and potential side effects.[33] Griseofulvin is generally well-tolerated, with mild side effects such as gastrointestinal upset or headache being the most common. While resistance to antifungal medications is a growing concern, griseofulvin has not seen

the same level of resistance development as some other antifungals like terbinafine in certain populations. [28]

The most common systemic therapy given to patients with tinea cruris is the same as most other tinea, which is griseofulvin. A total of 300 tinea cruris patients (77.7% of all tinea cruris patients) received griseofulvin therapy. The most common topical therapy was ketoconazole 2% cream, which was given to 49 patients (12.7%). In patients with tinea cruris, several types of therapy were different from the Clinical Practice Guidelines, namely 2% ketoconazole shampoo, 10% urea cream, and sodium fusidate. Urea cream is used to moisturize dry skin and reduce irritation, possibly given in tinea cruris in cases of dry, scaly skin, or hyperkeratosis in the area of tinea cruris infection. Sodium fusidate is a topical antibiotic used for bacterial infections, can be given if there is secondary infection due to bacteria in the tinea cruris area.

The most common therapy given to patients with tinea pedis is systemic therapy in the form of griseofulvin (n=6, 35.3%), followed by ketoconazole (n=4, 23.5%). The most common topical therapy given was 10% urea cream (n=3, 17.6%). Most systemic and topical therapies given to patients with tinea pedis are following the 2021 PERDOSKI clinical practice guidelines.

In patients with tinea manuum, systemic therapy of griseofulvin and itraconazole was given, as well as topical therapy of 2% ketoconazole cream. The most commonly given systemic therapy is different from the 2021 PERDOSKI Clinical Practice Guidelines, namely the griseofulvin tablets. The choice of griseofulvin rather than terbinafine tablets is also likely based on considerations of drug availability and cost while maintaining treatment effectivity.

Different from other tinea, the most common systemic therapy given to tinea unguium was itraconazole (n=9, 37.5%). The most common topical therapy given was 10% urea cream (n=5, 20.8%). Based on the 2017 PERDOSKI Clinical Practice Guidelines, the preferred therapy for tinea unguium is terbinafine tablets and alternative therapy is itraconazole tablets. In patients with tinea unguium, the systemic therapy given was in accordance with the 2017 PERDOSKI PPK. In tinea unguium, urea cream is not directly given to treat fungal infections, but as a supporting therapy to soften hard nails due to infection. Thus, it will facilitate the penetration of antifungal drugs into the infected nail area.

## STRENGTH AND LIMITATION

The strength of this study was comprehensive data collection over six years, providing a solid foundation for understanding dermatophytosis trends and patterns. It also examined a wide range of variables, such as demographics, clinical characteristics, diagnostic findings, and treatments, offering a holistic perspective on the disease. Furthermore, the study assessed adherence to Clinical Practice Guidelines, ensuring the therapies followed established standards of care.

The limitation of this study was eliance on medical records may introduce information bias or missing data, which affect the accuracy of the findings. The lack of results of fungal culture during the years 2018-2022 narrows potential identification of etiological agents and their resistance patterns over this period. Apart from being a single-center study, the findings may also not be generalizable in other settings or regions in the healthcare system. Lastly, the study did not evaluate treatment outcomes or rates of recurrence, which would be important in determining long-term effectiveness of the therapies.

## CONCLUSIONS

Tinea corporis and tinea cruris was the most common, while tinea manuum is the least common dermatophytosis. Female adults were the most affected group. Common clinical features for each type included alopecia for tinea capitis, erythematous macules for other types, and nail dystrophy for tinea unguium. *Trichophyton mentagrophytes* was the commonest pathogen in 2017. Most of the therapies followed Clinical Practice Guidelines with extensive use of griseofulvin and ketoconazole cream. Further research should explore therapeutic outcomes, preventive measures, and factors influencing recurrence and adherence to treatment.

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## ETHICAL CLEARANCE

This research has been reviewed by the Ethics Committee at Dr. Soetomo General Academic Hospital.

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## CONFLICT OF INTEREST

All authors have no conflict of interest

## AUTHOR CONTRIBUTION

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting, and approval for publication of this manuscript.

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